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Frontiers in Modern Life Science Research

VOLUME I

Editors:

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PREFACE

Frontiers in Modern Life Science Research presents a broad and contemporary perspective on the rapidly evolving landscape of life sciences. The field has undergone remarkable transformation through advances in molecular biology, biotechnology, genetics, microbiology, ecology, biomedical sciences, and interdisciplinary research. This book brings together diverse scholarly contributions that reflect emerging concepts, innovative methodologies, and current research directions shaping modern life science.

The chapters explore significant themes ranging from fundamental biological processes to applied and technology-driven approaches. Contemporary developments in molecular techniques, genomics, biotechnology, bioinformatics, environmental sciences, and health-related research demonstrate how life science knowledge is increasingly connected with technological innovation and societal needs. The contributions also highlight the importance of understanding biological systems at multiple levels, from molecules and cells to organisms, ecosystems, and human populations.

A key feature of modern life science research is its interdisciplinary character. Integration of biology with computational tools, data science, nanotechnology, biotechnology, and environmental studies has created new opportunities for addressing complex scientific challenges. The chapters included in this volume reflect this integration and emphasize the relevance of collaborative and innovative approaches in advancing scientific knowledge.

The book is intended for researchers, academicians, teachers, postgraduate and undergraduate students, and professionals seeking an overview of emerging developments in life sciences. It may also serve as a useful reference for identifying research opportunities and understanding the practical significance of contemporary biological discoveries.

We hope that Frontiers in Modern Life Science Research will encourage scientific curiosity, promote interdisciplinary thinking, and contribute to continued exploration and innovation in the life sciences. The collective contributions presented in this volume reflect the dynamic nature of biological research and its growing potential to address challenges related to health, environment, agriculture, biotechnology, and sustainable development.

- Editors

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CRISPR-CAS TECHNOLOGY: EMERGING APPLICATIONS IN MODERN MICROBIOLOGY

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Abstract

CRISPR-Cas systems, consisting of clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins, are among the most significant molecular discoveries in microbiology. Initially identified as an adaptive defence mechanism in bacteria and archaea against bacteriophages and other mobile genetic elements, CRISPR-Cas systems have become powerful programmable tools for genome engineering, gene regulation, microbial strain improvement, pathogen detection and sequence-specific antimicrobial development. The use of guide RNA for sequence-specific targeting enables precise modification of microbial genomes. The discovery of additional systems, including Cas12 and Cas13, has expanded the range of nucleic acids that can be targeted and has increased the potential applications of CRISPR technology. In microbiology, CRISPR-based approaches are particularly useful for investigating gene function in pathogenic, industrial and environmentally important microorganisms. Technologies such as CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), base editing and multiplex genome engineering have extended CRISPR applications beyond conventional gene knockout. CRISPR-based diagnostic systems also provide opportunities for rapid detection of infectious agents and antimicrobial resistance markers. However, challenges related to delivery, off-target activity, toxicity, DNA repair, genetic variation, biosafety and ethical considerations remain important. This chapter discusses the biological basis, major types, mechanisms and applications of CRISPR-Cas systems in modern microbiology, with emphasis on bacterial genome engineering, antimicrobial resistance, diagnostics, industrial microbiology and future research.

Keywords: CRISPR-Cas, Genome Editing, Microbiology, Bacteria, Antimicrobial Resistance, Diagnostics and Microbial Biotechnology.

Introduction

Modern microbiology has progressed from the observation of microorganisms to detailed analysis and manipulation of their genetic material. Developments in DNA sequencing, molecular cloning, synthetic biology and computational biology have created new approaches for

understanding microbial physiology and developing microorganisms with desirable characteristics. Among these advances, CRISPR-Cas technology has emerged as an important tool because of its sequence specificity, programmability and relatively simple experimental design (Barrangou *et al.*, 2007; Hille *et al.*, 2018).

CRISPR stands for clustered regularly interspaced short palindromic repeats. These sequences are commonly associated with CRISPR-associated proteins, known as Cas proteins. Together, they form adaptive immune systems in many bacteria and archaea. CRISPR-Cas systems recognize genetic material originating from bacteriophages, plasmids and other mobile genetic elements. Short fragments of foreign nucleic acids can be incorporated as spacers within CRISPR arrays. During subsequent exposure to the same or related genetic material, RNA produced from the CRISPR array guides Cas proteins to complementary target sequences, resulting in their interference or destruction (Marraffini, 2015; Makarova *et al.*, 2020).

The natural defence function of CRISPR-Cas systems provided the basis for their development as genetic engineering tools. In laboratory applications, guide RNA can be designed to target a specific DNA or RNA sequence. Depending on the Cas protein and experimental design, the target sequence can be cleaved, repressed, activated, precisely edited or detected through collateral nucleic-acid activity. This versatility has made CRISPR technology useful in medical, agricultural, environmental and industrial microbiology.

CRISPR-Cas System: An Overview

A typical CRISPR-Cas system contains a CRISPR array and one or more *cas* genes. The CRISPR array consists of repeated DNA sequences separated by unique spacer sequences, many of which originate from previously encountered mobile genetic elements. The *cas* genes encode proteins involved in spacer acquisition, CRISPR RNA processing and interference with target nucleic acids. The organization of these components varies among microorganisms and CRISPR-Cas types (Koonin *et al.*, 2017).

The CRISPR-Cas immune response can generally be divided into three stages: adaptation, expression and interference. During adaptation, a fragment of invading DNA is selected and incorporated into the CRISPR array as a new spacer. During expression, the CRISPR array is transcribed and processed to generate CRISPR RNAs (crRNAs). During interference, a crRNA-containing effector complex identifies a complementary nucleic-acid sequence and targets it for destruction or inhibition. These natural processes form the biological basis for programmable CRISPR applications.

Discovery and Development of CRISPR Technology

Unusual repeated DNA sequences were identified in bacterial genomes before their biological function was understood. Further investigations revealed that these repeats were separated by variable spacer sequences and were associated with cas genes. Studies examining the

relationship between CRISPR loci and bacteriophage resistance demonstrated that CRISPR-Cas systems provide adaptive immunity in bacteria. Barrangou *et al.* (2007) demonstrated that acquisition of new spacers could increase resistance to bacteriophage infection in *Streptococcus thermophilus*.

A major technological advance occurred with the development of the type II CRISPR-Cas9 system as a programmable RNA-guided DNA-cleavage platform. Jinek *et al.* (2012) demonstrated programmable DNA cleavage, and subsequent research established its potential for genome editing in cells. Cas9-based genome editing was followed by the discovery and engineering of additional CRISPR effectors, including Cas12 and Cas13. These systems differ in their target-recognition mechanisms, nucleic-acid substrates and cleavage activities, thereby expanding the CRISPR toolkit available for microbiological research.

Structure and Components of CRISPR-Cas Systems

Engineered CRISPR systems primarily consist of a CRISPR-associated effector protein and a guide RNA. Cas9 is a widely used DNA-targeting nuclease. It forms a complex with guide RNA and recognizes a target sequence located adjacent to a protospacer adjacent motif (PAM). Recognition of the guide-target sequence activates the nuclease domains of Cas9, resulting in cleavage of the DNA strands. The resulting DNA break can be repaired through cellular mechanisms, leading to targeted mutations or sequence replacement.

Cas12 proteins represent another important group of DNA-targeting CRISPR effectors.

Many Cas12 systems recognize different PAM sequences and generate staggered DNA breaks. Some Cas12 enzymes can also process their own guide-RNA arrays, making them useful for multiplex genome editing.

Cas13 proteins primarily target RNA. After binding to a complementary RNA sequence, certain Cas13 proteins activate collateral RNase activity. This property has been adapted for nucleic-acid detection and diagnostic applications (Wu *et al.*, 2024; Yang and Patel, 2024).

Mechanism of CRISPR-Cas-Mediated Gene Editing

In a conventional CRISPR-Cas9 editing system, a guide RNA is designed to complement a selected genomic sequence. The guide RNA associates with Cas9 and directs the nuclease to the target DNA. Successful targeting requires complementarity between the guide RNA and DNA sequence as well as the presence of an appropriate PAM. Cas9 then introduces a targeted double-strand DNA break.

Following DNA cleavage, the microorganism's DNA repair mechanisms determine the resulting genetic modification. Repair may produce small insertions or deletions that disrupt gene function. Alternatively, a donor DNA template can be provided to introduce a defined sequence change. In bacteria, editing efficiency and outcome can be affected by DNA repair pathways, cellular physiology and toxicity caused by prolonged nuclease activity. Therefore, successful

genome editing depends on appropriate guide design, expression levels, delivery methods and repair strategies (Arroyo-Olarte *et al.*, 2021).

CRISPR technology also enables gene regulation without DNA cleavage. Catalytically inactive Cas proteins, known as dead Cas or dCas proteins, can bind specific DNA sequences without cutting them. When dCas is directed to a promoter or coding region, transcription can be reduced or blocked. This approach is known as CRISPR interference (CRISPRi). Conversely, dCas fused with an activating domain can increase gene expression and is referred to as CRISPR activation (CRISPRa). These systems are particularly useful for studying essential genes because they allow gene expression to be reduced or increased without permanently deleting the gene.

Major Types of CRISPR-Cas Systems

CRISPR-Cas systems are broadly classified into two classes according to the organization of their effector machinery. Class 1 systems use multi-protein effector complexes, whereas Class 2 systems generally use a single large effector protein. Each class contains several types and subtypes with distinct molecular characteristics (Makarova *et al.*, 2020).

Type II systems containing Cas9 are among the most widely studied because of their relatively simple single-effector organization. Type V systems include Cas12 proteins and provide alternative DNA-targeting functions. Type VI systems contain Cas13 proteins that target RNA. The diversity of CRISPR systems is particularly important in microbiology because microorganisms that are difficult to modify using Cas9 may respond more effectively to alternative CRISPR effectors or their native CRISPR systems.

Applications of CRISPR-Cas Technology in Microbiology

CRISPR-Cas technology has a wide range of applications in microbiology, including targeted genome editing, functional genomics, gene regulation, microbial strain improvement, antimicrobial development, pathogen detection, microbial typing and synthetic biology. Precise genetic modification has improved the study of microbial genes and biological pathways that were previously difficult to investigate.

In bacterial functional genomics, CRISPR can be used to disrupt genes, modify promoters, regulate transcription and generate multiple genetic mutations. These approaches enable researchers to investigate relationships between genotype and phenotype more efficiently. CRISPRi is particularly useful for studying essential genes because partial repression can provide information about gene function without immediately causing cell death. CRISPR-based screening can also be used to evaluate multiple genetic targets simultaneously.

In industrial microbiology, engineered bacteria and fungi can function as microbial cell factories for producing enzymes, organic acids, amino acids, vitamins, biofuels and other valuable compounds. Multiplex CRISPR editing enables simultaneous modification of multiple metabolic pathways, helping redirect cellular resources towards desired products (Zhang *et al.*, 2019).

CRISPR-Cas in Antimicrobial Resistance

Antimicrobial resistance (AMR) is a major challenge in infectious disease microbiology. Conventional antibiotics may affect broad groups of bacteria and can create selective pressure that favours resistant populations. CRISPR-Cas systems provide an alternative approach based on sequence-specific antimicrobial activity. A CRISPR effector can be programmed to recognize resistance genes, virulence genes or strain-specific sequences. Depending on the bacterial system, targeting may result in plasmid elimination or bacterial cell death (Bikard and Barrangou, 2017).

CRISPR-based antimicrobials may therefore complement conventional antibiotic approaches. One important research objective is the selective removal of pathogenic or antibiotic-resistant bacteria while minimizing effects on beneficial microbiota. However, effective delivery remains a major challenge. Potential delivery approaches include bacteriophages, phage-derived particles and other engineered delivery systems. Because CRISPR targeting depends on sequence recognition, genetic variation among bacterial strains must also be considered during antimicrobial design.

CRISPR-Cas for Bacterial Pathogen Detection

CRISPR effectors have been adapted for molecular diagnostic applications. Cas12 and Cas13 systems can produce detectable signals after recognizing specific nucleic-acid targets. In some diagnostic platforms, target recognition activates collateral cleavage of reporter molecules. The resulting activity can be converted into fluorescence or other measurable signals.

CRISPR-based diagnostics have been investigated for detecting bacterial and viral pathogens, antimicrobial resistance determinants and other nucleic-acid targets. Their major advantages include high sequence specificity, programmable targeting and the potential for rapid testing.

When combined with isothermal amplification methods, CRISPR-based assays may be adapted for settings with limited laboratory infrastructure. However, sample preparation, target amplification, assay sensitivity, specificity and contamination control remain important factors that must be addressed before routine clinical or field use.

CRISPR-Cas in Microbial Biotechnology

Microbial biotechnology relies on understanding and modifying microbial metabolism. CRISPR-based genome engineering provides researchers with greater control over metabolic pathways. Genes involved in competing pathways can be reduced or removed, biosynthetic genes can be introduced, and regulatory sequences can be modified to achieve better control of metabolic flux. In food microbiology, CRISPR technologies are being investigated for improving starter cultures and other beneficial microorganisms. Potential applications include modifying fermentation performance, stress tolerance, flavour development and the production of beneficial metabolites.

In environmental microbiology, engineered microorganisms may be investigated for pollutant degradation, carbon conversion and production of environmentally useful compounds. Such applications require appropriate containment measures and careful assessment of ecological risks before any environmental release.

CRISPR is also an important technology in synthetic biology, where biological components are combined to construct predictable genetic circuits. Programmable gene repression and activation can be used to develop microbial systems that detect environmental signals, produce valuable compounds or perform defined biological functions. Continued discovery of new CRISPR effectors is expected to further expand microbial engineering strategies.

Advantages and Limitations

CRISPR-Cas technology offers several important advantages, including programmability, sequence specificity, flexibility and the availability of diverse effector systems. In many applications, a new target can be addressed by modifying the guide RNA rather than redesigning the entire effector protein. This simplifies experimental development and allows researchers to investigate multiple genes.

CRISPR also supports multiplex genome editing, enabling several genetic targets to be modified within a single experimental system. dCas-based systems allow reversible or adjustable gene regulation, while base editors and prime-editing approaches provide opportunities for precise sequence modification without depending on conventional double-strand DNA breaks. These characteristics are valuable for microbial genetics and synthetic biology.

However, CRISPR-Cas technology is not equally effective in all microorganisms. Off-target activity, guide-dependent variation, PAM requirements, delivery limitations and nuclease toxicity can affect editing efficiency. Some bacteria are difficult to transform or possess defence mechanisms that interfere with genome editing. Genetic variation within microbial populations may also reduce the effectiveness of a single guide sequence. Consequently, careful experimental validation is necessary.

Ethical and Biosafety Considerations

Genome editing of microorganisms raises important biosafety concerns. Genetic modifications may affect microbial growth, metabolism, interactions with hosts, antimicrobial susceptibility or environmental fitness. When engineered microorganisms are considered for use outside controlled laboratory conditions, their potential ecological effects must be carefully evaluated. Risk assessment should therefore be incorporated into experimental planning from the beginning. The development of CRISPR-based antimicrobials also requires careful consideration of target specificity and delivery. Researchers should minimize unintended genetic effects and follow appropriate containment procedures. Institutional biosafety guidelines, national regulations and

established laboratory practices should be followed when working with pathogenic or genetically modified microorganisms.

Future Perspectives

The future development of CRISPR technology in microbiology is expected to focus on increasingly precise and diverse molecular tools. New Cas proteins continue to be identified from the natural diversity of bacteria and archaea, while engineered variants are being developed with modified specificity, activity and PAM recognition. Cas12 and Cas13 systems have already expanded CRISPR applications beyond the conventional Cas9-based DNA-editing model.

Integration of CRISPR technology with artificial intelligence and computational biology may improve guide-RNA design, off-target prediction and analysis of large microbial datasets. Combining CRISPR with metagenomics and single-cell technologies may enable more detailed investigation of complex microbial communities. In clinical microbiology, sequence-specific pathogen detection and antimicrobial strategies may support improved responses to resistant microorganisms.

In industrial microbiology, improved multiplex editing and genome-scale regulation may accelerate the development of microbial cell factories. Future approaches may combine CRISPR editing, automated screening and metabolic modelling to develop microorganisms with optimized production characteristics. At the same time, technological advances should be accompanied by effective biosafety measures, quality control and ethical oversight.

Conclusion

CRISPR-Cas technology has transformed modern microbiology by converting a natural microbial defence mechanism into a versatile platform for genetic analysis and engineering. Its applications range from fundamental studies of bacterial gene function to microbial strain improvement, antimicrobial resistance research, pathogen detection and industrial biotechnology. Cas9, Cas12 and Cas13 provide complementary capabilities for targeting DNA and RNA, while CRISPRi, CRISPRa and newer precision-editing technologies have expanded the range of possible genetic and regulatory applications.

Despite its broad potential, CRISPR-Cas technology is not a universal solution. Challenges involving delivery, target specificity, microbial physiology, genetic diversity and biosafety continue to influence its application. Continued discovery of new Cas effectors and development of improved engineering strategies may enhance the accuracy, efficiency and accessibility of CRISPR systems. For microbiology, the integration of CRISPR with genomics, synthetic biology, diagnostics and computational approaches provides important opportunities for future research and practical applications.

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SYNTHETIC BIOLOGY AND BIOENGINEERING: ADVANCES, APPLICATIONS AND FUTURE PERSPECTIVES

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Abstract

Synthetic biology and bioengineering are swiftly advancing disciplines that amalgamate microbiology, molecular biology, genetics, biotechnology, and engineering concepts to create novel biological systems aimed at tackling contemporary issues. Synthetic biology facilitates the creation, alteration, and enhancement of biological elements and microbial systems to generate useful substances with greater efficiency and sustainability. Bioengineering further facilitates the advancement of modified microbes, biomaterials, biosensors, and bioprocesses for use in healthcare, agriculture, food technology, and environmental stewardship. Recent advances in genome engineering, metabolic engineering, CRISPR-based technologies and computational biology have enhanced the ability to understand and manipulate biological systems. These approaches contribute to the production of therapeutic molecules, enzymes, biofuels, biodegradable materials and other value-added products while reducing dependence on conventional chemical processes. Engineered microbial systems present advantageous approaches for biosensing, bioremediation, and sustainable industrial manufacturing. The amalgamation of synthetic biology and bioengineering offers a robust foundation for creating accurate, effective, and ecologically sustainable biological remedies. Continued research in these areas is expected to expand the applications of modern life sciences and contribute significantly to human health, industrial biotechnology and sustainable development.

Keywords: Synthetic Biology, Bioengineering, Microbial Biotechnology, Genome Engineering, and Industrial Biotechnology.

Introduction

Synthetic biology combines biology, engineering and computational approaches to design and modify biological systems for specific purposes. It draws on microbiology, molecular genetics and systems biology to treat living cells as programmable systems. Bioengineering applies these principles to develop useful products, devices, materials and processes for laboratory, medical and industrial applications.

Together, synthetic biology and bioengineering have expanded applications in healthcare, agriculture, energy, food technology and environmental management. Microbial organisms such as *Escherichia coli*, *Saccharomyces cerevisiae* and cyanobacteria can be engineered to carry out specific biochemical functions. This chapter discusses the major concepts, technologies, applications, challenges and future directions of synthetic biology and bioengineering.

Synthetic Biology: Concepts, Principles and Tools

Synthetic biology uses engineering principles such as standardization, modularity and abstraction to design biological systems. Genetic components, including promoters, ribosome-binding sites, coding sequences and terminators, can be combined to construct functional genetic devices and systems. Standardized biological parts facilitate the design, testing and reuse of genetic components.

Design-Build-Test-Learn Cycle

The Design-Build-Test-Learn (DBTL) cycle is a central approach in synthetic biology. First, computational tools are used to design genetic circuits or metabolic pathways. DNA synthesis and assembly methods, including Gibson assembly and Golden Gate cloning, are then used to construct the desired systems. The engineered organisms are experimentally tested, and the results are analysed and used to improve subsequent designs.

Automation, high-throughput screening and laboratory robotics have increased the speed and scale of this process, allowing many genetic variants to be evaluated simultaneously.

Genetic Circuits and Biological Parts

Synthetic gene circuits can control cellular responses to specific environmental or biological signals. Examples include toggle switches, oscillators and logic gates. These systems have been used to develop cellular memory devices, biological counters and biosensors for detecting metabolites, toxins and pathogens.

Advances in gene regulation, RNA-based control systems and orthogonal biological components have improved the performance of synthetic circuits while reducing interference with the host cell's natural processes.

Genome Engineering and CRISPR-Based Technologies

Genome engineering enables targeted modification of genetic material. CRISPR-Cas9 has become an important tool because it allows specific DNA sequences to be targeted using guide RNA. Cas9 can introduce targeted DNA breaks, which can be repaired through cellular repair mechanisms such as non-homologous end joining or homology-directed repair. These approaches can be used for gene disruption, insertion and sequence correction.

Expanding The Crispr Toolbox

CRISPR technology has developed beyond conventional gene editing. CRISPR interference (CRISPRi) can reduce gene expression, while CRISPR activation (CRISPRa) can increase gene

expression without changing the DNA sequence. Base editors and prime editors enable precise genetic changes with reduced dependence on double-strand DNA breaks.

Multiplex CRISPR systems can modify several genomic regions simultaneously, supporting the engineering of microorganisms with complex metabolic pathways.

Applications in Strain Engineering

Genome engineering is used for:

- Construction of microbial strains with improved metabolic production.
- Identification of genes associated with desirable characteristics through functional genomics.
- Investigation of genetic mutations relevant to gene therapy.
- Development of gene-drive systems for research on vector and pest population control.

Genome engineering therefore provides an important foundation for many applications of synthetic biology.

Metabolic Engineering for Bioproduction

Metabolic engineering modifies cellular metabolic pathways to increase the production of specific compounds. Applications include the production of pharmaceuticals, enzymes, biofuels and specialty chemicals. Common strategies include increasing the expression of rate-limiting enzymes, removing competing pathways and optimizing cofactor availability.

Systems biology and computational methods support metabolic engineering. Genome-scale metabolic models, flux balance analysis and machine-learning approaches can help predict suitable genetic modifications before laboratory testing.

Examples include microbial production of artemisinic acid, engineered yeast for the production of pharmaceutical and specialty compounds, and bacterial systems designed to produce biofuel-related compounds such as isobutanol and fatty-acid-derived hydrocarbons.

Bioengineering Applications in Healthcare

Bioengineering has extended synthetic biology into healthcare. Engineered microorganisms are being investigated as living therapeutic systems that can detect specific biological signals and release therapeutic molecules. Genetic engineering is also used to modify immune cells, including the development of chimeric antigen receptor (CAR) T-cell therapies for cancer treatment. Synthetic biology has contributed to the production of vaccines and therapeutic proteins. Cell-free protein synthesis systems use biological extracts to produce proteins and other biomolecules without maintaining living cells. These systems can support rapid production of vaccines, diagnostics and biologics, particularly when speed and portability are important.

Biosensors and Diagnostic Platforms

Synthetic biology can be used to construct biosensors that detect specific biological or chemical substances. These systems combine recognition components such as transcription factors,

riboswitches or aptamers with measurable outputs, including fluorescence, colour changes or electrical signals.

Biosensors can be designed to detect pathogens, environmental toxins, heavy metals and metabolic biomarkers. Whole-cell biosensors use living microorganisms as sensing systems and can provide low-cost detection platforms.

Paper-based and cell-free biosensors have further expanded the potential for field and point-of-care applications, including infectious disease diagnosis and water-quality monitoring.

Bioremediation and Environmental Applications

Environmental contamination by industrial effluents, plastics, heavy metals and agricultural chemicals is a major concern. Synthetic biology can support bioremediation by engineering microorganisms with metabolic pathways capable of degrading or sequestering pollutants.

Major areas of application include:

- Engineering bacteria to produce plastic-degrading enzymes such as PETase for polyethylene terephthalate degradation.
- Developing microbial systems for heavy-metal biosorption and bioaccumulation.
- Engineering rhizosphere bacteria to support phytoremediation of contaminated soils.
- Developing microorganisms capable of degrading pesticides, dyes and petroleum hydrocarbons.

These approaches may provide biological alternatives to conventional chemical and energy-intensive remediation methods.

Applications in Agriculture and Food Technology

In agriculture, synthetic biology can be applied to develop nitrogen-fixing microbial inoculants, biopesticides and microorganisms that support plant growth and stress tolerance. Engineered plant-growth-promoting rhizobacteria may improve nutrient availability and contribute to plant protection. In food technology, precision fermentation uses microorganisms to produce animal-free proteins, dairy alternatives, vitamins, flavour compounds and functional enzymes. These technologies provide alternative methods for producing food ingredients and may reduce some of the environmental impacts associated with conventional production systems.

Industrial Biotechnology and Sustainable Manufacturing

Industrial biotechnology uses microorganisms as biological production systems for chemicals, materials and fuels. Engineered microorganisms can produce platform chemicals, biodegradable polymers such as polyhydroxyalkanoates (PHAs) and biofuels. Such processes can reduce dependence on petrochemical raw materials. Bioprocess engineering is essential for converting laboratory research into industrial production. It includes fermentation optimization, product recovery, purification and process scale-up. Process monitoring and analytical technologies can improve production consistency and product quality.

Computational Biology and Systems-Level Design

Computational biology is an important component of modern synthetic biology. Genome-scale modelling, machine learning and artificial intelligence can assist in genetic circuit design, metabolic pathway optimization and analysis of large multi-omics datasets. Computer-aided design tools allow researchers to model and evaluate biological systems before constructing them experimentally. This reduces unnecessary experimental work and improves the efficiency of the Design-Build-Test-Learn cycle.

Challenges and Ethical Considerations

Synthetic biology and bioengineering face several technical, environmental and societal challenges. The expression of foreign genes can impose a metabolic burden on host organisms and affect their growth and stability. In addition, biological systems can behave differently from computational predictions because of their complexity.

Biosafety and biosecurity are also important considerations. Engineered organisms may have unintended effects if they persist or spread in natural environments. Some genome-engineering technologies also have potential dual-use concerns. Risk management requires appropriate regulatory systems, biological containment strategies and responsible research practices. Approaches such as genetic kill switches and auxotrophic dependency can be investigated to reduce the environmental persistence of engineered organisms. Public engagement and transparent governance are also important for responsible development.

Future Perspectives

The integration of synthetic biology with artificial intelligence, automation and materials science is expected to improve the design and construction of biological systems. Emerging areas include cell-free synthetic biology, minimal cell systems and xenobiology.

Xenobiology investigates biological systems based on alternative biochemical components, including non-natural nucleic acids and amino acids. These approaches may expand the range of biological systems that can be designed and engineered.

Continued advances in DNA sequencing, synthesis, editing, automation and computational modelling are expected to strengthen applications of synthetic biology in healthcare, food production, environmental management and industrial biotechnology.

Conclusion

Synthetic biology and bioengineering have changed the way biological systems are studied and developed by enabling their purposeful design and modification. Technologies such as genome engineering, CRISPR-based editing, metabolic engineering, biosensors and computational modelling have expanded the capabilities of engineered biological systems. The integration of biological knowledge with engineering and computational approaches provides opportunities for developing applications in healthcare, agriculture, environmental management and industrial

biotechnology. Continued research, appropriate biosafety measures and responsible governance will be important for the effective development and application of these technologies.

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FROM FOOT TO FLOOR: EXPLORING THE HIDDEN MICROBIAL WORLD OF EVERYDAY FOOTWEAR

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Abstract

The human foot harbors a diverse microbial community that interacts continuously with the skin, footwear, and surrounding environment. Everyday footwear provides a unique microenvironment characterized by moisture, warmth, sweat, and limited ventilation, which can support the survival and accumulation of microorganisms. Bacteria and fungi associated with the human foot may become transferred to footwear surfaces and persist within shoes, sandals, and other commonly used footwear. Over time, microbial attachment and biofilm formation may contribute to unpleasant odors and influence the microbial quality of footwear and surrounding surfaces. This chapter explores the hidden microbial world of everyday footwear, with emphasis on the human foot microbiome, microbial habitats within footwear, common bacteria and fungi associated with foot and footwear surfaces, and factors influencing microbial growth. It further discusses the potential movement of microorganisms from the foot to footwear and from footwear to floor surfaces, highlighting the possible role of footwear in microbial transmission and environmental contamination. The chapter also examines the potential health implications of footwear-associated microorganisms and emphasizes appropriate foot and footwear hygiene, cleaning, drying, and ventilation practices. Understanding the interactions among the foot, footwear, and environment provides valuable insights into personal hygiene, environmental microbiology, and public health. Further research using culture-independent and molecular approaches may improve our understanding of footwear-associated microbial communities and their significance in everyday life.

Keywords: Foot Microbiome, Footwear Microbiome, Bacteria, Fungi, Biofilm, Foot Hygiene.

1. Introduction

The human body is naturally inhabited by a diverse community of microorganisms, collectively known as the human microbiota. These microorganisms include bacteria, fungi, viruses, and other microbial groups that live on different body surfaces and interact continuously with the human host and the surrounding environment. The skin, as the largest organ of the human body,

provides different ecological niches that support distinct microbial communities. The composition of skin microbiota varies according to anatomical location and is influenced by factors such as moisture, temperature, pH, hygiene, lifestyle, and environmental conditions (Grice and Segre, 2011).

The human foot represents a unique microbial environment because it is frequently enclosed within footwear for prolonged periods. Sweat, warmth, moisture, and limited ventilation inside footwear can create conditions that support the survival and accumulation of microorganisms. The foot contains different anatomical regions with varying levels of moisture, oxygen availability, sweat gland distribution, and skin characteristics. These differences contribute to the formation of distinct microbial niches on the foot (Marshall *et al.*, 1987).

Everyday footwear, including shoes, sandals, slippers, and sports footwear, can act as a temporary habitat for microorganisms. Footwear surfaces may receive microorganisms from the skin of the foot, socks, sweat, dust, soil, and surrounding environmental surfaces. Depending on footwear material and environmental conditions, microorganisms may attach to inner and outer surfaces and persist for varying periods. Repeated use without adequate cleaning and drying may contribute to microbial accumulation.

The interaction between the foot and footwear is therefore a dynamic microbial exchange. Microorganisms can move from the human skin to footwear and may subsequently be transferred the potential movement of microorganisms from the foot to footwear and environmental surfaces.

2. Human Foot Microbiome

The human foot is considered one of the most diverse microbial niches of the body. The microbial community of the foot includes bacteria and fungi that normally live on the skin, together with microorganisms acquired from the environment. Different regions of the foot can possess different microbial communities because of variations in skin structure, moisture, sweat production, pH, and oxygen availability (Marshall, 1987). to floors and other environmental surfaces during walking. Similarly, footwear can acquire microorganisms from contaminated surfaces and transport them to new locations. This interaction creates a microbial pathway connecting the foot, footwear, and environment.

Microbial attachment to footwear surfaces may, under suitable conditions, lead to biofilm formation. Biofilms are organized microbial communities embedded in a self-produced extracellular matrix. Biofilm formation can promote microbial persistence and survival under environmental stress. Moisture, nutrients, temperature, ventilation, and surface characteristics may influence microbial attachment and persistence (Flemming and Wingender, 2010).

Therefore, exploring the hidden microbial world of everyday footwear is relevant to personal hygiene, environmental microbiology, and public health. This chapter focuses on the human foot

microbiome, footwear as a microbial habitat, microorganisms associated with footwear, microbial attachment and biofilm formation, and

Common bacterial groups associated with healthy foot skin include members of the families *Corynebacteriaceae* and *Micrococcaceae*, together with representatives of *Staphylococcaceae*, *Streptococcaceae*, and other bacterial groups. Some microorganisms are normal members of the skin microbiota and may contribute to the ecological balance of the skin. However, changes in the local environment can sometimes favor the growth of opportunistic microorganism (Marshall, 1987).

Fungal microorganisms are also important components of the foot microbiome. Yeasts and filamentous fungi may be present on healthy feet, while dermatophytes such as *Trichophyton*, *Microsporum*, and *Epidermophyton* are associated with superficial fungal infections. The presence of a microorganism on the foot does not necessarily indicate disease; rather, disease depends on factors such as host susceptibility, microbial characteristics, skin barrier condition, and environmental conditions (Berenji, 2020).

The foot microbiome is influenced by several factors. Moisture and perspiration can increase the availability of water and nutrients for microorganisms. Temperature and ventilation also influence microbial survival. Personal hygiene, frequency of washing, sock use, footwear type, and duration of footwear use may further affect microbial communities. Thus, the foot microbiome should be considered a dynamic ecosystem rather than a fixed microbial population.

3. Footwear as a Microbial Habitat

Footwear creates a distinct microenvironment that differs from the external environment. Shoes, slippers, sandals, and sports footwear vary considerably in their materials, construction, ventilation, and ability to retain moisture. Closed footwear generally provides greater protection from the external environment but may also create conditions favorable for microbial persistence when moisture and heat accumulate.

The internal microclimate of footwear is influenced by temperature, humidity, ventilation, physical activity, and footwear design. Research investigating footwear microclimate has shown that footwear type can influence temperature and humidity inside shoes. Higher humidity and temperature, together with reduced ventilation, may be associated with increased bacterial growth on plantar skin. This demonstrates the importance of footwear design in shaping the microbial environment of the foot.

Sweat is an important factor in the footwear microenvironment. Human sweat itself is not necessarily responsible for unpleasant odor, but microorganisms can metabolize components of sweat and other organic materials, producing volatile compounds associated with foot odor. Repeated use of footwear may allow microorganisms and organic residues to accumulate, particularly when footwear is not adequately dried between uses.

Footwear material may also influence microbial attachment. Fabric, leather, rubber, foam, and synthetic materials possess different surface properties and moisture-retention capacities. Rough or porous materials may provide suitable sites for microbial attachment, whereas smooth surfaces may be easier to clean. However, the microbial behavior of a particular footwear material depends on several interacting factors.

The external surface of footwear has a different microbial environment from the internal surface. The inner surface is more closely associated with the foot and sweat, whereas the outer sole is exposed to soil, dust, water, floors, and other environmental materials. Consequently, footwear can act as a bridge between the human body and the external environment.

4. Associated with Everyday Footwear Microorganisms

Microorganisms associated with footwear may originate from two major sources: the human body and the external environment. The inner surface of footwear is likely to receive microorganisms from the foot, socks, and perspiration. The outer surface, particularly the sole, may acquire microorganisms from floors, soil, dust, water, and other contaminated surfaces.

Bacteria commonly associated with human skin include members of the genera *Staphylococcus*, *Corynebacterium*, and *Micrococcus*. Some of these microorganisms are part of the normal microbiota, while others may act as opportunistic pathogens under suitable conditions. Environmental bacteria may also be introduced onto footwear surfaces during outdoor activities. Fungi associated with feet and footwear may include yeasts, molds, and dermatophytes. *Dermatophytes* are particularly important because they can cause superficial infections of keratinized tissues. Warm and moist conditions inside footwear may favor the persistence of some fungal organisms, especially when footwear is repeatedly used without sufficient drying.

The microbial composition of footwear can vary according to individual habits, geographical location, climate, footwear type, occupation, physical activity, and hygiene practices. Therefore, there is no single universal microbial community associated with all footwear.

Table 1: Examples of microorganisms associated with foot and footwear environments

Microbial Group	Example	General Significance
Skin associated bacteria	<i>Staphylococcus</i> , <i>corynebacterium</i> , <i>Micrococcus</i>	Normal microbiota, some may be opportunistic
Environmental bacteria	<i>Bacillus</i> and other environmental organism	May be acquired from soil and surface
Yeasts	<i>Candida</i> , <i>Malassezia</i>	May occur as part of skin microbiota
Filamentous fungi	<i>Aspergillus</i> and related molds	Environmental occurrence
Dermatophytes	<i>Trichophyton</i> , <i>Microsporum</i> , <i>Epidermophyton</i>	Associated with superficial fungal infections

The identification of microorganisms from footwear requires appropriate microbiological methods. Culture-based techniques can identify organisms capable of growing under selected laboratory conditions. Molecular approaches, including sequencing and metagenomics analysis, may provide a more comprehensive picture of microbial diversity (Grice, 2011).

5. Microbial Contamination and Biofilm Formation

Microbial contamination of footwear occurs when microorganisms become deposited on the inner or outer surfaces. The process may begin with direct contact between the foot and footwear or through contact with contaminated environmental surfaces. Once deposited, microorganisms may survive, become temporarily attached, or establish more persistent communities depending on environmental conditions.

Biofilm formation is an important mechanism of microbial persistence on surfaces. Biofilms are structured communities of microorganisms surrounded by an extracellular polymeric substance matrix. The general stages of biofilm development include initial attachment, irreversible adhesion, micro colony formation, maturation, and dispersal (Flemming, 2010).

In footwear, moisture and organic matter may support microbial attachment and growth. Repeated use may increase the availability of nutrients derived from sweat, skin cells, and environmental contaminants. Poor ventilation may further contribute to microbial persistence.

Biofilm formation can have practical consequences. Microorganisms within biofilms may be more resistant to environmental stress and may be difficult to remove completely through simple cleaning. However, the formation and persistence of biofilms on everyday footwear depend on the specific microorganisms, footwear material, moisture conditions, and duration of exposure.

6. From Foot to Floor: Microbial Transmission

Footwear provides a potential pathway for microbial movement between the human body and environmental surfaces. During walking, microorganisms present on the foot or footwear may come into contact with floors, carpets, soil, and other surfaces. At the same time, footwear may acquire microorganisms from the environment and transport them to other locations.

The microbial transmission pathway can therefore be represented as:

Human Foot → Footwear → Floor

Environmental Surface → Footwear → Human Foot

The extent of microbial transfer depends on several factors, including microbial load, surface characteristics, moisture, friction, walking activity, footwear material, and environmental conditions. Research on footwear and floor contamination has demonstrated that walking can contribute to the distribution of microorganisms on floors (Hambræus and Malmberg, 1979). This interaction may be particularly important in healthcare environments. Studies have demonstrated that microorganisms present on footwear or slipper socks can be transferred

between floors and other surfaces. However, the level of risk varies according to the microorganism, environment, cleaning practices, and susceptibility of individuals.

MICROBIAL TRANSMISSION DIAGRAM: FROM FOOT TO FLOOR AND BEYOND

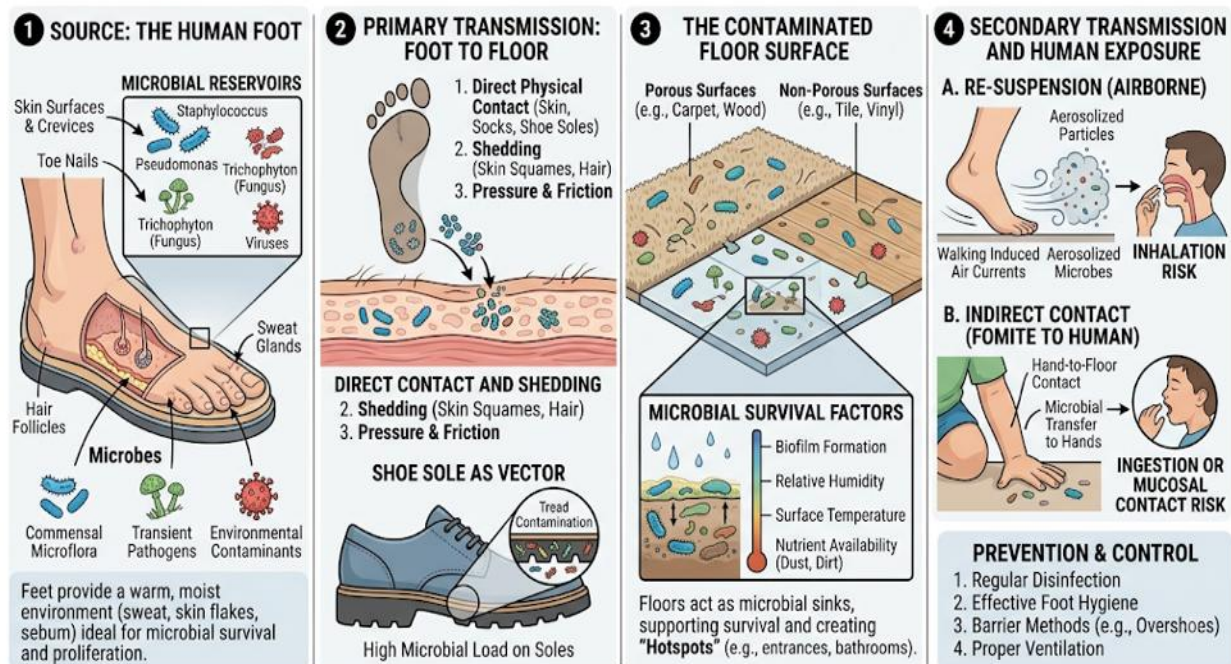


Figure 1: Microbial transmission

The concept of foot-to-floor transmission does not imply that all microorganisms transferred by footwear are harmful. Many microorganisms found on footwear are harmless environmental organisms or members of normal human microbiota. Nevertheless, understanding microbial movement through footwear can contribute to better hygiene practices and environmental infection-control strategies.

7. Health and Environmental Implications

The microbial communities associated with feet and footwear can have both beneficial and potentially harmful aspects. The normal foot microbiota forms part of the natural skin ecosystem and may contribute to microbial balance. However, disruption of the microbial community or favorable environmental conditions may contribute to microbial overgrowth or infection in susceptible individuals.

One common concern associated with the foot is fungal infection. Dermatophytes can cause infections of the skin and nails, while other microorganisms may be involved in different foot-related conditions. Warm and moist environments can favor fungal persistence, making foot hygiene and adequate drying important preventive measures (Berenji (2020)).

Footwear may also contribute to environmental microbial movement. Contaminated footwear soles may transfer microorganisms to floors and other surfaces. This may be relevant in hospitals, laboratories, schools, homes, and public buildings. However, environmental

contamination should be interpreted carefully because microbial presence alone does not necessarily indicate a health risk. The potential health significance of footwear-associated microorganisms depends on several factors, including the type and quantity of microorganisms, duration of exposure, environmental conditions, and individual susceptibility. Therefore, the presence of microorganisms should not automatically be considered evidence of disease.

8. Footwear Hygiene and Microbial Control

Proper foot and footwear hygiene can help reduce excessive microbial accumulation. Regular washing of the feet and thorough drying, particularly between the toes, are important personal hygiene practices. Clean socks should be used regularly, and damp footwear should be allowed to dry completely before reuse.

Footwear should be cleaned according to the manufacturer's recommendations and the material from which it is made. Where possible, footwear should be stored in a well-ventilated environment. Alternating between pairs of footwear may provide additional time for drying.

Ventilation is particularly important because moisture retention can promote microbial persistence. Breathable footwear materials may help reduce humidity around the foot. However, footwear choice alone cannot eliminate microorganisms because microbial exposure is a normal part of everyday life.

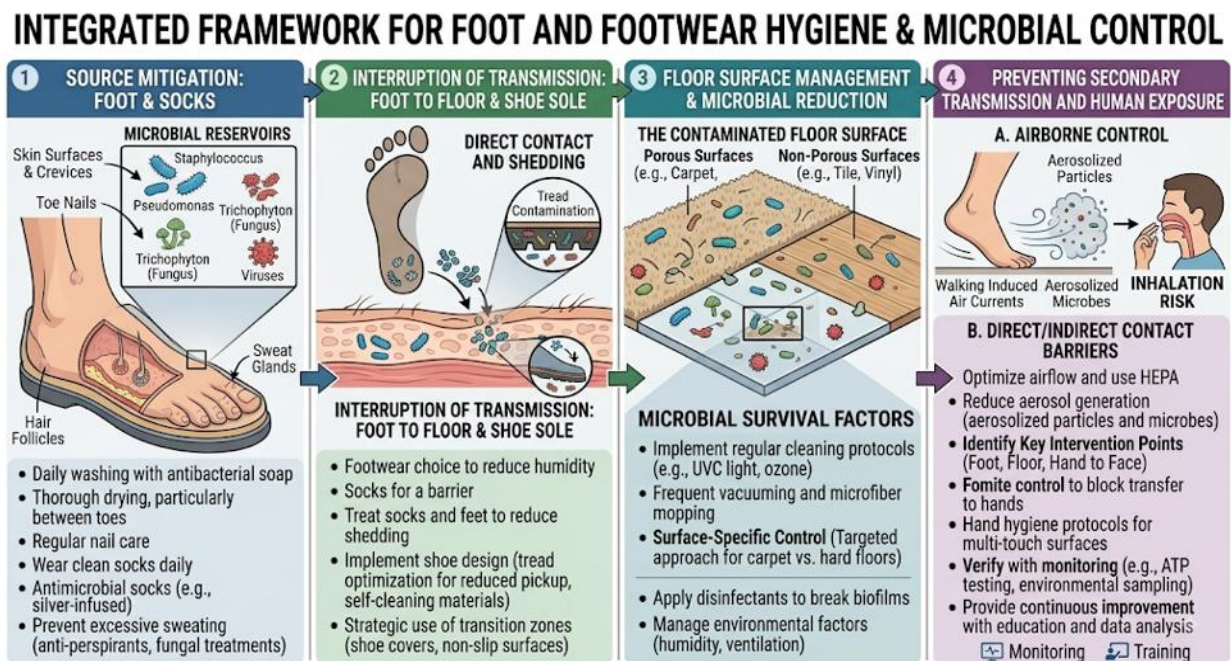


Figure 2: Footwear hygiene and Microbial control

In healthcare and other high-risk environments, footwear hygiene may be integrated with broader infection-control practices. Studies have investigated methods for reducing contamination of shoe soles and floors, including specialized decontamination approaches. Such approaches should be evaluated according to the environment and should not replace established infection-control practices.

9. Prevention and Recommendations

The following practices may help maintain a healthier foot and footwear environment:

- Maintain regular foot hygiene.
- Dry the feet thoroughly after washing.
- Change socks regularly.
- Avoid prolonged use of damp footwear. regular
- Allow footwear to dry completely between uses.
- Maintain adequate ventilation during footwear storage.
- Clean footwear according to its material and manufacturer's instructions.
- Avoid sharing personal footwear when possible.
- Pay attention to persistent foot symptoms and seek appropriate medical advice when needed.
- In healthcare and high-risk environments, follow institutional infection-control policies.

Public awareness regarding footwear hygiene can contribute to better personal hygiene practices. However, recommendations should be based on scientific evidence and should avoid unnecessary fear regarding normal environmental microorganisms.

10. Future Perspectives

Future research can provide a more detailed understanding of footwear-associated microbial communities. Traditional culture-based methods are useful for identifying microorganisms that can grow under laboratory conditions, but they may not capture the entire microbial diversity of footwear. Molecular techniques such as 16S rRNA gene sequencing for bacteria and internal transcribed spacer (ITS) sequencing for fungi can provide broader information about microbial communities.

Metagenomic approaches may further identify microorganisms and their functional potential. Comparative studies could investigate differences between footwear types, materials, climates, occupations, and patterns of use. Research could also examine microbial transfer between footwear and environmental surfaces under controlled conditions.

The development of antimicrobial or microbe-resistant footwear materials is another potential research area. However, the long-term effectiveness, safety, environmental impact, and possibility of selecting resistant microorganisms should be carefully evaluated. Understanding the footwear microbiome may ultimately contribute to improved hygiene practices, environmental monitoring, and infection-control strategies. More research is required to determine which microorganisms are persistent, which are transient, and which have genuine health significance.

Conclusion

The human foot is a complex microbial ecosystem that interacts continuously with footwear and the surrounding environment. Everyday footwear can provide a unique microenvironment influenced by temperature, moisture, sweat, ventilation, material, and frequency of use. These factors may influence the survival and accumulation of bacteria and fungi on footwear surfaces.

Microorganisms can potentially move between the foot, footwear, and environmental surfaces, creating a dynamic pathway of microbial exchange. Biofilm formation may contribute to microbial persistence under suitable conditions. Although many microorganisms associated with feet and footwear are harmless members of normal microbiota or environmental communities, some may have health significance under favorable conditions.

The study of the hidden microbial world of everyday footwear therefore provides valuable insights into microbiology, personal hygiene, and environmental health. Appropriate foot hygiene, adequate drying, regular cleaning, and proper footwear maintenance can help reduce excessive microbial accumulation. Future research using culture-independent and molecular approaches may provide a deeper understanding of footwear-associated microbial communities and their role in everyday microbial transmission.

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BEYOND TRADITIONAL TAXONOMY: AI AND DIGITAL APPROACHES TO PLANT TAXONOMY

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Introduction

Taxonomy and plant identification serve as the cornerstone of plant science. Accurate identification of plant species is important for vegetation surveys, ecological studies, assessment of biodiversity, medicine, ecosystem and environmental management, evolutionary studies etc. (Wäldchen and Mäder, 2018). Reliable identification skills are therefore essential for farmers, foresters, pharmacologists, taxonomists, conservation biologists, and technical personnel working in environmental agencies. They are also valuable for students, naturalists, and members of the public interested in exploring and documenting biological diversity (Austen *et al.*, 2016; Farnsworth *et al.*, 2013). In traditional approaches, identification of plant species was based on systematic morphological observations, comparing with standard floras and herbarium sheets and following taxonomic keys, etc. Although these approaches remain fundamental, the expanding scope of biodiversity observations has created a need for fast and authentic species identification.

Automating species identification and making reliable identification tools accessible to non-experts are increasingly important, particularly in the context of ongoing biodiversity loss (Ceballos *et al.*, 2015) and the declining availability of experienced taxonomists (Hopkins and Freckleton, 2002). Such approaches can help address the growing demand for species-level identification while supporting biodiversity documentation, monitoring, and conservation efforts. Rapid computational analysis is nowadays possible for large databases of plant images that are created by digital cameras, online biodiversity digital collections, smartphones and digitised natural history collections (Wäldchen and Mäder, 2018; Wäldchen *et al.* 2018).

The emergence of artificial intelligence (AI) and machine learning has consequently expanded the scope of plant taxonomy. Digital systems analyse the morphological characters of plants and aid in species identification. Recent advances have shifted from conventional image-processing techniques towards deep learning, convolutional neural networks (CNNs), transfer learning, few-shot learning and Vision Transformers. Zulfqar *et al.* (2025), in their review article, emphasised the demand and increasing usage of multi-organ datasets, self-supervised learning, and transformer-based approaches for biodiversity-related applications. However, the above-mentioned modern approaches cannot be considered a replacement for traditional taxonomy.

Instead, this provides an opportunity to bring together expertise, digital imaging, molecular information, geographical data and artificial intelligence. Recent studies have highlighted the intersection of AI-driven image recognition, molecular classification, remote sensing, and biodiversity assessment as a new approach to integrative plant identification (Nissima *et al.* 2025).

Digital Taxonomy: New Directions

Traditional plant taxonomy is based on the description of morphological, anatomical and reproductive characters coupled with the use of identification keys, floristic manuals and herbarium specimens. Although these are important tools in plant species identification, digitized herbarium collections, online biodiversity databases, georeferenced observations and plant images have improved the accessibility and availability of information for taxonomic studies (Bharati *et al.* 2023). Digital Botany is an offshoot of traditional botany which employs the use of digital devices and computers to further the study of plants. While the digitization of plant specimens mainly improves the storage, availability and distribution of botanical information, artificial intelligence enables data mining of large data sets to discover patterns and associations, perform specimen classification and identification tasks (Wäldchen and Mäder, 2018b). It is against this background that the digitalization of plant taxonomy is opening new directions for species identification, biodiversity documentation and taxonomy studies.

Digital Botany includes several digital resources, including both AI-based and non-AI-based tools. eFloraofIndia is a digital platform widely used in India for plant identification. It is a community-supported database of plant images that helps in plant identification through images, taxonomic information and contributions from plant enthusiasts. Other digital platforms widely used in India for recording biodiversity observations, taxonomy, geographical information, and citizen-science data include the India Biodiversity Portal and the Indian Biodiversity Information System (Vattakaven *et al.* 2016). At the global level, Plants of the World Online (POWO), World Flora Online and eFloras.org provide information on plant names, taxonomy, images, distribution and plant identification. AI-based platforms such as Pl@ntNet, iNaturalist and Seek have further expanded the use of digital tools for plant identification (Joly *et al.*, 2014; Joly *et al.*, 2016; Ueda, 2020). The iDigBio portal, a nationally funded aggregator of biodiversity and museum specimen data, provides access to a vast collection of digitized and georeferenced biological specimens, including vascular plant images (Willis *et al.* 2017). The availability of such large-scale image datasets, together with rapid advances in machine learning and computer vision, has created new opportunities for automating image-based species identification. These developments are making it increasingly feasible to develop identification tools that can assist both taxonomic experts and non-specialists in recognizing plant species. Biodiversity data systems such as GBIF provide access to records of species occurrence and biodiversity

assessment. These resources highlight that Digital Botany extends beyond AI-based identification, such as digital floras, citizen-science platforms, biodiversity databases etc.

Digital Herbaria

Herbaria, which are collections of dried plant specimens, form valuable sources of botanical information, and their digitisation has created new opportunities for the use of AI in plant research. Digitized herbarium specimens can provide information about plant distribution, historical vegetation, flowering and fruiting periods and changes in species occurrence over time, while AI can assist in specimen recognition, image classification and extraction of useful morphological information (Lorieul *et al.* 2020; Chulif *et al.* 2023; Guo *et al.* 2025). Combining herbarium images with photographs of living plants can help create more diverse datasets for AI-based identification. The accuracy of such systems depends greatly on the quality and regional diversity of the datasets, as AI models trained using plants from one region may not work equally well when identifying plants from other regions. Zulfiqar *et al.* (2025) highlighted geographical representation as an important challenge, emphasising the need for well-represented regional datasets in biodiverse countries such as India, particularly for its diverse tropical flora.

Table 1: Digital online platforms for plant identification

Platform	Main Purpose
eFloraofIndia	Photographic documentation, plant identification and taxonomic discussion
India Biodiversity Portal (IBP)	Biodiversity observations, species information, maps and citizen science
Indian Biodiversity Information System (IBIS)	Biodiversity information, observations, taxonomy, maps and e-People's Biodiversity Registers
Plants of the World Online (POWO)	Global plant names, descriptions, images, distributions and taxonomy
World Flora Online (WFO)	Global online flora and taxonomic information
eFloras.org	Online floras, identification resources and interactive keys
GBIF	Global biodiversity occurrence data
Seek by iNaturalist	Image-based organism identification

The comparison is based on findings reported in the literature on automated plant identification, machine learning, and AI-assisted plant classification (Wäldchen & Mäder, 2018; Nissima *et al.*, 2025; Zulfiqar *et al.*, 2025)

AI-Based Image Recognition for Plant Identification

Plant identification is challenging because closely related species can have very similar features, while plants of the same species may look different due to age, season, environment or disease. Differences in background, lighting, camera angle and image quality can make identification even more difficult. Zulfiqar *et al.* (2025) highlighted that reliable AI-based plant classification requires images covering different plant parts, species and geographical regions.

An important application of AI is Computer vision, which helps to identify and study plants from images. These systems mainly rely on characteristics such as leaf shape, colour, texture, venation, etc. These features are then compared using statistical methods or machine-learning techniques (Goëau *et al.* 2016; Carranza-Rojas *et al.* 2017). Plant identification became more efficient due to the advancement of deep learning systems, where computers now learn important characteristics directly from large image databases (Wäldchen and Mäder, 2018a). In AI based plant identification system, the collected images are processed and organized according to their species or groups. It then works on important features from the images, such as plant characteristics such as shape, colour and patterns and predicts the most likely species, often along with a confidence score.

Contemporary methods encompass semi-supervised learning, self-supervised learning, and few-shot learning, which are capable of operating with a limited quantity of labelled images. This feature enables them to detect rare plant species when extensive datasets are lacking. An additional novel approach is Vision Transformers, which can evaluate the connections among various elements of an image. These developments are enhancing the efficiency of AI systems for identifying plant species and contributing to biodiversity research. (Zulfiqar *et al.*, 2025).

By analysing plant observations together with geographical and ecological data, AI can support large-scale biodiversity assessment and environmental monitoring. Remote sensing technologies, including satellite, drone and hyperspectral imaging, enable monitoring of vegetation across large areas. Combined with machine-learning techniques, these data can support vegetation classification, habitat mapping and biodiversity monitoring (Nissima *et al.* 2025).

The approach of integrative digital taxonomy brings together images, morphological measurements, environmental information and molecular data to generate identifications. AI can help analyse these different sources of information, while botanists can review the evidence and confirm the identification. This integrated approach is especially useful for closely related or difficult species, where image-based identification may not be sufficient and molecular or other supporting evidence may be required.

Table 2: Digital technologies and their applications in modern plant science

Technology	Botanical Data Used	Major Application	Example of Use
Digital imaging	Leaves, flowers, fruits, whole plants	Plant identification	Species recognition
Computer vision	Plant images	Automated feature extraction	Leaf/flower classification
CNNs	Large image datasets	Species classification	Image-based identification
Vision Transformers	Complex image datasets	Fine-grained classification	Closely related species
Few-shot learning	Small labelled datasets	Rare species identification	Species with limited images
Self-supervised learning	Large unlabelled datasets	Feature learning	Large biodiversity datasets
Digital herbarium	Herbarium sheets + labels	Taxonomic documentation	Historical specimen analysis
DNA barcoding	DNA sequences	Species authentication	Difficult/cryptic taxa
GIS	Georeferenced observations	Distribution mapping	Species occurrence
Remote sensing	Satellite/drone/hyperspectral data	Vegetation and habitat assessment	Landscape-scale monitoring
Citizen science	Photographs + observations	Biodiversity documentation	iNaturalist/Pl@ntNet
Multimodal AI	Images + text + geography + molecular data	Integrative identification	Future digital taxonomy

The applications presented are based on findings reported in the literature by Wäldchen and Mäder (2018), Campbell *et al.* (2023), Lefort *et al.* (2025), Nissima *et al.* (2025), and Zulfiqar *et al.* (2025).

Limitations and Challenges in Digital Plant Identification

Despite rapid technological progress, AI-based plant identification has several limitations. AI works better when it is trained with good-quality and diverse data, while rare species may be difficult to identify because fewer records are available. Plant appearance may vary with age, season, habitat and environmental conditions, while closely related species may have similar features, making identification difficult in natural conditions (Kellenberger *et al.* 2018). Incorrectly labelled observations can reduce AI accuracy, particularly when data come from

users with varying levels of botanical knowledge (Lefort *et al.* 2025). Another important concern is that AI may provide a species name without explaining the botanical characters used for identification, highlighting the need for future systems to provide clear and understandable explanations that can support students, researchers and botanists.

The Continuing Role of the Taxonomist

AI is expected to support and strengthen taxonomic expertise rather than replace it. Even though AI can analyse many images and suggest plant identities, taxonomists are still needed to verify difficult species and make taxonomic decisions. Plant identification focuses on determining the identity of a specimen, while taxonomy involves describing, naming and classifying plants. Future Digital Botany is likely to focus on AI-assisted taxonomy. Zulfiqar *et al.* (2025) highlighted the potential of AI approaches to combine plant images with other information, such as text, geographical data, herbarium records and molecular data. They also noted that methods such as few-shot learning, self-supervised learning and Vision Transformers can be useful when labelled images of plant species are limited. The integration of AI with GIS, remote sensing, molecular data and conventional taxonomic expertise therefore represents an important direction for Digital Botany, enabling plant diversity to be documented, mapped and monitored at increasingly large spatial and temporal scales (Guisan and Zimmermann. 2000);

By combining traditional botanical knowledge with digital images, herbarium records, geographical information, molecular data and AI-based tools, plant identification and biodiversity documentation can become faster and more efficient. AI should therefore be used as a supporting tool for taxonomists, with the future of Digital Botany depending on the integration of botanical expertise, AI technologies and diverse sources of information to improve the understanding, documentation and conservation of plant diversity.

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ENVIRONMENTAL DNA (eDNA): A MOLECULAR TOOL FOR BIODIVERSITY MONITORING AND CONSERVATION

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Abstract

The accelerating loss of biodiversity has increased the need for reliable, sensitive and cost-effective approaches for species detection and ecological monitoring. Conventional biodiversity surveys based on visual observation, trapping, netting, camera trapping and morphological identification remain fundamental to ecological research; however, they can be labour-intensive, taxonomically demanding and less effective for rare, cryptic, nocturnal or low-abundance organisms. Environmental DNA (eDNA) has emerged as an innovative molecular approach that enables biodiversity assessment through the detection of genetic material naturally released by organisms into their surrounding environment. DNA-containing material can be collected from water, soil, sediment, air and other environmental matrices and analysed using polymerase chain reaction (PCR), quantitative PCR (qPCR), digital PCR, DNA metabarcoding and high-throughput sequencing. eDNA has demonstrated considerable potential for detecting rare and endangered species, monitoring invasive alien species, assessing aquatic and terrestrial communities, and evaluating biodiversity responses to environmental disturbances. Recent advances in high-throughput sequencing, bioinformatics, automated sampling, airborne eDNA and portable molecular technologies are further expanding the scope of eDNA-based biomonitoring. However, the reliability and interpretation of eDNA data can be influenced by DNA shedding, transport, persistence and degradation, environmental conditions, contamination, primer bias, sampling design and incomplete reference databases. Therefore, eDNA is best considered a complementary approach to conventional ecological monitoring rather than a universal replacement. This chapter examines the principles, sources, methodologies, applications, advantages, limitations and emerging technological developments associated with eDNA, highlighting its growing significance as a next-generation molecular tool for biodiversity monitoring, ecological assessment and conservation planning.

Keywords: Environmental DNA (eDNA); Biodiversity Monitoring; eDNA Metabarcoding; Molecular Ecology; Conservation Biology.

Introduction

Biodiversity monitoring is fundamental to understanding ecosystem structure, detecting ecological change and developing effective conservation strategies. However, biodiversity assessment is becoming increasingly challenging because species populations and habitats are being affected by habitat fragmentation, pollution, biological invasions, climate change and other anthropogenic pressures. Freshwater ecosystems are particularly vulnerable, with many species requiring intensive monitoring for effective conservation (Thomsen *et al.*, 2012). Traditional methods such as visual surveys, trapping, electrofishing, netting, acoustic surveys and morphological identification provide valuable ecological information but may require considerable field effort and specialist taxonomic knowledge.

The development of molecular approaches has transformed biodiversity assessment. Environmental DNA (eDNA) is one of the most important developments in this field. The concept involves detecting DNA released naturally by organisms into environmental matrices rather than obtaining DNA directly from a captured organism. eDNA can therefore provide evidence of species presence without necessarily requiring direct observation or collection of individuals (Taberlet *et al.*, 2012; Bohmann *et al.*, 2014).

The application of eDNA to biodiversity research has expanded considerably since early demonstrations of species detection from environmental water samples. Ficetola *et al.* (2008) demonstrated that DNA extracted from water could be used to detect an amphibian species, establishing an important foundation for aquatic eDNA monitoring. Subsequent studies demonstrated the potential of eDNA for rare species, endangered species and invasive species detection (Jerde *et al.*, 2011; Thomsen *et al.*, 2012).

The development of high-throughput sequencing further expanded eDNA from single-species detection to community-level biodiversity assessment. DNA metabarcoding enables multiple species to be identified from the same environmental sample and has consequently become an important approach in molecular ecology (Valentini *et al.*, 2016; Deiner *et al.*, 2017; Ruppert *et al.*, 2019; Creer *et al.*, 2016). Today, eDNA is applied across aquatic, terrestrial, soil and increasingly airborne environments.

Concept and Sources of Environmental DNA

Environmental DNA can broadly be defined as genetic material obtained from environmental samples rather than directly from an organism. The term has been used in different ways across molecular ecology, and Pawlowski *et al.* (2020) emphasized the importance of using the terminology carefully when describing eDNA-based biomonitoring. Organisms continuously release biological material into their surroundings. Sources of eDNA may include skin cells, mucus, scales, feathers, hair, saliva, urine, faeces, gametes, eggs and tissue fragments. After release, DNA may occur as cellular material or as extracellular DNA and can subsequently be

transported, adsorbed to particles, degraded or deposited in environmental matrices (Barnes & Turner, 2016).

Major Environmental Matrices

- **Water:** Water is the most extensively studied matrix for macro-organismal eDNA. Fish, amphibians, aquatic invertebrates and other organisms release DNA into surrounding water. Water sampling has therefore become a major approach for aquatic biodiversity assessment (Rees *et al.*, 2014; Takahashi *et al.*, 2023).
- **Soil:** Soil contains DNA from plants, fungi, microorganisms and soil-dwelling animals. Soil eDNA can provide information about both above-ground and below-ground biodiversity.
- **Sediment:** Sediments may preserve DNA from contemporary as well as historical biological communities and have been used for biodiversity reconstruction and environmental studies (Thomsen & Willerslev, 2015).
- **Air:** Airborne eDNA is an emerging research frontier. DNA-containing particles may originate from animals, plants, fungi and microorganisms. Air sampling therefore provides a potentially non-invasive approach for terrestrial biodiversity assessment (Johnson *et al.*, 2024).
- **Rainwater and plant surfaces:** Recent research has explored rainwash and other environmental pathways as alternative sources of biodiversity information. For example, Macher *et al.* (2023) demonstrated the potential of rainwash eDNA metabarcoding for assessing tree-canopy invertebrate diversity.

Types of eDNA Approaches

1. Species-Specific eDNA: Species-specific eDNA assays are designed to detect one particular target species. Primers and, in many cases, fluorescent probes are selected to amplify a species-specific DNA region. qPCR is commonly used because of its sensitivity and ability to quantify amplification. Species-specific eDNA is particularly useful for:

- Rare species detection
- Endangered species monitoring
- Invasive species surveillance
- Pathogen detection
- Early-warning systems

Jerde *et al.* (2011) demonstrated the value of eDNA for detecting rare aquatic invasive species, showing how molecular surveillance could complement conventional field-based approaches.

2. eDNA Metabarcoding: eDNA metabarcoding involves simultaneous identification of multiple taxa from environmental samples. Short DNA regions are amplified using broad-range

primers and sequenced using high-throughput sequencing platforms. The approach can provide information about species richness and community composition from relatively small environmental samples (Valentini *et al.*, 2016; Deiner *et al.*, 2017).

3. Metagenomics: Metagenomics involves broader sequencing of DNA present in an environmental sample rather than targeting a single barcode region. It can potentially provide information about organisms and their functional genetic characteristics. However, metagenomic approaches generally require greater sequencing and computational resources than targeted metabarcoding (Taberlet *et al.*, 2018).

Workflow of eDNA-Based Biodiversity Assessment

The reliability of an eDNA study depends on the complete workflow, from sampling through molecular analysis and ecological interpretation.

- 1. Study Design:** The first step is to define the biological question. Researchers must determine the target organisms, environmental matrix, sampling locations, number of replicates and temporal sampling strategy. Sampling design is particularly important because eDNA is not distributed uniformly within ecosystems (Taberlet *et al.*, 2018; Takahashi *et al.*, 2023).
- 2. Environmental Sampling:** In aquatic studies, a known volume of water is collected and filtered to capture DNA-containing particles. The selection of water volume, filter type, pore size and sampling location can influence DNA recovery. Turner *et al.* (2014) demonstrated that aquatic eDNA occurs across different particle-size fractions, emphasizing the importance of filtration and DNA-capture strategies. Sampling should include appropriate field replicates and controls. Taberlet *et al.* (2018) emphasized that sampling is a critical component of the entire eDNA workflow.
- 3. DNA Extraction:** DNA is extracted from filters, soil, sediment or other environmental material. Extraction protocols must maximize DNA recovery while removing substances that inhibit PCR. Soil and sediment may contain humic substances and other compounds capable of interfering with molecular amplification.
- 4. PCR Amplification:** The extracted DNA is amplified using selected primers. Depending on the objective, researchers may use conventional PCR, qPCR, digital PCR or metabarcoding PCR. Primer selection is particularly important in metabarcoding because primer bias can cause some taxa to be amplified more efficiently than others (Deiner *et al.*, 2017).
- 5. DNA Sequencing:** For metabarcoding, amplified DNA is generally subjected to high-throughput sequencing. Miya *et al.* (2015), for example, developed the MiFish primer system targeting a short 12S rRNA region for fish eDNA metabarcoding. Such marker systems have facilitated broad-scale fish biodiversity assessments.

- 6. Bioinformatics:** Sequencing produces large numbers of DNA reads that require computational processing. Typical steps include:
- i. Quality filtering
 - ii. Removal of primer sequences
 - iii. Removal of sequencing artefacts
 - iv. Generation of sequence variants or clusters
 - v. Taxonomic assignment
 - vi. Comparison with reference databases
 - vii. Biodiversity analysis

The quality of species identification is strongly dependent on the completeness and accuracy of reference databases (Taberlet *et al.*, 2018).

Environmental DNA and Biodiversity Monitoring

- 1. Detection of Rare and Cryptic Species:** One of the major strengths of eDNA is its potential to detect organisms that are difficult to observe directly. Rare, nocturnal, cryptic or low-density organisms can release detectable DNA into their environment. Jerde *et al.* (2011) demonstrated the use of eDNA to detect rare aquatic species, while Thomsen *et al.* (2012) applied eDNA to the monitoring of endangered freshwater biodiversity. Such studies established the importance of molecular detection for conservation-oriented monitoring.
- 2. Endangered Species:** Monitoring endangered species is essential for determining distribution, habitat occupancy and conservation status. eDNA may provide a minimally invasive method for detecting threatened organisms, particularly in aquatic habitats. However, a positive eDNA detection should not automatically be interpreted as evidence of a viable population. Detection may represent transient DNA transported from another location. Therefore, eDNA results should ideally be interpreted alongside ecological observations and repeated sampling (Goldberg *et al.*, 2016; Pilliod *et al.*, 2013).
- 3. Invasive Alien Species:** Early detection of invasive species is a major application of eDNA. When newly introduced species occur at low abundance, traditional surveys may fail to detect them. eDNA can potentially provide an early warning before populations become readily detectable through conventional methods (Jerde *et al.*, 2011; Rees *et al.*, 2014).
- 4. Aquatic Biodiversity:** Aquatic ecosystems have been central to the development of eDNA biomonitoring. Water samples can contain DNA from fish, amphibians, aquatic mammals and invertebrates. Valentini *et al.* (2016) demonstrated the potential of eDNA metabarcoding for simultaneously monitoring amphibian and fish communities. More recent reviews indicate that aquatic eDNA has developed into a broad biomonitoring field

encompassing diverse taxa, sampling approaches and molecular methods (Takahashi *et al.*, 2023).

- 5. Terrestrial Biodiversity:** Terrestrial eDNA research is expanding through the analysis of soil, water, rainwater, vegetation-associated material and air. Terrestrial applications include biodiversity surveys, monitoring of mammals, insects, plants and fungi, and assessment of ecological restoration. Recent reviews indicate that terrestrial eDNA monitoring is promising but requires sampling strategies specifically adapted to terrestrial conditions (Newton *et al.*, 2025; Cowgill *et al.*, 2025).

eDNA Metabarcoding and Community-Level Biodiversity

DNA metabarcoding represents one of the most significant advances in biodiversity monitoring. Rather than focusing on one species, metabarcoding can simultaneously characterize multiple taxa from a single environmental sample. For example, fish communities can be investigated using mitochondrial markers such as 12S. The MiFish primer system developed by Miya *et al.* (2015) demonstrated detection of more than 230 subtropical marine fish species, illustrating the potential of universal primer systems. Metabarcoding also enables biodiversity assessment at different taxonomic levels. Depending on the marker and reference database, sequences may be assigned to species, genus, family or higher taxonomic categories. However, the number of sequence reads should not automatically be treated as a direct measure of species abundance. Differences in DNA shedding, body size, metabolism, primer affinity and DNA degradation can influence sequence representation (Deiner *et al.*, 2017; Sassoubre *et al.*, 2016).

Environmental Processes Affecting eDNA Detection

Environmental DNA is not static. Its ecological interpretation requires understanding how DNA enters, moves through and disappears from the environment.

- 1. DNA Production:** DNA shedding differs among species, life stages and environmental conditions. Sassoubre *et al.* (2016) demonstrated differences in eDNA shedding and decay rates among marine fish species.
- 2. DNA Transport:** In aquatic ecosystems, DNA can move with water currents. Deiner and Altermatt (2014) demonstrated substantial downstream transport of invertebrate eDNA in a natural river. This has an important implication: detection of a species' DNA at a sampling location does not necessarily prove that the organism was physically present at exactly that point.
- 3. DNA Degradation:** Temperature, ultraviolet radiation, microbial activity, pH, salinity and other environmental factors can influence DNA persistence. Jane *et al.* (2015) demonstrated that water flow, distance and PCR inhibition can affect eDNA detection in streams. Understanding DNA persistence is therefore essential when interpreting positive and negative results.

Genetic Markers Used in eDNA Studies

Different taxonomic groups require different molecular markers.

Taxonomic Group	Common Markers
Fish	12S rRNA, COI
Amphibians	12S, 16S, COI
Mammals	12S, COI
Birds	COI, 12S
Invertebrates	COI
Plants	rbcL, matK, ITS
Fungi	ITS
Bacteria	16S rRNA

The choice of marker depends on taxonomic resolution, primer specificity, sequence length and reference database availability. Taberlet *et al.* (2018) provide extensive discussion of metabarcode selection and reference databases. For fish, the MiFish 12S primer system has become an important example of a broad-range marker strategy (Miya *et al.*, 2015).

Advantages of eDNA for Biodiversity Monitoring

The major advantages of eDNA include:

- **Non-invasive monitoring:** eDNA generally allows organisms to be detected without capture or physical handling.
- **High sensitivity:** Molecular assays can detect very low quantities of DNA, which is valuable for rare or low-density species (Ficetola *et al.*, 2008).
- **Detection of elusive species:** Organisms that are difficult to observe directly can potentially be detected from environmental samples (Bohmann *et al.*, 2014).
- **Multi-species detection:** Metabarcoding allows simultaneous assessment of multiple taxa (Deiner *et al.*, 2017).
- **Reduced field effort:** Environmental sampling can sometimes be faster than collecting and identifying individual organisms.
- **Repeated monitoring:** eDNA can facilitate repeated sampling over time, allowing temporal biodiversity changes to be investigated. Nevertheless, eDNA should be viewed as a complementary tool. Rees *et al.* (2014) and Goldberg *et al.* (2016) emphasized the importance of interpreting eDNA results within the broader ecological context.

Limitations and Challenges

Despite its advantages, eDNA has important limitations.

- **False Positives:** DNA contamination can produce positive results even when the target organism is absent. Contamination may occur during field sampling, filtration, DNA extraction, PCR or sequencing.

- **False Negatives:** Failure to detect DNA does not necessarily mean that the species is absent. Low DNA concentration, inadequate sampling, degradation or inefficient primer amplification may result in non-detection.
- **Primer Bias:** Metabarcoding primers may amplify some species more effectively than others, affecting estimates of community composition (Deiner *et al.*, 2017).
- **DNA Transport:** DNA may move away from the organism's actual location, particularly in rivers and other flowing systems (Deiner & Altermatt, 2014).
- **Reference Database Gaps:** Species identification depends on the availability of reference sequences. Poorly represented species may remain unidentified or may only be assigned to higher taxonomic levels.
- **Estimating Abundance:** The relationship between eDNA concentration and organism abundance is complex. DNA shedding and degradation vary among species and environmental conditions (Sassoubre *et al.*, 2016).
- **Lack of Standardization:** One of the major challenges is variation in sampling, filtration, DNA extraction, PCR and sequencing protocols. Takahashi *et al.* (2023) reviewed hundreds of aquatic eDNA studies and highlighted the considerable methodological diversity in the field.

Quality Control and Standardization

Because eDNA techniques are highly sensitive, strict quality-control procedures are essential.

Important controls include:

- Field blanks
- Filtration blanks
- Extraction blanks
- PCR negative controls
- Positive controls
- Technical replicates
- Biological replicates

Laboratories should maintain physical separation between pre-PCR and post-PCR areas to reduce contamination. Sampling equipment should be appropriately sterilized, and sample storage and transportation should be standardized. Goldberg *et al.* (2016) emphasized that the sensitivity of eDNA methods creates a corresponding need for rigorous quality assurance and quality-control procedures. Standardization is increasingly recognized as a major requirement for transforming eDNA from a research technique into a widely comparable biomonitoring tool. Recent work continues to emphasize standardized sampling, analytical workflows and reporting requirements (Blackman *et al.*, 2024; Çevik & Çevik, 2025).

Emerging Applications and Technological Frontiers

- **Airborne eDNA:** Airborne eDNA is one of the most exciting emerging applications. Biological particles released into the atmosphere can potentially provide information about terrestrial organisms without direct contact or capture. Johnson *et al.* (2024) reviewed the development of airborne eDNA and identified major opportunities as well as challenges involving sampling, atmospheric transport, contamination and interpretation.
- **Rainwash eDNA:** Rainwater can collect biological material from vegetation and other surfaces. Macher *et al.* (2023) demonstrated that rainwash eDNA metabarcoding can be used to investigate tree-canopy invertebrate communities, illustrating an innovative approach to terrestrial biodiversity assessment.
- **Automated Sampling:** Automation may enable continuous or repeated environmental sampling. Recent technological developments include autonomous sampling devices and in-situ filtration systems, which may improve temporal coverage while reducing field effort (Blackman *et al.*, 2024).
- **Portable Sequencing:** Portable sequencing technologies offer the possibility of performing molecular analyses closer to the field. This could shorten the time between sample collection and biodiversity assessment.
- **Artificial Intelligence and Bioinformatics:** The growing volume of eDNA sequence data requires increasingly sophisticated computational approaches. Machine learning and artificial intelligence may assist with sequence classification, taxonomic identification, biodiversity prediction and integration of molecular data with environmental variables.
- **eDNA and Remote Sensing:** Combining eDNA with GIS, satellite imagery and drone-based remote sensing could provide a powerful framework in which molecular evidence of species occurrence is linked to habitat characteristics.

eDNA in Conservation Planning

eDNA can contribute to conservation at several levels. At the species level, it can assist in determining distribution and occupancy. At the community level, metabarcoding can provide information about species richness and composition. At the ecosystem level, repeated molecular monitoring may help identify changes associated with pollution, habitat degradation, restoration or climate change. The potential of aquatic eDNA to contribute to large-scale biodiversity targets has recently received considerable attention. Altermatt *et al.* (2025) discussed how aquatic eDNA could support biodiversity assessment and monitoring associated with global biodiversity targets. Similarly, recent reviews of eDNA applications highlight its potential for ecosystem biodiversity assessment and conservation, while emphasizing the need for appropriate interpretation and methodological standardization (Çevik & Çevik, 2025).

Future Perspectives

The future of eDNA research is likely to move from isolated species detection toward integrated ecosystem surveillance.

Future developments may include:

- Real-time eDNA monitoring
- Automated environmental sampling
- Portable sequencing platforms
- Airborne eDNA monitoring
- Integration with artificial intelligence
- Integration with GIS and remote sensing
- Improved DNA reference databases
- Standardized international protocols
- Long-term biodiversity surveillance
- Integration of eDNA with conventional ecological surveys
- eDNA-based ecosystem health assessment
- Greater use of environmental RNA (eRNA)
- Application to climate-change monitoring
- Molecular monitoring of ecological restoration

Blackman *et al.* (2024) highlighted sampling, primer development, biomonitoring, quantification, DNA behaviour and reference databases among the areas requiring continued development. The future of eDNA therefore lies not only in improving molecular sensitivity but also in understanding how molecular signals translate into meaningful ecological information. Recent research on terrestrial vertebrates similarly emphasizes the need for best-practice sampling strategies as eDNA moves beyond aquatic ecosystems (Newton *et al.*, 2025). The development of terrestrial and airborne eDNA could substantially broaden the geographical and taxonomic scope of molecular biodiversity monitoring.

Conclusion

Environmental DNA has developed from an emerging molecular technique into an increasingly important approach for biodiversity monitoring and conservation. Its ability to detect DNA naturally released by organisms provides a non-invasive and sensitive means of investigating species presence across aquatic and terrestrial environments. Species-specific assays can support the detection of rare, endangered and invasive organisms, whereas metabarcoding enables simultaneous assessment of multiple taxa and community composition. However, eDNA is not without limitations. DNA transport, degradation, contamination, primer bias, incomplete reference databases and difficulties in relating DNA concentration to population abundance can

complicate ecological interpretation. Consequently, robust sampling design, appropriate controls, standardized laboratory procedures and careful bioinformatic analysis are essential.

The most promising future direction is the integration of eDNA with high-throughput sequencing, artificial intelligence, GIS, remote sensing, automated sampling and conventional ecological surveys. Such interdisciplinary integration can transform eDNA from a species-detection technique into a component of large-scale biodiversity surveillance. Thus, eDNA represents an important frontier in modern life science research, providing a molecular bridge between genomics, ecology, environmental science and conservation biology.

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HUMAN GUT MICROBIOME AND IT'S ROLE IN HEALTH AND DISEASE

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Abstract

The human gastrointestinal tract harbors a highly diverse and metabolically active community of microorganisms collectively known as the gut microbiome. This microbial ecosystem consists of bacteria, archaea, fungi, viruses, and protozoa that coexist with the host in a mutually beneficial relationship (Kho *et al.*, 2021). The gut microbiome performs numerous physiological functions, including digestion of dietary components, synthesis of vitamins, regulation of immune responses, maintenance of intestinal barrier integrity, and protection against pathogenic microorganisms (Valdes *et al.*, 2020).

Recent advances in molecular biology, high-throughput sequencing technologies, and bioinformatics have revolutionized our understanding of the gut microbiome. It is now recognized as an essential organlike system that contributes significantly to human health (Gibson *et al.*, 2021). Alterations in microbial composition, commonly referred to as gut dysbiosis, have been associated with numerous disorders, including inflammatory bowel disease, obesity, diabetes mellitus, cardiovascular disease, liver diseases, colorectal cancer, neurological disorders, allergies, and autoimmune diseases (Sanz *et al.*, 2025).

Therapeutic strategies such as probiotics, prebiotics, synbiotics, postbiotics, dietary interventions, and fecal microbiota transplantation are increasingly being explored for restoring microbial balance and improving health outcomes (Cani, 2021). This chapter provides a comprehensive overview of the human gut microbiome, its composition, development, physiological functions, clinical significance, and therapeutic applications (Lavelle and Sokol, 2020).

Keywords: Human Gut Microbiome, Dysbiosis, Probiotics, Prebiotics, Synbiotics.

1. Introduction

The human body is inhabited by a vast community of microorganisms that collectively form the human microbiota. These microorganisms colonize various body sites, including the skin, oral cavity, respiratory tract, urogenital tract, and gastrointestinal tract. Among these anatomical sites,

the gastrointestinal tract represents one of the most complex and densely populated microbial ecosystems in the human body (Joos *et al.*, 2025).

The gut microbiome refers to the microorganisms residing within the gastrointestinal tract together with their collective genetic information and functional capabilities (Sanz *et al.*, 2025). The microbial community of the gastrointestinal tract contains a vast number of microbial cells and an extensive repertoire of genes that considerably expands the metabolic potential of the human host. Consequently, the gut microbiome contributes substantially to nutrient metabolism and other physiological processes that cannot be performed by human cells alone (Ross *et al.*, 2024).

The human gastrointestinal tract provides different ecological niches for microbial colonization. Microbial communities vary considerably along the gastrointestinal tract because of differences in oxygen availability, gastric acidity, bile concentration, nutrient availability, intestinal motility, and other local environmental conditions (McCallum *et al.*, 2024). These spatial differences result in distinct microbial communities in different regions of the gastrointestinal tract, a phenomenon known as gut microbial biogeography (McCallum *et al.*, 2024).

Traditionally, microorganisms were primarily associated with infectious diseases and were frequently considered harmful to humans. However, advances in microbiology and molecular biology have demonstrated that the majority of microorganisms residing in the gut participate in beneficial or contextdependent interactions with the host (Zheng *et al.*, 2020). They contribute to digestion and nutrient metabolism, production of microbial metabolites, regulation of immune responses, maintenance of intestinal barrier function, and resistance against colonization by pathogenic microorganisms (Tilg and Adolph, 2020).

The gut microbiome also interacts with host metabolic and nervous systems. Gut microbial metabolites can influence host metabolism and immune pathways, while bidirectional communication between the gastrointestinal tract and the nervous system has been implicated in neurological and mental health processes (Cryan *et al.*, 2020). Therefore, the gut microbiome is increasingly considered an important functional component of human physiology rather than simply a collection of resident (Cani, 2021) Microorganisms. (Cani and Sanz, 2021).

The concept of humans as “superorganisms” emphasizes the intimate biological relationship between human cells and their associated microorganisms (Cani *et al.*, 2021). The composition and functional activity of the gut microbiome can influence physiological homeostasis, immune function, metabolism, and susceptibility to disease (Zheng *et al.*, 2020).

2. Historical Perspective of Gut Microbiome Research

The study of microorganisms inhabiting the human gastrointestinal tract has evolved considerably over several centuries. Early investigations were largely dependent on microscopic observation and culturebased techniques, whereas modern microbiome research relies

extensively on molecular sequencing, metagenomics, bioinformatics, and multi-omics approaches (Kho *et al.*, 2021).

Early Observations

The Dutch microscopist Antonie van Leeuwenhoek was the first to observe microorganisms using handmade microscopes during the seventeenth century. Although he described bacteria from oral samples, the understanding of intestinal microorganisms remained limited for several centuries. During the nineteenth century, the pioneering work of Louis Pasteur and b established the germ theory of disease, focusing primarily on pathogenic microorganisms. Beneficial microorganisms received comparatively little attention.

Metchnikoff's Hypothesis

In the early twentieth century, Nobel laureate Élie Metchnikoff proposed that consumption of fermented milk containing beneficial bacteria could improve health and prolong lifespan. His work laid the foundation for the modern concept of probiotics.

Culture-Based Era

Throughout much of the twentieth century, gut microbiology relied on culture-based methods. Although these methods identified many bacterial species, it became evident that a substantial proportion of gut microorganisms could not be cultivated under standard laboratory conditions.

Molecular Revolution

The introduction of 16S rRNA gene sequencing in the late twentieth century revolutionized microbial ecology by enabling identification of unculturable bacteria based on genetic markers. Subsequent advances in

- Next-generation sequencing (NGS)
- Metagenomics
- Metatranscriptomics
- etaproteomics
- Metabolomics have transformed our understanding of microbial diversity, functions, and host interactions

Human Microbiome Project

The Human Microbiome Project (HMP), launched in 2007 by the National Institutes of Health (NIH), aimed to characterize microbial communities associated with healthy individuals and determine their role in health and disease. The project significantly advanced microbiome research and established comprehensive microbial reference datasets. Today, gut microbiome research represents one of the fastest-growing fields in microbiology, immunology, nutrition, and precision medicine.

3. Human Gut Microbiome

The terms microbiota and microbiome are often used interchangeably, although they have distinct meanings.

- **Microbiota** refers to the collection of microorganisms inhabiting a specific environment.
- **Microbiome** encompasses the microorganisms, their genomes, metabolic products, structural

components, and interactions with the host.

The gut microbiome includes:

- BacteriaArchaea
- Viruses (especially bacteriophages)
- Fungi

4. Composition of the Human Gut Microbiome

The gastrointestinal tract contains one of the most diverse microbial ecosystems on Earth. Although thousands of microbial species have been identified, relatively few bacterial phyla dominate the healthy adult gut.

Major Bacterial Phyla

1. Firmicutes

Firmicutes constitute approximately 40–60% of gut bacteria.

Representative genera include:

- *Lactobacillus*
- *Clostridium*
- *Faecalibacterium*
- *Roseburia*
- *Ruminococcus*

Functions

- i. Fermentation of dietary fiber
- ii. Short-chain fatty acid (SCFA) production

2. Bacteroidetes

Representative genera:

- *Bacteroides*
- *Prevotella*
- *Alistipes*

Fuctions

- i. Breakdown of complex carbohydrates
- ii. Polysaccharide metabolism

- iii. Vitamin synthesis
- iv. Energy extraction

3. Actinobacteria

Important genus:

- *Bifidobacterium*

Functions

- i. Digestion of oligosaccharides
- ii. Production of acetate Immune regulation
- iii. Prevention of pathogen colonization

4. Proteobacteria

Representative genera:

- *Escherichia*
- *Salmonella, Helicobacter*

Normally present in relatively low abundance. Increased numbers may indicate intestinal inflammation or dysbiosis.

5. Verrucomicrobia

Representative species:

- *Akkermansia muciniphila* Functions:

Functions

- i. 1.Maintenance of intestinal mucus layer
- ii. 2.Metabolic health
- iii. 3.Anti-inflammatory activity

Table 1: Major Microbial Groups of the Human Gut

Phylum	Representative Genera	Major Functions
Firmicutes	<i>Lactobacillus</i> , <i>Faecalibacterium</i>	SCFA production, immune regulation
Bacteroidetes	<i>Bacteroides</i> , <i>Prevotella</i>	Carbohydrate digestion
Actinobacteria	<i>Bifidobacterium</i>	Vitamin synthesis, immune development
Proteobacteria	<i>Escherichia</i> , <i>Klebsiella</i>	Opportunistic metabolism; increased abundance may indicate dysbiosis
Verrucomicrobia	<i>Akkermansia</i>	Mucus degradation and intestinal health

5.1 Microbial Diversity of the Gastrointestinal Tract

Microbial populations vary considerably across different regions of the gastrointestinal tract due to changes in pH, oxygen concentration, nutrient availability, and intestinal motility.

Table 2: Microbial Diversity of the Gastrointestinal Tract

Region	Dominant Microorganisms	Approximate Microbial Density
Stomach	<i>Lactobacillus</i> , <i>Streptococcus</i>	10^2 – 10^3 cells/mL
Duodenum	<i>Streptococcus</i> , <i>Lactobacillus</i>	10^3 – 10^4 cells/mL
Jejunum	<i>Lactobacillus</i> , <i>Enterococcus</i>	10^4 – 10^7 cells/mL
Ileum	<i>Enterococcus</i> , <i>Bacteroides</i>	10^7 – 10^8 cells/mL
Colon	<i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Bifidobacterium</i>	10^{11} – 10^{12} cells/g

6. Factors Affecting the Gut Microbiome

Several factors influence the composition and diversity of the gut microbiome:

- **Diet:** A fiber-rich diet promotes the growth of beneficial bacteria, while processed foods may reduce microbial diversity.
- **Antibiotics:** Frequent or unnecessary use of antibiotics can kill beneficial bacteria and disrupt the gut microbiome.
- **Age:** The gut microbiome develops from birth and changes throughout different stages of life. **Lifestyle:** Regular exercise, adequate sleep, and low stress help maintain a healthy gut microbiome.
- **Environment:** Hygiene, living conditions, pollution, and geographical location can affect the types of microorganisms in the gut.

7. Role of the Human Gut Microbiome in Health

The human gut microbiome plays a vital role in maintaining overall health. It consists of trillions of microorganisms that live in the digestive tract and work together with the human body. These microorganisms help in digestion, nutrient absorption, immune system development, and protection against harmful microbes. A healthy and balanced gut microbiome is essential for normal body functions and disease prevention.

Digestion and Nutrient Absorption

One of the primary functions of the gut microbiome is to help digest food that the human body cannot break down on its own. Beneficial bacteria ferment dietary fibers and produce short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. These compounds provide energy to the cells lining the intestine and help maintain a healthy digestive system. The microbiome also improves the absorption of minerals such as calcium, magnesium, and iron.

Vitamin Synthesis

Certain gut bacteria produce essential vitamins that are important for human health. These include vitamin K, which is necessary for blood clotting, and several B-complex vitamins such as biotin, folate, and vitamin B12. Although the body obtains many vitamins from food, the gut microbiome contributes to meeting the body's nutritional needs.

Immune System Regulation

The gut microbiome plays an important role in the development and regulation of the immune system. Beneficial microorganisms help the immune system recognize harmful pathogens while preventing unnecessary immune responses against harmless substances. They stimulate the production of immune cells and antibodies, helping the body defend itself against infections.

Protection Against Harmful Microorganisms

The beneficial bacteria in the gut protect the body by preventing harmful microorganisms from growing. They compete with pathogens for nutrients and space in the intestine and produce antimicrobial substances that inhibit the growth of disease-causing bacteria. This process is known as colonization resistance and is an important defense mechanism.

Maintenance of the Intestinal Barrier

The gut microbiome helps maintain the integrity of the intestinal lining. A healthy intestinal barrier prevents harmful bacteria, toxins, and undigested food particles from entering the bloodstream. Beneficial bacteria strengthen the cells lining the intestine and reduce inflammation, thereby supporting overall gut health.

Metabolism and Weight Regulation

The gut microbiome influences metabolism by helping the body extract energy from food and regulating the storage of fat. It also affects hormones involved in hunger and appetite, such as ghrelin and leptin.

An imbalance in gut bacteria has been associated with obesity, metabolic syndrome, and type 2 diabetes.

Gut–Brain Axis

The gut and brain communicate through a complex network called the gut–brain axis. Gut microorganisms produce neurotransmitters and other signaling molecules that influence brain function, mood, and behavior. A healthy gut microbiome has been linked to improved mental health, better memory, and reduced stress and anxiety.

Other Diseases Associated with Gut Dysbiosis

Recent studies suggest that gut dysbiosis may also play a role in several other diseases, including: •

Cardiovascular disease

- Non-alcoholic fatty liver disease (NAFLD)
- Parkinson's disease
- Alzheimer's disease
- Rheumatoid arthritis

Although research is ongoing, maintaining a healthy gut microbiome may help reduce the risk or severity of these conditions.

Prevention of Gut Dysbiosis

- i. Healthy lifestyle practices can help maintain a balanced gut microbiome and reduce disease risk.
- ii. These include:
- iii. Eating a balanced diet rich in fruits, vegetables, whole grains, and dietary fiber.
- iv. Consuming probiotic foods such as yogurt and fermented foods.
- v. Including prebiotic foods like garlic, onions, bananas, and oats.
- vi. Avoiding unnecessary use of antibiotics.
- vii. Exercising regularly and managing stress.
- viii. Getting adequate sleep and drinking enough water.

Prevention of Diseases

A balanced gut microbiome helps reduce the risk of several diseases by supporting immune function and maintaining healthy metabolism. It may lower the risk of gastrointestinal infections, inflammatory bowel disease, allergies, cardiovascular disease, and certain types of cancer. Maintaining a healthy microbiome through a balanced diet and healthy lifestyle is important for disease prevention.

8. Role of Gut Microbiome in Disease

The human gut microbiome plays a vital role in maintaining health by supporting digestion, regulating immune responses, synthesizing essential vitamins, and protecting the body against harmful microorganisms. When the normal balance of the gut microbiota is disrupted, a condition known as dysbiosis occurs. Dysbiosis is characterized by a reduction in beneficial microorganisms, an increase in harmful or opportunistic microbes, and a loss of microbial diversity. This imbalance has been strongly associated with the development and progression of several gastrointestinal, metabolic, immunemediated, neurological, and cardiovascular diseases.

Dysbiosis

Dysbiosis refers to an imbalance in the composition and function of the gut microbiome. It can result from factors such as unhealthy dietary habits, excessive use of antibiotics, infections, chronic stress, aging, environmental exposures, and lifestyle changes. Dysbiosis disrupts the integrity of the intestinal barrier, allowing harmful bacteria and bacterial toxins to enter the bloodstream. This can trigger chronic inflammation, alter immune function, and contribute to the development of various diseases.

Common consequences of dysbiosis include:

- Reduced production of beneficial short-chain fatty acids (SCFAs)
- Increased intestinal permeability (“leaky gut”)
- Chronic low-grade inflammation
- Altered immune regulation

- Increased susceptibility to infections

Thus, dysbiosis is considered the underlying mechanism linking the gut microbiome to many chronic diseases.

Inflammatory Bowel Disease (IBD)

Inflammatory Bowel Disease (IBD), which includes Crohn's disease and ulcerative colitis, is a chronic inflammatory condition of the gastrointestinal tract. Studies have shown that individuals with IBD exhibit reduced microbial diversity and lower numbers of beneficial bacteria such as *Faecalibacterium prausnitzii* and *Bifidobacterium*, while harmful bacteria become more abundant. These microbial changes lead to:

- Excessive activation of the immune system
- Persistent intestinal inflammation
- Damage to the intestinal mucosa
- Reduced production of anti-inflammatory metabolites

Patients with IBD commonly experience abdominal pain, diarrhea, rectal bleeding, weight loss, fatigue, and nutritional deficiencies. Restoration of a healthy gut microbiome through dietary interventions, probiotics, and emerging microbiome-based therapies is being explored as a supportive treatment strategy.

Irritable Bowel Syndrome (IBS)

Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, bloating, constipation, diarrhea, or alternating bowel habits. Although IBS does not cause structural damage to the intestine, alterations in the gut microbiome are believed to contribute significantly to its symptoms. In IBS:

- Beneficial bacteria are often reduced
- Gas-producing bacteria may increase
- Between the gut and the brain (gut-brain axis) becomes disrupted

These changes contribute to increased sensitivity of the intestinal nerves, leading to pain and discomfort. Management includes dietary modifications, stress reduction, probiotics, and individualized treatment plans.

Obesity

The gut microbiome plays an important role in regulating energy metabolism and fat storage. Research has demonstrated differences in the gut microbial composition between obese and lean individuals. Certain bacterial communities can extract more energy from food, promoting increased calorie absorption and fat accumulation.

Mechanisms linking the gut microbiome to obesity include:

- Increased energy harvest from dietary carbohydrates

- Altered production of hormones regulating hunger and satiety
- Chronic low-grade inflammation
- Changes in lipid metabolism

Dietary interventions rich in dietary fiber, whole grains, fruits, and vegetables help promote beneficial bacteria that support healthy weight management.

Type 2 Diabetes Mellitus

The gut microbiome influences glucose metabolism and insulin sensitivity. Dysbiosis contributes to insulin resistance through chronic inflammation and impaired intestinal barrier function. Reduced levels of beneficial bacteria decrease the production of short-chain fatty acids, which normally improve glucose regulation.

Individuals with type 2 diabetes often exhibit:

- Reduced microbial diversity
- Increased inflammatory bacteria
- Elevated intestinal permeability
- Impaired metabolic regulation

Lifestyle modifications, dietary fiber, prebiotics, probiotics, and regular physical activity may positively influence the gut microbiome and improve metabolic health.

Colorectal Cancer

Alterations in the gut microbiome have been associated with the development of colorectal cancer. Certain bacteria, including *Fusobacterium nucleatum* and toxin-producing strains of *Escherichia coli*, promote inflammation, DNA damage, and tumor growth. Potential mechanisms include:

- Chronic intestinal inflammation
- Production of carcinogenic metabolites
- DNA damage in intestinal epithelial cells
- Suppression of anti-tumor immune responses

A diet rich in fiber and plant-based foods supports beneficial bacteria that produce protective short-chain fatty acids, particularly butyrate, which helps maintain colon health and may reduce cancer risk.

Allergic and Autoimmune Diseases

The gut microbiome plays a critical role in educating and regulating the immune system from early life. Reduced microbial diversity during infancy has been linked to allergic diseases such as asthma, eczema, and food allergies.

Dysbiosis may also contribute to autoimmune diseases including:

- Rheumatoid arthritis

- Multiple sclerosis
- Type 1 diabetes
- Systemic lupus erythematosus

Neurological and Mental Health Disorders

The gut and brain communicate through the gut-brain axis, involving neural, endocrine, immune, and metabolic pathways. The gut microbiome influences the production of neurotransmitters such as serotonin, dopamine, and gamma-aminobutyric acid (GABA), which affect mood, cognition, and behavior. Emerging evidence suggests associations between gut dysbiosis and:

- Depression
- Anxiety disorders
- Autism spectrum disorder
- Parkinson's disease
- Alzheimer's disease

Inflammation, altered neurotransmitter production, and microbial metabolites are believed to contribute to disease progression. Although research is ongoing, microbiome-targeted therapies may become important components of future neurological disease management.

Cardiovascular Disease

The gut microbiome influences cardiovascular health by producing metabolites that affect lipid metabolism, blood pressure, and vascular function. Some intestinal bacteria metabolize dietary choline and carnitine into trimethylamine (TMA), which is converted in the liver into trimethylamine N-oxide (TMAO). Elevated TMAO levels have been associated with an increased risk of atherosclerosis, heart attack, and stroke.

Maintaining a healthy gut microbiome through balanced nutrition and healthy lifestyle practices may contribute to improved cardiovascular health and reduced disease risk.

9. Methods to Improve the Gut Microbiome

Maintaining a healthy gut microbiome is essential for overall health and disease prevention. Several dietary, lifestyle, and therapeutic approaches can help restore and maintain a balanced gut microbial community.

Healthy Diet

Diet is one of the most important factors influencing the gut microbiome. A diet rich in dietary fiber, fruits, vegetables, legumes, whole grains, and fermented foods promotes the growth of beneficial bacteria. Fiber is fermented by gut bacteria to produce short-chain fatty acids (SCFAs), such as butyrate, acetate, and propionate, which improve intestinal health and reduce inflammation. Limiting processed foods, excessive sugar, and saturated fats also helps maintain microbial balance.

Probiotics

Probiotics are live microorganisms that provide health benefits when consumed in adequate amounts. Common probiotic bacteria include *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces boulardii*. They help restore the balance of gut microorganisms, strengthen the intestinal barrier, improve digestion, reduce diarrhea, and enhance immune function. Probiotic supplements and fermented foods such as yogurt, kefir, kimchi, sauerkraut, and fermented milk are common sources.

Prebiotics

Prebiotics are non-digestible food ingredients that stimulate the growth and activity of beneficial gut bacteria. Foods rich in prebiotics include garlic, onions, bananas, asparagus, oats, chicory root, and legumes. Prebiotics increase the production of SCFAs and improve gut health by selectively nourishing beneficial microorganisms.

Synbiotics

Synbiotics are combinations of probiotics and prebiotics that work together to improve gut microbial composition. The probiotic organisms colonize the intestine more effectively when supplied with prebiotics as a food source, resulting in improved digestive and immune health.

Postbiotics

Postbiotics are bioactive compounds produced by probiotic microorganisms during fermentation. These include enzymes, peptides, vitamins, organic acids, and short-chain fatty acids. Unlike probiotics, postbiotics do not contain live bacteria but still provide anti-inflammatory, antioxidant, and immunomodulating effects.

Fecal Microbiota Transplantation (FMT)

FMT involves transferring processed stool from a healthy donor into the gastrointestinal tract of a patient to restore normal gut microbiota. It is highly effective in treating recurrent *Clostridioides difficile* infection and is being investigated as a treatment for inflammatory bowel disease, irritable bowel syndrome, obesity, and metabolic disorders.

Judicious Use of Antibiotics

Antibiotics can disrupt the gut microbiome by eliminating both harmful and beneficial bacteria. Therefore, antibiotics should only be used when medically necessary and prescribed by healthcare professionals. After antibiotic therapy, consumption of probiotics and fiber-rich foods may help restore microbial diversity.

Regular Physical Activity

Exercise positively influences gut microbial diversity and increases beneficial bacteria associated with improved metabolism and immune function. Moderate physical activity also reduces inflammation and supports gastrointestinal health.

Adequate Sleep and Stress Management

Poor sleep quality and chronic stress negatively affect the gut microbiome through the gut-brain axis. Maintaining regular sleep patterns, practicing relaxation techniques such as yoga, meditation, and deep breathing, and managing stress contribute to a healthier microbiome.

Healthy Lifestyle Practices

Avoiding smoking, limiting alcohol consumption, maintaining adequate hydration, and consuming a balanced diet collectively support a diverse and healthy gut microbiome throughout life.

10. Recent Advances in Gut Microbiome Research

Recent technological developments have greatly expanded our understanding of the gut microbiome and its role in health and disease.

Next-Generation Sequencing (NGS)

Advanced DNA sequencing technologies allow researchers to identify and characterize thousands of microbial species present in the human gut without the need for traditional culture methods. These techniques have significantly improved our understanding of microbial diversity and function.

Personalized Nutrition

Researchers are developing personalized dietary recommendations based on an individual's gut microbiome profile. Personalized nutrition aims to optimize metabolic health, improve blood glucose control, and reduce the risk of chronic diseases.

Microbiome-Based Therapeutics

Scientists are developing targeted microbial therapies, including live biotherapeutic products, engineered probiotics, and microbial metabolites to treat inflammatory bowel disease, obesity, diabetes, and other chronic diseases.

Precision Probiotics

Precision probiotics represent an emerging approach in microbiome-based therapy that aims to provide targeted health benefits by selecting specific microbial strains according to their functional properties and disease-associated microbial alterations. Unlike conventional probiotics, which are generally administered to support overall gastrointestinal health, precision probiotics are designed to influence specific microbial, metabolic, immune, or host pathways associated with particular health conditions. These microorganisms may be selected for their ability to produce beneficial metabolites, strengthen intestinal barrier function, modulate immune responses, inhibit pathogenic microorganisms, or reduce the production of potentially harmful microbial metabolites. In some applications, microorganisms may also be genetically engineered to perform specific therapeutic functions. The development of precision probiotics generally involves characterization of microbial strains, identification of disease-associated pathways,

functional screening, and evaluation in preclinical and clinical models. Because gut microbiome composition and metabolic activity vary considerably between individuals, precision probiotics may also contribute to personalized microbiome-based interventions. However, their clinical application requires careful assessment of efficacy, safety, genetic stability, host–microbe interactions, and regulatory considerations.

Artificial Intelligence (AI) in Microbiome Research

Artificial intelligence and machine learning are increasingly used to analyze large microbiome datasets, predict disease risk, identify microbial biomarkers, and develop personalized treatment strategies.

Gut-Brain Axis Research

Recent studies continue to reveal strong connections between gut microorganisms and neurological conditions such as depression, anxiety, Parkinson’s disease, Alzheimer’s disease, autism spectrum disorder, and multiple sclerosis. Researchers are investigating microbiome-targeted therapies for improving mental health.

Cancer Immunotherapy

The gut microbiome has been shown to influence the effectiveness of cancer immunotherapy. Certain beneficial bacteria enhance immune responses against tumors, leading to improved treatment outcomes in patients receiving immune checkpoint inhibitors.

Microbiome and Precision Medicine

Microbiome analysis is increasingly being incorporated into precision medicine to predict disease susceptibility, treatment response, and drug metabolism, allowing more individualized healthcare approaches.

Organ-on-a-Chip and Gut Models

Researchers are using advanced laboratory models, including gut-on-a-chip technology and intestinal organoids, to study interactions between gut microbes and human tissues under controlled conditions.

Future Directions

Future research aims to identify disease-specific microbial signatures, develop standardized microbiome therapies, improve fecal microbiota transplantation protocols, and establish microbiome-based diagnostics for early disease detection.

Conclusion

The human gut microbiome is a highly complex and dynamic ecosystem that plays an essential role in maintaining human health. It contributes to digestion, nutrient metabolism, immune regulation, vitamin synthesis, protection against pathogens, and communication through the gut-brain axis (Kho *et al.*, 2021). Alterations in the gut microbiome, known as dysbiosis, have been associated with numerous diseases, including inflammatory bowel disease, irritable bowel

syndrome, obesity, diabetes mellitus, colorectal cancer, cardiovascular disease, autoimmune disorders, and neurological conditions (Valdes *et al.*, 2020).

Maintaining a healthy gut microbiome through a balanced diet rich in fiber, probiotics, prebiotics, regular physical activity, adequate sleep, stress management, and responsible antibiotic use can significantly improve overall health and reduce disease risk (Simpson *et al.*, 2021). Recent advances in sequencing technologies, artificial intelligence, personalized nutrition, microbiome-based therapeutics, and precision medicine have opened new opportunities for disease prevention, diagnosis, and treatment. Continued research into the gut microbiome promises to transform healthcare by enabling more personalized and effective therapeutic strategies in the future (Ross *et al.*, 2024).

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THE MICROPLASTIC: TINY PLASTIC BIG PROBLEM FOR TINY LIFE

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Abstract

Plastic pollution has become one of the most pressing environmental challenges of the 21st century, with microplastics emerging as a major threat to ecosystems and biodiversity. Microplastics are plastic particles smaller than 5 mm that originate either from the fragmentation of larger plastic waste or from intentionally manufactured products such as cosmetics, synthetic textiles, and industrial materials. Due to their small size, persistence, and widespread distribution, microplastics are now found in oceans, rivers, lakes, soil, air, drinking water, and food, making them a global environmental concern. Microplastics pose significant risks to tiny forms of life, including plankton, algae, bacteria, fungi, aquatic invertebrates, soil microorganisms, and fish larvae. These organisms often mistake microplastics for food, leading to reduced growth, impaired reproduction, oxidative stress, and disruption of normal physiological functions. Since these tiny organisms form the foundation of ecological food webs, the accumulation of microplastics can affect biodiversity, ecosystem stability, and the transfer of pollutants through the food chain. In addition, microplastics provide surfaces for microbial colonization, forming unique communities known as the plastisphere, which may contain both beneficial plastic-degrading microorganisms and potentially harmful pathogens. This provides a comprehensive overview of the classification, sources, environmental distribution, and ecological impacts of microplastics, with special emphasis on their interactions with microorganisms and their effects on tiny life. It also discusses recent advances in microbial biodegradation of plastics, sustainable management strategies, and future research directions aimed at reducing microplastic pollution. Understanding these aspects is essential for developing environmentally friendly solutions and promoting sustainable environmental management. Through increased scientific awareness, responsible plastic use, improved waste management, and innovative biotechnological approaches, the harmful effects of microplastics can be minimized, helping to protect ecosystems and ensure a healthier environment for future generation

Keywords: Microplastics, Plastic Pollution, Plastisphere, Biodegradation, Ecosystem, Tiny life.

Introduction

Plastic is one of the most widely used materials in modern society because of its durability, lightweight nature, versatility, and low production cost. It is extensively used in packaging, healthcare, agriculture, construction, transportation, textiles, and household products. Although plastics have greatly improved human convenience, their excessive production and improper disposal have created a serious environmental problem. Since most conventional plastics are non-biodegradable, they persist in the environment for hundreds of years, leading to the continuous accumulation of plastic waste.

Over time, larger plastic materials are broken down into smaller fragments by sunlight (ultraviolet radiation), heat, wind, wave action, and mechanical abrasion. These tiny particles, known as microplastics, are defined as plastic particles measuring less than 5 mm in diameter. Microplastics are generally classified into two categories: primary microplastics, and secondary microplastic. Today, microplastics have become ubiquitous in the environment. They have been detected in oceans, rivers, lakes, groundwater, agricultural soils, atmospheric dust, drinking water, seafood, table salt, and even human blood and lung tissue. Their widespread distribution has raised serious concerns because they are highly persistent and can easily enter natural ecosystems. Unlike larger plastic debris, microplastics are difficult to detect and remove, allowing them to accumulate over time.

From a microbiological perspective, microplastics provide an ideal surface for the attachment and colonization of microorganisms, leading to the formation of microbial communities known as the *plastisphere*. These communities consist of bacteria, fungi, algae, and other microorganisms that influence the environmental fate of plastic particles. Some microorganisms have shown the ability to degrade certain plastic polymers through enzymatic activity, offering a promising biological approach for reducing plastic pollution. However, microplastics can also transport harmful microorganisms and toxic pollutants, increasing environmental and public health risks.

Tiny organisms such as phytoplankton, zooplankton, algae, soil microbes, earthworms, aquatic insects, shellfish, and fish larvae are particularly vulnerable to microplastic pollution. These organisms often mistake microplastics for food, resulting in reduced feeding efficiency, impaired growth, reproductive failure, and physiological stress. Since these tiny organisms form the foundation of aquatic and terrestrial food webs, the effects of microplastics can spread throughout ecosystems and ultimately affect larger animals and humans.

The increasing presence of microplastics in food, drinking water, and air has also raised concerns regarding human health. Although research is still ongoing, studies suggest that long-term exposure to microplastics may contribute to inflammation, oxidative stress, and the transport of toxic chemicals within the body. Therefore, understanding the sources, distribution,

environmental impacts, and microbial interactions of microplastics is essential for developing effective pollution control strategies. (Thompson *et al.*, 2004).



2.Types of Microplastic

2.1 Primary Microplastics

Primary microplastics are plastic particles that are intentionally manufactured in microscopic sizes for industrial, commercial, and domestic applications. These particles are produced in small sizes before they are released into the environment. Due to their tiny size, they can easily pass through wastewater treatment systems and eventually enter rivers, lakes, and oceans.

Primary microplastics were widely used in personal care products, cosmetics, industrial cleaning agents, and synthetic textiles. Although several countries have restricted the use of plastic microbeads in cosmetics, they continue to enter the environment through various industrial and domestic activities.

Major Sources of Primary Microplastics

- Cosmetic products (face scrubs and exfoliating creams)
- Toothpaste
- Body wash and facial cleansers
- Industrial plastic pellets (nurdles)
- Synthetic textile fibres (polyester, nylon, acrylic)
- Paints and coatings
- Industrial abrasives
- Manufactured in microscopic sizes
- Usually spherical or unshaped
- Directly released into the environment
- Difficult to remove by wastewater treatment plants

2.2 Secondary Microplastics

Secondary microplastics are formed by the fragmentation of larger plastic materials due to environmental factors such as sunlight (UV radiation), heat, wind, waves, and mechanical abrasion. Unlike primary microplastics, these particles are not intentionally manufactured but are produced through the gradual degradation of plastic waste.

Plastic bottles, bags, food packaging, fishing nets, disposable containers, and agricultural plastics slowly break down into smaller fragments over time. Secondary microplastics constitute the largest proportion of microplastic pollution found in the environment. (Galloway *et al.*, 2017; Parata, 2018)

Major Sources of Secondary Microplastics

- Plastic bottles
- Plastic bags
- Food packaging materials
- Disposable cups and plates
- Fishing nets and ropes
- Agricultural mulch films
- Tyre wear particles
- Plastic household waste

2.3 Comparison Between Primary and Secondary Microplastics

Primary Microplastics	Secondary Microplastics
Intentionally manufactured	Formed by degradation of larger plastics
Uniform size and shape	Irregular size and shape
Used in cosmetics, industries, and textiles	Produced from plastic bottles, bags, fishing nets, and packaging
Directly released into the environment	Formed after exposure to sunlight, heat, and weathering
Comparatively easier to identify	More difficult to identify because of variable shapes

3. Sources of Microplastics

Microplastics originate from a wide variety of natural and human-related activities. However, the majority of microplastics found in the environment are produced through human activities. They are continuously released into water bodies, soil, and the atmosphere, where they persist for long periods because of their resistance to degradation. Understanding the major sources of microplastics is essential for developing effective strategies to reduce plastic pollution and protect environmental and public health (Thompson *et al.*, 2004; Andrady, 2017; Jembeck *et al.*, 2015).

3.1 Household Sources

Daily household activities are an important source of microplastic pollution. Washing synthetic clothes releases thousands of microscopic plastic fibres into wastewater. Household plastic items such as food containers, kitchen utensils, packaging materials, disposable products, and cleaning accessories gradually break down into tiny plastic particles during regular use and disposal.

Examples include Plastic food containers, synthetic clothing, Plastic packaging

3.2 Personal Care and Cosmetic Products

Many personal care products previously contained plastic microbeads that were used as exfoliating or cleansing agents. These particles are washed into drainage systems after use. Since they are extremely small, wastewater treatment plants often fail to remove them completely, allowing them to enter rivers and oceans.

Examples include Face scrub Body wash Cosmetic exfoliants.

3.3 Textile Industry

The textile industry is one of the largest contributors to microplastic pollution. Synthetic fabrics such as polyester, nylon, acrylic, and polypropylene release tiny plastic fibres during washing and manufacturing processes.

3.4 Industrial Activities

Plastic manufacturing industries produce large quantities of plastic pellets, powders, and fragments during production, transportation, and storage.

Example include plastic pellets (nurdles), plastic manufacturing waste, paint industries.

3.5 Agricultural Activities

Modern agriculture extensively uses plastic materials to improve crop production. Plastic mulch films, irrigation pipes, greenhouse covers, and fertilizer bags gradually degrade due to sunlight and weathering, releasing microplastics into agricultural soils.

Examples include plastic mulch films, greenhouse sheets fertilizer bags.

3.6 Fisheries and Marine Activities

Fishing and marine industries contribute significantly to plastic pollution. Lost or discarded fishing nets, ropes, floats, and aquaculture equipment gradually fragment into microplastics under the influence of sunlight and wave action.

3.8 Atmospheric Sources

Microplastics are also transported through the atmosphere. Tiny fibers released from clothing, household dust, industrial emissions, and vehicle movement become airborne and can travel long distances before settling on land or water surfaces

4. Distribution of Microplastics in the Environment

Microplastics are widely distributed across different environmental compartments due to their small size, lightweight nature, and resistance to degradation. They are transported by wind, water

currents, rainfall, and human activities, allowing them to spread from urban areas to remote ecosystems. Today, microplastics have been detected in marine environments, freshwater systems, agricultural soils, atmospheric dust, glaciers, polar regions, drinking water, and even living organisms. Their widespread presence has become a major environmental concern because they can persist for long periods and enter the food chain.

4.1 Distribution in Marine Ecosystems

Marine ecosystems are considered the largest sink for microplastics. Every year, millions of tonnes of plastic waste enter oceans through rivers, coastal activities, fishing operations, shipping, and tourism. Over time, larger plastic debris fragments into microplastics due to sunlight, wave action, and mechanical abrasion.

Microplastics are found in surface water, the water column, and deep-sea sediments. Marine organisms such as plankton, jellyfish, mussels, oysters, crabs, fish, and sea turtles often ingest these particles because they resemble natural food. This ingestion can lead to reduced feeding, impaired growth, and disruption of marine food webs (Cole *et al.*, 2011).

4.2 Distribution in Freshwater Ecosystem

Freshwater organisms, including algae, zooplankton, aquatic insects, mollusks, and freshwater fish, are exposed to microplastics, which may affect their growth, survival, and reproductive capacity (Cole *et al.*, 2011; Xiang *et al.*, 2023).

4.3 Distribution in Soil

Agricultural and urban soils have become important reservoirs of microplastics. Plastic mulch films, irrigation pipes, sewage sludge, compost, and plastic litter contribute to soil contamination. Since plastics degrade very slowly, these particles accumulate over time and interact with soil organisms. Microplastics can alter soil structure, reduce water-holding capacity, affect nutrient cycling, and influence the diversity of beneficial soil microorganisms.

4.4 Distribution in the Atmosphere

Microplastics are also present in the atmosphere in the form of tiny fibres and dust particles. These particles originate from synthetic textiles, tyre wear, road dust, industrial emissions, and household activities. Wind transports airborne microplastics over long distances, allowing them to reach mountains, forests, and polar regions (Parata, 2018).

Atmospheric deposition is now recognized as an important pathway through which microplastics enter terrestrial and aquatic ecosystems.

5. Microplastics and Microorganisms (Plastisphere)

Microplastics not only act as environmental pollutants but also provide a unique surface for the attachment and growth of microorganisms. Unlike natural materials, plastic surfaces remain stable in the environment for long periods, allowing microorganisms to colonize and form complex microbial communities. These communities are collectively known as the plastisphere.

The plastisphere has become an important area of research in environmental microbiology because it influences the transport, persistence, and ecological effects of microplastics.

The interaction between microorganisms and microplastics can have both beneficial and harmful consequences. Some microorganisms produce enzymes that help degrade plastic materials, whereas others may include pathogenic species capable of spreading through aquatic and terrestrial ecosystems (Zettler *et al.*, 2013).

5.1 Formation of the Plastisphere

When microplastics enter the environment, their surfaces are rapidly covered by organic matter such as proteins and polysaccharides. This conditioning layer attracts microorganisms, which attach to the plastic surface and begin forming a biofilm.

The process of plastisphere formation occurs in four stages:

- i. Surface Conditioning – Organic molecules coat the plastic surface.
- ii. Microbial Attachment – Bacteria and fungi attach to the conditioned surface.
- iii. Biofilm Formation – Microorganisms multiply and produce extracellular polymeric substances (EPS), forming a protective biofilm.
- iv. Community Development – A mature microbial community develops, containing bacteria, fungi, algae, protozoa, and other microorganisms.

Biofilms protect microorganisms from environmental stress and increase their survival in aquatic and terrestrial environments

5.2 Microorganisms Associated with Microplastics

Various microorganisms have been identified on the surfaces of microplastics.

- *Pseudomonas* spp.
- *Bacillus* spp.
- *Rhodococcus* spp.
- *Aspergillus* spp.
- *Penicillium* spp.

5.3 Ecological Importance of the Plastisphere

The plastisphere plays an important role in environmental processes. Certain microorganisms present in these communities participate in nutrient cycling and organic matter decomposition. Some bacteria and fungi produce enzymes capable of degrading plastic polymers, making them valuable for bioremediation research.

However, the plastisphere can also act as a carrier for harmful microorganisms. Pathogenic bacteria may attach to microplastics and be transported over long distances, increasing the risk of disease transmission. In addition, biofilms may facilitate the spread of antibiotic resistance genes among microbial populations

5.4 Plastic-Degrading Microorganisms

Recent studies have identified several microorganisms capable of degrading plastics through enzymatic activity.

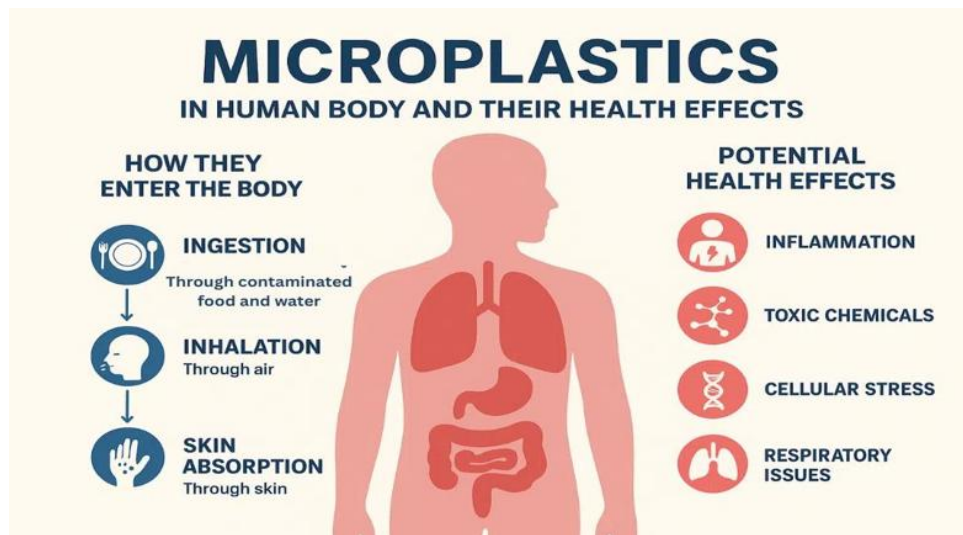
Examples

- *Ideonella sakaiensis* – Degrades polyethylene terephthalate (PET)
- *Pseudomonas* spp. – Degrades polyethylene (PE) and polyurethane (PU)
- *Bacillus* spp. – Contributes to polyethylene degradation
- *Aspergillus niger* – Degrades various synthetic polymers
- *Penicillium* spp. – Participates in plastic biodegradation

Although biodegradation is generally slow, these microorganisms offer promising eco-friendly solutions for plastic waste management.

6.Impact of Microplastics on Tiny Life and Human Health

Microplastics have emerged as one of the most significant environmental pollutants due to their widespread distribution and persistence in nature. Their small size enables them to be easily ingested or inhaled by a wide variety of organisms. Tiny organisms, including microorganisms, plankton, algae, aquatic invertebrates, and soil fauna, are particularly vulnerable microplastics in these organisms can disrupt biological functions, alter ecological interactions, and affect the to microplastics through food, drinking water, and air.



6.1 Impact on Tiny Life

Tiny organisms form the foundation of aquatic and terrestrial food webs. Any disturbance to these organisms can affect higher trophic levels and reduce ecosystem productivity (Wright *et al.*, 2017).

Impact on Plankton

Phytoplankton and zooplankton are the primary producers and primary consumers in aquatic ecosystems. They often mistake microplastics for food and ingest them.

Effects:

- Reduced feeding efficiency
- Impaired growth and development
- Decreased reproduction
- Increased oxidative stress

Algae are essential for oxygen production and nutrient cycling. Microplastics can adhere to algal surfaces, reducing light penetration and interfering with photosynthesis.

Effects:

- Reduced photosynthetic activity
- Slower growth
- Changes in nutrient absorption

Small aquatic animals such as mussels, oysters, shrimp, worms, and insects readily consume microplastics suspended in water.

Effects:

- Reduced energy availability
- Reproductive disorders
- Increased mortality
- Impact on Soil Organisms

Earthworms, nematodes, fungi, and beneficial bacteria are important for maintaining soil fertility. Microplastics can alter soil properties and affect these organisms.

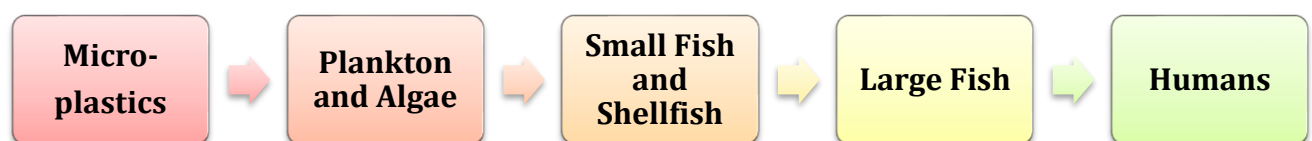
Effects:

- Reduced microbial diversity
- Disturbance of nutrient cycling
- Reduced earthworm

6.2 Impact on the Food Chain

Microplastics enter the food chain when tiny organisms ingest them. These particles are transferred from one trophic level to another through predation. As a result, fish, birds, marine mammals, and humans may accumulate microplastics in their bodies

Food Chain Pathway



This process is known as trophic transfer and may increase exposure to plastic particles and associated toxic chemicals.

6.3 Impact on Human Health

Humans are exposed to microplastics through seafood, drinking water, table salt, fruits, vegetables, packaged foods, and inhalation of airborne particles. Although research is still ongoing, scientists have identified several potential health concerns (Galloway *et al.*, 2017; Prata *et al.*, 2018).

Possible Health Effects

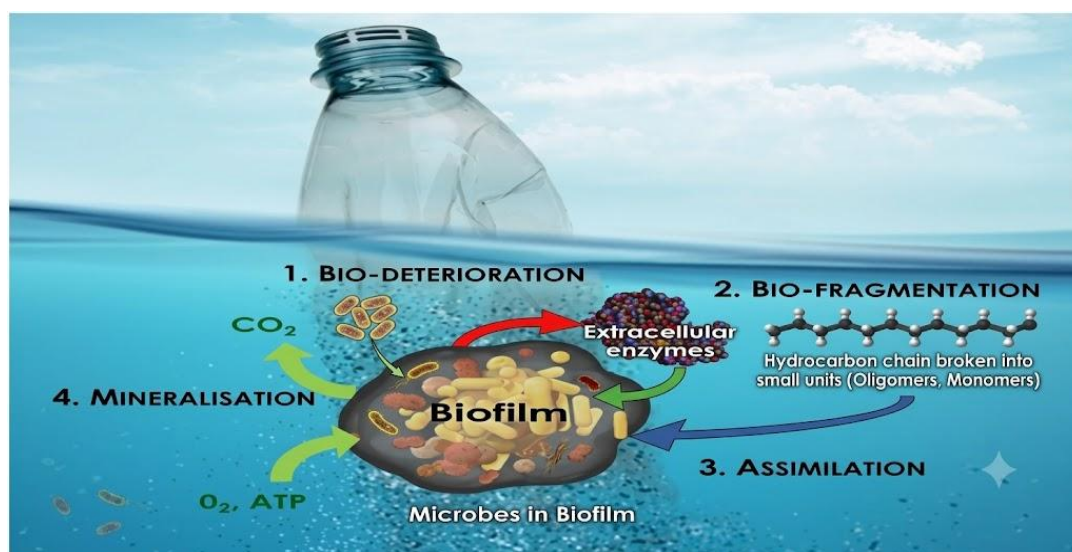
- Inflammation of tissues
- Oxidative stress
- Cellular damage
- Disruption of hormone (endocrine) function
- Transport of harmful chemicals and heavy metals
- Respiratory irritation from airborne microplastics

6.4 Ecological Consequences

- Loss of biodiversity
- Reduced ecosystem productivity
- Decline in aquatic and terrestrial ecosystem health

7. Plastic-Degrading Microorganisms

The persistence of plastic waste in the environment has encouraged researchers to explore biological methods for plastic degradation. Certain microorganisms possess the ability to colonize plastic surfaces and produce enzymes that gradually break down plastic polymers into simpler compounds. This process, known as microbial biodegradation, is considered an environmentally friendly and sustainable approach for managing plastic pollution. Although the degradation process is relatively slow, advances in environmental microbiology and biotechnology have shown promising results in enhancing microbial efficiency (Yoshida *et al.*, 2016; Cai *et al.*, 2023).



7.1 Common Plastic-Degrading Bacteria

Several bacterial species have demonstrated the ability to degrade different types of plastics under laboratory and environmental conditions. (Barnes *et al.*, 2009).

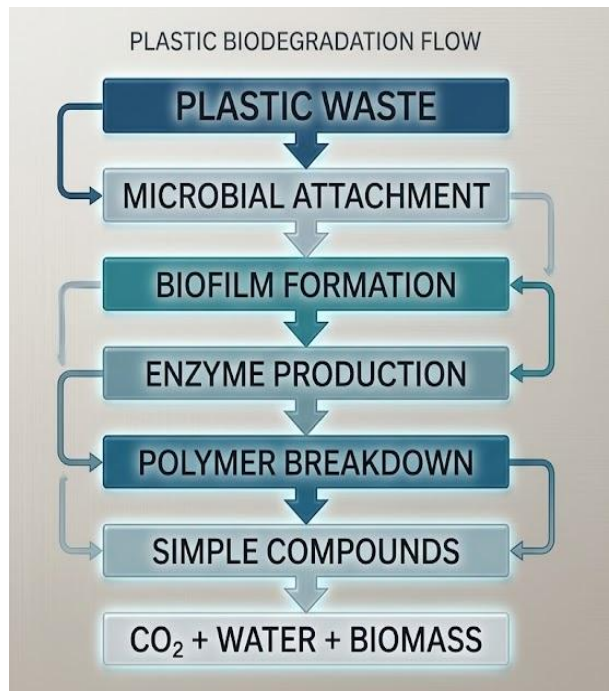
<i>Ideonella sakaiensis</i>	Polyethylene terephthalate (PET)
<i>Pseudomonas</i> spp.	Polyethylene (PE)
<i>Bacillus</i> spp.	Polyethylene (PE)
<i>Rhodococcus</i> spp	Polyethylene (PE)
<i>Acinetobacter</i> spp	Polyurethane (PU)

Fungi are also capable of degrading synthetic polymers by producing extracellular enzyme

7.2 Mechanism of Plastic Biodegradation

Plastic biodegradation occurs through several sequential steps:

- Attachment – Microorganisms attach to the plastic surface.
- Biofilm Formation – A microbial biofilm develops on the plastic.
- Enzyme Production – Microorganisms produce enzymes that break down plastic polymers.
- Polymer Degradation – Long polymer chains are converted into smaller molecules.
- Mineralization – The degraded products are converted into carbon dioxide, water, biomass, and other simple compounds.



8. Prevention and Control of Microplastic Pollution

Preventing microplastic pollution requires the combined efforts of individuals, industries, researchers, and governments. Reducing plastic production and improving waste management are essential for minimizing environmental contamination (Cole *et al.*, 2011).

Control Measures

- Reduce the use of single-use plastics.
- Improve plastic waste collection and recycling.
- Strengthen wastewater treatment technologies.
- Develop biodegradable polymers.
- Support research on microbial biodegradation and bioremediation

9. Future Scope

Although considerable progress has been made in understanding microplastic pollution several challenges remain. Future research should focus on developing sustainable technologies for monitoring, preventing, and removing microplastics from the environment.

Future Research Areas

- Discovery of new plastic-degrading microorganisms.
- Genetic engineering to improve biodegradation efficiency.
- Advanced wastewater treatment technologies.
- Long-term monitoring of microplastic pollution.

Continued research in environmental microbiology, biotechnology, and environmental engineering will play a crucial role in addressing the global challenge of microplastic pollution.

Conclusion

Microplastics have emerged as one of the most significant environmental pollutants of the modern era. Their persistence, widespread distribution, and ability to enter aquatic, terrestrial, and atmospheric ecosystems make them a serious threat to biodiversity and environmental sustainability. Tiny organisms such as plankton, algae, bacteria, fungi, aquatic invertebrates, and soil microorganisms are particularly vulnerable because they form the foundation of ecological food webs. The accumulation of microplastics in these organisms can disrupt growth, reproduction, nutrient cycling, and ecosystem stability.

Microplastics also provide surfaces for microbial colonization, leading to the formation of the plastisphere. While some microorganisms contribute to the spread of harmful pathogens, others possess the remarkable ability to degrade plastic materials through enzymatic processes. These plastic-degrading microorganisms offer promising opportunities for sustainable bioremediation and environmentally friendly waste management reducing microplastic pollution requires integrated approaches involving responsible plastic use, improved waste management, technological innovation, environmental education, and strong government policies. Continued scientific research and public awareness are essential for protecting ecosystems, conserving biodiversity, and ensuring a cleaner and healthier environment for future generations.

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GREEN MICROBIOLOGY AND CIRCULAR BIOECONOMY: MICROBIAL SOLUTIONS FOR SUSTAINABLE DEVELOPMENT

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Abstract

Green microbiology and circular bioeconomy are emerging interdisciplinary fields that combine microbiology, biotechnology, environmental science, and sustainable development. Green microbiology focuses on the use of beneficial microorganisms to reduce pollution, recycle waste, produce renewable energy, and replace harmful chemical processes with eco-friendly biological alternatives. The circular bioeconomy aims to minimize waste by converting biological resources into valuable products such as biofuels, bioplastics, biofertilizers, enzymes, and other biobased materials. Microorganisms such as bacteria, fungi, algae, and yeast play a central role in nutrient recycling, biodegradation, carbon sequestration, and waste valorization. This chapter discusses the principles of green microbiology, microbial applications in agriculture and industry, waste management, bioremediation, bioenergy production, sustainable agriculture, challenges, future prospects, and the importance of circular bioeconomy in achieving environmental sustainability (Atlas and Bartha, 2019).

Keywords: Green Microbiology, Circular Bioeconomy, Sustainable Development, Bioremediation, Sustainable Agriculture.

Introduction

Environmental pollution, climate change, depletion of natural resources, and increasing waste generation have become major global concerns. Conventional industrial and agricultural practices rely heavily on fossil fuels and synthetic chemicals, leading to environmental degradation. Therefore, sustainable alternatives are urgently needed (FAO, 2022).

Green microbiology is an innovative branch of microbiology that utilizes microorganisms and their metabolic activities to develop environmentally friendly technologies. Beneficial microbes naturally recycle nutrients, degrade pollutants, fix atmospheric nitrogen, produce renewable energy, and synthesize valuable bioproducts.

The circular bioeconomy complements green microbiology by promoting the reuse, recycling, and recovery of biological resources. Instead of following the traditional "take–make–dispose"

model, the circular bioeconomy emphasizes converting waste into valuable products, thereby reducing environmental pollution and conserving natural resources.

Together, green microbiology and circular bioeconomy contribute to achieving the United Nations Sustainable Development Goals (SDGs), particularly those related to clean energy, responsible consumption, climate action, and sustainable agriculture.

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Concept of Green Microbiology

Green microbiology is the application of microorganisms to solve environmental problems while promoting sustainable production systems. It replaces chemical-intensive processes with microbial-based biological processes that are environmentally safe and economically beneficial (Atlas and Bartha, 2019; Madigan *et al.*, 2021).

Objectives

- Reduce environmental pollution
- Improve soil fertility naturally
- Convert waste into useful products
- Produce renewable energy
- Replace synthetic chemicals with microbial products
- Support renewable energy
- Protect biodiversity
- Reduce greenhouse gas emissions
- Characteristics
- Eco-friendly
- Sustainable
- Cost-effective
- Renewable
- Energy-efficient
- Environmentally safe

Principles of Green Microbiology

Green microbiology is based on the sustainable use of microorganisms to protect the environment and promote efficient resource utilization. It encourages biological processes instead of chemical-intensive methods (Madigan *et al.*, 2021).

Principles

1. Sustainable Resource Utilization

Microorganisms help convert renewable biological resources into valuable products without exhausting natural resources.

2. Waste Minimization

Agricultural, industrial, and food wastes are converted into useful products such as compost, biogas, biofertilizers, and bioethanol instead of being discarded.

3. Pollution Prevention

Beneficial microbes degrade toxic pollutants in soil, water, and air, thereby reducing environmental contamination.

4. Renewable Energy Production

Microorganisms produce renewable energy sources such as biogas, biohydrogen, biodiesel, and bioethanol from biomass.

5. Natural Nutrient Recycling

Microbes recycle carbon, nitrogen, phosphorus, and sulfur through natural biogeochemical cycles.

6. Reduction of Chemical Usage

Microbial biofertilizers and biopesticides reduce dependence on synthetic fertilizers and pesticides.

7. Biodiversity Conservation

Green microbiology protects microbial diversity and maintains ecological balance.

8. Circular Economy Integration

Microorganisms transform waste into valuable bioproducts, supporting a circular economy.

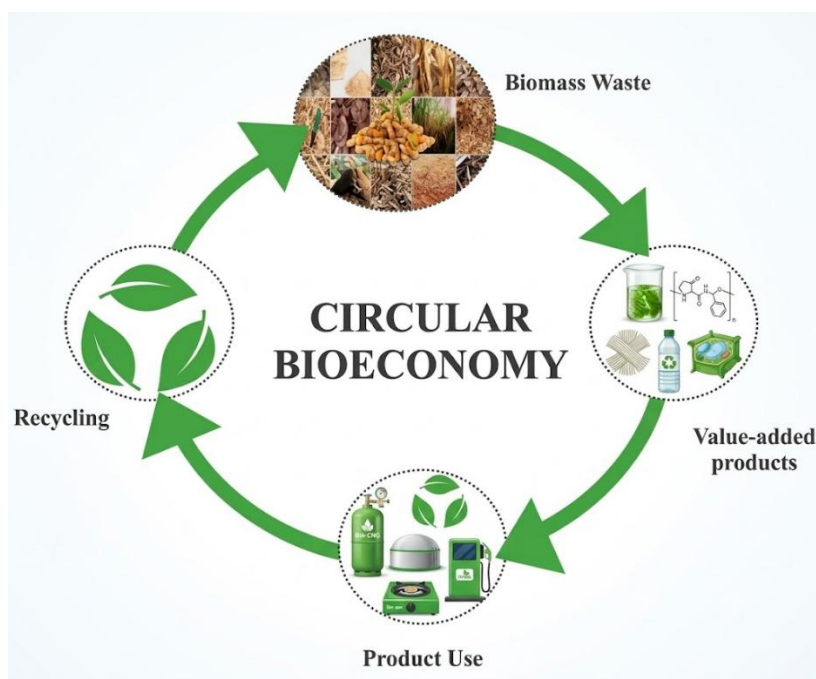
Importance

- Protects ecosystems
- Conserves natural resources
- Reduces greenhouse gas emissions
- Promotes sustainable industries
- Supports climate change mitigation

Circular Bioeconomy

The Circular Bioeconomy is a sustainable economic model that focuses on the efficient use of renewable biological resources to produce food, energy, and bio-based products while

minimizing waste and environmental pollution. Unlike the traditional linear economy, which follows the "take–make–dispose" approach, the circular bioeconomy follows the principles of Reduce, Reuse, Recycle, and Recover. It encourages the conversion of agricultural residues, food waste, forestry biomass, and industrial by-products into valuable products such as biofertilizers, biopesticides, biogas, bioethanol, bioplastics, enzymes, and animal feed through the action of microorganisms and biotechnology. This approach conserves natural resources, reduces greenhouse gas emissions, promotes resource efficiency, creates green jobs, and supports sustainable agriculture and industrial development. Therefore, the circular bioeconomy plays a vital role in achieving environmental sustainability, economic growth, and the United Nations Sustainable Development Goals (SDGs) (FAO, 2022; United Nations, 2015).



Role of Microorganism in Circular Bioeconomy

Microorganisms are the driving force of the circular bioeconomy because they convert waste into valuable products, recycle nutrients, and produce renewable energy. They help reduce pollution and improve environmental sustainability (Glazer and Nikaido, 2018).

Major Microorganisms

1. Bacteria

Bacteria decompose organic matter and convert it into useful products. They are used in composting, wastewater treatment, biofertilizer production, and biogas generation.

Examples:

- *Rhizobium*
- *Azotobacter*
- *Bacillus*
- *Pseudomonas*

2. Fungi

Fungi produce enzymes that degrade cellulose, lignin, plastics, and agricultural residues. They are widely used in composting and biodegradation.

Examples:

- *Penicillium*
- *Penicillium*
- *Trichoderma*

3. Algae

Algae absorb carbon dioxide and convert sunlight into biomass. They are used for biodiesel production, wastewater treatment, carbon capture, and biofertilizer production.

4. Yeast

Yeast converts sugars into ethanol and carbon dioxide during fermentation.

Major Microorganisms and Their Application

Microorganism	Type	Application
<i>Rhizobium</i>	Bacteria	Nitrogen fixation
<i>Azotobacter</i>	Bacteria	Biofertilizers
<i>Pseudomonas</i>	Bacteria	Bioremediation
<i>Bacillus</i>	Bacteria	Enzyme production
<i>Trichoderma</i>	Fungus	Biocontrol agent
<i>Aspergillus</i>	Fungus	Enzyme production
<i>Saccharomyces cerevisiae</i>	Yeast	Bioethanol production
<i>Chlorella</i>	Algae	Biodiesel production

Examples:

- Waste recycling
- Nutrient cycling
- Renewable energy production
- Pollution control
- Sustainable agriculture
- Climate change mitigation

Microbial Waste Management

Microbial waste management is the process of using microorganisms such as bacteria, fungi, algae, and yeast to decompose and convert organic waste into useful products. These microorganisms break down agricultural waste, food waste, industrial waste, and sewage through natural biological processes, reducing environmental pollution and minimizing landfill waste. The end products include compost, biofertilizers, biogas, bioethanol, enzymes, and other value-added products. Microbial waste management also improves soil fertility, supports nutrient

recycling, conserves natural resources, and reduces greenhouse gas emissions. It is an eco-friendly, sustainable, and cost-effective approach that plays a key role in the circular bioeconomy by transforming waste into valuable resources (Singh and Ward, 2018).

Biofertilizers

Biofertilizers are preparations containing beneficial microorganisms that improve soil fertility by increasing the availability of nutrients to plants (Singh and Singh, 2017).

Examples:

- *Rhizobium*
- *Azotobacter*
- *Azospirillum*
- Phosphate Solubilizing Bacteria (PSB)

Advantages

- Improves soil fertility
- Increases crop yield
- Reduces chemical fertilizer use
- Eco-friendly and cost effective

Biopesticides

Biopesticides are biological agents derived from microorganisms or natural sources that control pests and plant diseases without harming the environment (Kaur and Arora, 2020).

Examples:

- *Bacillus thuringiensis* (Bt)
- *Trichoderma* spp
- *Beauveria bassiana*
- *Pseudomonas fluorescens*

Advantages

- Eco-friendly
- Target-specific
- Safe for humans and animals
- Reduces chemical pesticide use
- Maintains biodiversity.

Bioenergy Production

Bioenergy production is the process of producing renewable energy from biological materials (biomass) using microorganisms. It helps reduce dependence on fossil fuels and supports sustainable development (Rittmann, 2018).

Types of Bioenergy

- **Biogas** – produced by anaerobic digestion of organic waste using methanogenic bacteria.

- **Bioethanol** – Produced by fermentation of sugars using *Saccharomyces cerevisiae* (Yeast).
- **Biodiesel** – Produced from vegetable oils, animal fats, or microalgae.
- **Biohydrogen** – Produced by certain bacteria and algae under suitable conditions.

Advantages

- Renewable and eco-friendly energy source
- Reduces greenhouse gas emissions
- Converts organic waste into useful energy
- Decreases dependence on fossil fuels
- Supports the circular bioeconomy

Application

- Electricity generation
- Cooking fuel
- Transportation fuel
- QIndustrial heating
- Rural energy supply

Microbial Enzymes and Industrial Applications

Microbial enzymes are biological catalysts produced by microorganisms such as bacteria, fungi, and yeast. They accelerate biochemical reactions and are widely used in various industries because they are efficient, eco-friendly, and cost-effective. Common microbial enzymes include amylase, protease, lipase, cellulase, and pectinase. These enzymes are used in the food and beverage industry for food processing, the detergent industry for stain removal, the textile and paper industries for fabric and pulp processing, the pharmaceutical industry for drug production, and the biofuel industry for biomass conversion. Thus, microbial enzymes play an important role in industrial biotechnology and sustainable manufacturing (Glazer and Nikaido, 2018).



Green Biotechnology and Sustainable Agriculture

Green biotechnology is the application of biotechnology in agriculture to improve crop productivity, soil fertility, and environmental sustainability while minimizing the use of harmful chemicals (Singh and Singh, 2017; FAO, 2022).

Applications

1. Biofertilizers

Enhance nutrient availability and improve soil fertility naturally.

2. Biopesticides

Protect crops from pests and diseases without harming the environment.

3. Composting

Microorganisms convert organic waste into nutrient-rich compost.

4. Plant Growth-Promoting Rhizobacteria (PGPR)

Promote root growth, nutrient uptake, and disease resistance.

5. Mycorrhiza

Improve phosphorus uptake and water absorption.

Challenges and Limitations

Although green microbiology and the circular bioeconomy offer many advantages, several challenges remain (FAO, 2022; United Nations, 2015).

1. High Initial Investment

Setting up biotechnological facilities requires significant financial resources.

2. Limited Public Awareness

Many farmers and industries are unaware of microbial technologies.

3. Technical Constraints

Large-scale production and maintenance of microbial cultures require skilled personnel.

4. Regulatory Issues

Strict government regulations may delay the commercialization of microbial products.

5. Climate Dependence

Microbial activity is affected by temperature, pH, moisture, and nutrient availability.

6. Market Acceptance

Some bio-based products face competition from conventional products.

Possible Solutions

- Increase government support and funding.
- Promote awareness and education.
- Strengthen research and development.
- Encourage public-private partnerships.
- Develop cost-effective technologies.

Future Perspectives

Green microbiology and the circular bioeconomy are expected to play a major role in achieving sustainable development in the future. Rapid advancements in microbial biotechnology, genomics, synthetic biology, artificial intelligence (AI), and bioengineering are creating new opportunities for eco-friendly production systems. The integration of these technologies will improve the efficiency of microbial processes and reduce dependence on fossil fuels (Glazer and Nikaido, 2018).

Future research is expected to focus on developing advanced microbial strains for faster biodegradation, enhanced biofuel production, carbon capture, and efficient waste recycling. The use of genetically engineered microorganisms may increase the production of biofertilizers, bioplastics, enzymes, pharmaceuticals, and renewable energy. Microalgae are also gaining attention as a sustainable source of biofuels, animal feed, nutraceuticals, and carbon sequestration.

Industries are increasingly adopting circular bioeconomy principles by converting agricultural residues, food waste, and industrial by-products into valuable products. Smart biorefineries, precision agriculture, and digital biotechnology are expected to further strengthen sustainable production systems.

Governments, research institutions, and industries must collaborate to encourage innovation, promote public awareness, and implement supportive policies. These efforts will accelerate the transition toward a greener economy and help achieve global sustainability goals.

Conclusion

Green microbiology and the circular bioeconomy provide sustainable solutions to some of the world's most pressing environmental challenges. Beneficial microorganisms play a crucial role in waste recycling, nutrient cycling, pollution control, renewable energy production, and sustainable agriculture. Their applications reduce environmental pollution, conserve natural resources, and minimize dependence on fossil fuels and synthetic chemicals.

The circular bioeconomy promotes efficient utilization of biological resources by transforming waste into valuable products such as biofertilizers, bioplastics, biofuels, enzymes, and other bio-based materials. This approach supports economic growth while protecting ecosystems and biodiversity.

Despite challenges such as high initial costs, technical limitations, and regulatory barriers, continued research, technological innovation, and government support will help overcome these obstacles. Green microbiology and the circular bioeconomy are expected to become essential components of sustainable development and contribute significantly to environmental protection, food security, clean energy production, and climate resilience (United Nations, 2015; FAO, 2022).

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THE HUMAN MICROBIOME: HOST–MICROBE INTERACTIONS, HEALTH, DISEASE AND EMERGING THERAPEUTICS

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Abstract

The human microbiome consists of a diverse collection of microorganisms and their genetic material that inhabit the body and interact with host tissues. These communities include bacteria, archaea, fungi, viruses and other microbial groups and are particularly prominent in the gastrointestinal tract, while also occurring in habitats such as the skin, mouth, respiratory system and urogenital tract. Current life science research indicates that these microbial ecosystems participate in nutrition, metabolism, immune regulation and resistance to disease. Gut microorganisms assist in the utilization of dietary substrates, formation of biologically active metabolites, maintenance of the intestinal barrier and communication with host cells. An imbalance in microbial composition or activity, generally termed dysbiosis, has been associated with inflammatory bowel conditions, metabolic disorders and susceptibility to some infections. Developments in sequencing, metagenomics, metabolomics, bioinformatics and computational biology have made it possible to investigate microbial communities at unprecedented levels of detail. Consequently, interventions involving probiotics, prebiotics, dietary modification, faecal microbiota transplantation and emerging microbial therapeutics are being explored. A further direction is precision microbiome medicine, where individual microbial characteristics may contribute to personalized approaches to prevention and treatment. Nevertheless, further experimental and clinical work is required to establish causality, clarify host–microbe mechanisms and ensure that microbiome-based interventions are safe, reproducible and clinically effective. The microbiome has therefore become an important area of modern life science research with implications for understanding health, preventing disease and developing new therapeutic strategies.

Keywords: Human Microbiome, Microbial Metabolites, Host–Microbe Interactions, Microbiome Medicine and Precision Health.

Introduction

The human body functions as an ecosystem in which human cells coexist and interact with numerous microbial populations. These microorganisms occupy distinct body habitats and

continuously communicate with host tissues and with each other. The term microbiota generally refers to the microorganisms themselves, whereas microbiome is commonly used to describe the microorganisms together with their collective genetic potential. Microbial composition differs considerably among anatomical sites because factors such as oxygen concentration, pH, nutrient availability, temperature and host defence mechanisms vary from one location to another.

Among these habitats, the gastrointestinal tract represents one of the richest and most functionally significant microbial environments. Intestinal microorganisms contribute to the processing of dietary substances, modification of bile acids and formation of metabolites including short-chain fatty acids. They also interact with epithelial cells and immune mechanisms. These functions demonstrate that the microbiome is an active component of human physiology rather than merely a collection of organisms living in the body.

The rapid expansion of microbiome research has been closely associated with molecular methods that permit the study of microorganisms that are difficult or impossible to cultivate using conventional laboratory techniques. Sequencing, metagenomic analysis, metabolomics and computational methods have enabled researchers to examine both community composition and functional potential. As a result, microbiological research has increasingly expanded from investigations of individual organisms to the study of complete microbial ecosystems.

Modern microbiome science also adopts a systems-oriented perspective. Rather than examining one biological component independently, researchers consider the relationships among microorganisms, host cells, metabolites, diet, immunity and environmental influences. This integrated approach provides a useful framework for investigating the ways in which microbial communities may affect human health.

Human Microbiome: Concepts and Diversity

The human microbiome encompasses microorganisms and their associated genetic information across the different microbial habitats of the body. Although bacteria constitute a major component, archaea, fungi, bacteriophages and other viruses are also important members of these ecosystems. The microbial profile of each body site is shaped by the local physical and chemical environment as well as by host-related factors.

Major Microbial Habitats

- Skin microbiome – microbial populations associated with the skin and its related structures.
- Oral microbiome – microorganisms inhabiting the teeth, tongue, saliva and other oral surfaces.
- Gut microbiome – a complex microbial ecosystem strongly involved in digestion, metabolism and interactions with immunity.

- Respiratory microbiome – microbial populations occurring throughout the respiratory environments, particularly the upper respiratory tract.
- Urogenital microbiome – microbial communities whose composition is influenced by local physiological and environmental conditions.

Development and Factors Shaping the Human Microbiome

Microbial communities begin to establish early in life and continue to change throughout an individual's development. Their composition can be affected by maternal influences, delivery conditions, feeding practices, environmental exposure, antibiotic treatment and dietary habits. During childhood and adulthood, additional changes occur in response to lifestyle, physiological status and environmental conditions.

- **Diet and Nutrition:** Diet is a major determinant of gut microbial composition and activity. Dietary fibre and other complex carbohydrates can provide substrates for microorganisms capable of fermenting them, resulting in the formation of metabolites that may influence intestinal and systemic physiology. Consequently, differences in dietary patterns can alter both the organisms present and the functions carried out by the microbial community.
- **Antibiotics and Medications:** Antibiotic treatment can reduce susceptible members of the microbiota and consequently modify the structure of the microbial community. The magnitude and duration of these effects depend on factors such as the antimicrobial agent, dose, treatment period and characteristics of the individual microbiome. Other medications may also affect microbial metabolism or modify the environment in which microorganisms reside.
- **Age and Lifestyle:** The microbiome changes over the course of life and is influenced by several lifestyle variables. Diet, sleep, physical activity, psychological stress, environmental exposure and medication use can all contribute to differences in microbial communities. Thus, the human microbiome should be regarded as a dynamic ecosystem rather than a permanently fixed population.

Gut Microbiome and Human Physiology

The gut microbiome contributes to a range of physiological activities. Intestinal microorganisms can transform dietary compounds that are not readily processed by human enzymes. Fermentation of suitable substrates generates short-chain fatty acids, including acetate, propionate and butyrate, which can interact with intestinal tissues and immune pathways.

Gut microorganisms also participate in the transformation of host-derived substances such as bile acids and in the metabolism of nutrients and xenobiotic compounds. Through these activities, they modify the chemical environment of the intestine and contribute to communication between the gastrointestinal tract and other organs.

- **Intestinal Barrier and Microbial Interaction:** The intestinal barrier provides separation between the internal tissues and the contents of the gastrointestinal tract. Microbial molecules and metabolites can influence epithelial activity, mucus production and immune signalling. A stable microbial ecosystem may contribute to barrier maintenance, whereas alterations in microbial composition or function may promote inappropriate host responses.

Microbiome and the Immune System

The microbiome has an important relationship with immune development and regulation. Microbial molecules and metabolites can interact with receptors on host cells and modify signalling pathways involved in immunity. Exposure to microorganisms early in life is particularly relevant to the development of immune tolerance and balanced responses to environmental substances.

Host and microbiota influence each other in a two-way relationship. The immune system regulates microbial populations through physical barriers, antimicrobial substances and immune responses, while microorganisms provide signals capable of shaping immune activity. This reciprocal communication is important for maintaining physiological equilibrium.

- **Microbiome and Inflammation:** Alterations in microbial communities have been associated with changes in inflammatory activity. However, an observed association between a microbial feature and a disease cannot by itself demonstrate that the microbial change caused the condition. Current research therefore emphasizes mechanistic studies and experimental approaches designed to determine whether particular organisms, pathways or metabolites have causal roles.

Dysbiosis and Human Disease

Dysbiosis is broadly used to describe a change in the composition or functional activity of a microbial community that occurs in association with an unhealthy state. It can include reduced representation of particular functions, expansion of selected microbial groups, changes in diversity or shifts in microbial metabolism. The interpretation of dysbiosis depends on the body site and the disease being considered.

- **Gastrointestinal Disorders:** Changes in intestinal microbial communities have been reported in association with inflammatory bowel diseases, including Crohn's disease and ulcerative colitis. Microbiome alterations have also been investigated in other gastrointestinal disorders. The contributions of diet, host genetics, inflammation and microbial activity remain subjects of continuing research.
- **Metabolic Health:** The gut microbiome has been studied in relation to obesity, insulin resistance and type 2 diabetes. Investigated mechanisms include alterations in microbial metabolite production, energy extraction, bile acid transformation and inflammatory

signalling. These relationships are complex and involve interactions among microbial, dietary and host factors.

- **Other Health Conditions:** Microbiome research has also examined possible relationships with cardiovascular conditions, liver disorders, neurological diseases, allergies and susceptibility to infection. The amount and strength of evidence are not uniform across these conditions, and ongoing studies are evaluating the reproducibility and clinical relevance of reported associations.

Microbiome and the Gut–Brain Axis

The gut–brain axis refers to the network of communication between the gastrointestinal tract and the nervous system. Communication can occur through neural, immune, endocrine and metabolic pathways, with microbial products representing one component of this interconnected system. Microorganisms can generate or modify biologically active compounds, while host tissues respond to microbial signals. Research is examining how these interactions may relate to stress, behaviour and neurological function.

- **Microbial Metabolites:** Short-chain fatty acids, transformed bile acids and other microbial products are being investigated as possible mediators of communication between microorganisms and host tissues. Their biological effects can depend on factors such as concentration, anatomical location, receptor activity and the broader physiological environment.

Microbiome and Cardiometabolic Health

Microbial metabolism can transform dietary and host-derived molecules that subsequently enter the circulation. Research has considered possible microbial contributions to lipid metabolism, glucose regulation, blood pressure and inflammatory processes. Certain microbial metabolites have been investigated as potential biomarkers or therapeutic targets, although their clinical utility requires rigorous validation.

Microbiome and Infectious Disease

Resident microbial communities can influence the ability of pathogens to establish themselves by competing for nutrients and attachment sites, producing inhibitory substances and interacting with host defence mechanisms. Disruption of the gut microbiota by antibiotics may alter the ecological environment and increase vulnerability to selected opportunistic infections.

Colonization resistance illustrates the protective role that resident microbial communities can provide against invading organisms. Improved understanding of these ecological interactions may contribute to microbiome-directed strategies that complement conventional antimicrobial therapies.

Technologies Used in Modern Microbiome Research

Contemporary microbiome studies rely on high-throughput molecular methods together with computational analysis. These approaches enable researchers to characterize microbial populations, identify functional genes, assess metabolites and compare microbial ecosystems across individuals, environments and health conditions.

- **16S rRNA Gene Sequencing:** Sequencing of the 16S rRNA gene is widely applied to characterize bacterial and archaeal communities. It can provide information about taxonomic composition and relative abundance, although the method may not reliably distinguish closely related organisms and does not directly describe the complete functional activity of a microbial community.
- **Shotgun Metagenomics:** Shotgun metagenomics examines DNA recovered from an entire microbial community rather than targeting a single marker gene. It can provide information about the organisms present and their functional genes and may achieve greater taxonomic resolution than targeted sequencing approaches.
- **Metatranscriptomics:** Metatranscriptomic analysis examines RNA transcripts generated by microorganisms within a community. It can therefore provide insight into genes that are actively expressed under particular environmental or physiological conditions.
- **Metaproteomics and Metabolomics:** Metaproteomics focuses on proteins associated with microbial communities, whereas metabolomics evaluates small molecules and metabolic products. The combined use of these approaches helps researchers progress from determining which organisms are present to investigating which biological activities may be occurring.
- **Bioinformatics and Computational Biology:** The scale of microbiome datasets requires computational workflows for sequence quality assessment, taxonomic assignment, functional annotation, statistical evaluation and data visualization. Machine-learning approaches and multi-omics integration are increasingly being applied to identify complex patterns and potential biomarkers.

Probiotics, Prebiotics and Synbiotics

Probiotics are live microorganisms that may provide a health benefit when administered in appropriate quantities and under suitable conditions. Their effects depend on the particular strain and context, so benefits observed with one preparation should not automatically be generalized to every probiotic product. Prebiotics are substrates selectively utilized by host microorganisms in ways that may provide a health benefit. Synbiotics combine live microorganisms with substrates intended to support their activity.

Mechanisms of Action

- Competition with potentially harmful microorganisms for nutrients or ecological niches.
- Production, transformation or regulation of microbial metabolites.

- Contribution to intestinal barrier maintenance.
- Modulation of host immune signalling.
- Alteration of the surrounding microbial ecosystem.

Faecal Microbiota Transplantation

Faecal microbiota transplantation (FMT) involves transferring processed material obtained from a screened donor to a recipient with the aim of restoring or altering the intestinal microbial community. It has an established medical role in appropriate cases of recurrent *Clostridioides difficile* infection, while its application to other disorders continues to be evaluated.

FMT highlights the possibility that therapeutic effects may result from modifying a complete microbial ecosystem rather than introducing one microorganism at a time. Donor screening, standardized processing, safety surveillance and long-term monitoring are important components of responsible implementation.

Next-Generation Microbiome Therapeutics

Emerging microbiome therapies seek to provide greater control than conventional probiotic products or FMT. Approaches under investigation include defined microbial consortia, genetically engineered microorganisms, purified microbial metabolites, bacteriophage-based interventions and targeted modification of microbial pathways. Although these strategies may permit more precise manipulation of microbial functions, many remain in experimental or clinical-development stages.

Microbiome-Based Diagnostics and Biomarkers

A biomarker is a measurable characteristic that can provide information about a biological or disease-related state. Microbial species, genes, metabolites and community-level features have all been investigated as potential indicators of disease status or treatment response. Microbiome-based measurements may eventually complement conventional clinical assessments, but microbial profiles can vary with diet, geography, age, medication, sampling location and laboratory procedures. Standardized sampling and rigorous validation are therefore essential.

- **Personalized and Precision Microbiome Medicine:** Individuals can differ substantially in both microbial composition and their responses to dietary or microbiome-directed interventions. Precision microbiome medicine seeks to incorporate information about an individual's microbial composition and function when designing prevention or treatment strategies. Potential applications include individualized dietary guidance, prediction of therapeutic response and selection of microbiome-targeted interventions. The field remains under development, and stronger evidence is needed before many proposed applications can enter routine clinical practice.

Microbiome In Cancer Research

The microbiome has attracted increasing interest in cancer research because microorganisms may interact with immune responses, drug metabolism and the tumour microenvironment.

Studies have explored relationships between particular microbial characteristics and responses to immunotherapy and other cancer treatments. Microbial metabolic activity may also influence the transformation, availability or effectiveness of therapeutic compounds. These observations have encouraged research into microbiome-informed cancer care, although the underlying mechanisms and clinical applications require further investigation.

Microbiome, Nutrition and Functional Foods

Food provides substrates that microorganisms can metabolize and is consequently a major environmental influence on the gut microbiome. Dietary fibres, resistant starch and a range of plant-derived compounds can be transformed by intestinal microorganisms and may alter the production of microbial metabolites. Functional foods and dietary interventions are therefore being examined as practical methods for influencing microbial ecosystems. Responses to the same food may differ between individuals because host physiology and microbial composition are not identical.

- **Fermented Foods:** Fermented foods contain microorganisms and compounds produced during fermentation. Their influence on the human microbiome depends on the food composition, microorganisms involved, processing conditions and patterns of consumption. Research continues to investigate whether fermented foods can affect microbial diversity and immune or metabolic outcomes.

Microbiome and Environmental Factors

The microbial communities associated with humans are also shaped by environmental conditions. Sanitation, antimicrobial exposure, dietary practices, urbanization, occupation and contact with animals may contribute to differences in microbiome composition. This area of research demonstrates the close relationship between environmental microbiology and human health, since changes in environmental exposure can modify the microbial ecosystems encountered by individuals.

Microbiome Research in Agriculture and One Health

The One Health concept emphasizes that human, animal and environmental health are interconnected. Microbial communities associated with animals, food systems and the environment can influence antimicrobial resistance, exposure to microorganisms and health outcomes. Studying these interconnected microbial networks may support integrated approaches to disease prevention and sustainable health management.

Challenges in Microbiome Research

Despite rapid advances, microbiome science faces important obstacles in interpretation and clinical translation. Microbial communities are highly complex and dynamic, and their characteristics are influenced by numerous host and environmental variables.

- **Correlation versus Causation:** The identification of a microbial association with a disease does not establish that the microorganism caused the disease. Disease processes,

medication use and dietary changes may themselves alter microbial communities. Longitudinal investigations and mechanistic experiments are therefore required to clarify causal relationships.

- **Standardization:** Differences in sample collection, storage, DNA extraction, sequencing technology, reference databases and analytical pipelines can produce variation between studies. Establishing standardized procedures is consequently important for improving reproducibility and enabling meaningful comparisons.
- **Inter-individual Variation:** Microbial composition differs among individuals because of factors including geographical location, diet, age, genetics, lifestyle and medication exposure. A microbial pattern identified in one population may therefore not be directly applicable to another population without further validation.
- **Safety and Regulation:** Microbiome-directed therapies require assessment of safety, manufacturing consistency and potential long-term effects. Live microbial interventions can present risks that differ from those associated with conventional small-molecule medicines, particularly for vulnerable individuals. Regulatory systems will need to adapt as new microbiome technologies move toward clinical use.
- **Ethical and Data Considerations:** Microbiome studies can generate large amounts of biological information that may also have personal implications. Ethical research therefore requires suitable informed consent, protection of privacy, transparent reporting and responsible use of predictive models. Scientific and public communication should distinguish preliminary associations from findings that have been firmly established.

Conclusion

The human microbiome is a dynamic biological ecosystem that contributes to important physiological processes, including digestion, metabolism, immune development and resistance to microbial colonization. Advances in metagenomics, metabolomics, bioinformatics and multi-omics analysis have substantially improved the ability to investigate these complex communities. Alterations in the microbiome have been associated with numerous health conditions, but major questions concerning causality, reproducibility and differences between individuals remain. Microbiome-directed approaches such as probiotics, prebiotics, FMT and newer microbial therapeutics are being investigated, with some having defined clinical applications and many others still undergoing evaluation.

Future progress will depend on functional characterization of microbial ecosystems, standardized methodologies, longitudinal research, computational modelling and well-controlled clinical studies. The integration of microbiome science with precision medicine and synthetic biology may support the development of more targeted microbial interventions. Continued interdisciplinary investigation and responsible governance will be important for translating microbiome discoveries into meaningful contributions to human health.

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A SURVEY ON OVER-THE-COUNTER (OTC) DRUG PRODUCTS AT SOME RETAIL PHARMACY

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1. Introduction

Over-the-counter (OTC) drugs are those that consumers can purchase and take without a doctor's prescription. Common ailments that can be treated with self-care are prevented, diagnosed, and cured using these medications. Over-the-counter drugs are considered high-quality, safe, and effective when used as directed on the package label. Typical examples include topical treatments for moderate skin diseases, laxatives, oral rehydration salts, antacids, antihistamines, cough and cold cures, and analgesics like ibuprofen and paracetamol.

Globally, the usage of over-the-counter medications has grown dramatically in recent years. Long wait times for medical appointments, rising healthcare expenses, easy access to medications, and increased public awareness of minor illness self-management are some of the factors that have contributed to this trend. The idea of self-medication has grown to be a crucial part of contemporary healthcare systems, allowing people to handle minor health issues on their own without adding to the strain on medical institutions. Consequently, over-the-counter medications help make healthcare services more affordable and accessible.

Over-the-counter medications are mostly distributed through retail pharmacies, which are essential in encouraging their responsible and safe use. Patients seeking treatment for common ailments frequently consult community pharmacists as their first healthcare provider. Pharmacists offer helpful advice on medication selection, dosage, administration, treatment length, potential adverse effects, and safety measures. Through their expert intervention, patients are able to take their prescriptions as prescribed.

Over-the-counter medications are not completely risk-free, despite their advantages. Adverse drug responses, drug-drug interactions, toxicity, dependency, and overdose can arise from improper or excessive usage of these products. Additionally, self-medication may conceal the signs of severe underlying illnesses, delaying identification and treatment. Antimicrobial resistance is a serious worldwide public health issue that can arise from the irrational use of antimicrobial drugs that are readily available without adequate oversight.

Therefore, research and surveys carried out at retail pharmacies are crucial for assessing consumer attitudes, behaviors, and knowledge about the use of over-the-counter medications.

These surveys assist in determining popular over-the-counter products, self-medication trends, consumer choice-influencing factors, and possible safety issues. The results can help legislators, regulators, and medical professionals create plans to encourage the sensible and responsible use of over-the-counter medications, which will enhance patient safety and public health outcomes. "Globally, approximately 50–80% of the population uses over-the-counter medicines for the management of minor illnesses, making them one of the most commonly used healthcare products worldwide."



Figure 1: Over-the-counter (OTC) Drugs

2. Definition of Over-the-counter (OTC) Drugs

Over-the-counter medicines are pharmaceutical products approved for sale directly to consumers without requiring a physician's prescription.

2.1 World Health Organization (WHO) Perspective

Over-the-counter medicines are drugs that can be used safely and effectively by consumers without the supervision of a healthcare professional when used according to labeled instructions.

2.3 Indian Perspective

In India, there is currently no separate legal category officially designated as Over-the-counter drugs under the Drugs and Cosmetics Act, 1940. Generally, medicines that are not included under prescription-only schedules such as Schedule H, H1, and X are considered Over-the-counter medicines and may be sold without a prescription.

3. Objectives of Survey on Over-the-counter (OTC) Drug Products

The survey may aim to:

- Determine commonly purchased Over-the-counter medicines.
- Assess self-medication patterns.

- Evaluate consumer awareness.
- Identify reasons for Over-the-counter use.
- Study pharmacist involvement.
- Assess adverse effects and misuse.

4. Characteristics of Over-the-counter (OTC) medicines:

- Safe when used according to instructions
- Intended for treatment of minor ailments
- Low toxicity
- Easily accessible in pharmacies
- Lower risk of abuse compared with prescription medicines
- Require proper labeling and dosage information
- Suitable for self-diagnosed conditions.
- Clear and accurate labeling

5. Historical Background of Over-the-counter (OTC) Medicines

Self-medication practices have existed for centuries through herbal remedies & traditional medicines. Modern Over-the-counter regulation developed after implementation of drug safety laws.

Table 1: Important milestones of over-the-counter

S.No.	Year	Development
1	Ancient Era	Use of medicinal plants and home remedies
2	1906	Pure Food and Drug Act introduced drug regulation
3	1938	Federal Food, Drug and Cosmetic Act strengthened safety requirements
4	1951	Durham-Humphrey Amendment separated prescription and OTC drugs
5	1972	OTC Drug Review Program initiated
6	1990s	Expansion of OTC switch programs
7	Present	Expansion of pharmacist-guided OTC therapy

Many drugs originally available only by prescription have been reclassified as over-the-counter (OTC) medicines after demonstrating adequate safety and effectiveness.

6. Classification of Over-the-counter (OTC) Drug Products

Over-the-counter products may be classified according to therapeutic use.

6.1 Analgesics and Antipyretics

Examples:

- Paracetamol
- Ibuprofen

- Aspirin

Uses:

- Fever
- Headache
- Body pain

6.2 Antacids

Examples:

- Calcium Carbonate
- Magnesium hydroxide

Uses:

- Gastric acidity
- Indigestion
- Heartburn

6.3 Cough and Cold Preparations

Examples:

- Cough syrups
- Nasal decongestants
- Lozenges

Uses:

- Cold
- Cough
- Nasal congestion

6.4 Antihistamines

Examples:

- Cetirizine
- Loratadine

Uses:

- Allergy
- Rhinitis
- Skin itching

6.5 Antidiarrheal Drugs

Examples:

- Loperamide
- Oral rehydration salts

6.6 Laxatives

Examples:

- Isabgol
- Bisacodyl

Uses:

- Constipation

6.7 Dermatological Products

Examples:

- Antifungal creams
- Antiseptic lotions

Uses:

- Skin infection
- Minor wounds

7. Role of Retail Pharmacy in Over-the-counter (OTC) Drug Dispensing

Retail pharmacies contribute significantly to healthcare through:

Pharmacist responsibilities:

- Patient counseling
- Dose recommendation
- Identifying contraindications
- Prevention of misuse
- Monitoring adverse effects
- Referral to physician when necessary

Importance:

Community pharmacists are often the first healthcare professionals consulted by patients seeking treatment for minor ailments.

8. Over-the-counter (OTC) Drug Labeling Requirements

A proper Over-the-counter drug label should contain:

- i. Product name
- ii. Active ingredients
- iii. Therapeutic indications
- iv. Dosage instructions
- v. Warnings and precautions
- vi. Contraindications
- vii. Adverse effects
- viii. Storage conditions

- ix. Manufacturing details
- x. Expiry date

Importance of Labeling

- Promotes safe use
- Prevents medication errors
- Improves patient compliance
- Supports informed decision-making

9. Methodology for Survey Study

Study Design: Cross-sectional observational survey

Study Area: Selected retail pharmacies

Study Population: Consumers purchasing Over-the-counter products

Sample Size: Depending on study design (50–500 participants)

Data Collection Tool: Structured questionnaire

Questions may include:

- Age
- Gender
- Education
- Disease condition
- OTC medicine purchased
- Reason for self-medication
- Frequency of use
- Awareness regarding adverse effects

10. Common Over-the-counter (OTC) Drugs Purchased in Retail Pharmacies

Table 2: Typical findings include of over-the-counter drugs in retail pharmacies

S.No.	Drug Category	Common Examples	Major Indication
1	Analgesics	Paracetamol	Fever/Pain
2	Antacids	Antacid tablets	Acidity
3	Cold remedies	Cough syrups	Cold
4	Antihistamines	Cetirizine	Allergy
5	Vitamins	Multivitamins	Weakness
6	GI products	ORS	Diarrhea

11. Advantages of Over-the-counter (OTC) Medicines

Advantages include:

- Easy availability
- Reduced treatment cost

- Time saving
- Immediate relief for minor illnesses
- Reduced burden on healthcare system

For Healthcare System

- Reduces burden on hospitals
- Minimizes physician workload
- Optimizes healthcare resources

12. Disadvantages and Risks of Over-the-counter (OTC) Drugs

- Self-Diagnosis Errors: Patients may incorrectly identify their illness.
- Drug Interactions: Over-the-counter medicines may interact with prescription drugs.
- Adverse Drug Reactions: Incorrect use may lead to toxicity.
- Overdose

Examples:

Excessive use of Paracetamol may cause liver damage.

- Delay in Medical Consultation: Serious diseases may remain undiagnosed.
- Drug Abuse: Certain Over-the-counter preparations may be misused when taken in excessive amounts.

13. Consumer Awareness Regarding Over-the-counter (OTC) Drugs

Awareness varies according to:

- Education level
- Economic status
- Health literacy
- Access to pharmacist counseling

Important awareness factors:

- Correct dose
- Storage conditions
- Expiry date
- Adverse effects
- Contraindications

14. Regulatory Aspects of Over-the-counter (OTC) Drugs

Drug regulatory authorities monitor:

- Safety
- Efficacy
- Labeling
- Advertising

- Post-marketing surveillance

Regulation helps ensure rational medicine use.

15. Responsible Self-Medication

The World Health Organization supports responsible self-medication as an important component of self-care.

Principles

- Use medicines only for approved indications.
- Follow label instructions carefully.
- Do not exceed recommended doses.
- Seek professional advice if symptoms persist.
- Avoid sharing medicines.
- Store medicines properly.

16. Over-the-counter (OTC) Drug Switch

An Over-the-counter switch refers to the conversion of a prescription drug into a non-prescription medicine after evaluation of its safety and effectiveness.

Criteria

- Established safety profile
- Low abuse potential
- Easy diagnosis of target condition
- Clear instructions for use

Examples

- Cetirizine
- Loratadine
- Omeprazole (in several countries)

17. Future Perspectives of Over-the-counter (OTC) Medicines

Future developments in Over-the-counter medicines include:

- Digital health integration
- Mobile health applications
- Improved patient education
- Expanded pharmacist-led services
- Enhanced Pharmacovigilance systems
- Personalized self-care approaches

The increasing emphasis on self-care and preventive healthcare is expected to expand the role of Over-the-counter medicines globally.

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ANTI-INFLAMMATORY FOODS IN CARDIOVASCULAR DISEASE: BIOACTIVE MECHANISMS AND RISK REDUCTION

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Abstract

Cardiovascular disease develops through complex interactions among dyslipidaemia, endothelial dysfunction, oxidative stress, thrombosis and persistent low-grade inflammation. Dietary strategies capable of modulating these processes have therefore become central to cardiovascular prevention. Anti-inflammatory foods—including fruits, vegetables, whole grains, legumes, nuts, seeds, fatty fish and extra-virgin olive oil—provide fibre, unsaturated fatty acids, polyphenols, carotenoids and other bioactive constituents that influence inflammatory and metabolic pathways. This chapter evaluates the mechanisms through which these foods may reduce cardiovascular risk. Polyphenols can regulate nuclear factor- κ B and NLRP3 inflammasome signalling, enhance endogenous antioxidant defences and preserve endothelial nitric oxide. Omega-3 fatty acids generate specialised pro-resolving mediators that may facilitate the controlled resolution of inflammation, while fermentable fibre supports microbial production of short-chain fatty acids and intestinal-barrier integrity. Nuts, seeds and olive oil additionally improve lipid profiles and replace pro-inflammatory saturated fats when incorporated appropriately. Anti-inflammatory dietary patterns, particularly Mediterranean-style diets, are associated with lower concentrations of inflammatory biomarkers, improved blood pressure and reduced cardiovascular events. Nevertheless, effects cannot be attributed to single foods because bioactive availability, processing, dose, baseline health and dietary substitution substantially influence outcomes. Foods should therefore be evaluated within complete dietary patterns rather than promoted as isolated “superfoods.” A varied, minimally processed, plant-forward diet containing appropriate sources of unsaturated fat offers a practical and sustainable strategy for attenuating vascular inflammation and reducing cardiovascular risk alongside established medical and lifestyle management.

Keywords: Anti-Inflammatory Foods, Cardiovascular Disease, Polyphenols, Omega-3 Fatty Acids, Dietary Fibre, Mediterranean Diet.

1. Introduction

Atherosclerosis is increasingly recognised as a chronic inflammatory disorder rather than a consequence of lipid accumulation alone. Endothelial injury, oxidised lipoproteins and metabolic

stress activate immune pathways that promote leukocyte recruitment, plaque formation and eventual thrombotic events. Dietary patterns can either amplify or attenuate these processes by altering oxidative balance, lipid metabolism, intestinal integrity and inflammatory signalling.

Anti-inflammatory foods are rich in polyphenols, carotenoids, fermentable fibre, unsaturated fatty acids, vitamins and minerals. Prominent examples include berries, leafy vegetables, legumes, whole grains, nuts, seeds, fatty fish and extra-virgin olive oil. Their benefits arise through complementary actions rather than from a single nutrient or food. However, processing, preparation, dose and the foods being replaced strongly modify physiological effects. This chapter examines the bioactive mechanisms linking anti-inflammatory foods with cardiovascular protection, evaluates recent evidence for risk reduction and identifies priorities for translating mechanistic knowledge into clinically meaningful, culturally adaptable dietary strategies.

2. Inflammation as a Cardiovascular Target

Vascular inflammation begins when hypertension, hyperglycaemia, smoking, visceral adiposity or oxidised LDL disrupts endothelial homeostasis. Activated endothelial cells express adhesion molecules that recruit monocytes into the arterial wall. These cells differentiate into macrophages, internalise modified lipoproteins and contribute to foam-cell formation. Continued inflammatory signalling promotes plaque progression, fibrous-cap degradation and thrombosis.

Dietary inflammatory potential appears relevant to this process. Updated evidence shows that diets with higher Dietary Inflammatory Index scores are associated with increased cardiovascular incidence and mortality (Ni *et al.*, 2025). Among individuals with established coronary heart disease, higher inflammatory dietary scores have also been associated with greater all-cause mortality (Wang *et al.*, 2024). The index reflects the combined contribution of dietary components rather than the action of one food.

Anti-inflammatory dietary patterns typically emphasise vegetables, fruits, legumes, whole grains, nuts, fish and olive oil while limiting refined carbohydrates, processed meat, trans fats and excess added sugar. Meta-analytic evidence suggests that these patterns can reduce high-sensitivity C-reactive protein and favourably influence blood pressure and lipids (Jiang *et al.*, 2025). These findings support inflammation as a modifiable nutritional target while emphasising that dietary patterns provide stronger evidence than isolated “anti-inflammatory” ingredients.

3. Polyphenol Rich Plant Foods and Vascular Protection

Polyphenols include flavonoids, anthocyanins, phenolic acids, stilbenes and lignans found in berries, grapes, cocoa, tea, coffee, olives, nuts, seeds and colourful vegetables. These compounds are often described as antioxidants, but their physiological effects extend beyond direct free-radical scavenging. They modulate cellular signalling, endogenous antioxidant enzymes, inflammatory gene expression and vascular nitric-oxide availability (Arshad *et al.*, 2025).

Polyphenols may inhibit nuclear factor- κ B activation, reduce pro-inflammatory cytokine production and limit oxidative modification of LDL. Anthocyanin-rich berries can potentially improve endothelial function by enhancing nitric-oxide signalling and reducing vascular oxidative stress. Blueberry polyphenols have been linked with improvements in blood pressure, arterial stiffness and endothelial responsiveness, although clinical trials vary in dose and duration (Onuh *et al.*, 2023).

Extra-virgin olive oil combines monounsaturated fatty acids with hydroxytyrosol, oleuropein and related phenolics. These constituents may reduce lipid oxidation, inflammatory signalling, platelet activation and endothelial dysfunction (Serreli *et al.*, 2024). Higher olive-oil consumption has been associated with reduced cardiovascular risk, with virgin varieties potentially providing greater benefit because of their higher phenolic content (Xia *et al.*, 2022).

Polyphenols also interact with the gut microbiota. Microbial transformation generates smaller metabolites that may be more bioavailable than their parent compounds. These reciprocal interactions could influence intestinal integrity, circulating inflammation and atherosclerotic progression (Morvaridzadeh *et al.*, 2025).

4. Omega - 3 Fatty Acids and Inflammatory Resolution

Fatty fish such as salmon, sardines and mackerel provide eicosapentaenoic acid and docosahexaenoic acid. Unlike conventional anti-inflammatory agents that suppress inflammatory initiation, these fatty acids can contribute to specialised pro-resolving mediators—including resolvins, protectins and maresins—which facilitate the controlled termination of inflammation and restoration of tissue homeostasis.

Omega-3 fatty acids may reduce hepatic triglyceride synthesis, alter cell-membrane composition, modulate cytokine production and influence platelet and endothelial activity. However, cardiovascular effects differ according to formulation, dose, background diet and clinical population. Recent evidence indicates that benefits may be more pronounced in selected high-risk groups, while high-dose supplementation can carry risks, including bleeding or atrial fibrillation in susceptible individuals (Djuricic & Calder, 2025).

Food sources should therefore be distinguished from concentrated supplements. Fish delivers omega-3 fatty acids within a matrix containing protein, selenium and vitamin D and may replace processed or fatty meat. Plant sources such as walnuts, chia and flaxseed provide α -linolenic acid, although its conversion to longer-chain omega-3 fatty acids is limited. The principal cardiovascular benefit may arise from regular food-based intake within a balanced dietary pattern rather than indiscriminate supplementation.

5. Fibre, Gut Microbiota and Metabolic Inflammation

Whole grains, pulses, vegetables, fruits, nuts and seeds provide fermentable fibres that escape digestion in the small intestine. Colonic microorganisms convert these substrates into acetate,

propionate and butyrate. These short-chain fatty acids support epithelial-barrier integrity and interact with receptors involved in immune regulation, appetite, glucose metabolism and vascular tone.

A strengthened intestinal barrier may restrict the passage of lipopolysaccharide and other pro-inflammatory microbial products into the circulation. This is clinically relevant because metabolic endotoxaemia may contribute to insulin resistance, endothelial dysfunction and vascular inflammation. Cereal fibre and whole-grain carbohydrates are also important markers of favourable carbohydrate quality (AlEssa *et al.*, 2026).

Whole grains further supply phenolic acids, resistant starch and minerals that complement their fermentable fibres. Oat β -glucan can lower total and LDL cholesterol through increased intestinal viscosity and bile-acid excretion (Yu *et al.*, 2022). Legumes provide resistant starch, polyphenols and plant protein, while displacing refined grains or processed meats. Consequently, fibre-rich foods can influence inflammation both directly through microbial metabolism and indirectly through improved glycaemic and lipid regulation.

6. Nuts, Seeds and Anti Inflammatory Lipid Replacement

Nuts and seeds provide monounsaturated and polyunsaturated fats, fibre, phytosterols, polyphenols, tocopherols, magnesium and plant protein. Their cardiovascular contribution depends substantially on substitution. Replacing foods rich in saturated fat or refined carbohydrates with nuts can improve lipid quality without necessarily increasing body weight when portions remain appropriate.

Recent evidence associates habitual nut and seed consumption with lower cardiovascular risk, particularly coronary heart disease, partly through improvements in atherogenic lipids (Arnesen *et al.*, 2023). Flaxseed contributes α -linolenic acid and lignans, walnuts provide α -linolenic acid and polyphenols, and almonds supply vitamin E and monounsaturated fat. A recent synthesis indicated that polyphenol-rich seed foods may improve triglycerides and HDL cholesterol in people with coronary heart disease, although effects on LDL cholesterol and inflammatory markers were inconsistent (Jia *et al.*, 2024).

These findings demonstrate why “anti-inflammatory” should not be treated as an absolute food classification. Benefits depend on portion size, processing, salt or sugar coatings and the dietary component replaced. Unsalted nuts and minimally processed seeds are more appropriate than heavily salted, sweetened or fried alternatives.

7. Anti Inflammatory Dietary Patterns and Risk Reduction

The Mediterranean diet provides the most extensively studied framework, combining vegetables, fruits, legumes, whole grains, nuts, fish and olive oil with limited processed meat and refined food. These components provide complementary fibres, polyphenols and unsaturated fats

capable of influencing inflammation, lipids, blood pressure and endothelial function (Pant *et al.*, 2024).

In secondary prevention, the CORDIOPREV randomised trial demonstrated that Mediterranean and low-fat dietary patterns could influence long-term cardiovascular outcomes, with a Mediterranean pattern providing meaningful protection among patients with coronary heart disease (Delgado-Lista *et al.*, 2022). Longer-term observational evidence also links sustained Mediterranean-diet adherence with lower cardiovascular incidence (Georgoulis *et al.*, 2024).

The emphasis should therefore remain on dietary synergy. Berries cannot counteract an otherwise ultra-processed diet, and olive oil cannot neutralise excessive energy, sodium or refined carbohydrate intake. Anti-inflammatory foods are most effective when they collectively replace pro-inflammatory dietary exposures within a sustainable pattern.

8. Challenges and Future Perspectives

The field must progress from attractive “superfood” claims towards measurable anti-inflammatory functionality. Food composition, cultivar, processing, dose and bioavailability vary considerably, while studies use inconsistent biomarkers and short intervention periods. C-reactive protein alone cannot capture the complexity of vascular inflammation (Jiang *et al.*, 2025).

Future trials should integrate interleukins, tumour-necrosis-factor signalling, adhesion molecules, lipid mediators, endothelial function, plaque imaging and microbiome-derived metabolites. Food-based interventions must be distinguished from concentrated supplements, particularly for omega-3 fatty acids and polyphenols. Objective biomarkers should strengthen adherence assessment.

Precision nutrition offers a bright translational pathway. Baseline microbiota, inflammatory phenotype, genetics, sex and cardiometabolic status may determine which dietary combinations provide the strongest response. Culturally adapted patterns—such as Mediterranean-style diets incorporating regionally available grains, pulses, spices, oils and fish—could improve affordability and adherence (Mantoo *et al.*, 2026). Future functional foods should preserve bioactive compounds without introducing excess sugar, sodium or saturated fat. The goal is no longer to identify one miraculous ingredient, but to design personalised food combinations that demonstrably resolve inflammation and reduce cardiovascular events.

Conclusion

Anti-inflammatory foods provide a scientifically credible foundation for cardiovascular prevention because they address several mechanisms underlying atherosclerosis simultaneously. Fruits, vegetables, whole grains, legumes, nuts, seeds, fatty fish and extra-virgin olive oil supply a diverse matrix of polyphenols, fibre, unsaturated fatty acids, phytosterols, vitamins and minerals. These constituents may regulate inflammatory signalling, reduce oxidative stress,

improve lipoprotein metabolism, preserve endothelial function and support intestinal-barrier integrity.

Their benefits are complementary. Polyphenols influence cellular signalling and nitric-oxide availability; omega-3 fatty acids support inflammatory resolution; fermentable fibres promote short-chain-fatty-acid production; and unsaturated fats improve dietary lipid quality when they replace saturated fats. This biological synergy explains why complete dietary patterns generally provide stronger cardiovascular evidence than isolated supplements or single “superfoods.”

Nevertheless, anti-inflammatory foods are not pharmacological substitutes. They should accompany lipid-lowering therapy, antihypertensive treatment, smoking cessation, physical activity and appropriate clinical monitoring when these interventions are indicated. Their effects also depend on preparation and substitution. Nuts coated with salt or sugar, highly processed whole-grain foods and vegetables prepared with excessive saturated fat may provide fewer benefits than their unprocessed counterparts.

The most effective strategy is therefore a minimally processed, plant-forward dietary pattern supplemented with appropriate fish and unsaturated oils. Such a pattern can be adapted to different cultures through locally available fruits, vegetables, pulses, grains, spices, seeds and oils. Future research must determine which combinations produce the strongest effects in specific inflammatory and metabolic phenotypes. Cardiovascular inflammation is not defeated by one celebrated superfood; it is progressively quietened by a dietary pattern in which every meal shifts the biological environment away from vascular injury and towards resolution.

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MORPHOTAXONOMIC STUDY AND ETHNOMEDICINAL USES OF SELECTED PLANTS FROM PURNA, MARATHWADA

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Abstract

The present investigation was carried out to document morphotaxonomy and medicinal uses of six common medicinal plants from Marathwada region. The flora was surveyed during 2025-2026. Six species viz., *Tinospora cordifolia* (Willd.) Miers ex Hook.f. (Gulvel), *Aegle marmelos* (L.) Corr. (Bael), *Mangifera indica* L. (Amba), *Pongamia pinnata* (L.) Pierre (Karanj), *Butea monosperma* (Lam.) Taub. (Palas) and *Tamarindus indica* L. (Chinch) were found to be most common and easily available medicinal plants. Detailed morphological description, classification, flowering-fruitlet period and ethnomedicinal uses were documented. The study provides baseline data for conservation and utilization of medicinal plants.

Keywords: Morphotaxonomy, Medicinal Plants, Ethnomedicine, Marathwada, Fabaceae.

Introduction

The present study deals with the morphotaxonomy and medicinal uses of six common medicinal plants found in Marathwada region. The campus has a botanical medicinal garden and natural vegetation. During preliminary survey, *Tinospora cordifolia*, *Aegle marmelos*, *Mangifera indica*, *Pongamia pinnata*, *Butea monosperma* and *Tamarindus indica* were found to be most common and easily available medicinal plants. The study of morphotaxonomy and medicinal uses of plants has become a critical need of today. There has been increasing interest in recent years in traditional herbal medicine, particularly the tribal medicines. India has rich heritage of medicinal plants used in Ayurveda, Unani and Siddha systems. Marathwada region of Maharashtra state. The documentation of local flora is essential for conservation, utilization and for providing recognition to the traditional use of medicinal plants.

Geology and Soil

The study area Purna is located in Parbhani district of Marathwada region. The soil is predominantly black cotton soil (Vertisol), derived from basalt. It is clayey, deep, and highly waterretentive. The soil is rich in calcium, magnesium, carbonates and moderately alkaline with pH 7.5 to 8.5.

Climate and Rainfall

The climate of Purna region is semi-arid, tropical monsoon type with three distinct seasons: Summer - March to May (35°C to 44°C), Monsoon - June to September, Winter - October to February (12°C to 28°C). Average annual rainfall is 700-800 mm, mostly received from South-West monsoon.

Research Work Related to Flora

Tilak (1963) has studied Flora of Aurangabad district and described morphology and uses of many medicinal plants. Naik (1969) explored and described 804 plants belonging to 472 genera and 106 families of Angiospermic plants from Marathwada. Naik (1998) in Flora of Marathwada and Almeida (1996) in Flora of Maharashtra have extensively documented the flora. Work on ethnobotany was carried out by Banerjee (1955), Jain (1963), Shah and Joshi (1971), Goel *et al.* (1984), Maheshwari *et al.* (1986), Arora (1988).

Present Research Work

The present work was undertaken with following objectives: a) To survey and collect six most common medicinal plants, b) To study their detailed morphotaxonomy for correct identification, c) To document their traditional and ethnomedicinal uses, d) To create awareness about conservation.

Materials and Methods

Field survey was conducted during 2025-2026 in different seasons. Collection: Fresh plant material with flower and fruit was collected. Photographs were taken in natural habitat. Identification: Identification was done with the help of standard floras - Flora of Marathwada (Naik, 1998), Flora of Maharashtra (Almeida, 1996), Flora of Bombay (Cooke, 1901-1908). Herbarium Preparation: Herbarium sheets were prepared by standard method of pressing, drying and mounting on herbarium sheets. The herbarium is deposited in Botany Department. Morphological Description: Detailed description was made under heads - Habit, Habitat, Root, Stem, Leaf, Inflorescence, Flower, Calyx, Corolla, Androecium, Gynoecium, Fruit, Seed, Flowering and Fruiting period. Medicinal Uses: Uses were confirmed from literature of Ayurveda, Unani.

Enumeration - Morphology and Medicinal Uses

1. *Tinospora cordifolia* (Willd.) Miers, Gulvel Family: Menispermaceae

Description: Habitat: Terrestrial; Habit: Large, glabrous, deciduous climber with succulent stem; Root: Tap root; Stem: Woody twiners with long pendulous aerial roots, covered with whitish green papery bark; Leaf: Simple, alternate, broadly ovate, 5-9x4-8.5 cm, deeply cordate at base, acuminate at apex, 7-nerved; Inflorescence: Axillary racemes; Flower: Yellowish-green, unisexual; Calyx: Sepals 6 in 2 whorls; Corolla: Petals 6, clawed; Fruit: Drupe 1-3, subglobose, 6-10 mm, red; Seed: White, bean-shaped. Fl. & Fr.: Jan to June.

Uses: 1. Stem is bitter, stomachic, tonic, enriches blood. 2. Aqueous extract useful in chronic low fever, Kala-azar and diabetes. 3. Tincture used in mild malaria. 4. Useful in jaundice, skin diseases, enlarged spleen.

2. *Aegle marmelos* (L.) Corrêa – Bael Family: Rutaceae

Description: Habit: Small to medium sized deciduous tree with stout axillary thorns; Root: Tap and branched; Stem: Woody, bark grey, fragrant; Leaf: Compound, 3-foliate, leaflets lanceolate to ellipticlanceolate, 4-6x2-3 cm, with fragrant glands; Inflorescence: Axillary cymes; Flower: Bisexual, greenish-white, scented; Calyx: Sepals 5, united; Corolla: Petals 5, 10-12 mm long; Androecium: Stamens numerous; Fruit: Amphisarca, globose, 5-10 cm diam, pulp sweet orange. Fl. & Fr.: March to July.

Uses: 1. Roots useful in diarrhoea, dysentery. 2. Leaves useful in ophthalmia, diabetes. 3. Unripe fruits useful in diarrhoea, dysentery. 4. Ripe fruits cooling, laxative.

3. *Mangifera indica* L. – Amba Family: Anacardiaceae

Description: Habit: Large evergreen tree; Root: Tap; Stem: Erect, woody; Leaf: Simple, oblonglanceolate, 8-20x4-6 cm, entire, acuminate; Inflorescence: Terminal panicles; Flower: Polygamous, pentamerous; Calyx: Sepals 5; Corolla: Petals 5, dull white; Androecium: 5 stamens, only 1 fertile; Fruit: Drupe, large, fleshy. Fl. & Fr.: Jan to June.

Uses: 1. Leaves useful in haemorrhages, wounds, ulcers, diarrhoea. 2. Ash of burnt leaves useful in scalds and burns. 3. Bark and roots useful in leucorrhoea, vomiting, dysentery.

4. *Pongamia pinnata* (L.) Pierre – Karanj Family: Fabaceae

Description: Habit: Medium sized tree; Root: Tap; Stem: Erect, bark smooth grey; Leaf: Compound, imparipinnate, 10-20 cm, leaflets 5-7, ovate-oblong, 4-9x3-5 cm; Inflorescence: Axillary racemes; Flower: Papilionaceous, pinkish-white; Calyx: Campanulate; Corolla: 10-12 mm; Fruit: Pod woody, oblong-ellipsoid, 1-seeded. Fl. & Fr.: March to Aug.

Uses: 1. Root juice used for cleaning foul ulcers and strengthening gums. 2. Bark used in bleeding piles. 3. Leaves infusion used for rheumatism. 4. Seed oil (Karanj oil) highly esteemed in scabies, herpes.

5. *Butea monosperma* (Lam.) Taub. – Palas Family: Fabaceae

Description: Habit: Deciduous tree, 12-15 m tall; Root: Tap; Stem: Bark brownish-ash, cracked; Leaf: Compound, trifoliate, leaflets ovate-rhomboid, 8-15x5-8 cm; Inflorescence: Rigid racemes 15-30 cm; Flower: Large, bright orange-red, 3-6 cm; Fruit: Pod stalked, flat, 1-seeded, silvery tomentose. Fl. & Fr.: March to June.

Uses: 1. Bark useful in diarrhoea, bone fractures, tumours, diabetes. 2. Leaves useful in boils, worm infestation. 3. Flowers useful in diarrhoea, fever, leprosy and as birth control. 4. Seeds anthelmintic. 5. Gum useful in diarrhoea.



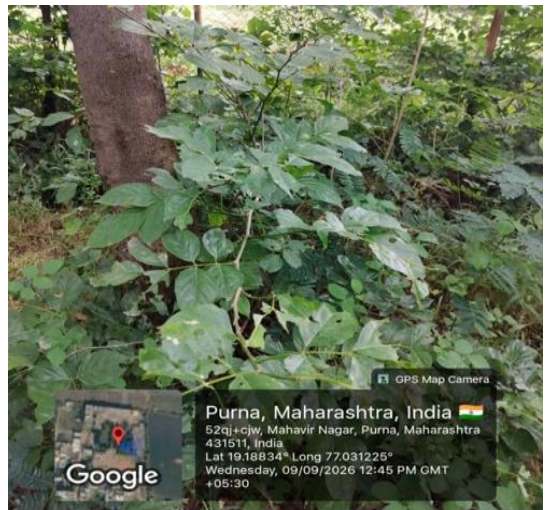
Tinospora cordifolia



Aegle marmelos



Mangifera indica



Pongamia pinnata



Butea monosperma



Tamarindus indica

6. *Tamarindus indica* L. – Chinch Family: Caesalpinaceae

Description: Habit: Large tree, 12-24 m high; Root: Tap; Stem: Bark light brownish, furrowed; Leaf: Paripinnate, leaflets 10-20 pairs, oblong, 1-2 cm; Inflorescence: Racemes; Flower: Yellow with red veins; Fruit: Pod, 7-15 cm long, curved, brown, pulpy. Fl. & Fr.: April to Dec.

Uses: 1. Bark used for loss of sensation in paralysis. 2. Leaves applied to reduce inflammatory swelling, tumours and ringworm. 3. Flowers cure vata and kapha. 4. Ripe fruit pulp laxative, useful in liver complaints.

Result and Discussion

In the present investigation, 6 medicinal plants belonging to 4 families were documented. Fabaceae was found to be dominant family with 3 species. All the plants are terrestrial trees or climbers and are well adapted to black cotton soil and semi-arid climate. The morphological studies confirmed their identification. The medicinal uses documented show that these plants are highly valuable in Ayurveda and Unani systems. Bark, leaves, fruits and seeds are the most used parts. The conservation of these plants in botanical garden is necessary.

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A REVIEW ON STATUS OF PRECISION MEDICINE IN INDIA: CURRENT SCENARIO, CHALLENGES, OPPORTUNITIES AND FUTURE PERSPECTIVES

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Abstract

Precision medicine is an emerging healthcare approach that aims to tailor disease prevention, diagnosis and treatment according to an individual's genetic, environmental, lifestyle and clinical characteristics. The rapid development of genomics, next-generation sequencing, bioinformatics, artificial intelligence and molecular diagnostics has accelerated the transition from conventional population-based medicine toward more individualized healthcare. India, with its large and genetically diverse population, has substantial potential to benefit from precision medicine. However, implementation remains at an evolving stage because of limitations in population-specific genomic data, high costs, inadequate infrastructure, shortage of trained professionals, limited clinical guidelines, data-privacy concerns and unequal access to advanced healthcare.

Several Indian initiatives have strengthened the country's precision-medicine ecosystem. The GenomeIndia Project, involving 20 institutions, aims to characterize genetic variation across Indian populations and create a reference resource for genomic medicine. Genome India+1 Precision medicine is also increasingly relevant to oncology, pharmacogenomics, rare diseases, infectious diseases, diabetes, cardiovascular diseases and therapeutic drug monitoring. Personalized medicine in India identified growing research activity and government interventions but also emphasized the need for an integrated policy framework for implementation.

Development of Indian-specific genomic databases, affordable sequencing, standardized clinical guidelines, reimbursement mechanisms, multidisciplinary training and robust ethical and data-governance frameworks will be essential. Precision medicine could significantly improve healthcare outcomes in India only when technological progress is accompanied by equitable access and appropriate health-policy interventions.

Keywords: Precision Medicine, Personalized Medicine, Genomics, Pharmacogenomics, Genomeindia, Genetic Diversity, India, Precision Oncology, Genomic Medicine.

1. Introduction

Traditional medicine generally uses population-level evidence to determine diagnosis and treatment. However, patients with apparently similar diseases can have substantially different disease mechanisms, treatment responses and adverse effects. Precision medicine attempts to

address this variation by incorporating information about an individual's genetic profile, environment, lifestyle, biomarkers and clinical characteristics into healthcare decisions.

The concept has become increasingly important with advances in human genome sequencing, next-generation sequencing (NGS), molecular diagnostics, transcriptomics, proteomics, metabolomics and computational biology. Precision medicine has already demonstrated considerable clinical utility in oncology, where molecular alterations can be used to select targeted therapies. It is also being applied to pharmacogenomics, rare diseases, infectious diseases and chronic disorders.

India represents a particularly important setting for precision medicine because of its enormous population and extensive genetic, ethnic and geographical diversity. Genomic studies that primarily represent European populations may not adequately capture genetic variants occurring in Indian populations. Consequently, Indian-specific genomic resources are important for accurate disease-risk prediction, genetic diagnosis and treatment selection.

The GenomeIndia Project represents one of the most significant national efforts in this area. The project involves 20 academic and research institutions and aims to create a comprehensive catalogue of genetic variation representing India's population diversity. Genome India The project's importance for precision medicine lies in improving the representation of Indian populations in genomic research and potentially enabling more appropriate genomic diagnostics and therapies.

Despite these developments, precision medicine in India is not yet routinely available across the healthcare system. Research and advanced genomic services remain concentrated in major academic institutions, specialized hospitals and private laboratories. Cost, infrastructure, workforce and regulatory limitations remain significant barriers.

Therefore, this review examines the current status of precision medicine in India, its major applications, research and policy developments, challenges, opportunities and future requirements.

2. Literature Review

2.1 Concept and Scope of Precision Medicine

Precision medicine integrates multiple types of patient information to improve disease prevention, diagnosis and treatment. Genomics is a major component, but precision medicine extends beyond genetic testing. It can incorporate:

- Genomic and whole-genome sequencing
- Pharmacogenomics
- Molecular biomarkers
- Transcriptomics and proteomics
- Metabolomics

- Electronic health records
- Artificial intelligence and machine learning
- Therapeutic drug monitoring
- Environmental and lifestyle information

A review published by researchers from India highlighted applications of precision medicine across oncology, infectious diseases, asthma, cardiovascular disease, diabetes, obesity and other conditions. The same review identified cost, data security, integration of information systems and shortage of trained personnel as major challenges.

2.2 Genomics and the GenomeIndia Project

The availability of population-specific genomic information is fundamental to precision medicine. The GenomeIndia Project was established as a national initiative supported by India's Department of Biotechnology. Its objective is to catalogue genetic variation across the Indian population and provide a foundation for genomic research and precision healthcare. Genome India, the project is particularly important because India contains numerous genetically differentiated populations. Under-representation of these populations in global genomic databases can result in inaccurate interpretation of genetic variants and potentially reduce the effectiveness of genomic testing. The GenomeIndia initiative has progressed to the publication of a marker paper describing India's genetic diversity, and the project's official resources report completion of sequencing of 10,000 genomes. Genome India This represents a major transition from isolated genomic research toward development of national genomic infrastructure.

2.3 Pharmacogenomics

Pharmacogenomics is one of the most clinically relevant components of precision medicine. Genetic differences can influence drug metabolism, efficacy and toxicity. Consequently, pharmacogenomic information can potentially help clinicians select an appropriate drug and dose for an individual patient. Indian populations possess genetic variants that may differ in frequency from those observed in European or other reference populations. This makes population-specific pharmacogenomic research particularly important. A recent scoping review focusing specifically on Indian clinical practice identified actionable pharmacogenomic variants relevant to 57 drugs and 24 major genes. The study emphasized that lack of Indian population-specific evidence and difficulties in interpreting genomic reports remain major obstacles to implementation.

The potential applications include:

- Anticancer drugs
- Anticoagulants
- Antiplatelet drugs
- Antiepileptic drugs
- Psychiatric medications

- Drugs used in transplantation
- Analgesics and commonly prescribed medications

However, routine pharmacogenomic testing is still limited in India.

2.4 Precision Oncology

Oncology is currently one of the most advanced areas of precision medicine. Molecular profiling can identify mutations, amplifications, fusions and other biomarkers that can guide targeted treatment.

Precision oncology has applications in cancers such as:

- Breast cancer
- Lung cancer
- Colorectal cancer
- Leukaemia
- Lymphoma
- Ovarian cancer
- Certain rare and paediatric cancers

Molecularly targeted therapies have demonstrated the clinical potential of precision medicine. Examples include therapies directed against HER2, EGFR, VEGF and RAS-associated pathways.

In India, however, access to comprehensive genomic profiling is uneven. Large tertiary hospitals and specialized cancer centres have greater access than district hospitals and rural healthcare facilities.

2.5 Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is another important component of individualized therapy. TDM measures drug concentrations in biological samples and uses the results to optimize dosing.

A review of TDM in India reported that although the approach has significant potential, its use remains limited largely to selected academic medical centres and teaching hospitals. Potential applications include immunosuppressants, anticancer drugs, antifungals, antibiotics and antitubercular medicines. This is particularly relevant in India because nutritional status, infectious diseases, polypharmacy and other population-specific factors may influence drug pharmacokinetics.

2.6 Government and Institutional Initiatives

The development of precision medicine in India has involved government agencies, universities, research institutes, hospitals and biotechnology organizations.

Important developments include:

- GenomeIndia Project
- Indian cancer-genomics initiatives
- Population genomics and biobanking
- Expansion of NGS facilities
- Development of molecular diagnostic laboratories
- Pharmacogenomics research
- Bioinformatics and computational biology programmes
- Increasing integration of artificial intelligence with healthcare

Research examining the institutionalization of personalized medicine in India found that the country's healthcare innovation system is dynamic and that government-funded interventions have contributed to development of the field. However, the authors also identified policy gaps and proposed an integrated framework for wider adoption.

2.7 Ethical, Social and Legal Considerations

Precision medicine involves the collection and analysis of highly sensitive genetic and health information. Consequently, privacy, informed consent, data ownership and potential genetic discrimination are important concerns.

A recent review specifically examining precision medicine in India identified ethical concerns relating to informed consent, privacy and genetic discrimination, while social barriers included cost, rural accessibility and differences in health awareness.

These issues are especially important in India because of:

- Large socioeconomic disparities
- Differences in health literacy
- Unequal access to healthcare
- Rural–urban differences
- Diversity of languages and cultures
- Variable availability of genetic counselling

Therefore, technological development must be accompanied by appropriate ethical and regulatory frameworks.

3. Current Status of Precision Medicine in India

The present status can be summarized as follows:

Area	Current Status in India
Population genomics	Rapidly developing
Genome sequencing	Increasing availability
GenomeIndia	Major national initiative
Precision oncology	Relatively advanced in tertiary centres

Pharmacogenomics	Emerging
Rare-disease genomics	Growing rapidly
Therapeutic drug monitoring	Available but underutilized
AI/health-data integration	Emerging
Rural accessibility	Limited
Standardized national clinical guidelines	Still developing
Insurance/reimbursement	Major limitation
Genetic counselling	Insufficient relative to need
Trained workforce	Significant gap
Ethical/data-governance framework	Developing

Overall, India has moved beyond the purely research-oriented stage, but precision medicine is not yet uniformly integrated into routine clinical practice.

4. Major Observations

The literature reviewed allows several important observations to be made as

4.1 India has strong potential for precision medicine

India's large population and genetic diversity provide an exceptional opportunity to develop population-specific genomic resources. Genome India is an important step toward this objective. Genome India+1

4.2 Research is progressing faster than clinical implementation

A considerable amount of genomic and pharmacogenomic research is being conducted, but translating research findings into routine patient care remains difficult.

4.3 Precision oncology is currently one of the strongest applications

Cancer care has benefited substantially from molecular diagnostics and targeted therapies. Nevertheless, access is concentrated in specialized centres.

4.4 Pharmacogenomics requires more Indian-specific evidence

Evidence from other populations cannot always be directly applied to Indian patients. Recent Indian research has emphasized the need to establish actionable pharmacogenomic variants relevant to Indian clinical practice.

4.5 Cost is a major barrier

The cost of sequencing, molecular testing, interpretation and targeted treatment can restrict access. Precision medicine therefore risks increasing healthcare inequality if implementation is limited to patients who can afford private-sector services.

4.6 There is a shortage of trained professionals

Successful implementation requires geneticists, molecular pathologists, bioinformaticians, genetic counsellors, clinical pharmacologists, oncologists and data scientists.

4.7 Data governance is increasingly important

Genomic data are uniquely identifiable and can have implications for biological relatives as well as the individual tested. Appropriate consent, privacy and security mechanisms are therefore essential.

4.8 Therapeutic drug monitoring is an underused precision-medicine tool

Evidence suggests that TDM could contribute to individualized therapy in several clinical areas but remains underdeveloped in India.

4.9 Rural and urban disparities remain significant

Advanced genomic diagnostics are more readily available in metropolitan and tertiary healthcare centres. Expanding precision medicine to rural and underserved populations will require decentralization, telemedicine, referral networks and affordable testing.

5. Challenges

The principal challenges facing precision medicine in India include:

- i. High cost of genomic testing and targeted therapies
- ii. Limited population-specific genomic datasets
- iii. Insufficient trained workforce
- iv. Limited genetic counselling services
- v. Unequal rural and urban access
- vi. Lack of standardized clinical guidelines
- vii. Limited insurance coverage and reimbursement
- viii. Difficult interpretation of variants of uncertain significance
- ix. Privacy and cybersecurity concerns
- x. Ethical and legal uncertainties
- xi. Fragmentation of healthcare and genomic data
- xii. Limited awareness among clinicians and patients

International literature on pharmacogenomics similarly identifies insufficient evidence, healthcare-provider training, ethical/legal concerns, patient awareness and high testing costs as major implementation barriers. +1

6. Future Perspectives

The future of precision medicine in India should focus on translating genomic discoveries into affordable clinical services.

Important priorities should include:

- Establishing comprehensive Indian genomic reference databases.
- Expanding GenomeIndia and related population-genomics resources.
- Developing national pharmacogenomic guidelines.
- Integrating genomic information with electronic health records.

- Establishing standardized genomic laboratories.
- Increasing genetic counselling services.
- Developing reimbursement mechanisms for clinically validated genomic tests.
- Training physicians, pharmacists, laboratory professionals and bioinformaticians.
- Strengthening genomic-data protection and ethical oversight.
- Developing public–private partnerships.
- Increasing representation of rural and underserved populations in genomic research.
- Using artificial intelligence responsibly for genomic-data interpretation.
- Conducting health-economic studies to demonstrate cost-effectiveness.

A key objective should be to move from "genomics research" to "genomics-enabled healthcare."

Conclusion

Precision medicine in India is at an important transitional stage. Significant progress has occurred in genomics, molecular diagnostics, precision oncology, pharmacogenomics and therapeutic drug monitoring. National initiatives such as the GenomeIndia Project have created an important foundation for developing Indian-specific genomic resources. Genome India+1
Nevertheless, precision medicine has not yet achieved widespread integration into routine healthcare. The major obstacles include cost, inadequate infrastructure, shortage of trained professionals, limited population-specific clinical evidence, insufficient genetic counselling, unequal access and unresolved ethical and data-governance challenges.

The future success of precision medicine in India will therefore depend not only on technological advances but also on affordability, accessibility, clinical validation, education, policy development and ethical governance. India's genetic diversity makes the country particularly valuable for precision-medicine research, but this diversity also requires Indian-specific evidence rather than simple extrapolation from Western genomic datasets.

In conclusion, India has the scientific capacity and population-scale opportunity to become a major contributor to global precision medicine. The next phase should emphasize translating genomic discoveries into evidence-based, affordable and equitable clinical practice. +1

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BIOFILM-BASED BIOFERTILIZERS: IMPROVED SURVIVAL AND EFFICIENCY UNDER FIELD CONDITIONS

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Abstract

Biofilm-based biofertilizers are microbial formulations designed to improve the persistence and functional activity of beneficial microorganisms in agricultural environments. Biofilms are surface-associated microbial communities embedded in an extracellular matrix that can support attachment, root colonization and protection from environmental fluctuations. This chapter reviews the biological basis of biofilm formation and its relevance to plant-growth-promoting microorganisms, with emphasis on root colonization, microbial survival, nutrient mobilization, stress tolerance, formulation, application, quality control and field evaluation. Biofilm formation may improve the persistence of inoculants near plant roots, but performance remains dependent on microbial strain, crop, soil, climate, formulation and interactions with the native microbiome. Accordingly, laboratory evidence of biofilm formation should be complemented by greenhouse and multi-location field evaluation before claims of improved agricultural efficiency are made.

Keywords: Biofilm, Biofertilizer, Plant-Growth-Promoting Rhizobacteria, Rhizosphere, Root Colonization, Nutrient Mobilization, Sustainable Agriculture.

1. Introduction

Biofertilizers contain living microorganisms that can contribute to plant nutrition, growth promotion or other beneficial biological activities in soil. Their practical performance depends on strain quality, formulation, survival during storage, establishment after application and interactions with the native soil microbiome [1,2].

Biofilm-based approaches seek to improve these characteristics by supporting microorganisms as surface-associated communities rather than relying exclusively on free-living cells. A biofilm is a structured microbial community associated with a surface and embedded in an extracellular polymeric matrix. This matrix can contain polysaccharides, proteins, extracellular DNA and other macromolecules that influence cell attachment and the local microenvironment [5].

Biofilm formation is dynamic and commonly involves initial attachment, development of microcolonies, maturation of the matrix-associated community and dispersal. In agricultural systems, plant roots, soil particles and carrier materials may provide surfaces on which beneficial

microorganisms attach. The ability to form a biofilm is, however, strain- and environment-dependent.

2. Concept and Biological Basis of Biofilms

Biofilm development changes the physiological and ecological behaviour of microbial cells. Cells within a biofilm experience localized gradients of nutrients, oxygen, water and metabolites, while the extracellular matrix can provide physical structure and protection. These features may improve persistence under some environmental stresses compared with planktonic growth [5].

In the rhizosphere, biofilm formation can be particularly relevant because microorganisms must remain associated with roots despite fluctuations in moisture, temperature, nutrient availability and competition with indigenous microorganisms. Root exudates containing sugars, amino acids, organic acids and other compounds can influence microbial attachment and activity [3,4].

Biofilm formation should therefore be viewed as one component of inoculant fitness rather than as a guarantee of plant-growth promotion. A strong biofilm phenotype in vitro does not necessarily predict successful establishment or agronomic performance in every soil or crop.

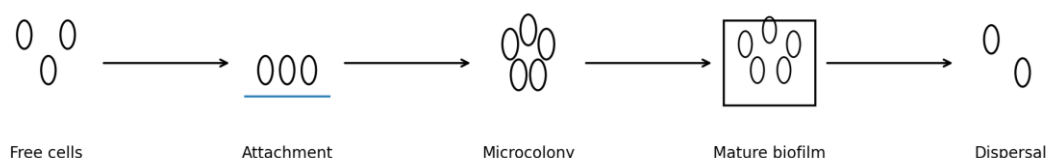


Figure 1: Generalized stages of microbial biofilm development

Table 1: Representative microbial groups and commonly studied agricultural functions

Microbial Group	Major Function	Potential Contribution
Rhizobium	Biological nitrogen fixation in compatible legume associations	Nitrogen nutrition and nodule-associated plant growth
Azospirillum	Associative nitrogen fixation and plant-growth promotion	Root development and nutrient acquisition
Bacillus	Phosphate solubilization, enzyme/metabolite production and stress-associated functions	Root-zone activity and multifunctional plant-growth promotion
Pseudomonas	Siderophore production, metabolite production and root colonization	Nutrient acquisition and suppression of selected pathogens

3. Microorganisms Used in Biofilm-Based Biofertilizers

Beneficial microbial groups investigated for plant-growth promotion include Rhizobium, Azospirillum, Bacillus and Pseudomonas. Depending on the strain and plant association, these microorganisms may contribute to biological nitrogen fixation, phosphate solubilization, potassium mobilization, siderophore production, modulation of plant-growth-related compounds and suppression of selected plant pathogens [2,4].

Selection of strains for biofilm-based inoculants should consider crop compatibility, root-colonization capacity, functional traits, formulation stability, genetic and phenotypic stability, safety and performance under the intended environmental conditions.

4. Biofilm Formation and Root Colonization

Plant roots release a complex mixture of compounds into the rhizosphere. These exudates influence microbial recruitment, attachment and activity. Beneficial microorganisms can attach to root surfaces and develop biofilms, increasing opportunities for persistent root association [3]. Root colonization is influenced by soil moisture, pH, temperature, nutrient status, root genotype, exudate composition and competition with resident microorganisms. Field persistence should therefore be measured rather than assumed. Culture-based enumeration, microscopy and molecular approaches such as strain-specific quantitative PCR can be combined to track inoculants and distinguish them from native populations [3].

5. Mechanisms of Plant-Growth Promotion

Plant-growth-promoting microorganisms can act through direct and indirect mechanisms. Direct mechanisms include biological nitrogen fixation, phosphate solubilization, potassium mobilization and production or modulation of compounds that influence root development. Indirect mechanisms include siderophore production, competition with harmful microorganisms, antimicrobial metabolite production, extracellular enzyme activity and stimulation of plant defence responses [2,4].

Biofilm formation may increase the persistence of these activities near plant roots by maintaining cells in a structured community. The magnitude of the benefit nevertheless depends on the specific microorganism, plant genotype and environmental conditions.

6. Improved Microbial Survival Under Field Conditions

Agricultural fields expose inoculated microorganisms to changing temperature, moisture, pH, osmotic conditions, ultraviolet radiation and competition or predation by native soil organisms. Formulation and biofilm-associated growth may create localized conditions that help cells tolerate some of these stresses [1,3].

Improved survival is particularly important between inoculation and successful establishment in the rhizosphere. However, survival alone does not establish agronomic efficacy; the inoculant must remain viable and express the intended functional traits in the target environment.

7. Nutrient Availability and Fertilizer Efficiency

Microbial inoculants can complement conventional fertilizer management. Phosphate-solubilizing microorganisms may increase the availability of poorly soluble phosphorus, potassium-mobilizing microorganisms may release potassium from mineral sources, and nitrogen-fixing microorganisms can contribute biologically available nitrogen in compatible plant associations [2,4].

Field responses vary with soil chemistry, crop genotype, climate, inoculant quality and native microbial communities. Biofertilizers should therefore be evaluated as components of integrated nutrient management rather than automatically considered replacements for mineral fertilizers.

Table 2: Major mechanisms associated with plant-growth-promoting microorganisms

Mechanism	Basic Principle	Potential Benefit
Nitrogen fixation	Biological conversion of atmospheric nitrogen into biologically useful forms	Supports nitrogen nutrition in compatible plant associations
Phosphate solubilization	Mobilization of poorly soluble phosphate	Improved phosphorus availability
Potassium mobilization	Release of potassium from mineral sources	Potentially improved potassium acquisition
Siderophore production	High-affinity binding and mobilization of iron	Improved microbial iron acquisition and competition
Phytohormone-related activity	Production or modulation of compounds influencing plant growth	Root growth and nutrient acquisition
Biological suppression	Competition, antimicrobial compounds, enzymes or induced plant responses	Reduced pressure from selected pathogens

Table 3: Common delivery approaches for microbial inoculants

Method	Main Advantage	Important Consideration
Seed treatment	Places inoculant near emerging roots	Coating uniformity and seed compatibility
Root dipping	Direct exposure of transplant roots	Handling and transplanting conditions
Soil application	Targets the root-zone soil	Soil moisture and microbial competition
Irrigation delivery	Can distribute inoculant across the crop root zone	Equipment and formulation compatibility

8. Biofilm-Based Biofertilizers and Stress Tolerance

Drought, salinity and temperature fluctuations can reduce microbial activity and plant productivity. Biofilm-associated growth may help beneficial microorganisms remain attached to roots and maintain activity during periods of environmental stress. Microbial effects on root development, nutrient acquisition and plant defence may also contribute indirectly to plant stress tolerance.

Because these responses are context-dependent, stress-related benefits should be demonstrated through controlled experiments followed by greenhouse and field validation. Claims should be linked to the particular strain, crop and stress condition tested.

9. Formulation Strategies

A practical biofilm-based biofertilizer requires an appropriate microbial strain and a formulation that preserves viability and functional performance. Carrier materials should support microbial survival, handling, storage and application. Important properties include moisture compatibility, shelf life, protection during transport, compatibility with agricultural inputs and maintenance of viable functional cells [1]. Formulation development may involve carrier-based systems, liquid formulations, immobilization or protective matrices. The appropriate strategy depends on the microorganism, target crop, delivery method, storage conditions and regulatory requirements. Formulation performance should be assessed using defined viability and stability criteria.

10. Application Methods

Biofilm-based inoculants may be applied by seed treatment, seedling root dipping, soil application or irrigation-associated delivery, depending on the formulation and crop. Seed treatment places the inoculant close to emerging roots, while root dipping provides direct exposure of transplant roots. Soil and irrigation applications can target the root zone but are strongly influenced by moisture, soil properties, equipment and microbial competition.

Application protocols should be standardized for inoculant concentration, carrier compatibility, timing and environmental conditions. The selected delivery method should be validated for uniform application and adequate establishment of the intended microorganism.

11. Quality Control and Field Evaluation

Quality control should include microbial identity, viable cell concentration, absence of unwanted contaminants, formulation stability and maintenance of intended functional traits. Biofilm-forming ability may be assessed using laboratory assays, but laboratory performance alone cannot establish field effectiveness. Field trials should measure microbial persistence, root colonization, plant growth, nutrient uptake and yield-related traits using appropriate controls, replication and, where possible, multiple locations or seasons. Assessment of both microbial survival and the target plant-beneficial function is important because persistence does not necessarily translate into measurable agronomic benefit [3].

12. Advantages and Limitations

Potential advantages of biofilm-based inoculants include improved surface attachment, greater opportunities for root colonization, persistence of beneficial microorganisms and sustained expression of plant-growth-promoting activities. These characteristics may improve the reliability of microbial inoculants under some field conditions. Important limitations include strain dependence, variation among soils and climates, formulation complexity, storage requirements, interactions with native microbiomes and the possibility that laboratory biofilm performance will not translate directly to field performance. Commercial development therefore requires evidence across the complete production and application pathway [1,3].

13. Future Prospects

Future research should integrate microbial ecology, formulation science and agronomy. Important areas include selection of robust strains, compatible microbial consortia, improved carrier systems, controlled biofilm development, longer shelf life and crop-specific delivery.

Molecular approaches may help identify traits associated with adhesion, extracellular matrix production, root colonization and plant-growth promotion. Combining genomic, physiological and field data could improve the selection of inoculants suited to particular crops, soils and climates. Multi-location testing and standardized performance criteria will also be important for reliable commercialization.

Conclusion

Biofilm-based biofertilizers represent a promising biological approach for improving the persistence and functional activity of beneficial microorganisms in agricultural systems. Biofilm formation can support surface attachment and root colonization and may increase tolerance to environmental fluctuations. Associated microorganisms can contribute to nutrient mobilization and plant-growth promotion through several direct and indirect mechanisms.

Because field performance depends on microbial strain, formulation, crop, soil, climate and resident microbiota, rigorous quality control and field validation are essential. Biofilm formation should therefore be considered a useful trait within a broader inoculant-development strategy rather than a stand-alone indicator of efficacy. When integrated with appropriate agronomic practices and validated under realistic field conditions, biofilm-based inoculants may contribute to more sustainable and efficient crop production.

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EFFECT OF pH ON THE COLOUR STABILITY OF CYANOBACTERIAL PHYCOCYANIN IN A MODEL FOOD SYSTEM

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Abstract

Phycocyanin is a natural blue pigment with potential use as a food colourant, but its stability is affected by acidic pH and heating. In the reported model system, the effect of different polysaccharides on the colloidal and colour stability of phycocyanin was investigated under acidic and heating conditions. Among the tested polysaccharides, ι-carrageenan, xanthan gum, and high-methoxyl pectin formed soluble and stable complexes with phycocyanin at pH 3.0. Among these, ι-carrageenan showed the highest colloidal and colour stability. These findings indicate that polysaccharide–phycocyanin interactions can reduce colour loss and improve the stability of phycocyanin during food and beverage processing. Polysaccharides, particularly ι-carrageenan, may therefore help enhance the application of phycocyanin as a natural alternative to artificial food pigments.

Keywords: Phycocyanin; ι-carrageenan; pH Stability; Colour Stability; Natural Food Pigment.

1. Introduction

Colour is an important quality attribute of foods and beverages because it influences visual acceptance and helps identify a product. The use of natural pigments has therefore received considerable attention in food research. Phycocyanin is a blue, water-soluble phycobiliprotein obtained from cyanobacteria such as *Arthrospira* (*Spirulina*). It has been investigated as a natural source of blue colour for food and beverage formulations.

The main limitation of phycocyanin is its sensitivity to processing conditions. Acidic pH can promote aggregation and changes in the protein–chromophore environment, while heating can accelerate colour loss. These limitations are particularly important in acidic beverages and other foods that undergo thermal processing. A published Food Hydrocolloids study specifically examined polysaccharide–phycocyanin complexation at pH 3.0 and after heating at 60 °C and 80 °C for 30 min. The study reported improved colloidal and colour stability, with ι-carrageenan showing the strongest performance among the six polysaccharides tested [7].

2. Cyanobacterial Phycocyanin

C-phycocyanin is a major phycobiliprotein in many cyanobacteria and is responsible for an intense blue colour. It is a protein–chromophore complex belonging to the light-harvesting phycobiliprotein family. Its visible absorption maximum is reported around 615–620 nm.

The colour of phycocyanin is closely related to the structure and environment of its chromophore. Changes in pH can alter protein association and the spectral behaviour of the pigment. Under acidic conditions, aggregation and precipitation may reduce the apparent intensity of the blue colour. Reviews of phycocyanin stability also describe strong effects of pH and temperature on pigment integrity and optical properties [2,6].

3. Effect of Acidic pH on Colour Stability

pH influences the ionization state of proteins and polysaccharides and can therefore change intermolecular interactions. Phycocyanin is relatively well dispersed around neutral conditions, whereas acidic conditions can reduce solubility and promote aggregation. At low pH, the optical properties of the chromophore may also change, which can appear as a reduction or shift in blue colour. A review reports lower absorbance at 620 nm for phycocyanin at pH 3.0 and 4.0 compared with less acidic conditions, consistent with precipitation and structural changes [6]. For the present chapter, pH 3.0 is the defined acidic condition because it is the value reported in the supplied study and should be treated as the experimental challenge condition, not as a general optimum pH for phycocyanin.

4. Polysaccharide Stabilization of Phycocyanin

The reported study evaluated six polysaccharides: κ -carrageenan, ι -carrageenan, λ -carrageenan, xanthan gum, high-methoxyl pectin, and guar gum. The complexes were examined at pH 3.0 before and after heating at 60 °C and 80 °C for 30 min. Soluble complexes with good colloidal stability were obtained with ι -carrageenan, xanthan gum, and high-methoxyl pectin. The ι -carrageenan–phycocyanin complex showed the best colloidal and colour stability [7].

5. Interaction Between Phycocyanin and Polysaccharides

The stabilization is associated with interactions between the pigment and polysaccharide chains. The published work identified electrostatic complexation as an important driving force and found that hydrophobic interactions also contributed to complex formation. The highly negatively charged carrageenans, particularly ι -carrageenan, showed high affinity and entrapment efficiency toward phycocyanin. Structural measurements further indicated changes in tertiary structure while native-like secondary structure was maintained [7]. This interaction can be understood as a protective formulation strategy. Instead of allowing pigment molecules to associate and precipitate freely under acidic conditions, the polysaccharide can help maintain a more dispersed complex. Better dispersion is important because visible precipitation and structural changes are closely connected with loss of the desired blue appearance.

6. Thermal Stability Under Food-Processing Conditions

Heating is another important factor affecting natural colourants. The study used 60 °C and 80 °C for 30 min to compare the behaviour of the complexes under thermal stress. The κ -carrageenan complex showed the strongest resistance to colour deterioration among the tested polysaccharide systems [7]. This is relevant to food and beverage processing because natural pigments may encounter heat during preparation and thermal processing.

The results should not be interpreted as showing that all foods will retain exactly the same colour under these temperatures. Food composition, oxygen, light, ionic strength, sugars, proteins, and storage conditions can alter pigment behaviour. The values discussed in this chapter therefore remain tied to the stated model-system conditions.

7. Colour Evaluation in a Model Food System

Colour stability may be assessed by direct visual observation and by instrumental methods. A simple model system can be prepared at the selected pH and compared before and after heating. Changes in blue intensity, turbidity, precipitation, and visible colour shift can be recorded. UV–Visible spectrophotometry can provide quantitative information, while a colourimeter can provide L^* , a^* , and b^* values when available.

The published work used colourimetry and particle-size analysis to evaluate the complexes. It also examined molecular interactions and structural changes. In a student or laboratory project, the most important point is to report the actual measured values obtained from the experiment rather than adding values from another study.

8. Food and Beverage Relevance

Phycocyanin is being explored as a natural blue colourant in beverages and other foods. A beverage study using C-phycocyanin from *Arthrospira platensis* evaluated the extract as a blue colourant using spectroscopy and colorimetry, supporting the relevance of phycocyanin to food and beverage colour development [3].

The importance of stabilization is practical: a pigment that has good colour initially but loses its appearance during acidification or heating may not perform adequately in a finished product. Polysaccharide complexation provides one formulation approach for improving dispersion and colour retention, particularly under acidic processing conditions.

9. Discussion

The main observation from the reported study is that the effect of pH cannot be separated from the composition of the surrounding food matrix. At pH 3.0, phycocyanin can experience aggregation and colour instability, but selected polysaccharides can form soluble complexes and improve its colloidal behaviour. Among the six polysaccharides evaluated, κ -carrageenan gave the strongest overall protection against the combined challenge of acidity and heating [7].

The result is particularly relevant to natural blue food colourants because acidic foods and beverages are common, while protein-based pigments can be sensitive to processing. The study therefore provides a useful scientific basis for investigating phycocyanin stabilization in model food systems.

Conclusion

Cyanobacterial phycocyanin is a promising natural blue food pigment, but its colour and colloidal stability can be affected by acidic pH and heating. In the reported model system, phycocyanin was examined at pH 3.0 and after heating at 60 °C and 80 °C for 30 min. Among six polysaccharides, ι-carrageenan, xanthan gum, and high-methoxyl pectin produced soluble and stable complexes, while ι-carrageenan showed the best overall colloidal and colour stability. These findings support polysaccharide–phycocyanin complexation as a practical approach for improving the use of natural blue pigments in food and beverage applications.

Table 1: Summary of the reported experimental conditions and findings

Parameter	Condition / Reported Value	Significance
Pigment	Cyanobacterial phycocyanin	Natural blue food pigment
Acidic pH	3.0	Main acidic test condition
Polysaccharides	κ-, ι-, λ-carrageenan; xanthan gum; high-methoxyl pectin; guar gum	Stabilization comparison
Heat treatment	60 °C and 80 °C, 30 min	Thermal challenge
Soluble stable complexes	ι-carrageenan; xanthan gum; high-methoxyl pectin	Good colloidal stability at pH 3.0
Best reported complex	ι-Carrageenan–phycocyanin	Highest reported colloidal and colour stability

Table 2: Suggested observations and measurements for colour-stability evaluation

Sample / condition	Observation to Record	Measurement
Phycocyanin at pH 3.0, before heating	Blue intensity, turbidity, visible precipitation	Visual observation / absorbance
Phycocyanin + selected polysaccharide at pH 3.0	Dispersion and colour compared with control	Visual observation / absorbance
After 60 °C, 30 min	Colour retention and precipitation	Colour or absorbance
After 80 °C, 30 min	Colour change under stronger thermal challenge	Colour or absorbance
Best reported formulation: ι-carrageenan–phycocyanin	Compare colour retention with control	Colour or absorbance

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PROBIOTIC MICROORGANISMS FROM TRADITIONAL FERMENTED FOODS: HEALTH BENEFITS AND ENTREPRENEURSHIP

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Abstract

Traditional fermented foods are important reservoirs of diverse microorganisms and represent a valuable source for the discovery of strains with probiotic potential. This chapter reviews the microbial diversity associated with traditional fermented foods and beverages, the principal criteria used to screen and characterize probiotic candidates, the mechanisms through which selected strains may interact with the host and other members of the gut microbiota, and the evidence for health-related effects. Particular attention is given to gastrointestinal, immune, metabolic and gut-brain outcomes, while emphasizing that probiotic effects are generally strain-specific and that fermented foods should not automatically be equated with probiotic products. The chapter also considers the pathway from microbial discovery to commercial product development, including strain characterization, safety assessment, process development, regulatory requirements, quality control and business models. Traditional knowledge and regional fermentation practices may provide opportunities for entrepreneurship, but commercialization should be accompanied by scientific validation, regulatory compliance, traceability and equitable consideration of traditional knowledge.

Keywords: Probiotics, Lactic Acid Bacteria, Traditional Fermented Foods, Fermented Beverages, Gut Microbiota, Functional Foods, Entrepreneurship.

1. Introduction

Fermentation is one of the oldest forms of food biotechnology. Across cultures, microorganisms have been used to transform milk, vegetables, cereals, legumes, fish, meat and beverages, improving sensory properties, preservation and, in some cases, digestibility. Traditional fermentation practices are diverse because the microbial communities involved are shaped by raw-material composition, environmental conditions, processing methods and local production practices [1,2].

The term probiotic has a more specific meaning than simply 'beneficial microorganism'. FAO/WHO guidance and subsequent ISAPP consensus define probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [3,4].

Therefore, the presence of a microorganism in a fermented food does not by itself establish that the organism is a probiotic. Demonstration of safety, identity, functional properties and a health benefit is required.

Traditional fermented foods have attracted renewed scientific interest because they contain complex microbial communities and may provide candidates adapted to particular food matrices. Advances in sequencing, whole-genome analysis and high-throughput screening now make it possible to characterize these communities and identify strains with potentially useful technological or functional properties [2,5]. At the same time, consumer interest in fermented foods and functional products has created opportunities for value-added food enterprises.

This chapter integrates the microbiological basis of probiotic discovery from traditional fermented foods with the practical considerations involved in converting a promising strain or fermented-food concept into a safe and commercially viable product.

2. Traditional Fermented Foods: A Global Microbial Heritage

Traditional fermented foods and beverages occur in many regions and can be grouped broadly according to their principal substrates: dairy products, vegetables, cereals and legumes, fish and meat products, and fermented beverages. Each category may contain characteristic microbial successions or consortia influenced by raw-material composition, salt concentration, temperature, oxygen availability, processing vessels and repeated use of fermentation cultures [2,5].

Dairy fermentations such as yoghurt, kefir and dahi commonly contain lactic acid bacteria (LAB), whereas kefir and related products involve more complex bacterial–yeast communities. Vegetable fermentations such as kimchi and sauerkraut commonly undergo microbial succession as acidity increases. Cereal- and legume-based foods such as idli, dosa and tempeh can involve mixed bacterial, yeast or fungal communities. Soybean fermentations such as natto and kinema may contain *Bacillus* species in addition to other microorganisms. Fermented beverages, including kombucha and boza, further demonstrate the breadth of microbial associations in traditional foods [2,5].

Microbial diversity in these foods is scientifically valuable because strains that survive repeated fermentation under local environmental conditions may possess technological characteristics such as acid tolerance, salt tolerance, carbohydrate utilization or metabolite production. However, claims of probiotic activity must be demonstrated for individual strains rather than inferred from the food source alone.

3. Probiotic Microorganisms: Diversity, Sources and Selection Criteria

Lactic acid bacteria remain among the most frequently investigated microorganisms associated with fermented foods. Relevant genera include *Lactobacillus* sensu lato and its reclassified genera, *Lactiplantibacillus*, *Lacticaseibacillus* and *Limosilactobacillus*, as well as *Lactococcus*,

Streptococcus, *Leuconostoc*, *Pediococcus* and *Weissella*. *Bifidobacterium* species are also important probiotic organisms, although they are taxonomically distinct from LAB. Selected yeasts, particularly *Saccharomyces* strains, and some *Bacillus* species have also been studied for probiotic applications [4,6].

Isolation from a fermented food is only the first stage of probiotic discovery. Candidate strains require systematic assessment of identity, safety, functional properties, technological performance and, ultimately, evidence of a benefit in the intended host [3,4].

Safety criteria include absence of relevant virulence determinants and unacceptable transferable antimicrobial-resistance traits, together with an appropriate safety history or formal safety assessment. Functional criteria may include tolerance to gastrointestinal conditions, relevant interaction with intestinal surfaces, production of antimicrobial substances and other strain-specific properties. Technological criteria include genetic and phenotypic stability, survival during processing and storage, compatibility with the food matrix, acceptable sensory characteristics and manufacturing feasibility.

A practical screening workflow may begin with isolation and phenotypic characterization, followed by molecular identification using appropriate methods such as 16S rRNA sequencing for bacteria and ITS sequencing for fungi and yeasts. Promising isolates can then undergo simulated gastrointestinal, acid and bile tolerance testing, antimicrobial susceptibility assessment and other safety assays. Whole-genome sequencing can strengthen strain-level characterization by identifying genes relevant to antimicrobial resistance, virulence and functional traits [4,6]. These laboratory findings should not be treated as evidence of clinical efficacy; human health claims require appropriate clinical substantiation.

4. Mechanisms of Probiotic Action

Probiotic effects are strain-dependent and may arise through several interacting mechanisms. Current evidence supports three broad, overlapping categories: interactions with other microorganisms, effects on the intestinal barrier, and modulation of host immune and metabolic pathways [6,7].

Probiotic microorganisms may compete with other microorganisms for nutrients or attachment sites and may produce organic acids, hydrogen peroxide, bacteriocins or related antimicrobial compounds. They can also influence the composition and metabolic activity of the resident microbiota, including interactions that affect short-chain fatty acid production [7].

Some probiotic strains can influence mucus production, epithelial-cell survival and tight-junction-associated pathways. These effects may contribute to maintenance of intestinal barrier integrity and reduction of inappropriate microbial translocation. The magnitude and reproducibility of such effects depend on the strain and experimental context [7].

Probiotic microorganisms can interact with host pattern-recognition systems and immune cells and may influence cytokine responses, regulatory T-cell pathways and mucosal immune functions. Certain microbial metabolites can also participate in host signalling. Mechanistic findings provide biological plausibility for some observed health effects, but they should not be interpreted as proof of clinical benefit without human evidence [7].

5. Health Benefits of Probiotics Derived from Fermented Foods

The health effects of fermented foods and probiotic products must be considered separately. Fermented foods contain microorganisms, food-derived metabolites and nutrients, but not every fermented food contains a defined probiotic strain, and not every strain present in a fermented food has demonstrated a health benefit. Current consensus literature emphasizes the distinction between fermented foods and probiotic products and the need to evaluate benefits at the appropriate strain and product level [5].

Specific probiotic strains have been investigated for outcomes such as antibiotic-associated diarrhoea, infectious diarrhoea and symptoms associated with some functional gastrointestinal disorders. Evidence varies according to strain, dose, population and clinical endpoint; efficacy should therefore not be generalized to all microorganisms within a genus [6,7].

Clinical studies of selected probiotic strains have examined respiratory infections, immune responses and allergic outcomes. Results are heterogeneous, and claims should be linked to the specific strain, formulation and population for which evidence is available [7].

Some studies have reported modest effects of selected probiotic strains on metabolic markers, lipid profiles or blood pressure. Proposed mechanisms include bile-acid metabolism, microbial metabolite production and modulation of inflammatory pathways. The evidence remains product- and strain-specific and should not be presented as a universal effect of fermented foods [7].

The gut–brain axis involves neural, endocrine, immune and microbial-metabolite signalling. Some probiotic interventions have reported changes in measures related to stress, anxiety or mood, but this area remains less clinically established than several gastrointestinal applications. Effects vary with strain, dose and study population, and further controlled trials are required [7]. Laboratory and animal studies have identified antimicrobial, antioxidant and other potentially beneficial properties in selected probiotic strains and their metabolites. Such findings are useful for hypothesis generation and product development, but in vitro or animal observations should not be presented as established human therapeutic effects [6,7].

6. From Strain to Product: The Development Pipeline

Commercial development requires more than finding an interesting microorganism. A potential probiotic must be traceable from source to final product and must meet appropriate safety, identity, technological and regulatory requirements.

Key stages include documented bioprospecting and isolation; taxonomic and strain-level characterization; functional and technological screening; culture-collection deposition where appropriate; assessment of intellectual-property options; pilot-scale fermentation; stabilization or encapsulation; appropriate preclinical and human evidence for proposed claims; regulatory assessment; formulation; sensory evaluation; packaging; shelf-life validation; and route-to-market planning.

7. Entrepreneurship in the Probiotics and Fermented-Food Sector

Probiotic entrepreneurship spans fermented foods, functional beverages, dietary supplements, microbial ingredients and related technologies. Market-size estimates vary substantially among commercial reports because they use different definitions, product categories and geographic boundaries. Precise market valuations should therefore be quoted only with the corresponding report, year, scope and methodology. Potential business models include ingredient or culture supply, branded consumer products, artisanal and heritage enterprises, contract fermentation and co-manufacturing, and platform or technology ventures.

An illustrative pathway begins with a documented traditional food source from which a candidate strain is isolated. After identity and safety characterization, the strain is evaluated for technological and functional properties. A promising candidate can then be deposited in a culture collection, assessed for intellectual-property protection, developed at pilot scale and incorporated into a suitable product format. Market testing, regulatory review, shelf-life assessment and evidence for any proposed health claim should precede broad commercialization.

Regulatory requirements depend on jurisdiction and product category. Conventional fermented foods, dietary supplements and products carrying specific health claims may be subject to different requirements. In the European Union, health claims are governed by Regulation (EC) No. 1924/2006 and the applicable authorization framework. In the United States, regulatory requirements depend on intended use, product category and claims. Across markets, strain identity, safety, viable-count verification, manufacturing controls, stability and substantiation of claims are central quality considerations. Entrepreneurs working with microorganisms obtained from traditional or indigenous knowledge systems should also consider access-and-benefit-sharing obligations and ethical sourcing. The Nagoya Protocol may be relevant where genetic resources and associated traditional knowledge fall within applicable national frameworks.

Opportunities include bioprospecting regional fermented foods, developing accurately represented provenance-based products, expanding plant-based fermented products, using digital distribution, and building research and manufacturing partnerships. Major barriers include the cost and duration of safety and clinical substantiation, loss of viability during processing and storage, regulatory differences among export markets, competition from established companies, and the risk of marketing claims exceeding the available scientific evidence.

Table 1: Representative traditional fermented foods and associated microorganisms

Food/Beverage	Typical Region	Main Substrate	Representative Microorganisms
Yoghurt / Dahi	South Asia, Middle East, Europe	Milk	Lactobacillus spp.; Streptococcus thermophilus
Kefir	Caucasus region	Milk	Lactobacillus/Lactiplantibacillus spp.; Lactococcus; Leuconostoc; yeasts
Kimchi	Korea	Cabbage, radish	Leuconostoc; Lactiplantibacillus; Weissella
Sauerkraut	Central/Eastern Europe	Cabbage	Leuconostoc; Lactiplantibacillus
Idli / Dosa	South India	Rice, black gram	Leuconostoc; Enterococcus; yeasts
Tempeh	Indonesia	Soybean	Rhizopus spp.; associated bacteria
Natto / Kinema	Japan; Nepal / Northeast India	Soybean	Bacillus subtilis and associated bacteria
Boza	Balkans, Turkey	Cereal	Lactic acid bacteria; yeasts
Kombucha	East Asia; now global	Sweetened tea	Acetic-acid bacteria; yeasts
Gundruk / Sinki	Nepal/Himalayan region	Leafy vegetables/radish	Lactic acid bacteria; Pediococcus; Leuconostoc

Table 2: Selected health domains and representative probiotic mechanisms

Health Domain	Representative Examples	Potential Mechanism
Gastrointestinal health	Lactobacillus rhamnosus GG; Saccharomyces boulardii	Competitive exclusion; metabolite production; barrier effects
Irritable bowel symptoms	Selected Bifidobacterium and Lactiplantibacillus strains	Barrier and microbiota modulation
Immune modulation	Selected Bifidobacterium and Lactobacillaceae strains	Interaction with immune signalling pathways
Metabolic/lipid outcomes	Selected Lactiplantibacillus and Bifidobacterium strains	Bile-acid metabolism; microbial metabolite signalling
Gut–brain axis	Selected Lactobacillus and Bifidobacterium strains	Microbial, immune, vagal and neuroendocrine signalling
Antipathogenic activity	Selected Weissella, Pediococcus and Lactiplantibacillus strains	Organic acids and bacteriocin-like compounds

8. Challenges and Future Perspectives

Probiotic effects are generally strain-specific, and results obtained with one strain should not automatically be generalized to other strains, even within the same species. Standardization of viable-count measurement, stability testing, clinical endpoints and study populations remains important for meaningful comparison among products.

Metagenomics, metabolomics and metatranscriptomics can provide a more detailed view of microbial communities in traditional fermentations. Genome-scale analyses may support safer and more rational strain selection. Personalized nutrition, postbiotics and next-generation microbial candidates are active research areas, but their development requires careful validation. Responsible stewardship of traditional knowledge, including recognition of communities and compliance with applicable access-and-benefit-sharing requirements, is equally important.

Conclusion

Traditional fermented foods represent an important intersection of microbiology, food technology, nutrition and entrepreneurship. They provide diverse microbial resources from which probiotic candidates may be discovered, but the term probiotic should be reserved for microorganisms for which appropriate safety, functional and health-benefit evidence is available. Commercial development requires strain-level characterization, process control, regulatory compliance, quality assurance and evidence appropriate to the proposed product and claim. Entrepreneurial opportunities are particularly relevant where scientific innovation can be combined with responsible use of regional fermentation knowledge, traceable sourcing and sustainable product development. Continued research, clinical validation and ethical commercialization will be essential for translating microbial diversity in traditional foods into reliable health-oriented products.

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