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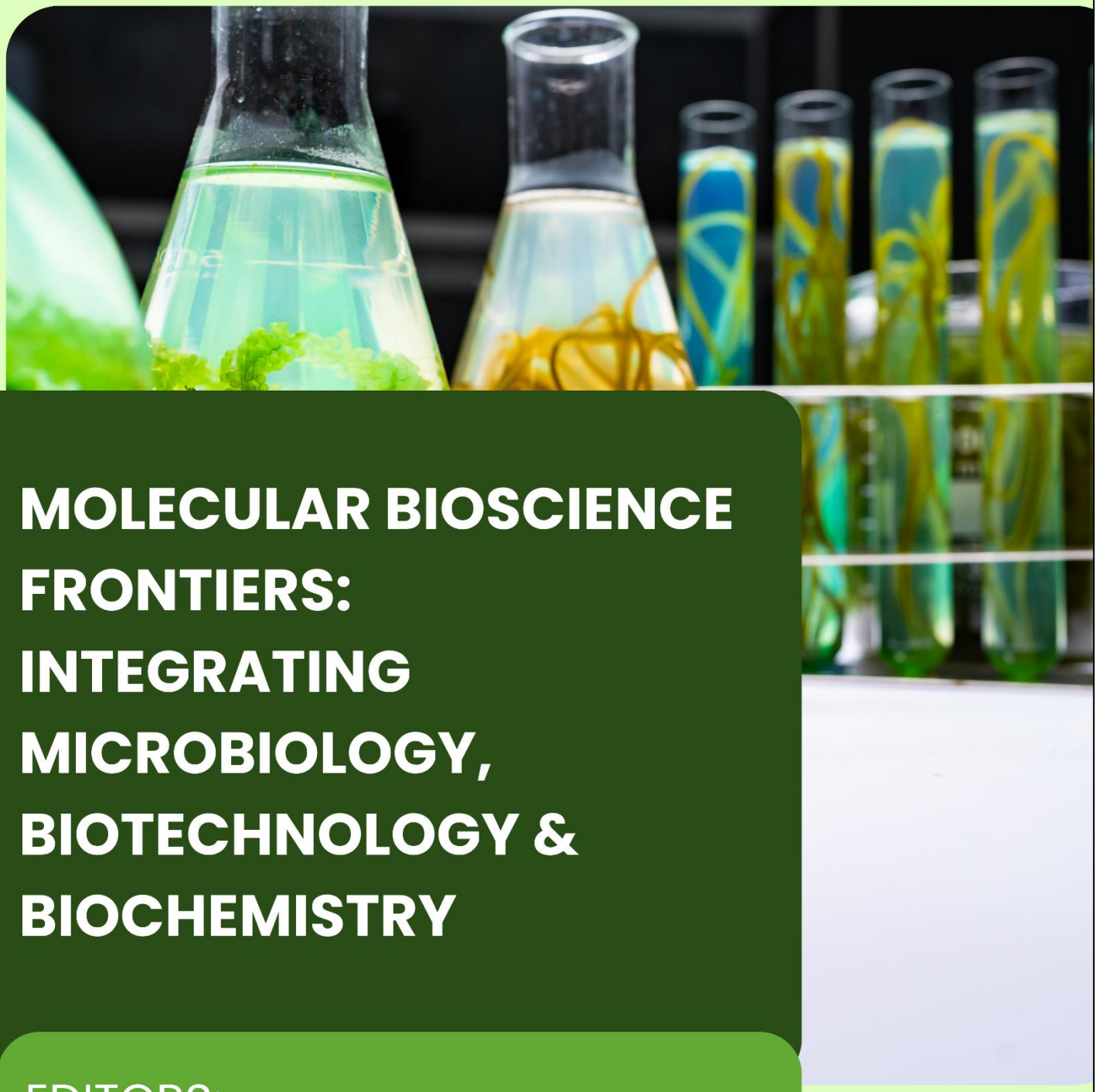

MOLECULAR BIOSCIENCE FRONTIERS: INTEGRATING MICROBIOLOGY, BIOTECHNOLOGY & BIOCHEMISTRY

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PREFACE

The rapid advancement of molecular biosciences has revolutionized our understanding of living systems, providing innovative solutions to challenges in healthcare, agriculture, environmental sustainability, and industrial development. The integration of microbiology, biotechnology, and biochemistry has created a multidisciplinary platform that bridges fundamental scientific discoveries with practical applications. *Molecular Bioscience Frontiers: Integrating Microbiology, Biotechnology and Biochemistry* is a comprehensive collection of scholarly works that highlights these dynamic intersections and their role in shaping the future of biological sciences.

This volume brings together contributions from distinguished researchers, academicians, and emerging scientists who present recent advances in microbial diversity, molecular genetics, enzyme technology, metabolic engineering, synthetic biology, industrial biotechnology, immunology, bioinformatics, environmental microbiology, and biochemical innovations. The chapters illustrate how interdisciplinary research is expanding our knowledge of biological processes while fostering sustainable technologies and novel therapeutic approaches.

The objective of this book is to provide readers with an updated understanding of contemporary developments in molecular biosciences and to encourage collaborative research across traditional disciplinary boundaries. By integrating theoretical concepts with practical applications, this volume serves as a valuable resource for students, educators, researchers, healthcare professionals, and industry practitioners seeking to explore emerging trends and innovative methodologies in life sciences.

We sincerely thank all the contributing authors for their valuable research contributions and commitment to scientific excellence. We are equally grateful to the reviewers whose insightful comments and constructive recommendations have significantly enhanced the quality of the chapters. Our heartfelt appreciation is extended to the editorial team and the publisher for their dedicated efforts in coordinating, editing, and publishing this volume.

We hope that *Molecular Bioscience Frontiers: Integrating Microbiology, Biotechnology and Biochemistry* will inspire scientific curiosity, promote interdisciplinary collaboration, and serve as a reliable reference for advancing research, innovation, and education in the rapidly evolving field of molecular biosciences.

- Editors

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AI-INTEGRATED MULTI-OMICS AND GREEN NANOBIOTECHNOLOGY FOR NEXT-GENERATION PHYTOCHEMICAL DISCOVERY AND ANTIMICROBIAL THERAPEUTICS

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Abstract

One of the most urgent global health concerns of our day is the emergence of antimicrobial resistance (AMR). It's endangering the efficacy of the antimicrobial medications we now use and increasing the demand for new, creative therapeutic approaches. Natural products derived from therapeutic plants have long been a wealth of bioactive substances. However, because of our inadequate understanding of the complex biological interactions at work, conventional approaches to finding these phytochemicals can be laborious, resource-intensive, and frequently unsuccessful. Fortunately, a new research strategy has been made possible by recent advances in green nanobiotechnology, multi-omics technologies, and artificial intelligence (AI). We can identify, optimize, and administer phytochemical-based antimicrobial medicines more quickly because to this integrated paradigm. Machine learning, deep learning, molecular docking, and predictive modeling are examples of AI-powered computer techniques that enable rapid bioactive drug screening, therapeutic target identification, and antimicrobial mechanism predictions. A comprehensive understanding of plant biosynthetic pathways, molecular signatures, and host-pathogen interactions is provided by multi-omics platforms that include genomes, transcriptomics, proteomics, and metabolomics. We can identify novel phytochemicals, metabolic pathways, and biomarkers associated with antimicrobial activity by integrating this information with AI algorithms. Furthermore, by facilitating the sustainable production of nanoparticles, nanoencapsulation, and targeted delivery systems, green nanobiotechnology increases the therapeutic potential of plant-based substances. This increases antibacterial efficacy while also improving bioavailability and stability. AI, multi-omics, and green nanotechnology together offer a novel approach to developing next-generation antimicrobial therapies that are more accurate, environmentally friendly, and sustainable. This chapter explores the most recent developments in green nanobiotechnological approaches for antimicrobial applications, multi-omics-driven molecular exploration, and AI-assisted phytochemical discovery. We also highlight the challenges associated with regulatory approval, clinical translation, toxicity evaluation, scalability, and computational validation. This integrated

approach opens up exciting possibilities for sustainable drug discovery and the creation of advanced antimicrobial solutions to tackle emerging infectious diseases.

Keywords: Artificial Intelligence, Multi-Omics Integration, Phytochemicals, Green Nanobiotechnology, Antimicrobial Resistance, Machine Learning, Nanoparticle-Based Therapeutics, Natural Product Discovery.

1. Introduction

Antimicrobial agents have been crucial in the fight against infectious diseases and have greatly improved healthcare outcomes around the globe. However, the swift rise and spread of antimicrobial resistance (AMR) among bacteria, fungi, and parasites have seriously undermined the effectiveness of our current treatments. The World Health Organization (WHO) has flagged AMR as a significant public health concern, noting that resistant microorganisms lead to higher mortality rates, longer hospital stays, and hefty economic costs worldwide (Murray *et al.,* 2022). With traditional antibiotics losing their punch, there's an urgent need for fresh antimicrobial strategies that include new chemical compounds, innovative delivery methods, and sustainable treatment options.

Medicinal plants are a treasure trove of diverse secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, and saponins, all of which boast impressive antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects. Throughout history, natural products have played a vital role in drug development, with many approved medications tracing their roots back to plant-derived substances. Take quinine, artemisinin, and paclitaxel, for instance; they highlight the therapeutic value of phytochemicals in today's medicine. Yet, despite their vast potential, traditional methods for discovering phytochemicals like extraction, isolation, purification, and biological screening often come with challenges, including extensive experimental demands, low yields, complex structures, and the difficulty of predicting how they work biologically (Atanasov *et al.,* 2021).

Recent advancements in artificial intelligence (AI) have truly transformed the landscape of natural product research, allowing for swift analysis of intricate biological and chemical datasets. With the help of AI techniques like machine learning (ML) and deep learning (DL), researchers can now predict molecular properties, pinpoint bioactive compounds, optimize drug candidates, and delve into complex biological networks. Unlike traditional screening methods, AI algorithms can seamlessly combine chemical structures, biological responses, genomic data, and clinical information to uncover promising therapeutic molecules with greater accuracy and lower experimental costs (Vamathevan *et al.,* 2019). The role of AI in drug discovery has become especially crucial in the realm of antimicrobial research, where it aids in predicting antibacterial activity, discovering new targets, and speeding up the repurposing of existing compounds. Moreover, multi-omics technologies have broadened our understanding of phytochemical biosynthesis and antimicrobial mechanisms by offering detailed molecular-level insights.

Genomics uncovers the genetic pathways that lead to the production of secondary metabolites, while transcriptomics sheds light on gene expression patterns tied to metabolic regulation. Proteomics helps us understand functional proteins and their interactions, and metabolomics allows for the identification and quantification of bioactive metabolites. By integrating these layers of omics data, we gain a comprehensive view of biological systems, paving the way for the discovery of new phytochemical candidates with therapeutic potential (Hasin *et al.,* 2017). However, the sheer volume and complexity of multi-omics datasets necessitate advanced computational methods for effective interpretation, making the integration of AI vital for efficient, data-driven discoveries.

Green nanobiotechnology is shaping up to be an exciting new avenue for boosting the therapeutic potential of phytochemicals. Traditional methods of synthesizing nanoparticles often rely on toxic chemicals, consume a lot of energy, and can harm the environment. On the flip side, green synthesis techniques harness the power of plant extracts, microbial systems, and biomolecules to create nanoparticles in an eco-friendlier way. For instance, plant-derived nanoparticles that include metals and metal oxides like silver (Ag), gold (Au), zinc oxide (ZnO), copper oxide (CuO), and iron oxide (Fe_2O_3) have shown impressive antimicrobial effects. They work through various mechanisms, such as generating reactive oxygen species, disrupting membranes, inhibiting enzymes, and causing genetic damage (Iravani, 2011). Additionally, nanoformulation strategies enhance the solubility, stability, bioavailability, and targeted delivery of phytochemicals, effectively addressing many of the challenges faced by free bioactive molecules. The combination of AI, multi-omics, and green nanotechnology creates a multidisciplinary approach that paves the way for the next generation of phytochemical discovery and antimicrobial therapy development. AI algorithms can sift through multi-omics datasets to pinpoint crucial biosynthetic pathways, forecast molecular interactions, and help design nano-enabled delivery systems. Meanwhile, multi-omics analysis offers molecular insights into antimicrobial mechanisms, and green nanotechnology ensures sustainable production while boosting the therapeutic efficacy of phytochemical-based formulations. This integrated platform holds great promise for speeding up the discovery of safer, more effective, and environmentally friendly antimicrobial agents.

Recent studies have shown just how powerful it can be to blend computational intelligence with natural product research. AI-driven models have made impressive strides in predicting antimicrobial peptides, uncovering new antibiotic candidates, and analyzing the intricate interactions between hosts and microbes (Stokes *et al.,* 2020). On a similar note, metabolomics-assisted techniques have allowed for the quick identification of plant metabolites that contribute to various biological activities, while nanoparticle delivery systems have enhanced the therapeutic effectiveness of compounds derived from plants (Newman & Cragg, 2020). However, despite these exciting developments, we still face hurdles like standardizing plant

extracts, ensuring the reproducibility of nanomaterial synthesis, validating computational results, assessing toxicity, and bridging the gap between lab research and clinical applications. This chapter aims to provide a thorough look at the promising integration of AI, multi-omics technologies, and green nanobiotechnology in the quest for next-generation phytochemical discoveries and antimicrobial treatments. We'll explore how computational methods help identify new bioactive compounds, the role of multi-omics platforms in unraveling molecular mechanisms, and how green nanotechnology can boost antimicrobial effectiveness. Additionally, we'll touch on future directions for sustainable drug discovery, precision therapeutics, and the development of AI-driven antimicrobial solutions.

2. Review of Literature

2.1 Evolution of Phytochemical Discovery and Natural Product-Based Antimicrobial Research

Natural products sourced from plants, microorganisms, and marine life have played a crucial role in shaping therapeutic agents throughout the history of pharmaceuticals. Secondary metabolites derived from plants, like flavonoids, alkaloids, terpenoids, phenolic acids, tannins, and glycosides, showcase a wide range of biological activities. These include antimicrobial, antioxidant, anticancer, anti-inflammatory, and immunomodulatory effects. The structural diversity of these compounds is something that's tough to replicate with synthetic chemical libraries, which is why natural products are such valuable assets in drug discovery (Newman & Cragg, 2020).

Traditionally, discovering phytochemicals involves a series of steps: extraction, fractionation, purification, structural characterization, and biological evaluation. While this method has led to the identification of significant drugs, it often suffers from low efficiency, the repeated isolation of known compounds, a lack of complete understanding of biological mechanisms, and a hefty time investment. Moreover, many phytochemicals struggle with poor water solubility, instability, and limited bioavailability, which can hinder their clinical use (Atanasov *et al.,* 2021).

With the rise of antimicrobial resistance, there's been a growing interest in exploring plant-based antimicrobial compounds. Phytochemicals can tackle resistance mechanisms through various actions, such as disrupting microbial membranes, inhibiting enzymes, interfering with nucleic acid synthesis, modulating quorum sensing, and inducing oxidative stress. Unlike traditional antibiotics that typically focus on a single microbial pathway, phytochemicals often target multiple pathways, which helps to minimize the chances of rapid resistance development (Cushnie *et al.,* 2014). However, sophisticated tools that can analyze intricate biological systems are needed for the effective identification of antimicrobial phytochemicals. By facilitating predictive discovery, molecular mechanism analysis, and enhanced therapeutic delivery, the recent merger of artificial intelligence (AI), multi-omics methods, and nanotechnology has revolutionized natural product research.

2.2 Multi-Omics Approaches in Phytochemical Discovery

Multi-omics is all about bringing together various layers of biological data, such as genomics, transcriptomics, proteomics, metabolomics, and epigenomics. Unlike the more traditional single-layer studies, multi-omics offers a richer, more detailed view of how molecules interact and the biological pathways at play. In the realm of phytochemical research, these multi-omics strategies help us uncover biosynthetic pathways, understand regulatory mechanisms, track metabolite production, and explore antimicrobial responses.

When we combine multi-omics datasets with computational intelligence, we create a robust platform for discovering new bioactive compounds and delving into their therapeutic mechanisms. This approach is especially beneficial for studying medicinal plants, where intricate metabolic networks control the production of valuable secondary metabolites.

2.2.1 Genomics-Based Identification of Biosynthetic Pathways

Genomics offers a deep dive into the complete genetic makeup of organisms, laying the groundwork for our understanding of how phytochemicals are produced. Within plant genomes, there are countless biosynthetic gene clusters (BGCs) that play a crucial role in generating specialized metabolites like alkaloids, terpenoids, flavonoids, and polyketides. Thanks to advancements in next-generation sequencing (NGS) technologies, we can now quickly sequence and annotate the genomes of medicinal plants.

Genome mining techniques empower researchers to pinpoint genes that code for enzymes such as polyketide synthases, terpene synthases, cytochrome P450 enzymes, and glycosyltransferases, all of which are key players in the production of secondary metabolites (Kautsar *et al.,* 2020). For instance, genomic research on medicinal plants has uncovered the metabolic pathways that lead to the creation of valuable compounds like artemisinin from Artemisia annua, vinblastine from Catharanthus roseus, and paclitaxel from Taxus species.

By identifying these biosynthetic pathways, we can develop metabolic engineering strategies that enhance production and promote the sustainable use of phytochemicals. Moreover, AI-driven genome analysis tools take biosynthetic pathway prediction to the next level by uncovering hidden gene clusters and forecasting enzyme functions. Machine learning algorithms can sift through genomic patterns and link genetic data with metabolite profiles, speeding up the discovery of new natural products.

2.2.2 Transcriptomics for Understanding Gene Expression Regulation

Transcriptomics is all about diving into the world of RNA transcripts that are produced under certain physiological or environmental conditions. RNA sequencing, or RNA-seq, has emerged as a powerful tool for unraveling how plants manage the production of secondary metabolites when faced with stress, pathogens, or various environmental factors.

In the realm of phytochemical research, transcriptomic analysis plays a crucial role in pinpointing genes that are expressed differently in relation to the production of antimicrobial

compounds. For instance, when plants encounter pathogens or experience environmental stress, they can trigger defense-related pathways that ramp up the synthesis of compounds like phenolics, flavonoids, and terpenoids. When transcriptomic studies are paired with metabolomics, they offer fantastic insights into how gene expression patterns relate to the accumulation of metabolites. Co-expression network analysis is particularly useful for identifying the regulatory genes that oversee key biosynthetic pathways. With the help of AI, transcriptomic analysis can automate the interpretation of extensive RNA sequencing datasets.

Deep learning models are capable of recognizing intricate gene expression patterns and predicting the regulatory networks that play a role in phytochemical production. This not only simplifies the computational process but also enhances the accuracy of identifying potential therapeutic targets (Subramanian *et al.,* 2017).

2.2.3 Proteomics in Functional Characterization of Phytochemical Targets

Proteomics is all about the large-scale identification and quantification of proteins that are expressed in biological systems. Since proteins are the key players in cellular activities, proteomics gives us valuable insights into biochemical pathways and how molecules interact with each other.

In the realm of antimicrobial research, proteomic techniques are invaluable for pinpointing microbial proteins that are influenced by phytochemicals. Methods like two-dimensional gel electrophoresis, liquid chromatography–mass spectrometry (LC-MS/MS), and quantitative proteomics are employed to study how microbial protein expression changes after exposure to natural compounds.

Phytochemicals may influence microbial proteins involved in:

- Cell wall synthesis
- Membrane transport
- Oxidative stress response
- Energy metabolism
- DNA replication
- Protein synthesis

By profiling proteins, we can uncover potential antimicrobial targets and gather mechanistic evidence for how these compounds work therapeutically. When we combine proteomic data with AI-driven prediction models, we can better identify interactions between proteins and compounds, enhancing our ability to predict drug targets. Recent strides in structural biology and artificial intelligence, especially in protein structure prediction models, have greatly enriched our understanding of how phytochemicals interact with biological targets. AI-generated protein models open up exciting possibilities for quickly screening natural compounds against microbial targets.

2.2.4 Metabolomics for Bioactive Compound Identification

Metabolomics stands out as a key player in the realm of phytochemical discovery, primarily because metabolites are a direct reflection of the chemical activities within biological systems. Thanks to cutting-edge analytical techniques like nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS), we can achieve a thorough profiling of plant metabolites.

Metabolomic approaches allow:

- Identification of unknown phytochemicals
- Comparative analysis of medicinal plant varieties
- Authentication of herbal materials
- Correlation of metabolites with biological activities
- Discovery of antimicrobial biomarkers

The combination of untargeted metabolomics and chemometric analysis has significantly sped up the identification of bioactive molecules by uncovering metabolic signatures linked to antimicrobial properties. For instance, metabolomics-guided fractionation helps researchers focus on the most promising active fractions before diving into more detailed purification processes. Artificial intelligence plays a crucial role in enhancing the interpretation of metabolomics data by sifting through complex spectral datasets and predicting the biological roles of unknown metabolites. With the help of machine learning algorithms, we can classify metabolic patterns, pinpoint biomarkers, and explore the connections between chemical structures and their antimicrobial effects (Wishart, 2019).

2.3 Artificial Intelligence-Assisted Phytochemical Discovery

Artificial intelligence is shaking things up in the world of pharmaceutical research, making it possible to quickly analyze vast amounts of biological and chemical data. When it comes to discovering new phytochemicals, AI plays a crucial role in various tasks, such as screening compounds, predicting molecular properties, analyzing drug-target interactions, forecasting toxicity, and fine-tuning potential therapeutic candidates.

2.3.1 Machine Learning-Based Compound Prediction

Machine learning algorithms tap into existing biological datasets to forecast how unknown molecules might behave. In the realm of phytochemical research, these ML models dive into chemical descriptors, molecular fingerprints, and biological activity data to pinpoint compounds that could have promising antimicrobial properties.

Common AI algorithms used include:

- Random Forest (RF)
- Support Vector Machine (SVM)
- Artificial Neural Networks (ANN)
- Gradient Boosting Models

- Deep Neural Networks

These models significantly reduce experimental screening requirements by prioritizing promising candidates for laboratory validation.

2.3.2 Deep Learning in Natural Product Drug Discovery

Deep learning methods have improved molecular prediction by analysing complex nonlinear relationships between chemical structures and biological functions. Convolutional neural networks (CNNs), recurrent neural networks (RNNs), and transformer-based models are increasingly applied in drug discovery.

Deep learning models can:

- Predict antimicrobial activity
- Generate novel molecular structures
- Identify drug-like properties
- Predict toxicity and pharmacokinetics

A landmark study demonstrated that deep learning-based screening successfully identified novel antibiotic candidates, highlighting the potential of AI in antimicrobial discovery (Stokes *et al.,* 2020).

2.4 Green Nanobiotechnology for Antimicrobial Therapeutics

Nanotechnology has truly transformed the field of antimicrobial therapy, making drug delivery more efficient, boosting bioavailability, and introducing innovative antimicrobial mechanisms. Yet, traditional methods for creating nanoparticles often rely on harmful chemicals and raise environmental issues. Enter green nanobiotechnology, which tackles these challenges by harnessing biological resources for the synthesis of nanoparticles.

Plant extracts contain natural reducing agents such as polyphenols, flavonoids, proteins, and sugars that facilitate nanoparticle formation. Green synthesis provides advantages including:

- Eco-friendly production
- Low toxicity
- Cost effectiveness
- Biocompatibility
- Sustainable processing

2.4.1 Plant-Mediated Nanoparticle Synthesis

Plant-mediated synthesis has been widely reported for producing silver, gold, zinc oxide, copper oxide, titanium dioxide, and iron oxide nanoparticles. Phytochemicals act as reducing and stabilizing agents during nanoparticle formation.

Green nanoparticles exhibit antimicrobial activity through multiple mechanisms:

- Reactive oxygen species generation
- Cell membrane damage
- Protein denaturation

- DNA interaction
- Enzyme inhibition

Silver nanoparticles synthesized using plant extracts have demonstrated broad-spectrum antimicrobial activity against bacterial and fungal pathogens (Iravani, 2011).

2.4.2 Nano-Encapsulation of Phytochemicals

Although phytochemicals possess excellent biological activity, poor absorption and rapid degradation limit their therapeutic application. Nanoencapsulation provides an effective strategy to overcome these challenges.

Nanocarriers including:

- Liposomes
- Polymeric nanoparticles
- Nanoemulsions
- Metal nanoparticles
- Mesoporous silica nanoparticles

improve:

- Solubility
- Stability
- Controlled release
- Cellular uptake
- Targeted delivery

Nanoformulated phytochemicals demonstrate enhanced antimicrobial activity compared with free compounds due to improved interaction with microbial cells.

2.5 Integration of AI, Multi-Omics, and Green Nanobiotechnology

The convergence of AI, multi-omics, and green nanobiotechnology represents a next-generation platform for antimicrobial therapeutic development. Multi-omics generates comprehensive biological information, AI provides advanced analytical capabilities, and nanotechnology enables efficient therapeutic delivery.

An integrated workflow involves:

- Genome sequencing to identify biosynthetic pathways.
- Transcriptomic analysis to determine active metabolic regulation.
- Proteomic studies to identify molecular targets.
- Metabolomic profiling to discover bioactive compounds.
- AI algorithms to predict activity and optimize candidates.
- Green nanotechnology to develop effective delivery systems.

This integrated strategy reduces discovery time, improves accuracy, and supports sustainable development of antimicrobial therapeutics.

3. Results and Discussion

3.1 AI-Driven Multi-Omics Platforms for Accelerated Phytochemical Discovery

The fusion of artificial intelligence (AI) with multi-omics technologies has really changed the game when it comes to discovering phytochemicals. It allows for a more systematic analysis of the intricate biological data we have. Traditionally, natural product research has relied on a trial-and-error method, which involves extracting, purifying, and evaluating biological samples. While this approach has led to the discovery of several therapeutic molecules, it can be quite time-consuming and resource-intensive. However, by integrating AI with multi-omics, we can bypass many of these hurdles, gaining predictive insights into molecular pathways, compound activities, and therapeutic potentials even before we hit the lab for experimental validation. By applying AI algorithms to datasets from genomics, transcriptomics, proteomics, and metabolomics, researchers can uncover hidden connections between genetic data, metabolite production, and antimicrobial activity. Machine learning models are capable of analyzing thousands of molecular features at once, helping to predict which phytochemicals might have the pharmacological traits we're looking for. This not only streamlines the experimental screening process but also speeds up the identification of promising antimicrobial candidates. Recent strides in deep learning have shown that neural network-based models can effectively pinpoint new antimicrobial molecules from extensive chemical libraries. These AI models sift through molecular fingerprints, structural traits, and biological activity profiles to forecast which compounds might have therapeutic benefits. The success of deep learning in antibiotic discovery highlights how computational intelligence can enhance traditional natural product research, significantly cutting down the time it takes to bring new drugs to market (Stokes *et al.,* 2020).

Moreover, AI-assisted multi-omics analysis opens up exciting avenues for grasping the intricate ways phytochemicals engage with microbial systems. Rather than zeroing in on just one molecular target, these integrated approaches uncover a web of pathways that natural compounds affect, such as regulating oxidative stress, maintaining membrane integrity, influencing metabolic pathways, and driving resistance-related mechanisms.

3.2 Genomics and Transcriptomics-Guided Identification of Antimicrobial Phytochemicals

Genomic and transcriptomic studies have shed light on how antimicrobial phytochemicals are made and regulated. By using genome mining techniques, scientists can pinpoint the biosynthetic gene clusters that are responsible for creating these specialized metabolites. This has not only deepened our understanding of the rich diversity in plant metabolism but also opened doors for the sustainable production of valuable compounds through metabolic engineering.

For instance, sequencing methods have uncovered the genes that play a role in the biosynthesis of terpenoids, flavonoids, alkaloids, and phenolic compounds. With this knowledge, researchers can tweak biosynthetic pathways to boost the production of important therapeutic metabolites.

Plus, AI-driven genome analysis enhances our ability to predict these pathways by identifying the functional genes and regulatory elements tied to the synthesis of bioactive compounds.

On the other hand, transcriptomic studies add another layer of insight by showing how gene expression shifts in response to environmental stress, pathogen attacks, and different developmental stages. Plants often ramp up their defense mechanisms by increasing the production of antimicrobial secondary metabolites. By combining RNA sequencing with computational network analysis, researchers can pinpoint key regulatory genes that play a role in these defense responses.

The combination of transcriptomics and metabolomics is incredibly effective because it creates a direct link between how genes are expressed and the levels of metabolites produced. This holistic approach enables researchers to pinpoint biomarkers associated with antimicrobial activity, offering strong molecular proof of the therapeutic benefits of plant extracts (Hasin *et al.,* 2017).

3.3 Proteomics-Based Understanding of Phytochemical–Microbial Interactions

Proteomic analysis has really deepened our understanding of the molecular mechanisms behind the antimicrobial activity of phytochemicals. Unlike genomic and transcriptomic studies that merely hint at possible biological processes, proteomics takes a more direct approach by examining the functional proteins that drive cellular responses.

When microorganisms are treated with plant-derived compounds, we often see significant changes in the proteins that play crucial roles in essential biological pathways. Proteomic studies have revealed that phytochemicals can influence: Cell membrane-associated proteins

- Energy metabolism enzymes
- Stress response proteins
- DNA repair proteins
- Protein synthesis machinery

These molecular alterations explain the broad-spectrum antimicrobial effects of natural compounds.

The use of AI in predicting protein structures and modeling molecular interactions has further clarified how phytochemicals work. These advanced computational models can forecast how natural compounds bind to microbial proteins, helping researchers pinpoint potential therapeutic targets. This method not only cuts down on the need for extensive experimental screening but also aids in the smart design of antimicrobial formulations.

By combining proteomics with metabolomics, we gain a well-rounded view of how specific metabolites impact microbial protein networks. This integrated approach is especially valuable for discovering synergistic combinations of phytochemicals that could effectively tackle antimicrobial resistance mechanisms.

3.4 Metabolomics and AI-Assisted Biomarker Discovery

Metabolomics has really taken center stage in the world of modern phytochemical research, and it's easy to see why metabolites are the final products that drive biological activity. With cutting-edge analytical tools like LC-MS, GC-MS, and NMR spectroscopy, researchers can generate vast amounts of metabolic data that need some serious computational savvy to make sense of. Thanks to AI-assisted metabolomics analysis, we can quickly spot chemical patterns that are linked to antimicrobial activity.

Machine learning models are great at classifying metabolite profiles, pinpointing important biomarkers, and even predicting what unknown compounds might do biologically. Take metabolomics-guided screening, for instance. It can help us tell apart highly effective plant extracts from those that don't quite cut it by identifying unique chemical signatures.

This method not only enhances quality control but also helps standardize herbal materials. Moreover, AI-driven biomarker discovery is a game changer for precision phytotherapy. It allows us to identify specific compounds that are tied to therapeutic responses. Instead of relying on complex plant extracts without a clear molecular understanding, researchers can create standardized formulations that feature optimized blends of bioactive molecules.

The fusion of metabolomics and artificial intelligence holds incredible promise for developing predictive models that connect chemical composition with antimicrobial effectiveness, toxicity, and pharmacological results (Wishart, 2019).

3.5 Green Nanobiotechnology for Enhanced Antimicrobial Activity of Phytochemicals

While phytochemicals are known for their impressive antimicrobial properties, their use in clinical settings often faces challenges like poor solubility, instability, quick degradation, and limited absorption. Enter green nanobiotechnology, which offers a promising way to enhance the physicochemical properties and therapeutic effectiveness of these natural compounds.

The synthesis of nanoparticles using plants has become a go-to method for creating antimicrobial nanomaterials in an eco-friendly manner. The bioactive molecules found in plant extracts act as both reducing and stabilizing agents, which means there's no need for harmful chemicals. Plus, these green-synthesized nanoparticles tend to have better biological activity thanks to their tiny size, larger surface area, and improved ability to interact with microbial cells.Metal and metal oxide nanoparticles such as silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), copper oxide nanoparticles (CuO NPs), and iron oxide nanoparticles (Fe_2O_3 NPs) exhibit strong antimicrobial effects through multiple mechanisms:

- Generation of reactive oxygen species (ROS)
- Damage to microbial cell membranes
- Leakage of intracellular components
- Protein dysfunction
- DNA damage

The multi-target mechanism of nanoparticles reduces the likelihood of microbial resistance development compared with conventional antibiotics.

Recent research has demonstrated that combining phytochemicals with nanoparticles produces synergistic antimicrobial effects. Phytochemicals act as natural antimicrobial agents while nanoparticles enhance cellular penetration and stability. Such nano-enabled phytochemical systems represent promising alternatives for combating resistant pathogens.

3.6 AI-Guided Design of Green Nanotherapeutics

The combination of AI with green nanotechnology represents a new frontier in antimicrobial therapeutic development. AI algorithms can predict nanoparticle characteristics, optimize synthesis parameters, and evaluate biological interactions.

Important nanoparticle parameters influenced by AI optimization include:

- Particle size
- Surface charge
- Morphology
- Stability
- Drug-loading efficiency
- Release kinetics

Machine learning models can analyse relationships between synthesis conditions and nanoparticle performance, reducing experimental requirements and improving reproducibility.

AI-based toxicity prediction models also assist in evaluating nanoparticle safety before biological application. This is particularly important because nanoparticle characteristics strongly influence cellular interactions and potential adverse effects.

The future development of antimicrobial nanotherapeutics will likely involve AI-designed nanoparticles combined with multi-omics-guided phytochemicals, creating personalized and highly efficient treatment platforms.

3.7 Challenges and Future Perspectives

Despite significant progress, several challenges must be addressed before AI-integrated phytochemical nanotherapeutics achieve widespread clinical application.

Standardization of Plant-Based Materials

Variability in plant species, geographical conditions, cultivation methods, and extraction procedures affects phytochemical composition. Development of standardized cultivation and analytical protocols is essential.

Data Integration Challenges

Multi-omics datasets are highly complex and generated from different analytical platforms. Development of standardized databases and advanced AI models is required for effective data integration.

Experimental Validation

Although AI models can predict promising compounds, experimental validation remains essential. Combining computational predictions with biological assays will improve reliability.

Nanotoxicity and Safety Assessment

Long-term toxicity, environmental impact, and biological accumulation of nanoparticles require detailed investigation before clinical translation.

Regulatory Considerations

Clear regulatory frameworks are required for approval of AI-designed phytochemical nanomedicines. Collaboration between computational scientists, pharmacologists, toxicologists, and regulatory agencies is essential.

Future research should focus on developing autonomous AI platforms capable of integrating multi-omics information, predicting therapeutic outcomes, and designing sustainable nanoformulations. The combination of precision biology, computational intelligence, and green nanotechnology may establish a new generation of antimicrobial therapies.

Conclusion

The merging of artificial intelligence, multi-omics technologies, and green nanobiotechnology is paving the way for a groundbreaking approach to discovering new phytochemicals and developing antimicrobial therapies. With AI, we can quickly analyze intricate biological and chemical data, while multi-omics technologies give us a detailed look into biosynthetic pathways, molecular targets, and how antimicrobial mechanisms work. Green nanotechnology takes it a step further by boosting the therapeutic potential of phytochemicals, enhancing their stability, bioavailability, and targeted delivery. By combining genomics, transcriptomics, proteomics, metabolomics, and AI-driven computational methods, we create a robust platform for uncovering new antimicrobial compounds and grasping their biological roles. Plus, green-synthesized nanoparticles and nano-enabled delivery systems present eco-friendly solutions to the challenges posed by traditional antimicrobial agents. Looking ahead, the future of antimicrobial discovery will increasingly rely on interdisciplinary strategies that blend computational intelligence, systems biology, and sustainable nanotechnology. AI-enhanced multi-omics platforms hold the promise of speeding up the creation of safer, more effective, and environmentally friendly antimicrobial therapies that can tackle the rising issue of antimicrobial resistance.

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ENDOPHYTIC FUNGI: MOLECULAR BASIS OF SECONDARY METABOLITE BIOSYNTHESIS AND BIOTECHNOLOGICAL APPLICATIONS

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Abstract

Endophytic fungi live inside plant tissue without causing disease, and some produce the same complex secondary metabolites as their host plants a link first drawn into focus by the 1993 discovery that Taxomyces andreanae makes paclitaxel independent of its yew host. This chapter examines the molecular basis of that chemistry biosynthetic gene clusters built around polyketide synthases, nonribosomal peptide synthetases, terpene synthases, and their hybrids, most of which stay transcriptionally silent under standard culture conditions due to chromatin state, regulatory cross-talk, and missing environmental cues. It surveys current strategies for activating these clusters epigenetic modifiers, heterologous expression, and CRISPR-based transcriptional activation using the taxol case to illustrate both progress and limits. It closes by reviewing biotechnological payoffs in pharmaceuticals, agriculture, and industrial enzymes, alongside recurring obstacles: culture attenuation, low yields, and genetic intractability.

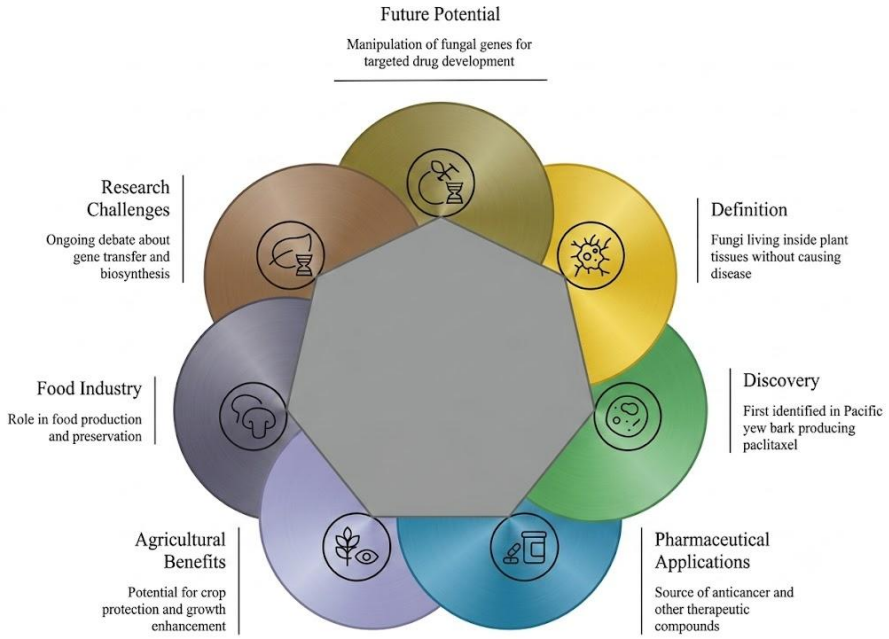
Keywords: Endophytic Fungi, Secondary Metabolites, Biosynthetic Gene Clusters, Polyketide Synthase, CRISPR Activation, Paclitaxel Biosynthesis.

1. Introduction

Every plant examined closely enough turns out to be carrying passengers. Endophytic fungi live inside root, stem, and leaf tissue for some or all of their life cycle without producing visible disease symptoms, and they do it in essentially every plant species that has been surveyed for them. What made this group interesting to chemists rather than just to mycologists was a 1993 paper reporting that a fungus isolated from Pacific yew bark, *Taxomyces andreanae*, produced small amounts of paclitaxel - the same anticancer diterpenoid the tree itself makes (Stierle *et al.,* 1993), discovered more than two decades before a wave of follow-up studies on other endophytes with similar biosynthetic potential. Since then, researchers have pulled taxol, camptothecin, podophyllotoxin, huperzine, and resveratrol out of fungi living in the tissues of the plants known for producing those exact compounds, work that has pushed endophytic fungi into pharmaceutical, agricultural, and food-industry research over the past three decades (Gupta *et al.,* 2023).

The pattern raises an obvious question: why would a fungus growing inside a plant carry genes for the same chemistry the plant already uses, the honest answer is that nobody has a complete explanation, and the field has spent three decades arguing about horizontal gene transfer,

convergent biosynthesis, and induction of the fungus's own dormant pathways by plant signals. What is clear is that the phenomenon is real, reproducible in enough species to matter, and tied to specific stretches of fungal DNA that can be studied, manipulated, and - with the right tools - switched on deliberately. That's the subject of this chapter: what those DNA stretches look like, how the enzymes they encode build complex molecules, and what people have managed to do with that knowledge so far.



1.1 Biosynthetic Gene Clusters: The Basic Unit of Fungal Chemistry

Fungal secondary metabolites almost never come from a single gene. They come from biosynthetic gene clusters (BGCs) runs of physically adjacent genes that together encode a full assembly line: a core synthase or synthase-like enzyme that builds the molecular scaffold, a set of tailoring enzymes that modify it, transporters that move the finished product out of the cell, and often a pathway-specific transcription factor that switches the whole cluster on or off. Once the scaffold is built, tailoring enzymes typically finish the job through methylation, epimerization, oxidative hydroxylation, and similar modifications, converting a rough core structure into the specific compound that eventually gets named and characterized (Zakariyah *et al.,* 2024). A typical filamentous fungal genome carries somewhere between thirty and eighty of these clusters, and that number keeps climbing as sequencing improves (Roux *et al.,* 2020). Four core enzyme families do almost all of the scaffold-building work, sometimes alone and sometimes stitched together into hybrids: polyketide synthases (PKS), nonribosomal peptide synthetases (NRPS), terpene synthases (TPS), and dimethylallyltryptophan synthases (DMATS).

1.1.1 Polyketide Synthases

Fungal PKS enzymes are iterative a single large multidomain protein loops back on itself, using the same set of catalytic domains over and over to add one acetate (or propionate, or other short acyl) unit at a time to a growing chain, the same basic chemistry cells use for fatty acid synthesis

but repurposed and diversified. The output ranges from simple aromatic polyketides to elaborate reduced polyketides depending on which optional domains - ketoreductase, dehydratase, enoyl reductase, methyltransferase - get used at each cycle and in what order. That combinatorial flexibility is a large part of why polyketides are such a chemically diverse class despite all coming from the same basic biochemical logic.

1.1.2 Nonribosomal Peptide Synthetases

NRPS enzymes build peptides without touching a ribosome. They're organized into modules, and each module handles one amino acid: it selects the residue, activates it, tethers it to a phosphopantetheine arm, and passes the growing chain to the next module for condensation, with the whole assembly acting as a large multifunctional megaenzyme carrying out this sequence one residue at a time. Because NRPS enzymes aren't limited to the twenty proteinogenic amino acids, the peptides they make routinely include unusual building blocks, D-amino acids, and cyclized or branched backbones that a ribosome could never produce.

1.1.3 Hybrid PKS–NRPS Pathways and Terpenoid Routes

Many of the more interesting endophyte metabolites come from clusters where a PKS and an NRPS are fused into one megasynthetase, or where a terpene synthase pathway feeds into an NRPS to attach a nonproteinogenic amino acid onto a terpenoid core, generating hybrid alkaloid scaffolds that neither enzyme family could build on its own. Fungal terpenoid biosynthesis runs largely on its own separate machinery, and this class matters enormously for the endophyte literature because taxol itself is a diterpenoid - its production requires both a terpenoid pathway to lay down the taxane skeleton and a separate phenylpropanoid route supplying the side chain that gets attached later, with the phenylpropanoid-derived benzoylphenylisoserine group specifically attached to carbon 13 of the baccatin III core (Subban & Kempken, 2023).

1.2 Why most of This Machinery Stays Switched Off

Sequence a fungal genome and you'll typically find dozens of BGCs. Grow that same fungus on a standard laboratory plate and most of those clusters will sit completely silent - the genes are there, but nothing gets transcribed, and no product ever accumulates, largely because BGCs are under tight regulatory control and most remain transcriptionally quiet under ordinary growth conditions (Verma *et al.,* 2023). This is the central practical problem in the field. A cluster's presence in a genome tells you almost nothing about whether you'll ever see its product in a flask.

Chromatin state matters enormously. Histone deacetylases and histone acetyltransferases add or remove acetyl marks on the histones packaging BGC DNA, and that acetylation state determines whether RNA polymerase can even reach the genes, with these enzymes responsible for establishing or silencing biosynthetic gene clusters and thereby controlling production of a wide range of secondary metabolites (Zakariyah *et al.,* 2024). Treating a culture with a histone deacetylase inhibitor - compounds like suberoylanilide hydroxamic acid or sodium butyrate are the most commonly used can open up chromatin at previously silent loci and trigger production of metabolites the fungus was never observed to make before (Verma *et al.,* 2023). It's worth

being precise about what this does and doesn't achieve: deleting or chemically inhibiting HDACs does not reliably switch every silent cluster on. The effect is closer to a broad reshuffling of the whole secondary metabolome, some clusters activate, others that were already active shut down, and the net result has to be checked experimentally rather than assumed.

Pathway-specific and global transcription factors sit on top of the chromatin layer. Most BGCs carry their own dedicated regulatory gene inside the cluster, but global regulators coordinate secondary metabolism across many clusters at once by influencing chromatin more broadly. Cross-talk between clusters can also flip a switch: in one striking case, forcing expression of a regulatory gene sitting next to a silent NRPS cluster in *Aspergillus nidulans* didn't just activate that NRPS cluster it also triggered transcription of an entirely separate, physically distant polyketide cluster, leading to production of the polyketide asperfuranone as an unexpected side effect (Bergmann *et al.,* 2010). Cross-cluster regulatory wiring like this means a fungus's true chemical output can't always be predicted just by looking at which genes sit next to which promoter.

Environmental and biotic cues are the third lever, and they're the one most directly relevant to the plant–endophyte relationship. Stress signals, co-culture with competing microbes, and specific host-derived cues can switch on clusters that stay dark in pure, unstressed culture. This is thought to be a major reason endophytic fungi often lose their ability to make a target compound after repeated subculturing away from the host plant (Zakariyah *et al.,* 2024) the environmental trigger that was switching the cluster on simply isn't present anymore.

1.3 Finding and Switching on Cryptic Clusters

Bioinformatic tools such as antiSMASH scan a genome and flag likely BGCs by matching known synthase domains and cluster architectures, and databases like MIBiG hold curated, experimentally validated reference clusters to compare against (Mozsik *et al.,* 2021). That combination has made BGC prediction and prioritization dramatically faster than it was even a decade ago, revealing that filamentous fungi carry a larger biosynthetic potential than researchers had previously assumed. Prediction alone doesn't get you a compound, though we still have to make the cluster express. Beyond epigenetic modifiers, three approaches dominate current practice. Promoter exchange replaces a cluster's native, tightly repressed promoter with a strong constitutive or inducible one, forcing transcription regardless of the fungus's normal regulatory logic. Heterologous expression moves an entire cluster, or a reconstructed version of it, into a well-characterized host — *Aspergillus nidulans* is the workhorse here, and it has already been used successfully by multiple groups as an expression chassis for compounds that gave no detectable product in their native, wild-type context (Roux *et al.,* 2020).

CRISPR-based transcriptional activation is the newest tool in this space. Rather than cutting DNA, a catalytically dead Cas protein fused to an activator domain (VPR is the common choice) is guided to a cluster's promoter region, where it recruits transcriptional machinery without altering the underlying sequence. Roux *et al.* (2020) built a CRISPR/dLbCas12a-VPR system in *Aspergillus nidulans* and used it to activate the previously silent NRPS-like gene *micA*, boosting

production of microperfuranone and leading to the discovery of a related cluster product, dehydromicroperfuranone that had never been observed before. A parallel dCas9-VPR system built for *Penicillium rubens* was targeted to the promoter of the transcription factor *macR*, switching on a silent macrophorin cluster and yielding detectable antimicrobial macrophorins as the product (Mozsik *et al.,* 2021). Because it doesn't require stable genome integration in every case, this method is considerably faster to deploy across many candidate clusters than classical gene replacement.

1.4 The Taxol Problem

Taxol is worth walking through in detail because it illustrates both the promise and the persistent limits of this field. *Taxomyces andreanae*, isolated from Pacific yew bark, was the first fungus shown to make paclitaxel and related taxanes on its own, in culture, independent of the plant (Stierle *et al.,* 1993). In the years since, roughly two hundred separate reports have described endophytic fungi associated with both *Taxus* and non-*Taxus* host plantsproducing taxol, though yields have consistently been far too low for commercial extraction to be practical (Subban & Kempken, 2023). The bottleneck isn't a lack of candidate organisms; it's that nobody has fully mapped the fungal biosynthetic pathway well enough to engineer around the low native yield.

Several strategies have been tried to push production higher. Feeding fungal cultures likely biosynthetic precursors acetate and phenylalanine among them has boosted yield, and pairing that with inhibitors of competing sterol biosynthesis pathways pushes more flux toward the taxane branch, since blocking sterol synthesis appears to redirect the shared isoprenoid precursor pool toward taxane production instead. Elicitation is a related tactic borrowed from plant cell culture work: methyl jasmonate, salicylic acid, and abscisic acid have all been used to trigger higher paclitaxel accumulation, with methyl jasmonate a well-known plant defense signal proving especially effective at inducing overproduction in *Taxus* suspension cultures (Kusari *et al.,* 2014). Co-culturing endophytic fungi directly with *Taxus* cells in the same bioreactor has produced some of the highest reported yields, consistent with the idea that some kind of ongoing biochemical cross-talk between host and fungus, not just the fungal genes alone, drives full-scale taxane production (Kusari *et al.,* 2014). Separate work mapping the correlation network between the taxoid and phenylpropanoid pathways in the endophyte *Cryptosporiopsis tarraconensis* has pointed to phenylalanine aminomutase as a specific rate-limiting bottleneck in that side-chain-supplying route (Ballakuti & Ghanati, 2023). Despite two decades of this kind of work, industrial-scale fungal taxol production has never materialized, and the field has had to reckon honestly with whether it's actually achievable given what's now understood about the chemical ecology of the producing fungi (Kusari *et al.,* 2014). Taxol is a useful cautionary case: a fungus having the right genes is necessary but nowhere near sufficient for a viable production platform.

1.5 Biotechnological Applications

1.5.1 Pharmaceuticals

Beyond taxol, endophytic fungi have yielded camptothecin and podophyllotoxin both used as anticancer drug scaffolds huperzine, an acetylcholinesterase inhibitor investigated for

Alzheimer's disease, and resveratrol, along with a long tail of antibacterial, antifungal, antitumor, and immunomodulatory compounds, many of them chemically identical or closely related to compounds first characterized in the host plants themselves (Gupta *et al.,* 2023). Antimicrobial resistance has given this work new urgency: PKS and NRPS pathways in endophytic fungi are now being screened specifically for novel antibacterial polyketides and peptides as the pipeline of new antibiotics from traditional sources has thinned, with endophytes proposed as an underused reservoir for exactly the kind of chemical novelty that resistant pathogens haven't yet encountered (Nazir *et al.,* 2024).

1.5.2 Agriculture

The same BGCs that make antimicrobial compounds for pharmaceutical screening double as candidates for biocontrol agents and biofertilizer components, since many endophyte metabolites function ecologically as chemical defenses against plant pathogens or competing microbes in the first place (Nazir *et al.,* 2024). CRISPR-Cas9 disruption of a MAP kinase kinase gene in the endophyte *Phomopsis liquidambaris* triggered accumulation of the flavonoids naringenin, kaempferol, and quercetin (Yang *et al.,* 2021). That kind of stress-linked metabolite production is directly relevant to using endophytes as seed treatments or soil amendments that help crop plants tolerate drought, salinity, or pathogen pressure.

1.5.3 Enzymes and Industrial Biotechnology

Endophytic fungi are also a source of extracellular enzymes cellulases, ligninases, proteases, and others — with applications in biofuel production, textile processing, and waste treatment, alongside their better-known role as secondary metabolite factories, and reviews of the field have specifically highlighted both the metabolites and the enzymes extracted from fungal endophytes as contributing to solutions for human, animal, and environmental problems (Gupta *et al.,* 2023).

1.6 Persistent Obstacles

Three problems recur across nearly every attempt to commercialize endophyte chemistry. Attenuation in culture is the most cited. Fungi that produce a target compound reliably when freshly isolated from the host plant frequently lose that ability after repeated subculturing on artificial media, a pattern documented across enough species that it's treated as close to a general rule rather than an occasional nuisance (Zakariyah *et al.,* 2024). The leading explanation ties back to the regulatory picture in Section 1.3 whatever host-derived or stress-related signal was keeping the relevant BGC active simply isn't present in a nutrient-rich lab flask, and the cluster quietly reverts to its default silent state. Low and inconsistent titers are the second problem, visible throughout the taxol literature and in most other endophyte-derived compounds — production in native fungal culture is almost always far below what would be needed for economical extraction, even after elicitation and precursor feeding (Subban & Kempken, 2023).

Genetic and physiological intractability is the third. Many endophytic fungal species are difficult to transform, lack established genetic tools, or grow too slowly for practical fermentation, which is exactly why heterologous expression in tractable hosts like *Aspergillus nidulans* has become

such a central strategy rather than a niche technique it sidesteps the native fungus's limitations entirely by rebuilding the pathway somewhere more cooperative (Roux *et al.,* 2020).

1.7 Where the field is headed

A few directions look likely to define the next stage of this work. Multi-omics profiling genomics, transcriptomics, and metabolomics run together on the same samples is becoming the standard way to link a candidate BGC to an actual detected compound, rather than relying on bioinformatic prediction alone (Verma *et al.,* 2023). CRISPR activation and other synthetic-biology tools are being extended beyond single-gene targets toward multiplexed activation of several clusters at once, which should speed up the rate at which cryptic BGCs get matched to real chemistry (Roux *et al.,* 2020; Mozsik *et al.,* 2021). And a growing number of groups are treating the host–endophyte relationship itself as the object of study, on the reasoning that the signals passing between plant and fungus may be the real key to unlocking production levels that isolated fungal culture alone has never been able to reach.

None of this makes endophytic fungi an easy or guaranteed source of new drugs and industrial compounds. But three decades after the taxol discovery, the toolkit for actually using what these fungi carry genome mining software, heterologous hosts, CRISPR activation, epigenetic modifiers have matured well past where it stood when the field started, and that alone makes the next round of cryptic-cluster discoveries considerably more likely to end in an isolated compound instead of just an interesting sequence.

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MOLECULAR APPROACHES IN FISH TAXONOMY: ADVANCES, APPLICATIONS AND FUTURE PERSPECTIVES

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Abstract

The fish taxonomy classification has witnessed dramatic changes since the advent of molecular biology in traditional taxonomy. Traditional fish taxonomy that uses morphological, meristic and morphometric properties has been the basis of fish identification over centuries. However, similar morphology between related fish species, phenotypic plasticity, ontogeny difference, sexual dimorphism, hybridization and presence of cryptic species make morphological identification unreliable. The recent development of genetic information has offered effective means to solve the problems and achieve more accurate fish species identification, reconstruct more realistic phylogeny and assess biodiversity. This chapter provides an overview of the current molecular techniques applied in fish taxonomy and examines their application, strength, limitation and future perspectives. This chapter focuses on the main molecular markers currently used in fish taxonomic research. These markers include mitochondrial DNA (mtDNA) markers like cytochrome c oxidase subunit I (COI), cytochrome b (Cyt b), 12S rRNA, 16S rRNA, as well as nuclear DNA (nDNA) markers including RAG1, RAG2, ITS, and S7 ribosomal protein genes. It is also important to pay attention to microsatellite and SNP markers and discuss their role in population genetics, stock identification, and conservation studies. In addition, particular attention is paid to DNA barcoding, an internationally recognized and widely used technique allowing for fast and efficient identification of fish species based on analysis of standardized COI genes and subsequent comparison with GenBank and BOLD reference databases. Moreover, the use of whole-genome sequencing, comparative genomics and phylogenomics becomes increasingly important for understanding complicated evolutionary patterns and finding cryptic diversity that cannot be revealed via morphological analysis.

Keywords: Fish Taxonomy, Molecular Taxonomy, DNA Markers, Mitochondrial DNA, Nuclear DNA, DNA Barcoding, Cytochrome C Oxidase Subunit I (COI), Species Identification, Fisheries Conservation.

Introduction

Taxonomy of fishes is a scientific discipline that deals with the discovery, identification, nomenclature and classification of fish species. Taxonomy allows us to know the species diversity, evolutionary and biogeographical relations between them. Proper species identification is important for documentation of biodiversity, recognition of invasive species, determination of

conservation status and development of appropriate management approaches (Eschmeyer & Fong, 2025). Species identification errors result in wrong data on biodiversity, ineffective measures for conservation, wrong statistical analysis of fisheries data and flawed ecological conclusions (Hubert & Hanner, 2015). For more than two centuries, fish taxonomy has been dominated by the morphological approach that uses body form, morphometric, meristic, color and osteological characteristics of fishes. Though the classical taxonomy cannot be bypassed in many respects, there is a list of problems with this method of species identification. First, phenotypic plasticity, ontogeny, sexual dimorphism, hybridization, geographical variation and cryptic species may affect correct species delimitation. Second, eggs, larvae, juveniles, processed fish samples and degraded specimens may not provide enough diagnostic morphological traits, which makes their species identification difficult (Hebert *et al.,* 2003).

Molecular biology has revolutionized the field of fish taxonomy through the introduction of DNA methods that make possible the development of highly efficient, objective and accurate approaches to species identification and evolution analysis. The molecular markers allow taxonomists to differentiate between morphologically similar species, identify cryptic species diversity, reconstruct phylogeny and determine species irrespective of their developmental state. Among these techniques, COI-based DNA barcoding became the worldwide standard for routine animal species identification because of its high level of discriminatory power and standardization of analysis (Ward *et al.,* 2005). Moreover, both mitochondrial and nuclear DNA markers greatly improved phylogenetics and population genetics (Avise, 2004).

The combination of molecular and morphological data has resulted in the formation of integrative taxonomy, where various types of information, including morphological, molecular, ecological, geographical and behavioral, can be combined to identify species and classify them. Finally, modern advances in the fields of artificial intelligence (AI), machine learning, and bioinformatics are bringing about revolutionary changes to the field of taxonomic research, including species recognition, genome data mining, image-based species classification and predictive biodiversity assessment. It is expected that these interdisciplinary methods will greatly advance the process of fish species identification in the upcoming decades (Höglund *et al.,* 2024).

In this chapter, we will analyze the existing molecular methods used to conduct research in fish taxonomy. The main focus will be placed on such molecular techniques as DNA barcoding, genotyping, sequencing technologies, whole genome sequencing, and phylogenomics. Moreover, we will review new technologies, problems and opportunities in the field of fish taxonomy.

Development of Molecular Taxonomy

DNA-based species identification became famous worldwide after the concept of DNA barcoding came into existence where the standardized fragment of mitochondrial cytochrome c oxidase subunit I (COI) gene is used for species identification in animals (Hebert *et al.,* 2003).

In comparison with conventional taxonomy, molecular methods offer more objectivity, repeatability and taxonomic resolution. They are much less influenced by phenotype plasticity, environment, development, etc., which makes molecular methods more useful when it comes to solving taxonomically complex issues (Avise, 2004).

1. DNA Markers Used in Fish Taxonomy

Molecular markers represent certain DNA sequences that differ in terms of genetic variation between individuals or taxa and are commonly used in the research of genetic diversity, species identification, phylogeny and population structure. Ideally, a molecular marker should be polymorphic, repeatable, inheritable, selectively neutral and amenable for amplification via molecular methods (Freeland *et al.,* 2011).

1.1 Mitochondrial DNA Markers

Mitochondrial DNA (mtDNA) markers are most commonly used molecular markers in fish taxonomy owing to their maternal inheritance, fast mutations, lack of recombination and multiple copies within cells. Genes such as cytochrome c oxidase subunit I (COI), cytochrome b (Cyt b), 16S rRNA, and 12S rRNA genes have shown to be highly efficient in species identification, phylogenetics and biodiversity evaluations. Among these genes, COI is the universal barcode used in identifying animal species due to its discriminative properties and reference databases (Ward *et al.,* 2005).

1.2 Nuclear DNA Markers

Nuclear DNA markers can supplement mtDNA markers by providing genetically informative data from both parents. The genes such as RAG1, RAG2, ITS, and S7 ribosomal protein introns are often used in fish systematics to establish deep phylogenies, detect hybridization events, and discriminate between closely related species. As opposed to mtDNA, the nuclear DNA can be subject to recombination and evolution in various rates, therefore, offering valuable information unattainable from mtDNA (Near *et al.,* 2012).

1.3 Microsatellites

Microsatellites or simple sequence repeats (SSRs) are defined as repeated sequences of nucleotides dispersed through the whole genome. Because of their polymorphism and co-dominance, microsatellite loci have become popular genetic markers in fields of population genetics, paternity testing, stock identification, conservation genetics and genetic diversity assessment. Despite being largely substituted with whole-genome markers recently, microsatellites can be used successfully in numerous fisheries and conservation projects (Selkoe & Toonen, 2006).

1.4 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms or SNPs refer to single nucleotide variations and are one of the most numerous genetic markers. With the help of high-throughput sequencing technologies, a number of SNPs can be identified in genomes of different fish species. SNPs allow gathering

highly informative genetic data in species delimitation, phylogeography, population structuring, adaptation and whole-genome association studies (Helyar *et al.,* 2011).

1.5Applications in Species Identification and Phylogeny

There has been a remarkable enhancement in fish taxonomy due to the use of DNA markers, which allow identifying accurately different species, discovering cryptic species, verifying commercially valuable fishes and inferring evolutionary relationships. DNA markers are extensively employed in biodiversity surveys, conservation biology, fisheries science, food certification, invasive species surveillance and molecular phylogenetics. The use of mitochondrial and nuclear DNA markers together with whole-genome information has made an excellent contribution to the reliability and accuracy of taxonomic and evolutionary analyses and has become the foundation of modern molecular taxonomy (Allio *et al.,* 2020).

2. Mitochondrial Genes Frequently Used for Fish Taxonomy

2.1 Cytochrome c Oxidase Subunit I (COI)

The COI gene is the universally recognized standard gene used for DNA barcoding in animal species. This gene provides enough inter-specific polymorphism to reliably identify the species yet is very conserved within each species. COI genes are widely available in different international databases like BOLD and GenBank and are crucial for fish taxonomy, biodiversity studies, food authenticity, and conservation studies (Hebert *et al.,* 2003; Ward *et al.,* 2005).

2.2 Cytochrome b (Cyt b)

Cytochrome b (Cyt b) is a mitochondrial protein coding gene used in the study of fish phylogenetics and phylogeography. Thanks to a relatively high evolutionary rate, Cyt b helps in resolving relationships between closely related species and populations. The gene is extensively used in studies of fish evolution, taxonomy, and biogeography (Kocher *et al.,* 1989).

2.3 Advantages of Mitochondrial DNA Markers

- There are several advantages of using mitochondrial DNA markers in fish taxonomy. These include a high mutation rate, which helps differentiate closely related species. Moreover, these markers undergo only maternal inheritance and no recombination, hence easing the analysis process.
- Additionally, having many mitochondrial genomes per cell enhances the DNA amplification process for small and processed tissues. In addition, there are many reference sequence databases available for mtDNA that help in identifying species and studying biodiversity (Boore, 1999).

2.4 Limitations of Mitochondrial DNA Markers

Although these markers have some useful traits, there are still some shortcomings associated with them. Being an indicator of only maternal ancestry, the information provided by mtDNA excludes the paternal genes and full evolutionary history of the species. Due to the hybridization, mitochondrial introgression, incomplete lineage sorting and the presence of NUMTs, mtDNA

can sometimes provide misleading results on phylogenetic analysis or species identification. Hence, mtDNA should always be used along with other markers like nuclear DNA and morphological characters (Toews & Brelsford, 2012).

2.5 Applications in Fish Taxonomy

Mitochondrial DNA markers have played an important role in fish taxonomy since they provide a great tool for proper species identification, revealing cryptic species, creating phylogeny and assessing genetic diversity. These markers are widely used in biodiversity assessments, conservation genetics, fishery management, seafood authentication, invasive species monitoring, and eDNA sampling. Combined with other DNA markers and morphology, mtDNA makes the basis for current fish taxonomy.

2.6 Advantages of Nuclear DNA Markers Over Mitochondrial DNA

There are several advantages of using nuclear DNA markers compared to mtDNA. They have biparental inheritance and can undergo recombination; thus, they represent full history of evolution of organisms. Nuclear markers are more efficient to detect hybridization, introgression, incomplete lineage sorting and gene flow that cannot be estimated by mtDNA alone. In addition, multiple independent nuclear loci increase the precision of phylogenetic and population geneticanalyses (Edwards *et al.,* 2016).

2.7 Combining Mitochondrial and Nuclear DNA Analyses

The combination of analyses of mitochondrial and nuclear DNA sequences is now a well-established approach in fish taxonomy studies. In combining analyses, the high level of mutability of mitochondria is used for species identification along with the bi-parental inheritance of nuclear DNA for gaining a full insight into evolutionary relationships. This multi-locus method enhances delimitation of species, solves taxonomic controversies, detects hybridization and enhances phylogenetic analysis. Thus, the combination of mtDNA and nuclear DNA serves as a foundation for modern integrative molecular taxonomy (Carstens *et al.,* 2013).

Table 1: Comparison of major molecular markers used in fish taxonomy

Marker	Genome	Major Application
COI	mtDNA	DNA barcoding
Cyt b	mtDNA	Phylogeny
16S rRNA	mtDNA	Phylogeny
12S rRNA	mtDNA	eDNA and metabarcoding
D- loop	mtDNA	Population genetics
ITS	Nuclear	Species delamination
RAG1/RAG2	Nuclear	Deep phylogeny
Rhodopsin	Nuclear	Teleost phylogeny
S7 Gene	Nuclear	Systematics

3. DNA Barcoding in Fish Taxonomy

One of the most powerful and reliable molecular methods for fish species identification is DNA barcoding. It utilizes the idea that short standardized DNA sequences can be used for distinguishing one species from another. Ever since the introduction of DNA barcoding in 2003 by Hebert *et al.,* this molecular tool has been significantly changing the face of taxonomy by making species identification quick, reliable and repeatable. In case of fish species, DNA barcoding has become a critical tool for biodiversity assessment, fisheries, conservation biology, seafood authenticity, and invasive species detection (Hebert *et al.,* 2003).

3.1 Principles of DNA Barcoding

The principle of DNA barcoding is founded upon the fact that species have a low genetic variability and high genetic distance between each other, and this phenomenon is known as the "barcode gap." Short DNA sequence is amplified with universal primers, sequenced and compared with authenticated databases of reference species for identification purposes (Ratnasingham & Hebert, 2007).

3.2 Laboratory Procedure

Barcoding procedure entails a standardized laboratory method that follows a series of procedures as outlined below:

- Specimen collection and storage.
- DNA extraction.
- PCR amplification of the COI gene using universal primers.
- amplification product confirmation using agarose gel electrophoresis.
- sequencing of the DNA.
- DNA editing and sequencing quality control.
- comparison with reference database followed by phylogenetic analysis.

3.3 GenBank

GenBank is a major publicly available nucleotide sequence database managed by NCBI and one of the largest databases in the world. This database is used to store sequences submitted by researchers from all over the world and provides similarity searches using Basic Local Alignment Search Tool (BLAST). GenBank is not specifically curated for DNA barcoding purposes; nevertheless, it is still used in studies where sequence comparisons are needed (Benson *et al.,* 2018).

3.4 Whole-Genome Sequencing (WGS)

Whole-genome sequencing (WGS) is a highly sophisticated technique that involves decoding the whole DNA of the genome of an organism at once. In contrast to DNA barcoding, where only one gene is being targeted, WGS provides a complete picture of the coding and non-coding parts of the genome of an organism. With the rapid development of high-throughput sequencing methods, WGS became one of the most important tools for studying fish taxonomy, evolution,

population genetics, and conservation due to the high-resolution genomic data provided by WGS (Ekblom & Galindo, 2011).

3.5 Principles of Whole-Genome Sequencing

Whole-genome sequencing entails the extraction of the quality DNA of the genome, construction of the library of sequences, high-throughput sequencing of the DNA, assembly of the sequences to complete genomes and further annotation and analysis of the genomes.

Genome Assembly

Genome assembly is the computational procedure of assembling whole genome sequences from millions of short or long DNA fragments obtained by sequencing. Long read sequencing platforms like PacBio and Oxford Nanopore, combined with hybrid approaches to genome assembly, have greatly enhanced genome completeness and accuracy. High-quality genome assemblies are key for gene discovery, comparative genomics, and molecular taxonomy (Rhie *et al.,* 2021).

Comparative Genomics

Comparative genomics involves analyzing similarities and differences between complete genomes in order to understand evolutionary relatedness and divergence. Within fish taxonomy, comparative genomics allows discovering conserved genes, adaptive changes, rearrangement events and species-specific genetic traits. This approach has greatly improved understanding of phylogenetic relationships and clarified confusing taxonomical relations of closely related fish species (Ellegren, 2014).

3.6 Identification of Cryptic Species

The advent of whole-genome sequencing has made the identification of cryptic species, which are not possible to differentiate morphologically or based on single locus genes, possible to be detected accurately. Genome-wide analysis detects divergence in the form of genetic differentiation of multiple loci and thus allows for species identification. The approach has made a great contribution to the recognition of fish diversity (Funk *et al.,* 2012).

3.7 Adaptive Evolution

Genome-wide analysis provides information about the molecular basis of adaptive evolution since genes or genomic regions that affect adaptation to environments, physiology and ecology can be found in the comparative analysis of genomes. Comparative genomic studies helped to understand the molecular basis of the adaptation of fishes to various aquatic environments (Bernatchez *et al.,* 2017).

3.8 Phylogenomics

Phylogenomics is a new field that integrates the two disciplines, namely phylogenetics and genomics and uses the genome level data for the reconstruction of evolutionary relationships between different groups. Phylogenomics differs from the traditional phylogenetics in that while the former uses hundreds to thousands of genes or even whole genomes for the creation of

evolutionary trees, the latter utilizes only one or few genetic markers for such purposes (Lemmon & Lemmon, 2013).

3.9 Phylogenetics vs. Phylogenomics

The method of phylogenetics estimates evolutionary relationships of organisms from a few molecular markers, like mitochondrial or nuclear genes. It is efficient in most cases of taxonomic analysis but can give inconsistent evolutionary signals based on the results obtained by a particular gene because of incomplete lineage sorting, hybridization or special gene evolution. On the other hand, phylogenomics uses genomic data which increase phylogenetic resolution and decrease uncertainty of evolutionary inference (Edwards *et al.,* 2016).

3.10 Genomic Data Sets

Phylogenomic studies employ huge genomic data sets, such as whole genomes, transcriptomes, ultra-conserved elements (UCEs), exon capture data and thousands of single nucleotide polymorphisms (SNPs). Such big sets of genomic data contain numerous genetic loci that allow estimating evolutionary relationships of organisms very accurately. Growing genomic resources make fish phylogenetic analysis more accurate (McCormack *et al.,* 2013).

3.11 Reconstructing Evolutionary Relationships

The use of genome-scale methods for reconstructing evolution has significantly improved our understanding of phylogenetic relationships in fishes by solving some phylogenetically ambiguous groups. Genomic data from multiple markers can increase the statistical power of constructing phylogenetic trees, increase accuracy in dating divergences and provide insight into rapid radiation, speciation, and adaptation events. Therefore, the phylogenomics approach became necessary in contemporary systematics (Jarvis *et al.,* 2014).

Applications

The field of phylogenomics has diverse applications in the areas of taxonomy of fishes, studies of biodiversity, evolutionary biology and conservation. Some of these applications include correct species delineation, cryptic species discovery, phylogenetic analysis, comparative genomics and divergence time estimation. In addition, phylogenomic data are useful in conservation genetics, fisheries management and the identification of evolutionarily significant units (ESUs) (Hughes *et al.,* 2018).

4. Integrative Fish Taxonomy

Integrative taxonomy represents a multilateral method of taxonomical investigation based on the integration of several different pieces of evidence for the purpose of obtaining correct species identification and reliable taxonomic classification. Contrary to conventional taxonomy, the basis of which is morphology, integrative taxonomy is supplemented by molecular, ecological, geographical, behavioral and computational information in order to solve taxonomic problems (Dayrat, 2005).

4.1 Morphology

Morphological analysis is the cornerstone of fish taxonomy and comprises morphometric measures, meristic features, coloration, osteology and exterior anatomical attributes. They serve as the primary pieces of evidence in species identification and are indispensable in species description and revision. However, morphological variability caused by environmental effects, developmental stages, and phenotypic plasticity frequently requires additional methods (Nelson *et al.,* 2016).

4.2 Molecular Markers

Molecular markers offer objective genetic evidence for species identification and phylogenetics. DNA barcoding, mitochondrially encoded genes such as COI and Cyt b, nuclear genes such as RAG1 and RAG2, as well as genome wide sequencing techniques have greatly helped to solve species delimitation problem through discovery of cryptic diversity, establishing of phylogenetic relationships and validating morphological identifications. Integration of molecular data with classical taxonomy greatly increased accuracy of fish taxonomy (Hebert *et al.,* 2003).

4.3 Ecology

Ecological data, such as habitat preference, feeding biology, reproduction biology, environmental tolerance and ecological niche represent important evidence for species identification. Closely related fish species living in different ecological niches usually possess various evolutionary traits which facilitate their distinction. Therefore, ecological data can be used together with morphological and molecular evidence in integrative taxonomy (Schlick-Steiner *et al.,* 2010).

4.4 Behaviour

Differences in behavioural traits, including courtship behaviour, reproduction and spawning, feeding habits, migrations, and communications may be found between closely related species. Differences in behavior are usually caused by reproductive isolation and adaptations to certain environments and serve as a criterion for species demarcation along with morphological and genetic features (De Queiroz, 2007).

4.5 Artificial Intelligence

Recently developed techniques of artificial intelligence and machine learning opened new ways in fish taxonomy. Image recognition tools based on AI, deep learning, computer vision, and automatic morphometrics allow fast identification of fish species from photos and other images. Moreover, the use of artificial intelligence speeds up work with genomic data, species distribution and biodiversity modeling (Wäldchen & Mäder, 2018).

4.6 Future Perspectives

The future of fish taxonomy is likely to involve the application of advanced genomics, artificial intelligence and digital resources for biodiversity. Third-generation sequencing tools such as PacBio and Oxford Nanopore have the potential of rapid and accurate genome assembly via

long-read sequencing. Meanwhile, artificial intelligence and machine learning will contribute towards automation of species identification, image-based classification, genomic analysis and biodiversity surveillance (Goodwin *et al.,* 2016).

Environmental DNA (eDNA) technology is becoming an effective method of biodiversity monitoring of water bodies by identifying rare, endangered and invasive species, without capturing specimens directly. Portable sequencing machines are expected to provide on-site species identification capabilities in particular for the remote and biodiverse areas. Moreover, the development of digital taxonomy using genomic databases and open-source bioinformatics tools as well as global biodiversity projects such as the International Barcode of Life (iBOL) project and Earth BioGenome Project will improve the identification of species, taxonomic standardization and conservation planning significantly.

Conclusion

Molecular methods have revolutionized the field of fish taxonomy by providing reliable and reproducible tools for species determination. Although traditional morphology-based taxonomy is essential, the shortcomings of such an approach in species identification and evolution studies can be effectively solved using molecular taxonomy. The use of DNA barcoding, mitochondrial and nuclear DNA markers, genome sequencing and phylogenomics has led to significant advancements in species delineation, phylogenetics, and biodiversity assessment.

The combination of molecular data with morphological, ecological, and behavioral data has resulted in the emergence of integrative taxonomy being the most advanced approach for fish taxonomy. The development of molecular methods of taxonomy has practical application in conservation, fishery management, detection of invasive species, seafood identification and conservation genetics. Environmental DNA (eDNA), artificial intelligence, machine learning and mobile sequencers are examples of new technologies that should advance taxonomic work even more. There still are challenges like high cost of technology, lack of bioinformatics skills and insufficient number of reference databases. However, constant improvement in technology allows dealing with those issues and makes the molecular taxonomy easier and more efficient. In conclusion, combining classical taxonomy with genomics and computational methods is the way forward in fish systematics.

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MICROBIAL AND BIOTECHNOLOGICAL INNOVATIONS IN THE TREATMENT OF MAMMARY TUMORS: FROM MOLECULAR MECHANISMS TO TRANSLATIONAL APPLICATIONS

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Abstract

Breast (mammary) cancer remains one of the most prevalent malignancies affecting women worldwide, and conventional treatment modalities continue to be constrained by systemic toxicity, incomplete tumor eradication, and the emergence of resistance. Over the past decade, insights from microbiology and biotechnology have converged to generate new therapeutic possibilities for mammary tumors. This chapter reviews the molecular underpinnings of mammary tumorigenesis, including hormonal signaling, oncogenic pathway dysregulation, and the tumor microenvironment, before examining the expanding body of evidence implicating the local and gut microbiome in breast cancer risk, progression, and treatment response. It then surveys microbial-based therapeutic strategies, including oncolytic bacteria, oncolytic viruses, bacterial toxins and peptides, and probiotic or postbiotic adjuvants, alongside biotechnological platforms such as synthetic gene circuits, bacterial outer membrane vesicle nanocarriers, and CRISPR/Cas-based tools. Clinical trial evidence and translational challenges, including safety, tumor targeting, and regulatory hurdles, are discussed. Collectively, the evidence indicates that microbial and biotechnological innovations offer complementary and mechanistically distinct avenues for improving the precision, efficacy, and tolerability of mammary tumor therapy, although substantial work remains before this promise is realized in routine clinical practice.

Keywords: Mammary Tumors, Breast Cancer, Oncolytic Bacteria, Tumor Microbiome, Translational Oncology.

1. Introduction

Breast cancer is the most commonly diagnosed cancer and one of the leading causes of cancer-related deaths among women worldwide, with approximately 2.3 million new cases reported each year (Sung *et al.,* 2021). Over the past few decades, significant advances have been made in its treatment through improved surgical techniques, radiotherapy, chemotherapy, hormone therapy, and HER2-targeted therapies (Burguin *et al.,* 2021). These developments have substantially improved patient survival and quality of life. However, many patients still experience tumor

recurrence, metastasis, or the development of resistance to drug therapy, which remains a major clinical challenge (Cao *et al.,* 2021; Masoud & Pagès, 2017). This is particularly true for aggressive forms of the disease, such as triple-negative and metastatic breast cancer, where effective treatment options are limited and long-term outcomes remain poor. As a result, there is growing interest in developing innovative therapeutic strategies that can overcome these limitations by targeting cancer through mechanisms different from those of conventional chemotherapy or hormone-based treatments (Masoud & Pagès, 2017; Burguin *et al.,* 2021).

To address these challenges, two rapidly advancing fields-microbiology and biotechnology have increasingly come together to develop innovative approaches for cancer treatment. The idea of using microorganisms to fight cancer is not entirely new. More than a century ago, William Coley observed that some cancer patients experienced tumor regression following certain bacterial infections, giving rise to the concept of bacterial cancer therapy. This early observation laid the foundation for what is now known as bacteriotherapy (Hoption *et al.,* 2003). Years later, the field gained renewed interest when researchers demonstrated that attenuated *Salmonella* strains could selectively accumulate and multiply within tumors, making them promising and genetically modifiable vehicles for targeted anticancer therapy (Chang & Lee, 2014). At the same time, remarkable advances in genome sequencing and metagenomic technologies have transformed our understanding of the human microbiome. It is now well established that the human body, including breast tissue, hosts a diverse community of microorganisms that influence cancer development, regulate immune responses, and affect the metabolism and efficacy of anticancer drugs, challenging the long-held belief that breast tissue is sterile (Wang *et al.,* 2026).

At the same time, rapid advances in biotechnology have greatly expanded the potential of microorganism-based cancer therapies. Technologies such as synthetic biology, nanotechnology, and genome editing now make it possible to design and modify living microorganisms with high precision, improving their safety, specificity, and therapeutic effectiveness. Engineered bacteria can be programmed with genetic circuits that detect the unique characteristics of the tumor microenvironment and selectively deliver therapeutic molecules to cancer cells (Wang *et al.,* 2025). Similarly, bacterial outer membrane vesicles have emerged as promising biocompatible nanocarriers for the targeted delivery of drugs, proteins, and nucleic acids (Ma *et al.,* 2026). In addition, CRISPR/Cas-based genome editing has become a powerful tool for identifying genetic vulnerabilities in mammary tumors and for engineering safer and more effective microbial therapeutic platforms (Chehelgerdi *et al.,* 2024). Together, these advances have created an exciting interdisciplinary field where microbiology, biotechnology, and genetic engineering are integrated to develop next-generation strategies for the treatment of mammary tumors.

This chapter provides a comprehensive overview of the emerging role of microbiology and biotechnology in the treatment of mammary tumors. It begins by describing the molecular mechanisms involved in mammary tumor development, providing the biological foundation for

understanding targeted therapeutic strategies. The chapter then explores the role of the breast and gut microbiome in tumor initiation, progression, and response to treatment. Subsequently, it discusses a range of microorganism-based therapeutic approaches, including oncolytic bacteria and viruses, bacterial toxins and peptides, probiotics, and postbiotics. The following section highlights recent advances in biotechnology, such as synthetic biology, nanotechnology, and genome editing, which have improved the precision, safety, and effectiveness of these therapies. Finally, the chapter reviews the current status of preclinical and clinical research, discusses the major challenges limiting clinical translation, and outlines future perspectives for the development of innovative microbiology- and biotechnology-based treatments for mammary tumors.

2. Molecular Mechanisms Underlying Mammary Tumorigenesis

2.1 Genetic and Hormonal Drivers

Mammary tumorigenesis is complex process which reflects the key features of classical hallmarks of cancer, including uncontrolled cell proliferation, evasion of growth suppressors, resistance to apoptosis, replicative immortality, sustained angiogenesis, and ability to invade surrounding tissues and metastasize to different organs (Hanahan and Weinberg, 2011). In breast cancer, these hallmarks are influenced by few clinically important molecular drivers. Around 15 to 20 % of mammary tumors overexpress the human epidermal growth factor receptor 2 (HER2) which promotes aggressive proliferation but also targeted effectively by antibodies such as trastuzumab while majority of tumors are hormone receptor-positive and depends on estrogen and progesterone receptor signaling for growth but can be treated with endocrine therapy (Waks & Winer, 2019). Inherited germline mutations in BRCA1 and BRCA2 genes which plays importanrt role in homologous recombination DNA repair, markedly increase lifetime breast cancer risk and are particularly associated with triple-negative and basal-like types of breast cancer, which are characterized by aggressive clnical behaviour and limited therapeutics (Chen *et al.,* 2018; Peshkin *et al.,* 2011). Recent studies have shown that loss of BRCA1 in mammary tumors activate transforming growth factor beta receptor 2 (TGFβR2) signalling which promotes epithelial-to-mesenchymal transition (EMT) further enhance cancer stem like properties and increasing resistance to chemoteherapy (Bai *et al.,* 2022). Similarly, activation of fibroblast growth factor receptor 2 (FGFR2) signaling has been reported to suppress BRCA1 expression thus driving the development of triple-negative mammary tumors while simultaneously increasing their sensitivity to immunotherapy (Lei *et al.,* 2021). These findings highlight the complex interactions between genetic mutations and cellular signaling pathways in mammary tumorigenesis and identify molecular vulnerabilities that can be targeted through emerging microbiological and biotechnological therapeutic approaches

2.2 Oncogenic Signaling Pathways

Mammary tumor progression is driven not only by individual genetic mutations but also by a complex network of interconnected signaling pathways. Most important are the estrogen receptor (ER), progesterone receptor (PR), and HER2 pathways, which interact with downstream cascades such as PI3K/AKT/mTOR, RAS/MAPK. These pathways promote uncontrolled cell proliferation, inhibit apoptosis, and contribute to both intrinsic and acquired resistance to endocrine therapy and HER2-targeted treatments (Rusquec *et al.,* 2020). In particular, aberrant activation of the PI3K/AKT/mTOR pathway often triggered by HER2 overexpression, has been strongly associated with resistance to trastuzumab and other HER2-directed antibodies. This has led to the development of combination treatment strategies that pair HER2-targeted regimens with PI3K or mTOR inhibitors to improve therapeutic efficacy (Nahta *et al.,* 2006). However, extensive cross-talk between these signaling networks enables tumor cells to activate alternative survival pathways when one pathway is inhibited, allowing resistance to develop over time. These limitations have prompted growing interest in alternate therapeutic approaches, including engineered bacteria and oncolytic viruses, which can exert antitumor effects through mechanisms that are independent of conventional oncogenic signaling pathways, thereby offering a potential strategy to overcome treatment resistance (Harrington *et al.,* 2019; Niu *et al.,* 2026).

2.3 Tumor Microenvironment and Immune Evasion

Mammary tumors do not develop as isolated masses but in a complex tumor microenvironment which consists stromal fibroblasts, immune cells, vasculature, and extracellular matrix and all of them coevolve with malignant epithelial cells (Sushma *et al.,* 2025). As tumors enlarge, their rapid growth often exceeds the capacity of the abnormal tumor vasculature to supply oxygen and nutrients, leading to the formation of hypoxic and poorly perfused regions thus reduce the effectiveness of conventional chemotherapy which relies on adequate drug delivery, and radiotherapy whose cytotoxic effects depend largely on oxygen-mediated DNA damage (Tao *et al.,* 2023). The tumor microenvironment promotes immune evasion by recruiting immunosuppressive cells such as regulatory T cells, tumor-associated macrophages, and myeloid-derived suppressor cells, while also upregulating immune checkpoint molecules that inhibit antitumor immune responses (Haist *et al.,* 2021). Interestingly, these hostile conditions for conventional therapies create an ideal niche for anaerobic and facultative anaerobic bacteria. Such microorganisms preferentially colonize and proliferate within the hypoxic core of tumors where they can survive and replicate while being efficiently eliminated from healthy, well-oxygenated tissues (Yu *et al.,* 2012). This unique tumor-targeting ability provides the biological basis for the development of bacterial therapeutics and represents one of the key principles underlying the microbial treatment strategies discussed in the following sections of this chapter.

3. The Mammary Tumor Microbiome: An Emerging Determinant of Disease

3.1 Gut and Local Breast Tissue Dysbiosis

Advances in multi-omics technologies confirms that breast tissue which once assumed to be sterile hosts a unique resident microbiota and also confirms that both the gut and local breast microbiota differs significantly between healthy individuals, patients with benign breast conditions, and those with malignant tumors. Moreover, microbial composition varies among different molecular subtypes of breast cancer and across populations with diverse ethnic backgrounds suggesting that these microbial communities may either contribute to tumor development or reflect the underlying biology of specific disease subtypes (Wong *et al.,* 2025). Obesity associated alterations in the gut microbial composition have been linked to breast cancer pathogenesis and prognosis, with gut dysbiosis proposed as one mechanistic link between metabolic dysfunction, chronic inflammation and tumor progression (Zhang *et al.,* 2026). Evidence from preclinical mice studies further supports this relationship. Antibiotics induced gut dysbiosis has been shown to accelerate mammary tumor growth by impairing antitumor immune responses and inducing structural changes in the mammary gland that create a more favorable environment for carcinogenesis (American Society for Microbiology, 2025).

3.2 Mechanistic Links: Estrogen Metabolism, Inflammation, and Genotoxicity

Growing evidence suggests that microbial communities influence mammary tumor development through multiple interconnected mechanisms. One of the best-characterized pathways involves the estrobolome, the collection of gut bacteria that produce β-glucuronidase enzymes. These enzymes deconjugate estrogens that have been inactivated by hepatic glucuronidation thus restoring them to their biologically active form and allowing their reabsorption into the circulation. Since the majority of breast cancers are hormone receptor-positive and rely on estrogen signaling for growth, alterations in the estrobolome can increase systemic estrogen exposure and thereby promote tumor initiation and progression (Hu *et al.,* 2023). Beyond hormonal regulation, gut dysbiosis contributes to carcinogenesis by inducing chronic low-grade inflammation, compromising the integrity of the intestinal and mammary epithelial barriers and in some cases producing genotoxic metabolites that directly damage host DNA (Lechuga *et al.,* 2023; Urbaniak *et al.,* 2016). Importantly, the influence of the gut microbiota extends beyond the gastrointestinal tract. Gut dysbiosis can reshape the breast tumor microenvironment by increasing circulating pro-inflammatory cytokines and chemokines, stimulating infiltration of myeloid cells, and promoting tissue fibrosis (Zhang *et al.,* 2022).

3.3 Intratumoral Microbiota and Clinical Correlates

The presence of bacterial DNA and microbially derived metabolites within breast tumor tissue have provided evidence that tumors harbor their own localized microbial communities. These intratumoral microbes are increasingly recognized as active participants in the tumor microenvironment where they can influence local metabolism, immune responses, and tumor

progression. Several studies have identified distinct intratumoral microbial signatures that correlate with disease outcome and therapeutic response. For example, particular immune associated intratumoral microbiome profiles have shown prognostic value and linked with resistance to chemotherapeutic agents such as tamoxifen and docetaxel, while elevated intratumoral *Fusobacterium nucleatum* in combination with high microRNA-21 expression has been linked to more aggressive tumors (Yang *et al.,* 2017; Li *et al.,* 2024). On the other hand, beneficial gut bacteria, including *Faecalibacterium* and *Akkermansia*, have been reported to exert protective effects and lowering the risk of breast cancer development (Kang *et al.,* 2025). These findings collectively indicates that the tumor and gut microbiome are not only a passive biomarker of the disease. Instead, they represent modifiable components of the tumor ecosystem with direct relevance to breast cancer prevention, prognosis and the development of microbiome based therapeutic strategies which are discussed in the following sections of this chapter.

4. Microbial-Based Therapeutic Innovations

4.1 Oncolytic Bacteria

Oncolytic bacteria exploit the unique characteristics of the tumor microenvironment, particularly the hypoxic and immunosuppressed regions described in Section 2.3, to selectively colonize tumors. Both naturally occurring and genetically engineered strains of *Clostridium*, *Salmonella*, *Escherichia coli*, and *Listeria* have been extensively investigated for their ability to localize within tumors and the production of cytotoxic toxins, enzymes, and bioactive metabolites while also stimulating innate and adaptive immune responses against the tumor (Khormi *et al.,* 2025). A notable example is an engineered *Salmonella* strain capable of depleting methionine within the tumor microenvironment further achieves oncolysis and significantly reduce metastasis in a variety of animal tumor models (Zhou *et al.,* 2023). More recently, advances in bacterial engineering have further enhanced the therapeutic potential of these microbial platforms. For example, an attenuated *Salmonella typhimurium* strain (VNP20009) coated with a coordination-guided therapeutic nanolayer was developed to promote pyroptosis, a highly inflammatory form of programmed cell death resulting in synergistic suppression of triple-negative breast cancer, bypassing the immunosuppressive and molecular heterogeneous nature of this subtype (Tian *et al.,* 2025). In parallel, synthetic biology has enabled the development of programmable gene circuits that allow engineered bacteria to sense their location and activate therapeutic genes expression only within the tumor. These systems may respond to externally applied signals, bacterial quorum-sensing mechanisms or tumor microenvironment conditions such as hypoxia and altered metabolism. By restricting therapeutic gene expression to the tumor site these programmable bacterial platforms significantly improve treatment precision while minimizing off-target toxicity (Niu *et al.,* 2026).

Clinical development of oncolytic bacterial therapy has progressed further with *Clostridium novyi*-NT, a non-toxigenic strain having alpha toxin gene deletion that selectively germinate within the

hypoxic tumor regions. In the first-in-human dose-escalation clinical trial, intratumoral administration of *C. novyi*-NT spores resulted in significant tumor lysis in a considerable proportion of patients with treatment-refractory solid malignancies. But toxicities like sepsis and gas gangrene occurred in patients receiving higher spore doses or those with larger tumors, enabling the determination of a maximum tolerated dose for this therapeutic approach (Janku *et al.,* 2021). Based on these promising findings, a subsequent phase Ib clinical trial evaluated *C. novyi*-NT in combination with the immune checkpoint inhibitor pembrolizumab. The combination reported an acceptable safety profile and produced objective clinical responses including a complete response in patients with several advanced solid tumors (Nelson *et al.,* 2025). Although these early clinical studies included patients with breast cancer as well as other accessible solid tumors, the underlying therapeutic principles remain broadly applicable across different mammary tumor settings. Consequently, this bacterial platform is now being actively investigated in preclinical breast cancer models and is considered a promising candidate for future microbiome-based therapeutic strategies against mammary tumors.

4.2 Oncolytic Viruses

Oncolytic viruses are another important class of microbial therapeutics but their mechanism of action differ fundamentally from oncolytic bacteria. These viruses are designed to infect, replicate within, and destroy cancer cells. Their tumor selectivity is majorly related to defects in antiviral defense pathways that are commonly present in malignant cells. In addition to direct oncolysis, viral infection triggers the release of tumor-associated antigens which promote the recruitment and activation of both innate and adaptive immune cells within the tumor microenvironment (Xiao *et al.,* 2026). In breast cancer, oncolytic virotherapy is being actively explored as a strategy primarily to overcome the immunosuppressive tumor microenvironment that often limits the efficacy of immune checkpoint inhibitors and other immunotherapies. Recent advances in genetic engineering have enabled oncolytic viruses to serve as targeted delivery vehicles for immuomodulatory genes (Xianshu *et al.,* 2025). By combining direct tumor destruction with immune activation and targeted gene delivery, oncolytic viruses complement bacterial therapies and represent an integral component of emerging tumor microenvironment-directed treatment strategies for breast cancer.

4.3 Bacterial Toxins and Anticancer Peptides

In addition to live microbes microbial therapeutics also include purified bacterial proteins and peptides that possess intrinsic anticancer properties while avoiding the challenges associated with administering live microorganisms. Azurin, a copper-containing redox protein produced by *Pseudomonas aeruginosa* is a widely studied example. Azurin primarily penetrates cancer cells where it stabilizes the tumor suppressor protein p53, inhibits tumor angiogenesis by interfering with vascular endothelial growth factor receptor-2 (VEGFR-2) signaling further restricting tumor growth and progression. Building on these findings researchers identified a 28-amino acid cell-

penetrating fragment of azurin known as p28 which retains the protein's ability to selectively enter cancer cells and stabilize p53. Preclinical studies suggest p28 effectively inhibits the proliferation of breast cancer cells while exhibiting minimal toxicity toward normal tissues (Yaghoubi *et al.,* 2020). These promising safety and efficacy findings subsequently led to the designation of p28 as both an orphan drug and a rare pediatric disease therapy, highlighting its potential as a novel targeted anticancer peptide.

4.4 Probiotics and Postbiotics

The growing recognition of the gut & breast microbiota based mammary carcinogenesis described in secton 3, bring researchers interest in probiotics and their non-viable derivatives known as postbiotics as supportive therapies for breast cancer. Postbiotics are primarily explored as adjuvant interventions that can enhance the efficacy of conventional treatments. Postbiotics comprise a diverse group of biologically active molecules including cell wall fragments, metabolites, bacterial lysate, extracellular vesicles, short-chain fatty acids, and other microbial metabolites. These compounds have been shown to modulate immune responses, suppress chronic inflammation, strengthen epithelial barrier integrity and exert selective cytotoxic effects against cancer cells (Balendra *et al.,* 2024). Experimental evidence further supports their therapeutic potential. In a preclinical breast cancer model postbiotics derived from *Lactobacillus rhamnosus* & *L. plantarum* cultures significantly enhanced the antitumor activity of tamoxifen and an experimental aziridine–hydrazide hydrazone compound resulting in greater apoptotic cell death in estrogen receptor-positive MCF-7 breast cancer cells than either agent achieved alone (Wasiak *et al.,* 2024). These findings suggest that postbiotics may improve the responsiveness of breast tumors to existing therapies while simultaneously modulating the tumor microenvironment in favor of antitumor immunity. Furthermore, recent reviews of the gut–breast microbiota axis have proposed that future postbiotic interventions could be personalized according to an individual's microbial composition thereby supporting precision medicine approaches to breast cancer management (Ghosh *et al.,* 2025).

5. Biotechnological and Nanotechnological Platforms as therapeutics

5.1 Synthetic Biology and Engineered Gene Circuits

Recent advances in synthetic biology have improved the precision, controllability and safety of microbial cancer therapeutics. Instead of relying solely on the natural tumor targeting ability of bacteria researchers now engineer sophisticated genetic circuits that enable microbes to sense their surroundings and regulate therapeutic functions in a highly controlled manner. One such approach involves exogenous input-responsive circuits in which therapeutic gene expression can be switched on or off using externally administered molecules such as oral small-molecule inducers. Another strategy employs autonomous bacterial signal-responsive circuits which can be based on quorum sensing. In these systems bacteria monitor their own population density and activate therapeutic functions such as lysis or drug release only after accumulating to a critical

concentration within the tumor. This minimizes premature activation and reduces the risk of damage to surrounding healthy tissues. A third approach utilizes tumor microenvironment-responsive circuits in which therapeutic gene expression is triggered by characteristic features of the tumor microenvironment including hypoxia, acidic pH or altered metabolite concentrations (Niu *et al.,* 2026). Collectively, these innovations have transformed oncolytic bacteria from naturally tumor-targeting microorganisms into programmable therapeutic platforms capable of integrating environmental sensing with precisely regulated therapeutic responses. As synthetic biology continues to evolve, these intelligent microbial systems are expected to enable increasingly personalized treatments with bacterial behavior tailored to the unique molecular and microenvironmental characteristics of individual mammary tumors thereby improving both therapeutic efficacy and safety.

5.2 Bacterial-Derived Nanocarriers

Gram-negative bacteria naturally secrete nanosized lipid bilayer structures known as bacterial outer membrane vesicles (OMVs) which have recently emerged as a microbial platform for targeted breast cancer therapy. Unlike live bacteria, OMVs are non-replicating which makes them inherently safer while still preserving intrinsic immunostimulatory properties responsible for the therapeutic potential. Owing to their small size, biocompatibility and efficient cellular uptake, OMVs can be utilized as carriers for the targeted delivery of anticancer drugs and immunomodulators. Advances in bioengineering further enabled OMVs to display tumor-targeting ligands or loaded with therapeutic cargo thereby enhancing their specificity and treatment efficacy. In a preclinical study, OMVs were fused with breast cancer cell membranes to create a hybrid membrane nanoparticle that combined the tumor-homing ability of cancer cell membranes with the intrinsic immunogenicity of bacterial vesicles. This biomimetic platform was subsequently used to deliver a sonodynamic therapeutic agent for the treatment of breast cancer bone metastasis resulting in improved tumor accumulation and superior antitumor efficacy through the combined effects of sonodynamic therapy and immune activation compared with conventional nanoparticle systems (Wang *et al.,* 2024). Consequently, OMVs are increasingly viewed as a next-generation drug delivery platform with significant potential for enhancing the precision, safety and effectiveness of microbial-based therapies for mammary tumors.

5.3 CRISPR/Cas-Based Approaches

CRISPR/Cas gene-editing technology has emerged as a powerful tool in breast cancer therapeutics, contributing through two complementary approaches. The first involves the use of genome-wide CRISPR screening to identify breast cancer subtypes-specific genetic dependencies and synthetic lethal interactions providing valuable insights for the development of targeted therapies. For example, CRISPR-based screening has identified novel vulnerabilities in endocrine-resistant breast cancers thus enabling the rational design of combination treatments to

enhance the effectiveness of existing hormonal therapies (Samuels *et al.,* 2025). The second which is more relevant to the microbial and biotechnological focus of this chapter is the integration of CRISPR technology into engineered therapeutic microorganisms like bacteria and bacteriophages to improve their therapeutic precision, safety, and functionality. A notable example is the development of CRISPR-Cas3-engineered bacteriophages which have entered early clinical evaluation as highly selective tools for eliminating specific bacterial populations. This strategy demonstrates the feasibility of precision microbiome engineering and raises the possibility of selectively modifying the gut or tumor associated microbiota to create a microbial environment that is less supportive for mammary tumor development and progression (Ghazaei *et al.,* 2026; Cheng *et al.,* 2025). Although no CRISPR-edited therapy has yet entered dedicated clinical trials specifically for breast cancer. CRISPR-engineered T-cell receptor therapies have already progressed to phase I clinical trials in patients with other solid tumors highlighting the growing clinical potential of gene-editing approaches. At the same time CRISPR is being used to engineer oncolytic bacteria thus improving biocontainment, enhanced tumor specificity and precisely regulated therapeutic payload expression. Together, these developments position CRISPR/Cas technology as a key enabling platform for the future of precision microbial therapeutics and personalized treatment strategies for mammary tumors.

6. Translational Applications and Clinical Trial Landscape

Although microbial and biotechnology-based therapies for mammary tumors are still in the early stages of clinical translation but their development has progressed considerably over the past decade. Several complementary therapeutic strategies are now advancing simultaneously, reflecting the growing interest in exploiting microorganisms and their derivatives for cancer treatment. Among these approaches, live oncolytic bacterial therapy has made the greatest clinical progress. *Clostridium novyi*-NT has been the most extensively investigated candidate with first-in-human clinical studies demonstrating the feasibility of intratumoral spore administration, establishing the maximum tolerated dose and showing measurable tumor regression in a substantial proportion of patients with treatment-refractory solid tumors (Janku *et al.,* 2021). Based on these positive results subsequent clinical evaluation of *C. novyi*-NT in combination with immune checkpoint blockade demonstrated durable partial and complete responses in a subset of patients with advanced solid malignancies further supporting the potential of bacterial therapy as an immunomodulatory anticancer strategy (Nelson *et al.,* 2025). In parallel, purified bacterial protein therapeutics have reached a more advanced stage of clinical development. The azurin derived peptide p28 successfully completed a phase I clinical trial where it exhibited an excellent safety profile and showed preliminary evidence of antitumor activity in patients with advanced, treatment-resistant solid tumors. These promising findings led to the granting of orphan drug and rare pediatric disease designations by regulatory authorities

highlighting the clinical potential of bacterial-derived peptides as targeted anticancer therapeutics (Warso *et al.,* 2013).

Probiotic and postbiotic therapies are at an earlier stage of clinical translation than other microbial-based approaches. Most of their anticancer potential evidences comes from in vitro experiments and preclinical animal studies with relatively few well-designed randomized clinical trials available. This slower clinical progress is due to challenges associated with standardizing live biotherapeutic products, including variations in bacterial strains, dosing regimens, viability during storage and administration and the limited bioavailability of many postbiotic formulations (Ghosh *et al.,* 2025). Similarly, OMV based drug delivery systems and synthetic biology-engineered bacterial platforms are still predominantly at the proof-of-concept stage. Although these technologies have demonstrated encouraging therapeutic efficacy, targeted drug delivery and immune activation in murine models of breast and other solid tumors, no completed clinical trials specifically evaluating their use in mammary tumors have been reported to date. Nevertheless, the successful clinical evaluation of *Clostridium novyi*-NT and the azurin-derived peptide p28 has established an important regulatory foundation for microbial therapeutics. Rather than replacing existing treatment modalities microbial therapeutics appear to be most effective when used in combination with established anticancer agents. In particular, bacterial and viral oncolytic therapies can remodel the immunosuppressive tumor microenvironment which become more susceptible to immune checkpoint inhibitors and other immunotherapies. Consequently, the future clinical application of microbial therapeutics for mammary tumors is likely to rely on rational combination strategies that integrate microbial platforms with conventional chemotherapy, targeted therapy, and modern immuno-oncology approaches to achieve more durable and effective clinical responses.

7. Challenges and Future Perspectives

Several challenges must be resolved before incorporation of microbial and biotechnological innovations in routine clinical treatment of mammary tumor. Safety remains the first concern for live bacterial therapeutics as toxic effects like sepsis and gas gangrene observed at higher doses of C. novyi-NT in larger tumors (Janku *et al.,* 2021). To improve the safety of these therapies future bacterial platforms will likely incorporate genetically encoded biocontainment strategies which includes engineered auxotrophies and inducible kill switches. Another major challenge is the substantial variability in the composition of both the gut and breast microbiota among individuals. Factors such as diet, ethnicity, lifestyle, antibiotic exposure, and breast cancer molecular subtype all influence microbial composition making it difficult to develop standardized probiotic, postbiotic, or microbiome-targeted therapies. It further highlights the need for microbiome profiling to precede personalized treatment selection. The poor bioavailability and short biological half-life of several postbiotic preparations reduced their current clinical reliability and will require improved encapsulation and delivery technologies (Ghosh *et al.,* 2025). Beyond these biological challenges the manufacturing, quality control, and

regulatory pathways for living or bacterially derived therapeutics remain less standardized than those for conventional small-molecule or biologic drugs, creating practical hurdles for scale-up and multi-center clinical evaluation.

Looking forward, several emerging technologies are likely to shape the next generation of microbial and biotechnological therapeutics approaches for mammary tumor treatment. The continued convergence of synthetic biology, computational modelling and machine learning-assisted genetic circuit design produce sophisticated bacterial platforms capable of sensing multiple tumor-specific signals and delivering therapeutic payloads with exceptional spatial and temporal precision. Bacterial OMV and other bacterially derived nanocarriers are likely to be further diversified as modular platforms capable of combining chemotherapeutic, immunomodulatory, and imaging payloads within a single biocompatible particle. CRISPR based tools are applied to engineer safer and more selective oncolytic bacteria and potentially reshape the dysbiotic tumor associated or gut microbial communities. Finally, larger and more rigorously controlled clinical trials ideally stratified by breast cancer molecular subtype and baseline microbiome composition will be necessary to move these largely preclinical and early-phase innovations toward evidence-based integration into standard mammary tumor care.

Conclusion

The treatment of mammary tumors is reshaped by the convergence of microbial biology and biotechnological engineering. Molecular characterization of mammary tumorigenesis has revealed the genetic drivers, signaling networks, and microenvironmental features including hypoxic and immunosuppressive niches that create specific vulnerabilities exploitable by microbial agents. Emerging understanding of the local and gut microbiome has repositioned resident bacterial communities from incidental bystanders to active participants in breast cancer risk, progression, and treatment response. Building on these insights, oncolytic bacteria and viruses, bacterial toxins and peptides, and probiotic or postbiotic adjuvants constitute a diverse portfolio of microbial therapeutics, while synthetic biology, bacterial nanocarriers, and CRISPR/Cas-based tools provide the biotechnological infrastructure needed to render these agents safer, more selective, and more controllable. Early clinical trials of C. novyi-NT and the azurin-derived peptide p28 demonstrate that this convergence can be translated into patients with acceptable safety and preliminary evidence of antitumor activity, even as most other platforms remain in preclinical development. Continued interdisciplinary collaboration among microbiologists, synthetic biologists, oncologists, and clinical trialists will be essential to fully realize the translational potential of microbial and biotechnological innovations in the treatment of mammary tumors.

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MICROBIAL EXTRACELLULAR VESICLES: TINY NANOSTRUCTURES DRIVING INNOVATIONS IN MICROBIOLOGY, BIOTECHNOLOGY, AND BIOMEDICINE

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Abstract
Microbial extracellular vesicles (MEVs) have emerged as a transformative area of research in molecular microbiology, revealing that microorganisms communicate through sophisticated nanoscale lipid bilayer vesicles rather than solely through soluble signaling molecules or direct cell-to-cell interactions. Produced by Gram-negative bacteria, Gram-positive bacteria, fungi, archaea, and some protists, MEVs typically range from 20 to 400 nm in diameter and carry a diverse cargo of proteins, lipids, nucleic acids, metabolites, toxins, enzymes, and signaling molecules. Their selective cargo composition enables microorganisms to coordinate quorum sensing, biofilm formation, nutrient acquisition, stress adaptation, antimicrobial resistance, and host–microbe interactions. Recent advances have demonstrated that MEVs are central mediators of the gut microbiota–host axis, where they regulate epithelial barrier integrity, immune homeostasis, and systemic inter-organ communication. Beyond their biological significance, MEVs are increasingly recognized as promising platforms for vaccine development, targeted drug delivery, cancer immunotherapy, diagnostics, and precision microbiome engineering due to their intrinsic biocompatibility and natural targeting capabilities. The integration of synthetic biology, nanotechnology, multi-omics, and artificial intelligence has further expanded opportunities to engineer vesicles with customized cargo and enhanced therapeutic efficacy. Despite significant progress, challenges related to vesicle heterogeneity, standardized isolation protocols, large-scale manufacturing, and biosafety remain major barriers to clinical translation. This review summarizes current knowledge on the biogenesis, molecular composition, biological functions, and emerging biomedical and biotechnological applications of microbial extracellular vesicles while highlighting future directions that may establish MEVs as next-generation tools in precision medicine, sustainable biotechnology, and microbiome-based therapeutics.

Keywords: Microbial Extracellular Vesicles, Outer Membrane Vesicles, Microbiome, Antimicrobial Resistance, Biotechnology, Drug Delivery, Postbiotics, Host–Microbe Interaction.

1. Introduction

Microorganisms have traditionally been viewed as unicellular organisms that interact with their environment primarily through the secretion of soluble metabolites, toxins, enzymes, and quorum-sensing molecules. However, discoveries over the past two decades have fundamentally

transformed this concept by demonstrating that bacteria, fungi, archaea, and other microorganisms actively secrete membrane-bound extracellular vesicles (MEVs) that function as highly organized intercellular communication systems. These nanoscale vesicles are now recognized as universal biological structures that enable microorganisms to exchange complex molecular information with neighboring microbes and host cells in both local and distant environments.

Microbial extracellular vesicles are spherical phospholipid bilayer structures ranging from approximately 20 to 400 nm in diameter. Initially described in Gram-negative bacteria as outer membrane vesicles (OMVs), subsequent studies have confirmed vesicle production in Gram-positive bacteria, fungi, and archaea, establishing vesiculation as a conserved phenomenon across all major microbial domains. Unlike random membrane debris generated during cell lysis, MEVs are produced through tightly regulated biogenetic pathways involving membrane remodeling, selective cargo loading, and controlled vesicle release. Their cargo includes proteins, enzymes, virulence factors, lipids, metabolites, DNA, messenger RNA, transfer RNA, small regulatory RNAs, and quorum-sensing molecules, reflecting the physiological state of the producing microorganism. The biological significance of MEVs lies in their ability to deliver protected molecular cargo directly to recipient cells. Encapsulation within a lipid bilayer shields cargo molecules from enzymatic degradation and facilitates targeted delivery through membrane fusion, receptor-mediated endocytosis, macropinocytosis, or phagocytosis. Consequently, vesicle-mediated communication is often more efficient than diffusion-based signaling, particularly in complex environments such as biofilms, mucosal surfaces, soil ecosystems, marine habitats, and the gastrointestinal tract.

Recent research has revealed that MEVs participate in a wide spectrum of biological processes, including quorum sensing, horizontal gene transfer, nutrient acquisition, stress adaptation, antimicrobial resistance, biofilm development, immune modulation, and host colonization. In pathogenic microorganisms, vesicles serve as delivery vehicles for toxins and virulence determinants that enhance infection while evading host immune defenses. Conversely, vesicles derived from probiotic and commensal microorganisms contribute to intestinal homeostasis by strengthening epithelial barrier integrity, regulating inflammatory signaling, and promoting beneficial host–microbiota interactions. These findings have established MEVs as central mediators of the microbiota–host axis and important determinants of human health and disease.

Beyond their physiological roles, MEVs have attracted considerable attention as versatile nanobiotechnological platforms. Their natural biocompatibility, ability to encapsulate diverse biomolecules, intrinsic targeting properties, and capacity for genetic engineering make them attractive candidates for vaccine development, targeted drug delivery, cancer immunotherapy, diagnostics, precision microbiome engineering, and postbiotic formulations. The convergence of synthetic biology, systems biology, nanotechnology, and artificial intelligence is further accelerating the development of engineered vesicles with enhanced therapeutic efficacy and safety.

This chapter provides a concise overview of the current understanding of microbial extracellular vesicles, focusing on their biogenesis, molecular cargo, biological functions, and emerging applications in microbiology, biotechnology, and biomedicine. In addition, current challenges and future research opportunities are highlighted to emphasize the growing importance of MEVs as next-generation tools in molecular biosciences.

2. Biogenesis and Molecular Cargo of Microbial Extracellular Vesicles

The formation of microbial extracellular vesicles is a genetically regulated process that differs among microbial groups but ultimately results in the release of nanoscale membrane-bound structures carrying highly selective molecular cargo. In Gram-negative bacteria, OMVs originate from localized outward bulging of the outer membrane. Disruption of interactions between the outer membrane, peptidoglycan layer, and Braun's lipoprotein facilitates membrane curvature and vesicle budding. Environmental stressors such as oxidative stress, antibiotic exposure, nutrient limitation, and quorum-sensing signals further stimulate vesicle production. In *Pseudomonas aeruginosa*, the Pseudomonas Quinolone Signal (PQS) promotes membrane curvature, thereby enhancing OMV biogenesis.

For many years, vesicle production by Gram-positive bacteria was considered unlikely because of their thick peptidoglycan cell wall. It is now well established that these organisms actively produce membrane vesicles through localized membrane protrusion accompanied by transient remodeling of the peptidoglycan layer by autolysins and peptidoglycan hydrolases. Similar mechanisms have been reported in *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, and several lactic acid bacteria, indicating that vesiculation is a conserved strategy among Gram-positive species. In fungi, extracellular vesicles are generated through both conventional Golgi-mediated secretion and endosomal pathways involving multivesicular bodies. Vesicles subsequently traverse the complex fungal cell wall composed of β-glucans, chitin, and mannoproteins through mechanisms that are still being elucidated. Archaeal vesicles are similarly produced through membrane budding, with several archaeal ESCRT-like proteins participating in membrane remodeling and vesicle release.

The biological activity of MEVs is determined primarily by their molecular cargo, which is selectively packaged during vesicle biogenesis rather than randomly incorporated. Proteomic analyses have identified structural proteins, porins, adhesins, transport proteins, molecular chaperones, hydrolytic enzymes, toxins, virulence factors, antimicrobial resistance proteins, and stress-response proteins within vesicles. Lipid components—including phosphatidylethanolamine, phosphatidylglycerol, cardiolipin, lipopolysaccharide, lipoteichoic acid, sphingolipids, and ergosterol—maintain vesicle stability while contributing to immune recognition and cellular uptake.

A particularly significant discovery has been the identification of nucleic acids within microbial vesicles. MEVs transport chromosomal DNA, plasmid DNA, messenger RNA, transfer RNA, ribosomal RNA fragments, CRISPR-associated RNAs, and small regulatory RNAs capable of modulating gene expression and facilitating horizontal gene transfer. Additionally, vesicles

contain metabolites, siderophores, vitamins, cofactors, quorum-sensing molecules, and secondary metabolites that contribute to microbial communication and ecological adaptation.

The selective enrichment of specific proteins and RNAs within vesicles indicates the existence of sophisticated cargo-sorting mechanisms, although the molecular determinants governing this process remain incompletely understood. Deciphering these mechanisms represents one of the major challenges in extracellular vesicle biology and will be crucial for the rational engineering of MEVs as therapeutic and biotechnological delivery systems.

3. Biological Functions and Biomedical Applications of Microbial Extracellular Vesicles

Microbial extracellular vesicles (MEVs) have emerged as highly specialized nanostructures that coordinate microbial physiology and mediate interactions between microorganisms and their hosts. Unlike passive membrane fragments, MEVs function as targeted delivery systems capable of transporting biologically active molecules across cellular barriers, thereby influencing gene expression, immune responses, microbial ecology, and disease progression. Their biological functions extend from microbial communication to host immunomodulation, making them central regulators of both microbial communities and host health.

One of the primary functions of MEVs is intercellular communication. Microorganisms inhabit highly competitive and dynamic environments where efficient communication is essential for survival. While classical quorum sensing depends on diffusible signaling molecules, vesicle-mediated communication provides several advantages by protecting signaling compounds from enzymatic degradation and delivering them directly to recipient cells. MEVs transport quorum-sensing molecules, transcriptional regulators, enzymes, metabolites, and small regulatory RNAs that coordinate collective microbial behaviors such as biofilm formation, virulence expression, nutrient utilization, and stress adaptation. This targeted mode of communication is particularly important within polymicrobial communities where multiple microbial species coexist and compete for ecological niches.

Another important biological role of MEVs is nutrient acquisition and environmental adaptation. Vesicles contain numerous extracellular enzymes, including proteases, lipases, glycosidases, phosphatases, and nucleases, which hydrolyze complex substrates into assimilable nutrients. Many bacterial vesicles also carry siderophores that efficiently scavenge iron from nutrient-limited environments, thereby enhancing microbial survival during host nutritional immunity. Furthermore, environmental stresses such as oxidative stress, antibiotic exposure, osmotic fluctuations, and nutrient deprivation stimulate vesicle production, enabling microorganisms to export stress-response proteins and maintain cellular homeostasis under adverse conditions.

MEVs also play indispensable roles in biofilm development, a major survival strategy adopted by microorganisms in clinical, environmental, and industrial settings. Vesicles contribute to the initial stages of biofilm formation by delivering adhesins and extracellular DNA that facilitate microbial attachment to biotic and abiotic surfaces. During biofilm maturation, they become integral components of the extracellular polymeric matrix, transporting enzymes, structural proteins, polysaccharides, and signaling molecules that strengthen biofilm architecture and

regulate nutrient distribution. In mature biofilms, vesicles disseminate antimicrobial resistance determinants, stress-response proteins, and quorum-sensing molecules, thereby enhancing community resilience against antibiotics, disinfectants, and host immune defenses. They also participate in biofilm dispersal by transporting matrix-degrading enzymes that enable bacterial cells to colonize new environments.

An equally significant function of MEVs is their involvement in horizontal gene transfer and antimicrobial resistance (AMR). In addition to classical mechanisms such as conjugation, transformation, and transduction, extracellular vesicles facilitate the transfer of plasmids, chromosomal DNA, integrons, transposons, antimicrobial resistance genes, and regulatory RNAs between bacterial populations. Encapsulation within vesicles protects genetic material from extracellular nucleases, thereby improving transfer efficiency in complex ecosystems. Moreover, vesicles transport antibiotic-degrading enzymes, particularly β-lactamases, which hydrolyze β-lactam antibiotics before they reach bacterial cells. This extracellular detoxification not only protects the producing bacterium but also neighboring susceptible microorganisms, creating a phenomenon known as community-level antibiotic resistance. Additionally, vesicles can function as decoys by binding antimicrobial peptides and bacteriophages, further contributing to microbial survival under antimicrobial pressure.

4. Microbial Extracellular Vesicles in Host–Microbe Interactions

One of the most transformative discoveries in microbial vesicle research is their ability to mediate long-distance communication between microorganisms and host tissues. Following their release into the extracellular environment, MEVs readily penetrate mucus layers and epithelial barriers before being internalized by epithelial cells, macrophages, dendritic cells, neutrophils, endothelial cells, and lymphocytes through membrane fusion, receptor-mediated endocytosis, macropinocytosis, or phagocytosis. Once internalized, vesicular cargo activates intracellular signaling pathways that regulate inflammation, immune differentiation, apoptosis, and tissue repair.

Host cells recognize MEVs through pattern recognition receptors (PRRs) that detect conserved microbial molecules such as lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan fragments, bacterial lipoproteins, flagellin, and microbial nucleic acids. These pathogen-associated molecular patterns activate Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors, initiating downstream signaling cascades involving NF-κB, MAPK, and inflammasome pathways. Activation of these pathways stimulates the production of cytokines including TNF-α, IL-1β, IL-6, IL-8, and IL-12, thereby orchestrating innate and adaptive immune responses.

Importantly, the immunological consequences of MEV exposure depend largely on their microbial origin and molecular cargo. Vesicles released by pathogenic microorganisms frequently contain toxins and pro-inflammatory molecules that promote infection and tissue damage. Conversely, vesicles derived from commensal and probiotic bacteria often exhibit anti-inflammatory properties by enhancing regulatory T-cell responses, promoting interleukin-10

secretion, and strengthening epithelial barrier function. These contrasting activities underscore the dual role of MEVs as both mediators of disease pathogenesis and maintainers of host immune homeostasis.

5. Microbial Extracellular Vesicles in the Gut Microbiota–Host Axis

The gastrointestinal tract harbors a complex microbial ecosystem that profoundly influences host metabolism, immunity, and health. Recent studies have established MEVs as major mediators of communication within the gut microbiota–host axis. Owing to their nanoscale size, vesicles readily diffuse through the intestinal mucus layer and interact with epithelial cells and immune cells of the gut-associated lymphoid tissue (GALT). Through these interactions, MEVs regulate epithelial barrier integrity, mucosal immunity, and inflammatory homeostasis.

Beneficial bacteria such as *Bacteroides fragilis*, *Lacticaseibacillus rhamnosus*, and *Lactiplantibacillus plantarum* produce vesicles enriched with immunomodulatory molecules that reinforce tight junction proteins, stimulate mucin production, and induce regulatory T-cell differentiation. These activities reduce intestinal permeability and maintain mucosal homeostasis. In contrast, vesicles released by enteric pathogens may disrupt epithelial barriers by delivering toxins and proteases, leading to increased intestinal permeability and chronic inflammation. Remarkably, gut-derived MEVs can enter systemic circulation and reach distant organs, giving rise to the concept of the microbiota–organ axis. Emerging evidence implicates microbial vesicles in regulating the gut–brain, gut–liver, gut–lung, and gut–adipose tissue axes, where they influence neuroinflammation, metabolic regulation, hepatic immunity, and systemic inflammatory responses. These findings position MEVs as central mediators of host physiology beyond the gastrointestinal tract.

6. Biomedical and Biotechnological Applications

The intrinsic biological properties of microbial extracellular vesicles have generated significant interest in their application across medicine, biotechnology, and food science. Their nanoscale dimensions, natural biocompatibility, membrane stability, and efficient cargo delivery distinguish them from many synthetic nanocarriers.

One of the most successful translational applications of MEVs is vaccine development. Outer membrane vesicles naturally display multiple microbial antigens together with potent immunostimulatory molecules, enabling robust activation of both innate and adaptive immunity. Licensed meningococcal vaccines based on bacterial outer membrane vesicles have demonstrated the clinical feasibility of this approach. Current research is expanding vesicle-based vaccines against tuberculosis, cholera, *Helicobacter pylori*, multidrug-resistant bacteria, and several viral pathogens.

MEVs are also emerging as promising drug delivery systems. Their lipid bilayer protects encapsulated therapeutic molecules—including antimicrobial peptides, nucleic acids, proteins, CRISPR-Cas components, and small-molecule drugs—from degradation while facilitating efficient uptake by target cells. Advances in synthetic biology now permit the engineering of

vesicles with customized cargo and improved tissue specificity, opening new possibilities for precision medicine and targeted antimicrobial therapy.

In cancer immunotherapy, bacterial vesicles stimulate dendritic cell maturation, antigen presentation, and cytotoxic T-cell activation through their pathogen-associated molecular patterns. Engineered vesicles carrying tumor-associated antigens or anticancer drugs have demonstrated encouraging preclinical efficacy with reduced systemic toxicity. Likewise, probiotic-derived vesicles are increasingly being investigated as cell-free postbiotics capable of reproducing many beneficial effects of live probiotics while minimizing biosafety concerns associated with viable microorganisms.

Beyond medicine, MEVs offer promising opportunities in food and agricultural biotechnology. Vesicles produced by lactic acid bacteria contribute to microbial communication during dairy fermentation, influence stress adaptation, and may enhance product quality. Their incorporation into functional foods and nutraceutical formulations as next-generation postbiotics represents an emerging area of research. Additionally, vesicle-based biosensors are being developed for rapid pathogen detection, food safety monitoring, and antimicrobial resistance surveillance.

7. Future Perspectives

Research on microbial extracellular vesicles (MEVs) has progressed rapidly over the past decade, transforming them from a biological curiosity into promising tools for medicine, biotechnology, and microbiome engineering. Recent advances indicate that MEVs are not merely by-products of microbial metabolism but highly regulated nanostructures that mediate intercellular communication, host–microbe interactions, and environmental adaptation. Their unique ability to transport proteins, lipids, nucleic acids, metabolites, and signaling molecules positions them at the interface of microbiology, immunology, biotechnology, and nanomedicine.

Despite these advances, several challenges must be addressed before MEVs can be translated into routine clinical and industrial applications. A major limitation is the lack of standardized protocols for vesicle isolation, purification, quantification, and characterization. Differences in culture conditions, purification methods, and analytical techniques often produce heterogeneous vesicle populations, making comparison between studies difficult. Establishing internationally accepted guidelines and reference standards will therefore be essential for improving reproducibility and accelerating translational research.

Another important research priority is understanding the molecular mechanisms governing cargo selection and vesicle biogenesis. Although proteomic and transcriptomic studies have demonstrated selective enrichment of proteins and RNAs within MEVs, the precise regulatory pathways responsible for cargo sorting remain largely unknown. Elucidating these mechanisms will facilitate the rational design of engineered vesicles with customized therapeutic payloads.

The convergence of synthetic biology, nanotechnology, multi-omics, and artificial intelligence (AI) is expected to redefine the future of MEV research. Engineered vesicles with enhanced targeting specificity, reduced immunogenicity, and programmable cargo are being developed for precision drug delivery, vaccine platforms, and microbiome modulation. Simultaneously, AI-

assisted analysis of proteomic, lipidomic, and transcriptomic datasets is expected to improve cargo prediction, biomarker discovery, and therapeutic optimization. These interdisciplinary approaches are likely to accelerate the translation of MEVs from laboratory research to precision medicine and sustainable biotechnology.

Conclusion

Microbial extracellular vesicles have fundamentally reshaped our understanding of microbial communication by demonstrating that microorganisms actively exchange complex biological information through nanoscale membrane-bound structures. Produced by bacteria, fungi, and archaea, MEVs selectively transport proteins, lipids, nucleic acids, metabolites, and signaling molecules that regulate microbial physiology, antimicrobial resistance, biofilm formation, and host–microbe interactions. Their central role in maintaining gut microbiota homeostasis, modulating immune responses, and mediating interkingdom communication has established them as key determinants of health and disease.

Beyond their physiological importance, MEVs have emerged as versatile platforms for vaccine development, targeted drug delivery, cancer immunotherapy, diagnostics, postbiotic formulations, and precision microbiome engineering. Their natural biocompatibility, intrinsic targeting capability, and capacity for molecular engineering provide significant advantages over many conventional nanocarrier systems. However, challenges associated with vesicle heterogeneity, scalable production, safety evaluation, and regulatory standardization remain major barriers to widespread clinical implementation.

Continued integration of molecular microbiology with systems biology, synthetic biology, nanotechnology, and computational sciences will further expand the therapeutic and industrial potential of MEVs. As our understanding of vesicle biology continues to evolve, microbial extracellular vesicles are expected to become indispensable tools for developing innovative strategies in precision medicine, biotechnology, agriculture, and food science, ultimately contributing to improved global health and sustainable biotechnological solutions.

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PLANT MICROPROTEINS: SMALL REGULATORY MOLECULES WITH MAJOR ROLES IN DEVELOPMENT, SIGNALING, AND CROP IMPROVEMENT

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Abstract

The major goals of modern agriculture are optimization and improvement of crops to sustain high productivity with minimal environmental impact. A variety of molecular methods have been developed for the regulation of trait-specific genes at the transcriptional and translational levels. Targeted post-translational modification for trait regulation is precisely done by microProteins which are involved in fine-tuning protein activity without altering overall plant structure and metabolism. MicroProteins (miPs) are small regulatory proteins with a single domain and size ranging from 5 to 20 kDa and they have their role in post-translational regulation of gene expression through protein-protein interactions. These proteins are referred to as 'microProteins' because of their small size and negative regulatory actions analogous to microRNAs (miRNAs). Deletions and duplications within genes encoding multidomain proteins have driven the evolution of single-domain proteins, which subsequently interact with their ancestral proteins from which they originated. It is hypothesized that microProteins and the proteins with which they interact have evolutionary relations.

Photoperiod-dependent flowering in rice is regulated by transcription factor HEADING DATE 1 (Hd1), which acts both as an activator and repressor of flowering in a daylength-dependent manner. An investigation was carried out using microProteins as a tool to modify rice sensitivity to the photoperiod by designing a synthetic Hd1 microprotein (Hd1miP) capable of interacting with Hd1 protein and overexpressing it in rice. Transgenic OX-Hd1miP plants flowered significantly earlier than wild-type plants when grown in long-day conditions. These results show the potential of microProteins to serve as tools for modulating crop traits and unravelling protein function. Using the synthetic microProtein approach an investigation was carried out on multidomain proteins of different classes. It has revealed that multidomain proteins like DCL1, BRI1, and CRY1 can be regulated by overexpressing their protein-protein interaction domains as synthetic microProteins. These results show that microProteins can inhibit any multidomain protein containing a protein-protein interaction domain.

The advances in analytical, molecular and computational methods have helped to expand the pool of miPs characterized across species. However, there is a lot to know about the cellular dynamics and specificities concerning the miPs. MicroProteins offer exciting potential for plant

breeding due to their targeted regulation and small size, allowing for easier manipulation. However, their precise functions and effectiveness in complex traits remain a challenge. Ongoing research focuses on identifying and characterizing novel microProteins and developing techniques for their precise manipulation in breeding programs.

Keywords: Microproteins, Regulatory Factors, Little Ninja, Little Zipper, *Arabidopsis thaliana*, Protein-Protein Interaction.

Introduction

Microproteins (miPs) are a recently recognized class of small regulatory proteins, generally ranging from 7-20 kDa in size and consisting of fewer than 150 amino acids. Despite their small size, miPs play important regulatory roles by containing specialized protein-protein interaction (PPI) domains that enable them to modulate the activity of larger proteins. Unlike conventional proteins, miPs typically lack enzymatic domains and function primarily by forming heterodimers with their target proteins, thereby altering or disrupting their normal activities. These proteins act as important post-translational regulators, and a majority of identified miPs have been found to originate from transcription factor families, where they regulate diverse cellular and developmental processes.

Origin of Microproteins

Microproteins (miPs) can originate through different evolutionary and molecular mechanisms, including gene duplication, alternative transcript generation, and post-translational processing.

The first microprotein identified was the DNA-binding inhibitor (Id) protein, which was isolated from a cDNA library generated from murine erythroleukemia cells. Id was characterized as a negative regulator of helix-loop-helix (HLH) DNA-binding proteins and was shown to interact with MyoD1 (Myoblast Determination Protein 1), a key transcriptional regulator involved in muscle differentiation in mice. The Id protein contains a helix-loop-helix domain of approximately 16 kDa and regulates the activity of several basic helix-loop-helix transcription factors, including E2A/TCF3, HEB/TCF12 and E2-2/TCF4 (Ke *et al.,* 2018). Among these, E2A/TCF3 plays a crucial role in regulating cell proliferation and differentiation in both normal and cancerous cells, whereas HEB/TCF12 contributes to lineage-specific differentiation processes, including the osteogenic differentiation of bone marrow mesenchymal stem cells (Yi *et al.,* 2012). These findings established miPs as important regulators of transcription factor activity and cellular differentiation.

In plants, the first microprotein family identified was the LITTLE ZIPPER (ZPR) protein family (Wenkel *et al.,* 2007). ZPR proteins contain a conserved leucine zipper domain that allows them to interact with homeodomain-leucine zipper III (HD-ZIPIII) transcription factors. Through these interactions, ZPR proteins regulate key developmental processes, including leaf patterning, shoot apical meristem maintenance and stem cell regulation. In Arabidopsis, ZPR proteins were shown to promote abaxial leaf development by modulating HD-ZIPIII activity, highlighting their role as

important regulatory components in plant development. The discovery of ZPR proteins expanded the understanding of miP functions beyond animals and demonstrated that microproteins are conserved regulatory molecules involved in diverse biological processes across different organisms.

Trans-microproteins (Trans-miPs)

Trans-miPs arise through the duplication of ancestral genes followed by evolutionary reduction, resulting in the retention of only the domain required for protein-protein interaction and dimerization. These microprotein-encoding genes exist as independent transcriptional units that are evolutionarily related to larger, multi-domain protein-coding genes. During evolution, the duplicated gene loses most functional domains while preserving the interaction domain, allowing the resulting miP to regulate the activity of its larger protein counterpart through competitive or inhibitory interactions.

Cis-microproteins (Cis-miPs)

Cis-miPs are generated from the same genomic locus as their larger parent proteins. They are produced through mechanisms such as alternative polyadenylation, alternative splicing or the use of alternative transcription start sites, which generate shorter mRNA transcripts encoding only the protein-protein interaction or dimerization domain. These truncated proteins retain the ability to interact with and modulate the activity of the full-length protein encoded by the same gene.

In addition to transcriptional mechanisms, microproteins can also be generated through post-translational processing. Proteolytic cleavage of larger precursor proteins can produce smaller protein fragments that retain functional interaction domains and interfere with the activity of their unprocessed parent proteins. Together, these mechanisms contribute to the diversity and regulatory functions of microproteins across different organisms.

Types of Microprotein Interactions

Microproteins regulate the activity of their target proteins primarily through protein-protein interactions, which can be classified into homotypic and heterotypic interactions.

- **Homotypic Interactions** occur between proteins that possess the same or highly similar protein-protein interaction domains. In this type of interaction, microproteins form homo or heterodimers with closely related proteins, often competing with the formation of functional protein complexes. As a result, they can inhibit or modify the activity of their target proteins by preventing the assembly of active multimers or by altering their DNA-binding ability, subcellular localization, or protein stability.
- **Heterotypic Interactions** involve the association of a microprotein with proteins containing different interaction domains or with unrelated regulatory proteins. These interactions enable microproteins to influence diverse signaling pathways by recruiting, sequestering, or modulating the activity of proteins outside their own family. Through heterotypic interactions, microproteins can regulate multiple cellular processes, including

signal transduction, transcriptional regulation, protein degradation and developmental pathways thereby expanding their functional versatility.

Modes of MicroProtein Regulation

Mechanisms of Microprotein-Mediated Regulation

Microproteins regulate the function of their target proteins through several distinct mechanisms, including sequestration, repressor complex formation, cytoplasmic retention and ion channel inhibition.

1. Sequestration

In sequestration, microproteins interact with their target proteins and prevent them from binding to their normal partners or DNA target by forming inactive protein complexes. miPs reduce the availability of functional target proteins and inhibit their downstream activity. For example, Id/Id-like proteins and LITTLE ZIPPER (ZPR) proteins regulate transcription factor activity through sequestration. Id proteins interact with basic helix-loop-helix (bHLH) transcription factors, such as E proteins and MyoD, preventing their DNA-binding activity. Similarly, ZPR proteins interact with HD-ZIPIII transcription factors and modulate their activity during plant development.

2. Repressor Complex Formation

Microproteins can promote the formation of transcriptional repressor complexes by recruiting inhibitory proteins or modifying the composition of transcription factor complexes. This mechanism prevents target proteins from activating gene expression. For example, MIF2 (mini zinc finger protein 2) and miP1a/miP1b act by interacting with transcriptional regulators and promoting repressive complexes that regulate gene expression.

3. Cytoplasmic Retention

Some microproteins regulate target proteins by altering their subcellular localization. Through direct interaction, miPs can retain transcription factors or signaling proteins in the cytoplasm, preventing their transport into the nucleus and thereby inhibiting their regulatory functions. MIF (mini zinc finger protein) family members have been shown to regulate target proteins through cytoplasmic retention mechanisms.

4. Ion Channel Inhibition

Microproteins can also regulate membrane-associated proteins, including ion channels. In this mechanism, miPs interact with ion channel proteins and alter their activity, affecting ion transport across membranes. A well-characterized example is the viral microprotein Vpu from human immunodeficiency virus (HIV), which interacts with host ion channels and modulates membrane protein activity.

Types of Microprotein Functions

Microproteins regulate the activity of their target proteins through specific protein-protein interactions and function as important modulators of cellular processes. Depending on the nature

of their interaction with target proteins, microproteins can either maintain proteins in an inactive state, disrupt functional complexes or regulate the transition between inactive and active forms.

Stable Repressed Complex

Microproteins can interact with target proteins to form a stable repressed inactive complex, in which the target protein remains unable to perform its normal function. Activation of the system requires additional regulatory factors or environmental signals that disrupt the inhibitory interaction and allow the formation of an active protein complex. This mechanism enables microproteins to act as molecular regulators by controlling the availability and activity of key regulatory proteins.

Stable Active Complex

In some cases, target proteins exist as stable active complexes that are essential for normal cellular function. Microproteins can interfere with these functional complexes by binding to one of their components, disrupting the interaction network, and converting the active complex into an inactive state. Through this mechanism, microproteins function as negative regulators by preventing target proteins from carrying out their downstream activities.

Microproteins may also regulate the stability and activity of protein complexes by influencing the equilibrium between active and inactive states. By selectively interacting with specific components, microproteins can either stabilize an active complex or prevent unnecessary activation, thereby providing precise control over cellular pathways.

PIF – Phytochrome Interacting Factor

A well-studied example of microprotein-mediated regulation in plants involves Phytochrome-Interacting Factors (PIFs), which are basic helix-loop-helix (bHLH) transcription factors that regulate light-responsive developmental processes, including shade avoidance responses. PIF-type bHLH proteins activate downstream target genes involved in light signaling and plant growth regulation.

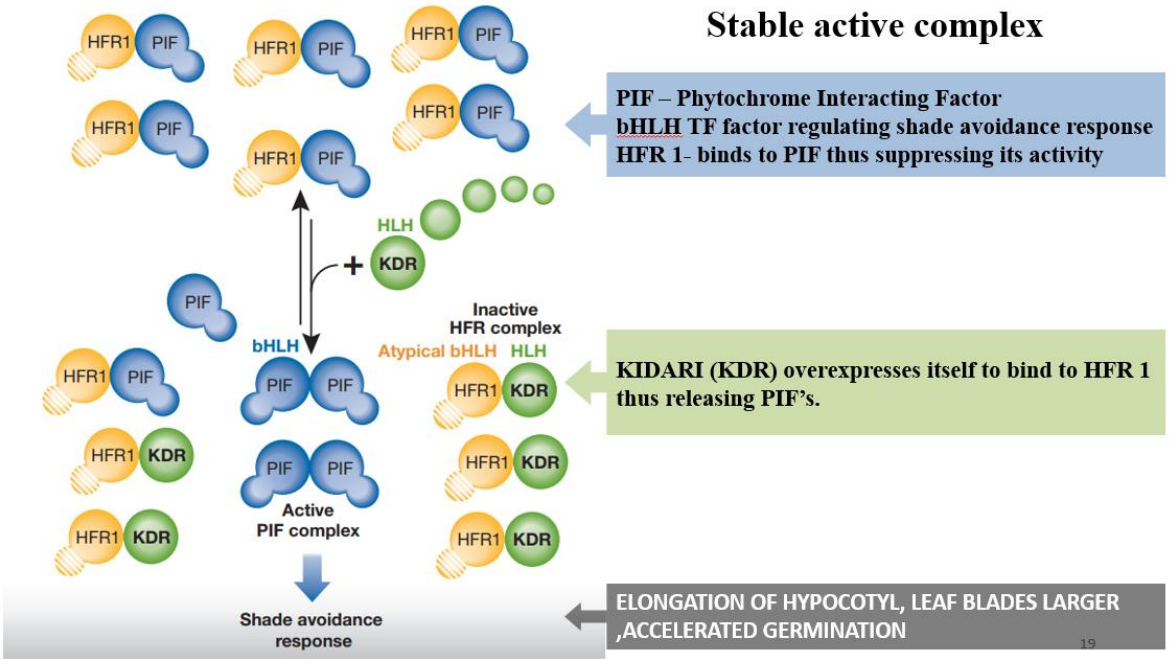
HFR1 – LONG HYPOCOTYL IN FAR-RED 1

The atypical bHLH protein HFR1 functions as a microprotein-like regulator by interacting with PIF proteins. HFR1 forms non-DNA-binding heterodimers with PIFs, thereby preventing PIFs from binding to target DNA sequences and suppressing their transcriptional activity. This interaction maintains PIFs in an inactive state and negatively regulates light response pathways.

KIDARI (KDR)

The microprotein KIDARI (KDR) regulates this pathway by interacting with HFR1. Overexpression of KDR results in the sequestration of HFR1, reducing its ability to inhibit PIF proteins. The release of PIFs restores their transcriptional activity, leading to increased expression of downstream light-responsive genes. Consequently, enhanced PIF activity results in developmental changes such as elongated hypocotyls, larger leaf blades, and accelerated germination.

This PIF–HFR1–KDR regulatory module demonstrates how microproteins control biological processes by regulating the formation, disruption, and stabilization of protein complexes, thereby acting as molecular switches in plant development and environmental responses.



Functional Role of Microproteins in Plants

Microproteins have emerged as important regulatory molecules in plants, where they modulate diverse developmental and physiological processes by interacting with key transcription factors and signaling proteins. Although they lack functional domains required for enzymatic activity, their conserved protein-protein interaction domains enable them to regulate target proteins through sequestration, inhibition and modulation of protein complex formation. Plant microproteins play crucial roles in processes including shade avoidance, light responses, leaf and shoot development and hormone signaling.

1. Shade Avoidance Response in Plants

Plants growing under dense vegetation experience reduced red (R) to far-red (FR) light ratios due to the absorption of red light by neighbouring plants. This change in light quality is perceived by phytochrome photoreceptors and serves as an environmental signal indicating competition for light resources. In response, plants initiate a series of morphological and developmental changes collectively known as the shade avoidance response (SAS).

The shade avoidance response is characterized by rapid elongation of stems and leaf petioles, reduced leaf blade expansion, and altered resource allocation toward vertical growth. During this process, energy and resources that would normally support leaf expansion and storage organ development are redirected toward stem elongation, allowing plants to compete more effectively for available light. Understanding SAS mechanisms is particularly important for agriculture, where high-density planting systems frequently expose crops to shade-like conditions.

Phytochrome-interacting factors (PIFs), particularly PIF4 and PIF5, are bHLH transcription factors that act as central regulators of shade avoidance responses. Under shade conditions, activated PIFs promote the expression of downstream genes involved in elongation growth. However, excessive PIF activity can lead to uncontrolled growth responses. Therefore, atypical bHLH microproteins regulate PIF activity to maintain an appropriate balance between growth and resource allocation.

The microprotein LONG HYPOCOTYL IN FAR-RED 1 (HFR1) interacts with PIF proteins and forms non-DNA-binding heterodimers, preventing PIFs from activating their target genes. Another microprotein, KIDARI (KDR), regulates this pathway by interacting with HFR1 and releasing PIFs from HFR1-mediated inhibition. This regulation fine-tunes shade avoidance responses and ensures optimal plant growth under changing light environments.

2. Light-Mediated Responses in Plants

Seed germination and early seedling development are strongly influenced by light availability. Seeds buried beneath the soil germinate in darkness by utilizing stored energy reserves. The developmental program adopted by seedlings under dark conditions is known as skotomorphogenesis, which is characterized by elongated hypocotyls, closed cotyledons, and the formation of an apical hook. This growth strategy enables seedlings to rapidly emerge from the soil while protecting the delicate shoot apical meristem.

Dark-grown seedlings rely on transcription factors such as PIFs and EIN3 to maintain skotomorphogenic development. However, these transcriptional regulators must be rapidly suppressed upon exposure to light to initiate photomorphogenesis, including cotyledon opening and chloroplast development.

This transition is regulated by microproteins miP1a and miP1b, which are approximately 13 kDa in size and are predominantly expressed in hypocotyls and cotyledons. Upon the transition from dark to light conditions, miP1a/b rapidly accumulate and interact with transcription factors such as PIF3 and EIN3. These interactions interfere with their oligomerization and reduce their transcriptional activity, thereby promoting the shift from dark-adapted seedling development to light-dependent growth.

3. Leaf and Shoot Development

Microproteins also play important roles in regulating leaf polarity and shoot development. A major example involves the homeodomain-leucine zipper III (HD-ZIPIII) transcription factor family, which is essential for establishing adaxial-abaxial polarity during leaf development. Leaf polarity refers to the differentiation between the adaxial region (upper surface of the leaf) and the abaxial region (lower surface of the leaf). Proper establishment of this polarity is essential for normal leaf architecture, cellular specialization and functional development of photosynthetic tissues.

HD-ZIPIII proteins normally function by forming homodimers that bind DNA and regulate genes involved in shoot apical meristem maintenance and leaf patterning. The dynamic balance between active HD-ZIPIII complexes and inactive complexes formed through interaction with microproteins is critical for maintaining normal plant development.

The LITTLE ZIPPER (ZPR) microprotein family regulates HD-ZIPIII activity by interacting with these transcription factors through their leucine zipper domains. Overexpression of ZPR proteins disrupts HD-ZIPIII homodimer formation and reduces their DNA-binding ability. This leads to developmental defects, including arrested shoot apical meristem activity and abnormal downward curling of leaves. Thus, ZPR proteins act as important modulators of HD-ZIPIII mediated developmental pathways.

4. Hormone Signaling

Microproteins are also involved in regulating plant hormone signaling pathways. A recent example is LITTLE NINJA (LNJ), a NINJA-related microprotein identified as a regulator of jasmonic acid (JA) signaling. The NINJA protein functions as a negative regulator of JA signaling by interacting with JAZ (JASMONATE ZIM-DOMAIN) repressors and recruiting transcriptional corepressors. The microprotein LITTLE NINJA modulates this repression mechanism and fine-tunes JA-responsive gene expression.

Ectopic overexpression of LNJ microprotein (LNJmiP) in barley and rice resulted in plants with altered architecture, including reduced height and increased branching/tillering compared with wild-type plants (Shin-Young Hong *et al.* 2020). The tillering phenotype was transferable between grass species, suggesting that manipulation of LNJ activity could provide potential strategies for modifying crop architecture.

Synthetic Microprotein Approach

The synthetic microprotein (syn-miP) approach is an emerging strategy used to study and manipulate protein-protein interaction networks by designing artificial microproteins that specifically target interaction domains of larger multidomain proteins. In this approach, well-characterized multidomain proteins from *Arabidopsis thaliana* are selected as templates and their individual protein-protein interaction domains are used to generate synthetic microproteins. These engineered microproteins retain the ability to interact with specific target proteins but lack the additional functional domains present in the full-length proteins, allowing researchers to investigate the role of individual protein interactions in biological processes.

For the generation of synthetic microproteins, cDNA sequences encoding selected interaction domains are cloned into a binary expression vector pJAN33 under the control of the constitutive Cauliflower Mosaic Virus 35S promoter (CaMV35S). The construct contains the BAR gene as a selectable marker for transformation and selection of transgenic plants. The recombinant binary vector carrying the synthetic microprotein construct is introduced into Arabidopsis thaliana ecotype Columbia (Col-0) using the floral dip transformation method.

One of the synthetic microproteins generated through this approach targeted the PAZ domain of DICER-LIKE 1 (DCL1), a type III RNase III enzyme involved in plant microRNA (miRNA) biogenesis. DCL1 is a multidomain protein that plays a central role in processing primary miRNAs (pri-miRNAs) into precursor miRNAs (pre-miRNAs). During miRNA processing, DCL1 forms a multiprotein complex with other regulatory proteins, including HYPONASTIC LEAVES 1 (HYL1), SERRATE (SE), and DAWDLE (DDL).

The PIWI/ARGONAUTE/ZWILLE (PAZ) domain of DCL1 functions as an important protein-protein interaction domain that mediates the recruitment and assembly of components required for efficient pri-miRNA processing. Based on this function, researchers hypothesized that expressing a synthetic microprotein specifically targeting the PAZ domain of DCL1 could interfere with the formation of the DCL1-HYL1-SE-DDL processing complex and thereby disrupt miRNA biogenesis.

Effect of Synthetic DCL1 Microprotein Expression

Transgenic Arabidopsis plants ectopically expressing the DCL1 PAZ domain synthetic microprotein (miP-DCL1) exhibited multiple developmental defects, demonstrating the effectiveness of the synthetic microprotein approach in manipulating endogenous protein complexes. The observed phenotypes included:

- Abnormally shaped cotyledons
- Altered leaf morphology
- Reduced rosette size
- Floral developmental defects

These developmental abnormalities indicate that disruption of DCL1 protein interactions through synthetic microproteins affects miRNA-mediated regulatory pathways.

Techniques to Identify and Characterize Microproteins and Short Peptides in Plants

The identification and characterization of plant microproteins remain challenging due to their small size, low abundance and similarity to other short peptides or degraded protein fragments. Therefore, integrated approaches combining proteomics, transcriptomics and computational prediction tools are widely used to discover and validate novel microproteins.

1. Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid chromatography coupled with mass spectrometry (LC-MS/MS) is one of the most powerful approaches for detecting and characterizing small proteins and peptides. In this method, protein samples are separated based on their chemical properties using liquid chromatography and subsequently identified according to their mass-to-charge ratio through mass spectrometry. Specialized proteomic workflows optimized for small proteins enable the detection of low molecular weight proteins that are often missed in conventional proteomic analyses. These approaches provide direct evidence of microprotein expression, sequence confirmation and post-translational modifications.

2. Next-Generation Sequencing (NGS)

Next-generation sequencing technologies integrated with transcriptomic and proteogenomic databases have significantly expanded the discovery of novel microproteins. RNA sequencing (RNA-seq) and ribosome profiling approaches allow researchers to identify small open reading frames (sORFs) that have the potential to encode functional microproteins. By integrating transcript data with peptide databases and mass spectrometry results, previously unannotated microprotein-coding genes can be identified and validated. These approaches have revealed that many small translated products previously considered non-functional may possess important regulatory roles.

3. Computational Prediction and Validation Tools

Computational approaches play an essential role in distinguishing functional microproteins from other small proteins, random peptides or protein degradation products. Bioinformatic tools such as miPFinder are used to predict and validate potential microproteins based on specific characteristics, including protein size, conserved protein-protein interaction domains, evolutionary relationships and sequence similarity with known microproteins. These computational predictions are often combined with experimental approaches to confirm microprotein expression and biological function.

Conclusion

The increasing challenges to global food security caused by climate change, environmental stresses and unsustainable agricultural practices highlight the urgent need for innovative and sustainable approaches to improve crop productivity and resilience. Microproteins have emerged as important regulatory molecules that control diverse physiological and developmental processes in plants through precise modulation of protein-protein interactions. Due to their small size, structural simplicity and ability to specifically regulate target proteins, microproteins represent promising candidates for the development of synthetic microproteins (syn-miPs) aimed at improving plant stress tolerance, growth regulation and productivity. Advances in understanding microprotein-mediated regulatory mechanisms, including their interactions with transcription factors and signaling components, provide valuable opportunities for utilizing them as biotechnological tools in crop improvement. A comprehensive understanding of microprotein evolution, function and regulatory networks is therefore essential for harnessing their potential in plant bioengineering. Future applications of synthetic microproteins may contribute to the development of climate-resilient crops and sustainable agricultural strategies to address global food security challenges.

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MOLECULAR BASIS OF ZOONOTIC PATHOGEN EMERGENCE AND CROSS-SPECIES TRANSMISSION

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Abstract

The emergence of zoonotic pathogen is a complex and multistage process that shaped by interaction between pathogen biology, host susceptibility, ecological condition and opportunities for interspecies contact. Various conditions like environmental disruption, agricultural intensification, livestock-wildlife interaction, globalization and change in human behaviour may increase the opportunities for pathogen exposure and cross species transmission but requires a pathogen to overcome multiple molecular and cellular barriers. These barriers include receptor incompatibility, restricted cellular entry, innate immune response and inefficient pathogen shedding. Various genetic mechanisms can facilitate the changes in this barrier and can influence the emergence and cross species transmission of zoonotic pathogens. This chapter reviews the molecular determinants of zoonotic pathogen emergence, mechanisms involved in cross species transmission. Recent technologies which can be useful for investigation of cross species transmission and emergence of disease are also discussed in this chapter. Integration of molecular evidence with ecological and epidemiological information through a One Health framework are also included in this chapter.

Keywords: Zoonotic Emergence, Disease Spillover, Cross Species Transmission, Molecular Epidemiology, Genomic Surveillance, One Health.

1. Introduction

Zoonotic diseases are infectious diseases that can be naturally transmitted between animal and humans. Emergence of these diseases play important role in human health, animal health, food security and socioeconomic stability of any country. Most of the emerging infectious diseases of humans are zoonotic and animal origins. (Jones *et al.,* 2008; Taylor *et al.,* 2001). Wildlife species are most important reservoirs of microbial pathogens; however, domestic animals may also act as reservoir, amplifying, bridging or intermediate hosts which can facilitate pathogens transmission to humans (Plowright *et al.,* 2017).

Human activities like land use change, deforestation, agricultural expansion, wildlife trade, urbanization also modify the frequency and intensity of interaction between wildlife, livestock and humans which leads to alter the disease emergence process and increase opportunities for

pathogen exposure (Allen *et al.,* 2017; Gibb *et al.,* 2020; Jones *et al.,* 2013). Only increased exposure alone does not ensure successful infection or sustained transmission but pathogen should have to overcome the host specific molecular, cellular, physiological and immunological barriers.

Transmission of pathogen from one species to another is prevented by various factors which are collectively known as species barriers. These barriers operate at several stages of infection, including receptor recognition, cellular entry, genome replication, protein synthesis, evasion of innate immunity, tissue dissemination and pathogen shedding. Cross-species transmission depends on compatibility between pathogen characteristics and biological environment of recipient host (Long *et al.,* 2019; Parrish *et al.,* 2008). Sustained cross species transmission generally requires the pathogen to process or acquire, characteristics that support efficient replication, tissue trophism, shedding and transmission among individual of recipient species (Wolfe *et al.,* 2007).

Genetic diversity facilitates biological material upon which natural selection acts during host adaption. Various genetic mechanism like mutation, recombination and genome reassortment leads to change in receptor specificity, cellular trophism, replication efficiency, virulence and transmissibility. However, the effect of genetic change mainly depends on genome of pathogen and biological characteristics of host. Successful emergence of pathogen is therefore rarely attributable to single mutation and more commonly results from interactions among multiple pathogen, host and environmental factors (Holmes, 2009; Parrish *et al.,* 2008).

Advances in molecular biology have substantially improved the investigation of zoonotic emergence. Various techniques like whole genome sequencing, metagenomics, comparative genomics, phylogenetics and molecular epidemiology enable researchers to characterize various pathogens and investigate the molecular mechanisms associated with cross species transmission and emergence. Integration of genomic information with epidemiological and ecological data can also assist in identification of potential pathways of emergence and transmission (Gardy & Loman, 2018).

This chapter discuss the molecular mechanism involved in zoonotic pathogen emergence and cross species transmission. Particular emphasis is placed on species barriers, host receptor recognition, pathogen genetic evolution, adaption to host cellular environment and immune invasion. This chapter also discusses molecular technologies used to investigate pathogen emergence and highlights the importance of integrating molecular, epidemiological and ecological information within One health frameworks.

2. Zoonotic Spillover and Species Barrier

Zoonotic spillover is complex process which involve the interaction between pathogen, host and environment. For occurrence of spillover, pathogen must have to overcome the ecological and biological barriers to transmit into new host (Plowright *et al.,* 2017). This process starts with

maintenance and circulation of the pathogen within one or more reservoir host population, which act as stable ecological systems where pathogen can permanently persist without any clinical disease (Haydon *et al.,* 2002). Once pathogen released or shed from the reservoir host through respiratory droplets, blood, saliva, urine or reproductive fluids, it's stability and dissemination are depends on environmental variables and transmission routes. The overall probability of human or recipient host exposure is ultimately determined by a combination of pathogen abundance, shedding, intensity, environmental persistence, host population density and interspecies contacts (Plowright *et al.,* 2017).

Following the exposure, pathogen have to enter in recipient host in adequate infectious dose and overcome some physiological barriers to cause infection by reaching specific target cell. The susceptibility of host depends on various factors like age, genetics, immune status, physiological condition. Many time exposures occurs but pathogen is unable to cause infection due to incompatible cellular entry, restricted replication, low receptor binding, immediate elimination by local immune defenses. When successful infection is established in new host it either transmitted to others or it become self-limiting "dead-end" spillover. Zoonotic emergence occurs only when pathogen acquires biological characteristics which are necessary for efficient replication, appropriate tissue trophism, high shedding and sustainable population transmission among individuals of recipient species (Wolfe *et al.,* 2007). Consequently, zoonotic emergence can be in the spectrum of occasional, isolated spillover event to long term host adaption and permanent establishment of pathogen within the new host population (Plowright *et al.,* 2021).

Various species factors which interfere the zoonotic spillover are known as species barriers. These can be anatomical, physiological, cellular, genetic and immunological factors which restrict the host range of pathogen (Parrish *et al.,* 2008). At cellular level, these barriers restrict the pathogen lifecycle by receptor binding, endosomal membrane fusion, intracellular replication, protein translation and virion release. The probability of overcome these barriers by pathogen is influence by host phylogenetic relationships. Species which are closely related to natural reservoir have high chances of transmission of infection. However, ecological contact can alter the host range of pathogen by characterizing broad receptor usage or genetic flexibility (Davies and Pedersen, 2008; Longdon *et al.,* 2014). Species barrier can be viewed as interaction between multi host compatibility of pathogen and cellular restriction factors of host species (Long *et al.,* 2019). Every pathogen doesn't require genetic mutation to break these barriers, some pathogen have characteristics of utilization of host energy source, broad receptor binding affinity, suppression of host immune response pathway, which facilitates the transmission to other individual and host adaption (Parrish *et al.,* 2018). Capability to pathogen to cause infection in various host species is described as host range. (Woolhouse *et al.,* 2001). Pathogen with narrow host range can only infect restricted species, which are highly related to each other. While pathogen with broad host range can infect different taxonomic species which may not

related to each other. This host range is usually due to cellular mechanism of host. Which restrict the pathogen entry, replication and shedding.

3. Molecular Determinants of Cross-Species Transmission

First biological barrier come across pathogen while entering into new host species is cellular attachment. This process relies on binding of host receptors. Differences in receptor sequence, structure, distribution and expression among various host species blocks cellular entry of pathogen. Incompatibility with host receptor act as major component of species barrier, though only receptor expression alone cannot lead to infection because various other factors like host activating proteases, specific membrane compositions, endosomal conditions and intracellular factors are required. A classic example of receptor barrier can be seen in influenza A virus, where viral hemagglutinin binds to terminal sialic acid residues on host cell glycoconjugates. Avian influenza viruses generally bind to sialic acid linked to galactose through α2,3 linkage, while human adapted viruses bind with α2,6 linked sialic acids. This shows that different anatomical distribution of same receptor within same respiratory tissues heavily dictates viral attachment and tissue trophism (Imai and Kawaoka, 2012; Long *et al.,* 20198). Corona virus uses similar receptor binding strategy, relying on specific domain within the outer spike protein to bind with host cell to mediate membrane fusion. Small mutation or recombination of gene can modify the receptor binding capacity and alter the ability of coronavirus to infect the different host species, provided spike protein is appropriately activated and cleaved by host specific proteases (Graham and Baric, 2010). Nipah virus uses its attachment glycoprotein to bind with ephrin-B2 and ephrin-B3 receptors. These receptors are highly conserved in mammalian species and widely expressed in arterial endothelial cells and neural tissue, therefore virus have naturally broad host range and systemic tissue trophism (Bonaparte *et al.,* 2005; Negrete *et al.,* 2005). Molecular changes which increase receptor binding affinity can improve cellular entry in new host but excessive binding, altered protein stability or incompatibility with cellular processes can reduce overall pathogen stability. Therefore, host adaptation always requires fine balance among receptor recognition, cellular entry, replication and virion release.

As pathogen establish in new host range, genetic changes occur in the pathogen as part of natural selection and create new variants which have fundamental biological material to infect new host species. Mutation cause alteration of genome structure and function of pathogen protein. RNA virus has high mutation rate due to lack proofreading capacity of RNA dependent RNA polymerase, which cause production of diverse population of related variants, which increase the chances that some variants posses pre-existing characteristics compatible with new host. In coronaviruses presence of proofreading exonuclease increases replication fidelity relative to other RNA viruses, but substantial genetic variation and recombination still occur within their populations (Denison et al, 2011). Other than small point mutation, pathogen uses larger genetic mechanisms to adapt into new environment. Genetic recombination involves exchange of genetic

information between related genomes, like template switching during RNA synthesis in coronavirus. (Wells *et al.,* 2023). Gene recombination sometime generates novel combinations that alter receptor usage, antigenicity or host range. Virus like influenza A which poses segmented genome, genome reassortment occurs when segments are exchanged during co-infection of single cell (Webster *et al.,* 1992). Reassortment can rapidly produce viruses with new combinations of surface glycoproteins and internal genes that alter antigenicity, host range, replication efficacy or transmission potential. Respiratory tissues of pig can support concurrent infection with influenza viruses from different host origins and act as potential mixing host. Reassortment can occurs in other susceptible animal hosts but its success depends on functional compatibility in newly combined genome segments (Ma *et al.,* 2009). In bacterial pathogens, horizontal gene transfer act as major evolutionary mechanism which utilize mobile genetic elements like plasmids, bacteriophages, transposons and integrins to facilitate rapid movement of antimicrobial resistance genes, virulence determinants, metabolic pathways and host adaptive traits among bacterial populations (Frost *et al.,* 2005).

After cellular entry, pathogen have to interact with host mechanism for genome replication, protein synthesis, acquire nutrients and exit from cell. These processes depend on multiple interaction between pathogen and host molecules. Host range is thus influenced by ability of pathogen to exploit host dependency factors which required to complete replication cycle, while simultaneously neutralizing host restriction factors which act as intracellular defenses (Long *et al.,* 2019). Efficient transmission requires appropriate tissue tropism, as pathogen replicating in deep tissue may cause sever clinical disease but have limited shedding, whereas adaptation of pathogen in upper respiratory tract enhance aerosol transmission and replication in intestinal tissue promotes fecal shedding and environment dissemination. Molecular adaption within host tissue not only increase ability to infect new species but also increase overall probability of establishing onward transmission.

4. Host Immune Responses and Pathogen Immune Evasion

Innate immunity of host act as most important barrier for pathogen. Host cell receptor such as, Toll like receptors, RIG-I like receptors, NOD like receptors and various cytosolic nucleic acid sensors serve as molecular network which detect conserved pathogen associated molecular patterns. After activation, receptor release intracellular signaling cascades that stimulate rapid production of Type I and Type III interferons, inflammatory cytokines, chemokines and various cellular defense responses (Akira *et al.,* 2006).

Type I and Type III interferon play important role in early antiviral activity by binding to cellular receptors and triggering interferon stimulating genes. Activation of genes suppress the early infection by blocking pathogen entry, restrict the genome replication, inhibiting protein synthesis and disrupt assembly and release particles. Immune genes and signaling pathways are different in different species, pathogen are adapted to natural reservoir’s immune pathway but it may unable

to counter the immune pathways of new host species. However, pathogens that have capacity to target highly conserved and evolutionarily stable immune mechanisms can cause infection across multiple species due to higher biological potential to cross the species barriers.

Zoonotic pathogen has highly specialized mechanisms to evade or suppress host defenses, replication and survival. Viral pathogens produce accessory proteins which mask pathogen detection, block initial induction of interferon, interfere with signaling pathways, alter cytokine responses and lower antigen presentation. Bacterial pathogen uses different mechanisms such as modifying their outer surface structures, survive and replicate into host phagocytic cells, interfering with normal phagosome maturation, blocking inflammatory response or continuously varying their surface proteins. Most common example of this mechanism is in Nipah virus, where specific protein encoded in viral phosphoprotein gene directly interfere with host interferon induction and signaling pathways. (Basler, 2012; Lawrence *et al.,* 2022). By blocking these host pathways, virus significantly suppress the initial antiviral activity of cell, which facilitates rapid viral replication and systemic spread of pathogen into host tissues.

Variation in antigenic structures promotes immune escape. Pathogens may alter their antigenic molecules by various mechanisms like mutation, recombination, reassortment, gene conversion or differential gene expression. For example, Influenza A viruses undergo gradual antigenic changes by accumulating the mutation in haemagglutinin and neuraminidase. Larger antigenic changes may occur when reassortment of genes introduce in genome segments of circulating viral populations (Webster *et al.,* 1992). The relationship between virulence and transmission is also complex process. Severe disease does not necessarily have greater transmission potential. Pathogen which rapidly immobilize or kill their host may reduce the opportunities of spread, whereas mild or asymptomatic infections may have high transmission possibilities by allowing infected host to remain active. The emergence of pathogen depends on route and timing of transmission, host behavior, pathogen shedding and ecological conditions.

5. Selected Examples of Molecular Adaptation in Zoonotic Pathogens

Influenza A viruses

Influenza A viruses infect diverse range of avian and mammalian hosts. Wild aquatic birds, mostly species of Anseriformes and Charadriiformes order are major natural reservoirs of viruses (Webster *et al.,* 1992). Transmission from wild birds to poultry and animals leads to host adaption, genetic diversification and reassortment of pathogenic genes.

Influenza A virus genome has eight negative sense RNA segments. These segments can be reassorted during co-infection. Reassortment may generate new combination of haemagglutinin, neuraminidase, polymerase protein and other viral components. Haemagglutinin mediates receptor binding and membrane fusion. Avian adapted virus prefer α2,3-linked sialic acid receptors, whereas human adapted virus bind with α2,6 linked receptor Imai and Kawaoka,

2012). Substitution of various amino acids in the haemagglutinin receptor binding region can alter receptor preference in mammalian respiratory tissues.

Receptor adaption only not sufficient for transmission of disease. Viral polymerase protein has to function efficiently within mammalian cells, various substitution in polymerase proteins, particularly polymerase basic protein 2, enhance the replication of pathogen in mammalian cells. Polymerase adaptation may improve interaction with host cellular factors and support viral replication at lower temperatures in mammalian airways (Long *et al.,* 2019).

Influenza emergence involves interaction between receptor binding specificity, polymerase compatibility, genome reassortment, protein stability, immune evasion, tissue tropism and ecological exposure. Therefore, multiple viral traits together should be considered for epidemiological evidence of disease.

Coronaviruses

Coronaviruses are positive sense RNA, enveloped viruses which infect broad range of mammalian and avian hosts. Large genomes, genetic diversity, capacity for recombination and ability to adapt receptor usage is responsible for cross species transmission of coronaviruses (Graham and Baric, 2010). Host range and tissue tropism of corona virus are determined by the spike glycoprotein. It mediates attachment to host cell receptor and fusion between viral and cellular membranes. Change in receptor binding domain alter the receptor affinity and provide recognition of receptor in new host species but spike activation by host proteases and availability of appropriate intracellular factors are also requires for entry of virus.

Recombination in coronavirus occurs through template switching during RNA replication. This process combines genomic regions from different viral lineages and changes the receptor usage, antigenicity, replication or host range of virus (Wells *et al.,* 2023).

Bats act as substantial natural reservoir, which maintain coronavirus diversity and contribute in evolution of several zoonotic coronaviruses. Viral characteristics, reservoir population, environmental conditions and contact with bats, intermediate animals and human together influence the spillover risk of virus (Ruiz-Aravena *et al.,* 2022).

Nipah Virus

Nipah virus is member of paramyxoviridae and zoonotic pathogen associated with severe encephalitis and respiratory disease. Fruit bats of genus *Pteropus* are natural reservoir of virus. Disease can transmit from bats to human, indirectly through domestic animal or contamination or in some outbreaks, through human-to-human transmission (Luby, 2013). Nipah virus uses attachment glycoprotein to bind ephrin-B2 and ephrin-B3 receptors. Ephrin B2 is commonly present in mammalian species and expressed in arterial endothelial cells and neural tissues. Conversion and distribution of these receptors is responsible for endothelial and neurological tropism o virus and determination of host range. (Bonaprte *et al.,* 2005; Negrete *et al.,* 2005). After receptor binding, glycoprotein mediates the fusion of viral envelop with host cell

membrane. Attachment and fusion protein together are responsible for viral entry. Tissue susceptibility of pathogen is depended on receptor distribution while viral replication and disease depend on host immune responses and other cellular factors. Nipah virus protein interferes with antiviral activity of interferon. Phosphoprotein gene disrupt signaling pathways which involves in innate immunity, thereby promotes viral replication and systemic spread (Basler, 2012; Lawrence *et al.,* 2022). Ecological condition is main factor for Nipah emergence. Alteration in habitat, agriculture practices, livestock and human movement and food contamination may increase the bat-animal-human contact. The emergence of Nipah demonstrates that how ecological exposure interacts with receptor compatibility, tissue tropism and immune evasion to determine spillover risk in new host.

6. Molecular Tools for Investigating Zoonotic Emergence

Advancement in molecular biotechnology have transformed process of investigation or detection of emerging zoonotic pathogens. Molecular methods is useful for determination of pathogen identity, genetic diversity, evolutionary relationships, host adaptation, transmission patterns and antimicrobial resistance of pathogen. Here are some advanced methods which can be useful for detection or identification of emerging zoonotic pathogens.

Molecular Method	Primary Application & Methodological Scope	Advantages over Traditional Tools	Methodological Limitations & Validation Requirements
Whole-Genome & Next-Generation Sequencing	Maps full pathogen genomes to capture detailed genetic variation. Tracks large-scale variations across massive numbers of DNA or RNA fragments analysed in parallel.	Provides high-resolution datasets for outbreak analysis. improves the identification of specific transmission clusters, adaptive mutations, recombination events, virulence factors, and resistance genes (Gardy & Loman, 2018).	Proper interpretation requires integrating sequence data with field epidemiological data regarding sampling times, locations, host contact networks, pathogen evolutionary rate (Gardy & Loman, 2018).
Phylogenetics & Molecular Epidemiology	Builds evolutionary trees to trace ancestral origins. Reconstructs geographic spread and evaluates potential transmission networks.	Integrates pathogen molecular structures directly with field data (e.g., host species, sampling date, location, clinical presentation, exposure history). Provides complete, real-time understanding of transmission pathways.	Phylogenetic reconstructions remain highly dependent on adequate sampling densities, appropriate evolutionary models, and reliable sequence data.

Metagenomic Sequencing	Analyses total genetic material directly extracted from clinical, animal, food or environmental samples.	Offers unbiased diagnostic approach for testing. Capable of discovering unexpected, novel, or difficult-to-culture pathogens in wildlife and at high-risk ecological interfaces without requiring prior knowledge of the specific organisms present (Chiu & Miller, 2019).	Data interpretation is complicated by sample contamination, low pathogen abundance, incomplete genomic coverage and complex background microbial communities (Chiu & Miller, 2019).
Comparative Genomics	Aligns and evaluates pathogen sequences across multiple factors. Compares data across divergent host lineages, distinct geographic zones, time periods or epidemiological settings.	Helps to isolate conserved genomic regions across different groups. Identifies genetic changes or specific mutations associated with host shifts, altered tissue tropism, virulence or enhanced immune evasion.	A statistical association identified between host-adapted lineages does not demonstrate biological causation. Genetic signatures strictly require validation through functional studies using cell culture, structural analysis, reverse genetics or appropriate experimental models.
Structural Biology & Computational Modelling	Resolves the atomic-level structures of pathogen proteins. Models physical interactions with host-cell surface molecules.	Utilizes structural methods like X-ray crystallography and cryogenic electron microscopy (cryo-EM) to characterize receptor-binding interfaces. Employs computational modelling and machine learning to predict how amino-acid substitutions affect protein structure, stability, antigenicity, or host range.	All *in silico* models and computational predictions must be interpreted strictly as working hypotheses rather than definitive evidence. They require thorough validation through empirical experimental and epidemiological testing.

7. Future Perspectives and Conclusion

Emergence of zoonotic disease is complex process involves animal, human and environmental system. Historical surveillance strategy limited to only one sector is insufficient for early detection. Integrated One Health approach is require for genomic surveillance across human clinics, livestock systems, wildlife population and environmental reservoirs for early detection and risk assessment is better for zoonotic emergence (One Health High-Level Expert Panel *et al.,*

2023). Phenotypic changes and transmission risk cannot be predicted by genome data alone, as expression of biological characteristic is depends on pathogen's genetics, host biology, exposure patterns and environmental condition. Future surveillances aim to synthesize real-time pathogen genomic data with portable sequencing machines, animal and human diseases records, wildlife distributions, livestock movements, land-use patterns, climate variable and spatial epidemiology. For advancement of these systems laboratory infrastructure, bioinformatics capacity, interdisciplinary training, equitable technology access and timely data sharing is required. Furthermore, integration of multi-omics tools like transcriptomics for characterizing gene expression changes, proteomics for identification of protein interactions and metabolomics for identification of metabolic alteration along with artificial intelligence and machine learning frameworks can improve identification of various molecular mechanism responsible for host susceptibility and spillover potential.

Successful cross species transmission occurs only when pathogen overcome the species barriers and invade, replicate and shed from host. Genetic mechanisms mutation, recombination, genome reassortment and horizontal gene transfer can generate diverse variant of pathogen which can cross species barrier. Successful emergence only occurs when coordinated interactions happens across multiple viral loci rather than isolated genetic changes. Various pathogen like influenza, corona virus, Nipah virus has produced various variant which can infect new host species.

Early detection of these variants is essential for control of zoonotic emergence. For that various advanced methodology like whole genome sequencing, comparative genomics, phylogenetics and computational modelling can be useful. Interpretation of molecular evidence with epidemiological, ecological and experimental data is most important aspect to compact with new emergence of pathogen. An integrated One health approach is essential for understanding and addressing emerging zoonotic threats. Future development in technology and integration among various sectors may strengthen the ability to identify the molecular changes associated with zoonotic emergence and cross species transmission and their early detection.

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NANOBIOTECHNOLOGY IN MODERN BIOMEDICAL RESEARCH

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Abstract

Nanobiotechnology has emerged as one of the most transformative interdisciplinary fields, integrating nanotechnology, biology, chemistry, medicine and engineering to develop innovative solutions for modern healthcare challenges. The unique physicochemical properties of nanomaterials, including their high surface-to-volume ratio, tunable optical characteristics, enhanced mechanical strength, and superior biocompatibility, have significantly advanced biomedical research. Nanobiotechnology has revolutionized drug delivery, disease diagnosis, tissue engineering, regenerative medicine, biosensor development, medical imaging, antimicrobial therapy and precision medicine. Nanoparticles can selectively target diseased tissues while minimizing adverse effects on healthy cells, thereby improving therapeutic efficacy and reducing toxicity. Furthermore, nanomaterials have facilitated the development of rapid diagnostic platforms capable of detecting diseases at very early stages. Recent advances in artificial intelligence, machine learning, and smart nanomaterials have further accelerated personalized medicine and next-generation biomedical applications. Despite remarkable progress, challenges such as toxicity, biodegradability, regulatory approval, manufacturing scalability and ethical considerations remain significant barriers to widespread clinical translation. This chapter presents a comprehensive overview of nanobiotechnology, discussing fundamental concepts, types of nanomaterials, biomedical applications, current challenges, future prospects and recent research developments. The chapter also highlights emerging technologies expected to shape the future of biomedical sciences.

Keywords: Nanobiotechnology, Nanomedicine, Drug Delivery, Biosensors, Tissue Engineering, Regenerative Medicine, Biomedical Imaging, Precision Medicine, Nanoparticles, Artificial Intelligence.

1. Introduction

Healthcare has undergone remarkable transformation over the past few decades owing to rapid advancements in biotechnology, materials science, molecular biology and computational technologies. Among these developments, nanobiotechnology has become one of the fastest-growing research areas due to its enormous potential to revolutionize diagnosis, treatment, and prevention of diseases. Nanobiotechnology combines nanoscale engineering with biological

systems to create functional materials, devices, and therapeutic agents capable of interacting with cells and biomolecules at the molecular level.

Nanotechnology generally deals with materials ranging from 1 to 100 nanometers in size. At this scale, materials exhibit unique optical, magnetic, electrical, thermal, and biological properties that differ significantly from their bulk counterparts. These unique characteristics enable precise interaction with biological systems, making nanomaterials highly suitable for biomedical applications.

The integration of artificial intelligence (AI), machine learning (ML), Internet of Medical Things (IoMT), and wearable healthcare technologies has further expanded the scope of nanobiotechnology. AI-assisted nanoparticle design and computational drug screening have significantly accelerated biomedical research while reducing development costs.

2. Fundamentals of Nanobiotechnology

Nanobiotechnology involves designing nanoscale materials capable of interacting with biological molecules such as

- DNA
- RNA
- Proteins
- Enzymes
- Lipids
- Cells
- Tissues

Major Characteristics

- High surface area
- Excellent drug loading capacity
- Controlled drug release
- Improved cellular uptake
- Enhanced stability
- Target-specific delivery
- Reduced toxicity
- Improved bioavailability

Nanomaterials can cross physiological barriers such as the blood-brain barrier, making them highly attractive for neurological disease treatment.

3. Types of Nanomaterials Used in Biomedical Research

3.1 Lipid-Based Nanoparticles

Examples include

- Liposomes

- Solid lipid nanoparticles
- Nanostructured lipid carriers

Applications

- Cancer chemotherapy
- Gene delivery
- Vaccine development
- mRNA therapeutics

Advantages include excellent biocompatibility, biodegradability, and low toxicity.

3.2 Polymeric Nanoparticles

Common polymers include

- PLGA
- PEG
- Chitosan
- Alginate

Applications

- Controlled drug release
- Tissue engineering
- Gene therapy
- Protein delivery

Applications

- Drug delivery
- Biosensors
- Neural interfaces
- Cancer diagnostics

4. Nanobiotechnology in Drug Delivery

Drug delivery represents one of the most successful biomedical applications of nanotechnology.

Conventional Drug Delivery Limitations

- Poor water solubility
- Rapid degradation
- Low targeting efficiency
- High systemic toxicity
- Frequent dosing

Drug Delivery Systems

- Liposomes
- Polymeric nanoparticles
- Dendrimers

- Nanogels
- Micelles
- Exosomes

Cancer treatment has greatly benefited from nanoparticle-based chemotherapy, allowing selective accumulation within tumor tissues through the enhanced permeability and retention (EPR) effect.

5. Nanobiotechnology in Cancer Therapy

Cancer remains one of the leading causes of mortality worldwide. Nanotechnology has significantly improved cancer diagnosis and treatment through precision medicine.

Major Applications

Chemotherapy

Nanoparticles encapsulate anticancer drugs to reduce toxicity.

Photothermal Therapy

Gold nanoparticles convert laser energy into heat to destroy tumor cells.

Photodynamic Therapy

Photosensitizer-loaded nanoparticles generate reactive oxygen species to eliminate cancer cells.

Immunotherapy

Nanocarriers improve immune checkpoint inhibitor delivery.

Gene Therapy

Nanoparticles deliver siRNA, mRNA, and CRISPR-Cas systems directly into cancer cells.

6. Nanobiotechnology in Medical Imaging

Medical imaging has become increasingly accurate through nanotechnology.

Imaging Modalities

- MRI
- CT
- PET
- Fluorescence imaging
- Photoacoustic imaging
- Ultrasound imaging

Iron oxide nanoparticles serve as MRI contrast agents, while gold nanoparticles enhance CT imaging due to their high X-ray attenuation.

7. Biosensors and Diagnostics

Nano biosensors provide rapid, accurate, and highly sensitive disease detection.

Components

- Bioreceptor
- Nanomaterial
- Signal transducer

- Data processor

Applications

- COVID-19 detection
- Glucose monitoring
- Cancer biomarkers
- Cardiac biomarkers
- Infectious diseases
- Food safety

Gold nanoparticles and graphene-based sensors exhibit excellent sensitivity owing to enhanced electron transfer and surface plasmon resonance.

8. Tissue Engineering and Regenerative Medicine

Nanotechnology supports tissue regeneration by mimicking the extracellular matrix (ECM).

Major Components

- Nanofibers
- Hydrogels
- Bioceramics
- Stem cells
- Growth factors

Applications include

- Bone regeneration
- Skin repair
- Cartilage engineering
- Neural tissue repair
- Cardiac tissue engineering

Electro spun nanofibers facilitate cell adhesion, proliferation, and differentiation.

9. Nanobiotechnology in Antimicrobial Therapy

The rise of antimicrobial resistance has prompted the development of nanoparticle-based antimicrobial agents.

Mechanisms include

- Cell membrane disruption
- Reactive oxygen species generation
- DNA damage
- Protein denaturation

These nanomaterials exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses.

Future Perspectives

Future research is expected to focus on

- Smart stimuli-responsive nanoparticles
- AI-guided nanomedicine design
- CRISPR-enabled nano-gene therapy
- Personalized nanomedicine
- Nano-enabled vaccines
- Wearable nano biosensors
- Organ-on-chip platforms
- Nanorobotics for targeted therapy
- Biodegradable nanomaterials
- Sustainable green nanotechnology

The convergence of nanotechnology with artificial intelligence, synthetic biology, and precision medicine is anticipated to drive the next generation of biomedical innovations.

Conclusion

Nanobiotechnology has transformed modern biomedical research by providing innovative solutions for disease diagnosis, targeted drug delivery, regenerative medicine, biosensing, medical imaging and precision therapeutics. The exceptional properties of nanomaterials enable highly efficient interactions with biological systems, resulting in improved treatment efficacy and reduced adverse effects. Emerging technologies such as artificial intelligence, machine learning, CRISPR gene editing and smart nanomaterials are further expanding the capabilities of nanobiotechnology. Although challenges related to toxicity, regulatory approval, scalability and ethical considerations remain, continuous advancements in interdisciplinary research are accelerating the clinical translation of nanobiotechnology. As personalized medicine becomes increasingly important, nanobiotechnology will continue to play a pivotal role in shaping future healthcare systems and improving global health outcomes.

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NANOBIOTECHNOLOGY: MOLECULAR APPROACHES FOR MICROBIAL AND BIOMEDICAL APPLICATIONS

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Abstract

Nanobiotechnology creates novel approaches to cancer treatment and antibiotic resistance by fusing nanotechnology with biological systems. The molecular processes by which bacteria, fungus, and algae serve as organic "nanofactories" for the creation of nanoparticles via enzymatic reduction and biomineralisation pathways are examined in this chapter. While synthetic biology makes it easier to design microbes for specific therapeutic uses, advanced characterisation techniques allow the visualisation of microbial-nanomaterial interactions. Nanozyme-based antimicrobials that break down bacterial membranes and biofilms, bacteria-based tumour therapy that takes advantage of selective colonisation of hypoxic tumour microenvironments, and nanobiohybrid systems that combine bacterial targeting with nanomaterial functionality for immunotherapy and controlled drug delivery are some important biomedical applications. Artificial intelligence integration speeds up the design of nanomaterials and customised treatment methods. Microbial nanobiotechnology offers revolutionary potential for precision medicine despite obstacles in biocompatibility, scalability, and regulatory approval. Future directions include biohybrid microrobots, smart hybrid nanomaterials, and sustainable green nanotechnology platforms for global health applications.

Keywords: Synthetic Biology, Cancer Treatment, Nanobiotechnology, Microbial Synthesis, Antimicrobial Resistance.

1. Introduction

The rapidly developing multidisciplinary topic of nanobiotechnology combines the concepts of nanotechnology with biological systems to create novel instruments, materials, and treatment approaches. This convergence takes use of the special qualities of nanoscale structures, which are frequently different from their bulk counterparts. These traits include higher surface area-to-volume ratios, greater reactivity, and new optical or magnetic characteristics [1]. These characteristics have made it possible for revolutionary developments in biosensors, antibacterial treatments, drug delivery systems, and diagnostic instruments. Nanobiotechnology is important because it has the ability to transform existing technologies by providing focused, effective, and sustainable substitutes for traditional methods. Nanobiotechnology has made it easier to build

medication delivery systems based on nanoparticles that can release therapeutic chemicals with amazing accuracy and penetrate biological barriers. The World Health Organization estimates that antimicrobial resistance (AMR) causes at least 700,000 deaths each year; if left unchecked, that figure is expected to increase to 10 million by 2050. Similarly, its applications in antimicrobial strategies address this developing challenge. Because of their natural capacity to interact with metals and other elements at the nanoscale, microorganisms such as bacteria, fungus, yeast, algae, and viruses have become essential components of nanobiotechnology [2,3]. Through biologically mediated processes such enzymatic reduction, biomineralisation, and extracellular secretion of reducing agents, these microbial systems function as natural "nanofactories" that create nanoparticles. Microbial nanoparticle production, in contrast to physical or chemical approaches, is highly adjustable and energy-efficient, enabling fine control over particle size, shape, and composition, frequently at ambient settings without hazardous chemicals or high-energy inputs [4,6]. The molecular foundations of microbial-nanomaterial interactions are examined in this chapter, with particular attention paid to synthesis methods, functionalisation techniques, and biological applications such as cancer treatment, targeted drug administration, and antimicrobial therapy. The area has been further transformed by the combination of genetic engineering, synthetic biology, and sophisticated characterisation methods, which has made it possible to create next-generation nanobiohybrid systems for precision medicine.

2. Microbial Systems as Nanofactories

2.1 Bacteria-Mediated Nanoparticle Synthesis

Because of their quick growth rates, easy culture needs, and genetic manipulability, bacteria are among the microorganisms that have been investigated the most in the creation of nanoparticles. Numerous bacterial species have been shown to be capable of producing a range of nanoparticles, including silver, gold, iron oxide, zinc oxide, and copper oxide. These species include Pseudomonas sp., Bacillus sp., Escherichia coli, and Corynebacterium sp. To reduce metal ions to their nanoscale metallic forms, these bacteria usually use enzymatic pathways including electron transfer mechanisms. There are two main processes by which bacteria produce nanoparticles: intracellular and extracellular [7].

- **Intracellular Synthesis:** Bacterial cells collect metal ions, which are then reduced by enzymes or metabolites to create nanoparticles in the cytoplasm or periplasmic region. Although this method frequently produces nanoparticles with narrow size distributions, recovering the nanoparticles may need further cell disruption procedures.
- **Extracellular Synthesis:** Metal ions are transformed into external nanoparticles by reductive enzymes or proteins released into the surrounding media. Gold and silver nanoparticles have been produced on a massive scale using this process. By changing growing conditions, different strains of Bacillus sp. and Pseudomonas sp. may produce nanoparticles with unique characteristics, such size and form.

For instance, it has been documented that Pseudomonas stutzeri uses nitrate reductase activity to produce silver nanoparticles with precise dimensions. Gold, zinc oxide, and selenium nanoparticles with regulated properties have also been demonstrated to be produced by Bacillus subtilis and Lactobacillus sp. To increase colloidal stability and stop aggregation during storage or application, these bacterially produced nanoparticles frequently need additional capping agents.

2.2 Fungal and Algal Nanoparticle Synthesis

Algae and fungus provide unique benefits for the creation of nanoparticles. Because of their high metal tolerance, simplicity of handling, and effective extracellular enzyme secretion, fungi like Aspergillus niger and Fusarium oxysporum are particularly appealing. Fungal enzymes like reductases and phytochelatins aid in the creation of nanoparticles, and the mycelial network offers a sizable surface area for metal ion interaction. Diatoms and other algae belong to a special class of microbial nanofactories. Mesoporous silica frustules, which are highly organised nanoporous structures that make good prospects for drug delivery applications, are naturally produced by photosynthetic unicellular microalgae called diatoms. Internalisation of soluble orthosilicic acid is the first step in the creation of biogenic silica, which is then condensed and deposited inside specialised vesicles known as silica deposition vesicles (SDVs). These naturally occurring silica structures are useful platforms for nanomedicine because of their exceptional homogeneity, large surface area, and biocompatibility.

3. Molecular Mechanisms of Nanoparticle-Microbe Interactions

3.1 Enzymatic Reduction Pathways

Enzymatic reduction reactions are the main mechanism by which microorganisms synthesise metal nanoparticles. The reduction of metal ions to elemental nanoparticles is mostly dependent on nitrate reductase enzymes. These enzymes help reduce metal ions to zero-valent metal atoms, which then nucleate and develop into nanoparticles by transferring electrons from NADH to the metal ions. The process for silver nanoparticles is that nitrate reductase reduces Ag^{+} ions to Ag_2, using the quinone redox-active core of the enzyme as an electron donor. Similar to this, reductases and other cellular metabolites reduce $AuCl_4^{-}$ to Au_4 during the creation of gold nanoparticles. Different microbial species have different specialised routes, which adds to the observed variety of nanoparticle characteristics [8].

3.2 Biomineralization and Magnetosome Formation

One specific method for creating nanoparticles is biomineralisation, which is especially noticeable in magnetotactic bacteria. These bacteria have special cellular organelles called magnetosomes that contain tiny magnetic iron mineral crystals (magnetite, Fe_3O_4, or greigite, Fe_3S_4). These crystals provide the bacteria magnetic characteristics and serve as a "biological compass" to direct their movement. The size, shape, and crystallographic orientation of the magnetic nanoparticles are carefully regulated by certain proteins (Mam proteins) found in the magnetosome membrane. Numerous genes and proteins that are essential to the production and biomineralisation of bacterial magnetic particles have been discovered via recent molecular

research. Fusion proteins, in which foreign protein domains can be attached onto naturally occurring transmembrane proteins linked to the magnetosome (such as Mms13, Mms16, and MamC), have been made possible by genetic engineering approaches, opening up applications in targeting and biomarker identification. Because genetic engineering allows for the expression of functional proteins directly on the magnetosome membrane, this method has many advantages over chemical alterations that could have harmful effects.

3.3 Surface Interactions and Biofouling

The biological reactions that follow when nanoparticles come into contact with microbial surfaces are determined by intricate molecular interactions. Through hydrophobic interactions, particular receptor-ligand binding, and electrostatic forces, the nanoparticle surface can interact with the microbial cell membrane [9]

- **Membrane Disruption:** When microbial cell membranes are physically damaged by nanoparticles, cellular contents seep out and the cell dies. By imitating phospholipase enzymes, nanozymes like materials based on cerium oxide destroy the connections that hold the membrane bilayer together. Bacteria are less likely to become resistant to these nanozymes because they specifically target the chemical integrity of phospholipids.
- **Generation of Reactive Oxygen Species (ROS):** Metal and metal oxide nanoparticles have the ability to catalyse the generation of ROS, which causes oxidative stress in microorganisms. This harms proteins, DNA, and other biological components.
- **Protein Corona Formation:** In biological settings, nanoparticles quickly adsorb proteins onto their surface, creating a "protein corona" that influences their interactions and biological identity. Depending on the makeup and structure of the adsorbed proteins, this corona can either increase or decrease antibacterial action.

4. Advanced Characterization Techniques

4.1 Imaging Microbial-Nanomaterial Interactions

The study of microbial-nanomaterial interactions at the molecular level has been transformed by advanced imaging techniques.

- **Confocal Microscopy:** Nanoparticle interactions with microbial cells can be seen using fluorescence microscopy. For instance, biofilm imaging displays the dispersion of bacteria (green) inside the biofilm matrix (red), whereas confocal microscopy images have shown that nanozyme treatment breaks bacterial cell membranes, allowing DNA to "ooze out" of treated bacterial cells.
- **Scanning Electron Microscopy (SEM):** The effects of antimicrobial treatments and the ultrastructure of biofilms can be seen by high-resolution SEM imaging. Both untreated and micro-robotically treated S. aureus biofilms have been visualised using SEM, displaying intricate architectural alterations.
- **HR-TEM:** Atomic-scale resolution of nanoparticle structures is possible using scanning transmission electron microscopy and high-resolution transmission electron microscopy.

The nanostructural architecture of TiO_2/Pt microrobots has been shown using STEM imaging, allowing for the link of structure and function.

4.2 Spectroscopic and Biophysical Methods

Complementary spectroscopic techniques provide information on nanoparticle composition, surface chemistry, and biological interactions:

- **Surface-Enhanced Raman Spectroscopy (SERS):** Enables ultrasensitive detection of biomolecules at nanoparticle surfaces
- **Dynamic Light Scattering (DLS):** Characterizes nanoparticle size distribution and aggregation state in biological media
- **Zeta Potential Measurements:** Assess surface charge, predicting colloidal stability and biological interactions
- **X-ray Diffraction (XRD):** Determines crystal structure and phase composition of nanoparticles

5. Biomedical Applications: Antimicrobial Nanobiotechnology

5.1 Nanozymes as Membrane-Disruptive Antimicrobials

Novel antimicrobial techniques have been developed in response to the advent of antibiotic resistance. A possible strategy for getting beyond resistance mechanisms is the use of nanozymes, which are nanomaterials with enzyme-mimetic activity. Using a chemical co-precipitation technique, researchers at the Indian Institute of Science created a cerium oxide-based nanozyme. To improve dispersibility and activity, the polymer was coated with sodium polyacrylate. By severing the chemical links that hold the phospholipid bilayer together, this nanozyme disrupts cell membranes by imitating phospholipase. Salmonella Typhi, Shigella flexneri, Escherichia coli, Vibrio cholerae, and Klebsiella pneumoniae were among the harmful bacteria against which the nanozyme was tested; it successfully inhibited their development and prevented the formation of biofilms. Crucially, the nanozyme's small size allowed it to break through well-formed biofilms (up to 10 days old), resolving a significant drawback of many traditional antibiotics. The nanozyme dramatically decreased bacterial attachment to the catheter surface when tested on urinary catheters, indicating possible uses in reducing infections linked to devices [10].

5.2 Biofilm Eradication with Micro-Nanorobotics

Because of their capacity for immune evasion, metabolic adaptability, and antibiotic resistance, biofilm-associated infections pose a serious threat. Pathogens including P. aeruginosa and S. aureus acquire slow-growth phenotypes and change gene expression within the biofilm matrix, improving their resistance to host defences and medications. Micro- and nanorobotic devices, which are distinguished by autonomous navigation, remote control, and programmable design, offer a novel paradigm for managing biofilm-associated infections. These tiny robotic beings work together in a synergistic way:

- Antimicrobial surfaces that actively eradicate microorganisms

- Systems for delivering drugs precisely for targeted treatment
- Applying calibrated shear force to physically disturb the structure of biofilms

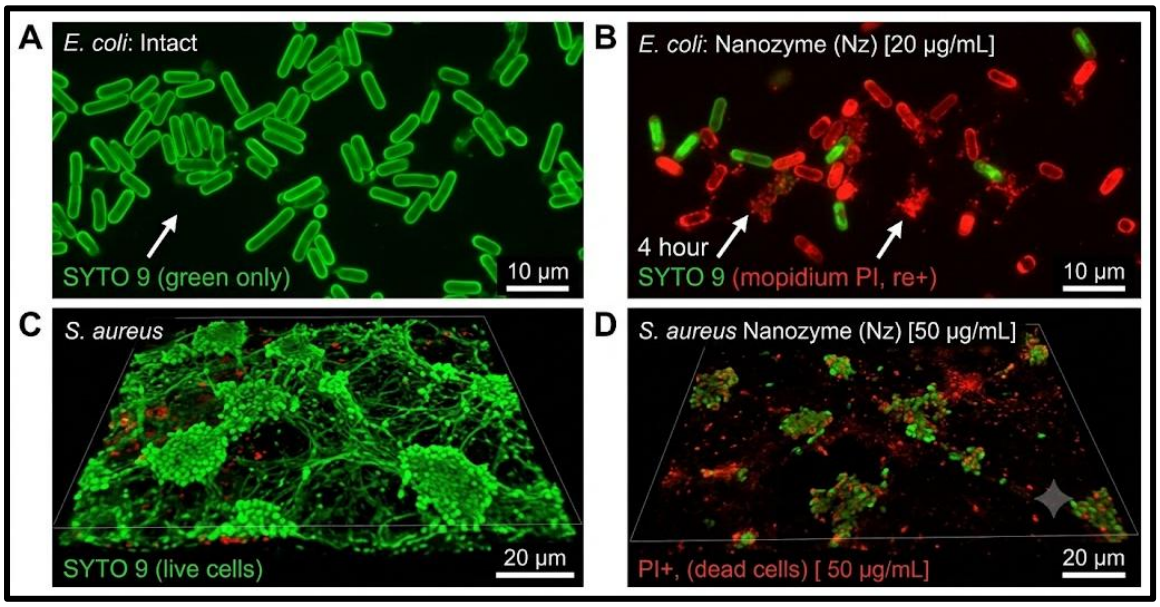

Figure 1: A) Confocal microscopy of untreated bacterial cells with intact membranes; B) Confocal microscopy of nanozyme-treated cells showing DNA leakage; C) Biofilm structure before treatment; D) Biofilm after nanozyme treatment showing disruption

Precise kinematic manipulation is made possible by stimuli-responsive remote control, and spatiotemporal localisation is provided by sophisticated imaging methods. These robotic methods greatly increase therapeutic efficacy by simultaneously inducing mechanical destabilisation of the biofilm and specifically eliminating persister (dormant) bacterial subpopulations. The mechanism of membrane breakdown in bacterial cells caused by nanozymes is depicted in Figure 1. Confocal microscopy images of untreated bacterial cells with intact cell membranes (green) and DNA (magenta) are displayed in Panel A, whereas treated cells with DNA leaking due to membrane breakdown are displayed in Panel B. Panel D shows the same biofilm following nanozyme treatment, indicating bacterial mortality and biofilm suppression, while Panel C shows biofilm imaging without nanozyme treatment (bacteria in green, biofilm matrix in red).

6. Biomedical Applications: Cancer Therapy

6.1 Bacteria-Based Tumor Therapy

One intriguing approach to precision cancer treatment is bacteria-based tumour therapy. Disordered vascular systems, hypoxia, acidity, and nutrient-rich environments are features of the tumour microenvironment (TME) that promote selective bacterial colonisation. Because of these characteristics, certain bacterial species preferentially accumulate in tumours at ratios greater than 10,000:1 as compared to normal tissues, making tumours ideal targets for bacteria. Certain receptors that can recognise chemicals generated by dying tumour cells direct bacteria to target tumour tissue. The dysfunctional vascular system allows germs to enter the bloodstream and

permeate tumour tissue, where lower oxygen levels provide an environment that is favourable for their growth. They may enter tumours deeply thanks to their small size and strong flagella, and necrotic areas offer plenty of nutrients for bacterial development. Because of their innate immunogenicity, bacteria can be used to treat cancer. When they colonise, they cause a large number of neutrophils to enter the tumour. These neutrophils then release extracellular traps made of chromatin fibres and antimicrobial proteins, which keep bacteria inside the tumour. By modifying microorganisms to release immunomodulatory substances, this localised immune response can be strengthened even further [11].

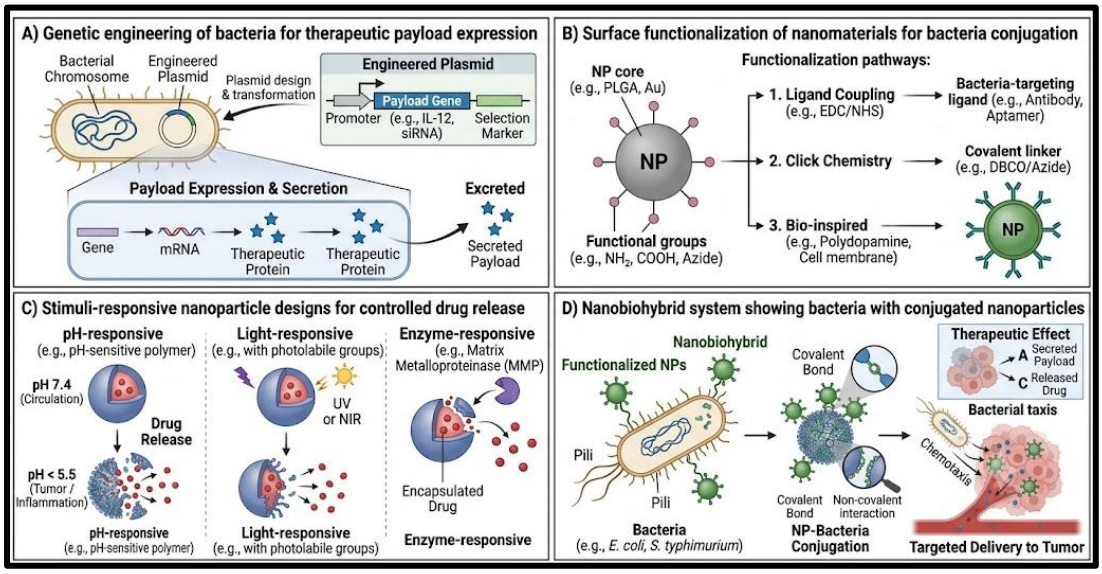

Figure 2: A) Genetic engineering of bacteria for therapeutic payload expression; B) Surface functionalization of nanomaterials for bacteria conjugation; C) Stimuli-responsive nanoparticle designs for controlled drug release; D) Nanobiohybrid system showing bacteria with conjugated nanoparticles

6.2 Bacterial-Nanomaterial Nanobiohybrids

Complementary benefits for cancer treatment are provided by the combination of nanomedicine and microorganisms. The amazing optical, magnetic, and acoustic capabilities of nanomaterials greatly improve the controllability and efficacy of tumour therapy based on microorganisms. Nanobiohybrid Systems: These systems integrate:

- Selective tumour accumulation using bacterial tumour targeting
- Utilising nanomaterials for environmental responsiveness, therapy, or imaging
- Genetic circuitry for regulated release of therapeutic payloads

6.3 Synthetic Biology Approaches for Targeted Therapy

Bacterial gene expression in the tumour microenvironment can be precisely controlled thanks to synthetic biology. Numerous induction systems have been created:

- **Hypoxia-inducible System:** The oxygen transcription factor fumarate and nitrate reduction regulatory protein (FNR), which are found naturally in *Salmonella*, are the

main components of hypoxia-inducible systems. FNR forms a transcriptionally active homodimer with $[4Fe\text{-}4S]^{2+}$ in hypoxic conditions, which triggers downstream gene activation. Hypoxia-inducible promoters such as pflE, ansB, and pepT, as well as synthetic promoters like FF+20, HIP-1, and nirB, are frequently employed.

- **Acid-Inducible Systems:** Researchers have discovered promoters like STM1787 in *Salmonella* (90-fold induction following acid exposure) and acid-induced promoters adiA and asr from *E. coli* by taking advantage of the acidic pH of the TME (5.8–6.6).
- **Lactate-Inducible Systems:** Based on the natural lldPRD operon, these systems allow induction in the lactate-rich TME because the LldR repressor dimer suppresses gene expression unless it is coupled to lactate.
- **NO-Inducible Systems:** Micromolar NO is produced at tumour locations as a result of iNOS overexpression brought on by Salmonella colonisation. The foundation of the NO-inducible system is NorR, an E. coli regulatory protein that is σβ-dependent and activates the Pnorv promoter when NO binds to it.

6.4 Microbial Therapeutics for Immunomodulation

Advancements in synthetic biology and nanotechnology have propelled engineered microorganisms to the forefront as an emerging platform for cancer therapy. Upon colonization of tumor sites, these microorganisms can be programmed to release therapeutic agents or immunomodulatory factors, thereby remodeling the tumor microenvironment and enhancing antitumor efficacy.

- **Precision Tumour Targeting:** Based on distinct TME traits, engineered bacteria can be created to colonise particular tumour types. Precise medication distribution and spatiotemporal management of therapeutic actions are made possible by the combination of microorganisms and intelligent control systems.
- **Immunotherapy Enhancement:** Bacteria have a natural adjuvant effect by stimulating the host immune system. Bacteria-based therapy can greatly increase immunotherapeutic efficacy when paired with immune checkpoint inhibitors (such as anti-CTLA4 or anti-PD-1 antibodies). Antibiotic-loaded F. nucleatum-mimetic nanovehicles have shown promise in overcoming immunosuppression caused by F. nucleatum and restoring the therapeutic effectiveness of immune checkpoint blockade.
- **Treatment Modalities:** Chemotherapy, photothermal treatment (PTT), photodynamic therapy (PDT), radiotherapy (RDT), chemodynamic therapy (CDT), sonodynamic therapy (SDT), and immunotherapy can all be enhanced by engineering microorganisms.

7. Smart Hybrid Nanomaterials for Chronic Infections

7.1 Microbiome-Responsive Therapeutic Platforms

The complicated biology of chronic wounds, which includes prolonged inflammation, microbial biofilms, and delayed tissue regeneration, continues to be a persistent clinical challenge. An

estimated 8 million people have chronic sores that frequently lead to serious complications like sepsis or amputation, and 6.3% of diabetics worldwide have diabetic foot ulcers. Promising solutions to these problems are provided by recent developments in sustainable and intelligent nanotechnology. Bio-based nanomaterials can reduce inflammation, speed up healing, and overcome multidrug resistance by combining microbiome-sensitive processes with tailored delivery systems. The complicated biology of chronic wounds, which includes prolonged inflammation, microbial biofilms, and delayed tissue regeneration, continues to be a persistent clinical challenge. An estimated 8 million people have chronic sores that frequently lead to serious complications like sepsis or amputation, and 6.3% of diabetics worldwide have diabetic foot ulcers. Promising solutions to these problems are provided by recent developments in sustainable and intelligent nanotechnology. Bio-based nanomaterials can reduce inflammation, speed up healing, and overcome multidrug resistance by combining microbiome-sensitive processes with tailored delivery systems [12].

7.2 Sustainable and Green Nanomaterials

Table 1: Various microbial-fabricated nanomaterials and their biomedical applications

Organism/Material	Nanoparticle Type	Formulation	Application
Magnetotactic bacteria	Bacterial magnetosome	Drug-loaded magnetosome	Anticancer drug delivery
Magnetospirillum magneticum AMB-1	Bacterial magnetosome	Protein-functionalized	Tumor marker labeling
Diatom	Modified diatomite NP	PEG + CPP functionalized	Anticancer drug delivery
Lactobacillus plantarum	AuNP	Antibiotic-AuNP-EPS	Drug delivery against MDR
Aspergillus niger	-	Glucose oxidase-NP	Biosensor
Yeast cells	nHAP-yeast	Folic acid functionalized	Active targeting
Bacterial EPS	Magnetic NP	MNP-gellan gum/mauran	Drug delivery and targeting
Oscillatoria limnetica	AgNPs	-	Biomedical applications
Ustilago maydis	AuNPs	Mannosylerythritol lipid	Biomedical applications

Sustainability has emerged as a central theme in biomedical innovation. While traditional synthetic nanomaterials demonstrate strong therapeutic efficacy, their persistence and toxicity raise significant environmental concerns. Green nanomaterials derived from renewable resources such as chitosan, cellulose, and marine polysaccharides provide safer alternatives with excellent

biocompatibility and antimicrobial efficacy against resistant pathogens while minimizing ecological harm (Table 1).

8. Integration of AI and Machine Learning

Advanced treatment platform design and optimisation have been further revolutionised by artificial intelligence (AI). AI makes it possible to find and refine nanomaterials that are suited to particular biological settings by utilising massive information and prediction algorithms. Machine learning (ML) models are very effective at forecasting interactions between nanomaterials and cells, which speeds up the creation of tailored treatments.

- **Design Optimisation:** AI techniques can forecast the characteristics of nanoparticles based on synthesis parameters, which eliminates the need for intensive experimental screening. The best synthesis conditions for desired size, shape, and surface features can be found using machine learning models that have been trained on nanoparticle characterisation data.
- **Biological Interaction Prediction:** By predicting how nanomaterials will interact with microbial and mammalian cells, predictive models can help designers create biocompatible and antimicrobial nanomaterials.
- **Real-Time Monitoring:** AI enables dynamic treatment modification and real-time monitoring of therapeutic efficacy, guaranteeing that interventions stay flexible and successful throughout infection.

The AI-driven nanomaterial design pipeline, from data gathering and model training to prediction and experimental validation, is depicted in Figure 3.

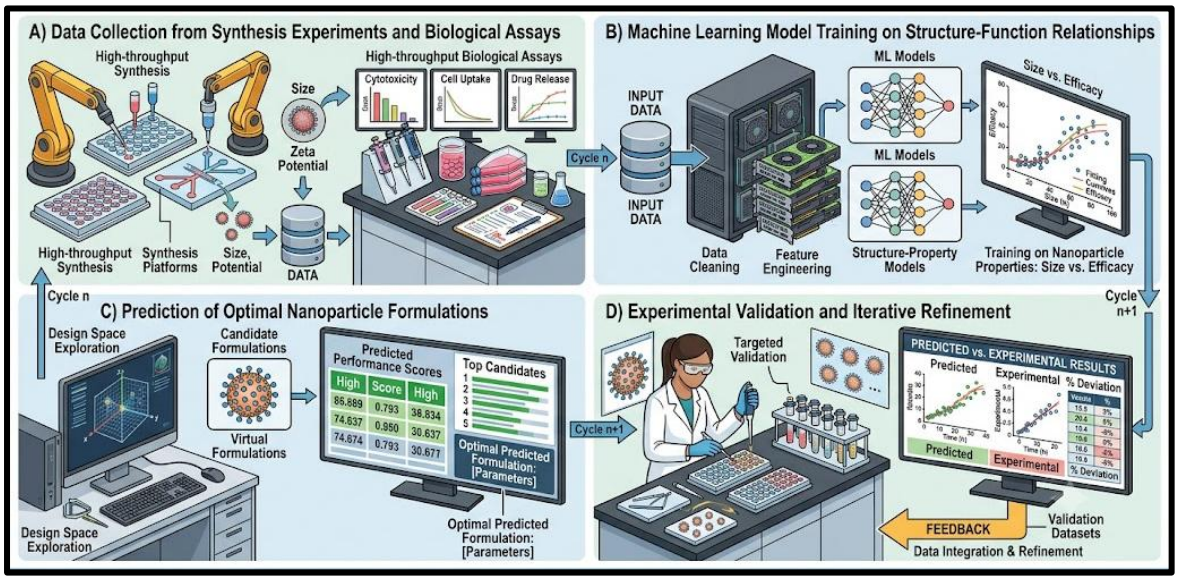

Figure 3. A) Data collection from synthesis experiments and biological assays; B) Machine learning model training on structure-function relationships; C) Prediction of optimal nanoparticle formulations; D) Experimental validation and iterative refinement.

9. Clinical Translation and Challenges

9.1 Current Challenges

Despite encouraging preclinical findings, there are a number of obstacles in the way of applying microbial nanobiotechnology techniques in clinical settings.

- **Biocompatibility and Toxicity:** Although biogenic nanoparticles often show strong biocompatibility, possible toxicity is still an issue. In healthy tissues, nanoparticles may cause genotoxicity, inflammation, or oxidative stress. Clinical translation requires a thorough safety assessment.
- **Targeting Efficiency:** While bacteria are naturally able to target tumours, the effectiveness of this ability varies greatly. The immune system quickly eliminates a variety of bacterial species, which lessens their accumulation at specific locations. To maintain functionality, extend circulation time, and reduce immunogenicity under systemic circumstances, surface engineering techniques like PEGylation, hybrid membrane integration, or selective masking of immunogenic epitopes may be necessary.
- **Scalability and Reproducibility:** Consistent yields and batch-to-batch reproducibility are difficult to achieve in microbial nanoparticle manufacturing. Nanoparticle characteristics can be greatly impacted by differences in bacterial strains, purification techniques, and growth conditions.
- **Regulatory Obstacles:** The regulatory framework for treatments based on nanotechnology is still unclear and complicated. For regulatory approval, issues like safety assessment procedures, quality control specifications, and nanoparticle characterisation standards must be specified.
- **Ethical Issues:** The use of genetically modified microbes presents ethical issues with relation to biosafety and environmental release. Strict safety regulations are necessary for contained use in clinical applications.

9.2 Future Perspectives

It is anticipated that interdisciplinary cooperation and technical advancement will further advance the incorporation of microbes and nanomaterials in precision medicine. Future paths consist of:

- **Personalised medicine:** AI-driven nanomaterial design may make it possible to create formulations tailored to each patient's unique genetic profile, microbiome makeup, and illness features.
- **Multifunctional Nanobiohybrids:** Comprehensive cancer treatment may be made possible by combining several therapeutic modalities (drug delivery, immunotherapy, phototherapy, and imaging) into a single nanobiohybrid device.
- **Advanced Biohybrid Microrobots:** Targeted therapy in previously unreachable locations may be made possible by autonomous navigation systems with accurate control and real-time imaging.

- **Sustainable Platforms:** Therapeutic development is in line with global sustainability objectives when green nanoparticles made from renewable resources are continuously developed.

Conclusions

A revolutionary strategy for tackling important issues in microbial and biomedical applications is nanobiotechnology. Microorganisms function as natural "nanofactories" that may create nanoparticles via economical and ecologically safe biochemical processes. These biogenic nanoparticles show promise in cancer immunotherapy, targeted drug delivery, and antimicrobial treatment due to their regulated size, shape, and functional characteristics. The rational design and optimisation of therapeutic platforms is based on the molecular processes that underlie microbial-nanomaterial interactions, such as enzymatic reduction, biomineralisation, and surface contacts. While synthetic biology and genetic engineering improve bacterial capacities for precision medicine, advanced characterisation techniques allow for a thorough analysis of these interactions. When paired with microbiome-targeted strategies, smart hybrid nanomaterials that react to certain biological stimuli present intriguing treatments for cancer and persistent infections. Personalised medicine is made possible by the rapid creation and optimisation of nanomaterials with the integration of AI and machine learning. The combination of microbiology, nanotechnology, and AI-driven innovation offers therapeutically useful, scalable, and ecologically sustainable solutions, even if biocompatibility, targeted efficiency, scalability, and regulatory approval continue to be major obstacles. Microbial nanobiotechnology is a new area of science and technology that has the potential to solve worldwide health issues.

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BIOCHEMICAL FUNCTIONS AND CLINICAL SIGNIFICANCE OF VITAMINS

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Abstract

Vitamins are essential organic micronutrients required in small amounts for normal growth, metabolism, and physiological function. Although they do not provide energy, they serve as coenzymes, antioxidants, and regulators of cellular processes, playing critical roles in energy metabolism, gene expression, immune function, hematopoiesis, bone health, vision, and nervous system function. Based on their absorption and storage, vitamins are classified as water-soluble (B complex & vitamin C) and fat-soluble (A, D, E, K). Water-soluble vitamins primarily function as coenzymes in carbohydrate, protein, and lipid metabolism, whereas fat-soluble vitamins are involved in vision, calcium homeostasis, antioxidant defense, and cell signalling. Vitamin deficiencies can adversely affect health, leading to disorders such as anemia, impaired immunity, neurological dysfunction, skeletal abnormalities also excessive intake of fat-soluble vitamins can cause toxicity. This chapter provides an overview of the biochemical functions, clinical significance, deficiency states, toxicity, and therapeutic applications of vitamins, emphasizing their essential role in maintaining health and preventing disease.

Keywords: Vitamins, Coenzymes, Metabolism, Deficiency Disorders, Antioxidants, Fat-Soluble Vitamins, Water-Soluble Vitamins, Micronutrients.

1. Introduction

Vitamins are chemical substances with biological activity that are needed in trace amounts to maintain regular physiological and metabolic processes. The majority of vitamins must be received through diet or supplementation because the human body is unable to efficiently manufacture them (1). Early in the 20th century, the idea of vitamins came into being after it was discovered that some illnesses were caused by dietary deficiencies. Conditions such as scurvy, beriberi, rickets, and pellagra demonstrated that specific micronutrients are indispensable for maintaining health (2). In general, vitamins fall into two groups (3).

2. Fat-Soluble Vitamins

Fat-soluble vitamins (A, D, E, and K) are absorbed with dietary fats and stored mainly in the liver and adipose tissue, allowing long-term reserves. They play essential physiological roles, including vision and immune function (vitamin A), calcium and phosphorus homeostasis, bone health (vitamin D), antioxidant protection (vitamin E), blood coagulation and bone metabolism

(vitamin K). Because they are stored in body tissues, both deficiency and excessive intake can result in significant clinical consequences (4).

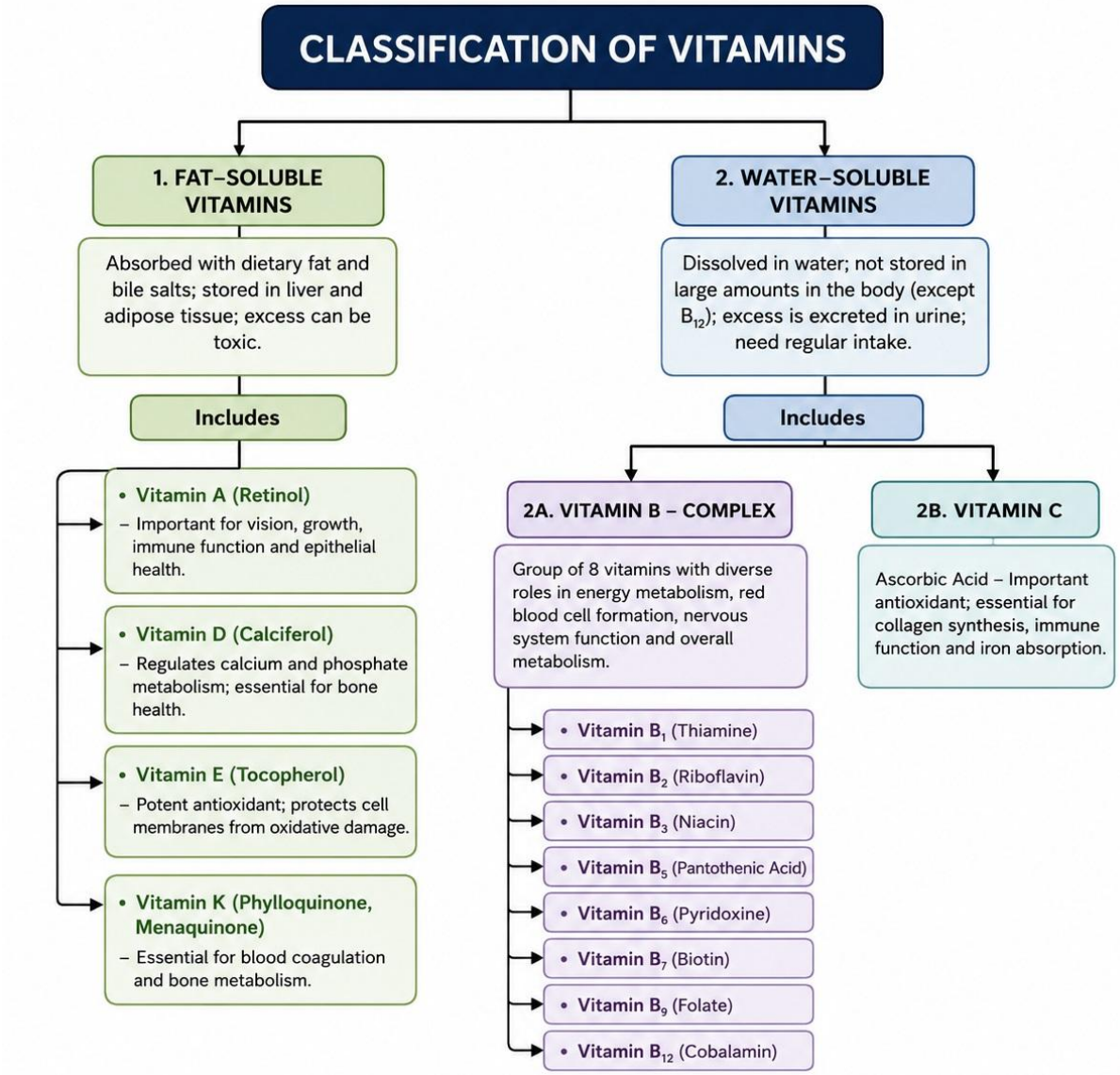

Figure 1: Classification of Vitamins

A. Vitamin A (Retinoids)

a) Sources: Liver, Fish oils, Eggs, Dairy products, Carrots, Spinach, Sweet potatoes

b) Biochemical Functions

i. ***Vision:*** Vitamin A is essential for normal vision, particularly in low-light conditions, by participating in the visual cycle through its active form, 11-cis-retinal. It supports phototransduction, retinal function, and visual acuity. Deficiency impairs dark adaptation, leading to night blindness and other progressive eye disorders (5).

ii. ***Formation of Rhodopsin in Retinal Photoreceptors:*** Vitamin A is essential for dim-light vision by forming rhodopsin, the light-sensitive pigment in retinal rod cells. Light converts 11-cis-retinal to all-trans-retinal, triggering nerve impulses to the brain.

Continuous vitamin A supply is required for rhodopsin regeneration and normal visual function (6).

iii. ***Cellular Differentiation:*** Tissue remodelling, cellular proliferation, and differentiation are all regulated by vitamin A. Retinoic acid, its active metabolite, and functions as a signalling molecule that affects the expression of genes related to cell specialization. This control promotes controlled cell proliferation and guarantees the proper growth and upkeep of epithelial tissues (7).

iv. ***Regulation of Gene Transcription and Epithelial Maintenance:*** Retinoic acid regulates gene expression by binding to retinoic acid receptors (RARs) and retinoid X receptors (RXRs), promoting epithelial cell differentiation and tissue integrity. This helps maintain healthy skin, respiratory epithelium, and gastrointestinal mucosa (8).

v. ***Immune Function:*** Both innate and adaptive immunity depend on vitamin A. As the body's initial line of defense against infections, it maintains the structural integrity of mucosal barriers. Additionally, vitamin A modifies immunological responses by affecting immune cell activity, differentiation, and proliferation (9).

vi. ***Maintenance of Mucosal Integrity and Immune Cell Function:*** By maintaining the epithelial barriers in the gastrointestinal, genitourinary, and respiratory systems, vitamin A lowers the risk of infection. It helps produce antibodies and enhances the activity of neutrophils, lymphocytes, macrophages, and natural killer cells. Additionally, retinoic acid contributes to immunological homeostasis and the control of inflammatory reactions (9).

vii. ***Growth and Development:*** Vitamin A is required for normal growth and development throughout life. It contributes to cellular proliferation, protein synthesis, and tissue maturation. Adequate vitamin A status supports normal growth in children and proper functioning of multiple organ systems (10).

viii. ***Role in Embryogenesis and Skeletal Development***: Vitamin A regulates embryonic development, organ formation, cell differentiation, and skeletal growth by supporting bone remodelling (11).

c) Vitamin A Deficiency

i. ***Night Blindness (Nyctalopia):*** Night blindness is the earliest sign of vitamin A deficiency, resulting from impaired rhodopsin regeneration and reduced dark adaptation. Prolonged deficiency can lead to severe ocular complications (12).

ii. ***Xerophthalmia:*** Xerophthalmia is an eye disorder caused by prolonged vitamin A deficiency, characterized by conjunctival and corneal dryness, Bitot's spots, and progressive vision loss, and remains a major cause of preventable childhood blindness(12).

iii. ***Keratomalacia:*** Keratomalacia is a severe form of vitamin A deficiency characterized by corneal softening, ulceration, and potential irreversible blindness, requiring urgent treatment (13).

iv. ***Impaired Immunity:*** Vitamin A deficiency impairs innate and adaptive immunity by weakening epithelial barriers and immune function, increasing susceptibility to respiratory and gastrointestinal infections and delaying recovery (14).

d) Vitamin A Toxicity (Hypervitaminosis A)

Excessive intake of vitamin A, especially through prolonged supplementation or high-dose preparations, may result in toxicity because vitamin A is stored in the liver and eliminated slowly headache, hepatotoxicity, bone pain and skeletal abnormalities, teratogenic effects (15).

B. Vitamin D (Calciferol)

a) Sources: Sunlight exposure, Fish liver oil, Fortified milk, Egg yolk.

b) Biochemical Functions of Vitamin D: The main role of vitamin D, a fat-soluble vitamin, is as a hormone that controls the balance of calcium and phosphate. It is essential for preserving skeletal integrity, promoting neuromuscular function, and regulating immunological and cellular functions. Before being physiologically active, vitamin D via sunshine exposure, food, or supplements passes through a series of metabolic activations (16).

i. ***Hepatic Activation:*** Formation of 25-Hydroxyvitamin D: Vitamin D undergoes hepatic 25-hydroxylation to form 25-hydroxyvitamin D [25(OH)D] (calcidiol), the major circulating form and the primary marker of vitamin D status (17).

ii. ***Renal Activation:*** Formation of 1,25-Dihydroxyvitamin D: In the kidneys, 25(OH)D is converted by 1α-hydroxylase into $1,25(OH)_2D$ (Calcitriol), the active form of vitamin D, which regulates gene expression through vitamin D receptors (18).

iii. ***Enhancement of Intestinal Calcium Absorption:*** Calcitriol enhances intestinal calcium absorption by inducing calcium-binding proteins and transport channels, thereby maintaining serum calcium levels and supporting bone mineralization and neuromuscular function (19).

iv. ***Regulation of Phosphate Metabolism:*** Vitamin D maintains phosphate balance by increasing intestinal absorption and regulating renal handling of phosphate, supporting hydroxyapatite formation, bone strength, and mineral homeostasis (20).

v. ***Maintenance of Bone Remodelling and Skeletal Integrity:*** Vitamin D is essential for bone growth, remodelling, and mineralization by maintaining calcium and phosphate balance. It also supports immune regulation, cellular differentiation, and overall metabolic health, while preventing rickets and osteomalacia(21).

c) Vitamin D Deficiency

i. ***Rickets (Children):*** Rickets is the classic manifestation of vitamin D deficiency in children, characterized by poor bone mineralization, skeletal deformities, delayed growth, and delayed tooth eruption (22).

ii. ***Osteomalacia (Adults):*** Osteomalacia is the adult manifestation of vitamin D deficiency, characterized by defective bone mineralization, bone pain, muscle weakness, and increased fracture risk (23).

iii. ***Osteoporosis and Increased Fracture Risk:*** Vitamin D deficiency contributes to osteoporosis by reducing calcium absorption, increasing bone resorption, and lowering bone mineral density, thereby raising the risk of fragility fractures, especially of the hip, spine, and wrist (24) .

iv. ***Muscle Weakness and Fall:*** Vitamin D supports muscle function by regulating calcium-mediated muscle contraction. Deficiency causes muscle weakness, impaired balance, reduced mobility, and increased fall risk, particularly in older adults (25).

v. ***Impaired Immune Function:*** Vitamin D modulates innate and adaptive immunity, helping maintain immune homeostasis and regulate inflammation. Deficiency increases susceptibility to infections, particularly respiratory infections (26) .

d) Vitamin D toxicity: Vitamin D toxicity is usually caused by excessive supplementation and results in hypercalcemia. Symptoms include nausea, vomiting, constipation, weakness, polyuria, and confusion. Severe or prolonged toxicity may lead to kidney stones, nephrocalcinosis, and soft tissue calcification.

C) Vitamin E (Tocopherol)

a) Sources: Vegetable oils, Nuts, Seeds, Green vegetables

b) Biochemical Functions of Vitamin E: Vitamin E (α-tocopherol) is a fat-soluble antioxidant that protects cell membranes from oxidative damage, maintains cellular integrity, and supports immune function, cell signalling, and tissue health (27).

i. ***Primary Lipid-Soluble Antioxidant:*** Vitamin E is the major lipid-soluble antioxidant that protects cell membranes from oxidative damage by neutralizing free radicals, particularly in the brain, muscles, liver, and cardiovascular system (28).

ii. ***Protection of Membrane Phospholipids:*** Vitamin E protects membrane phospholipids from lipid peroxidation, preserving membrane integrity, fluidity, and normal cellular function. (29) .

iii. ***Prevention of Oxidative Damage:*** Vitamin E protects lipids, proteins, and DNA from oxidative damage by neutralizing reactive oxygen species and working with other antioxidant systems to maintain redox balance(30).

iv. ***Maintenance of Erythrocyte Integrity:*** Vitamin E protects red blood cell membranes from oxidative damage, preventing hemolysis and maintaining normal erythrocyte

lifespan and oxygen transport (31).Beyond its antioxidant role, vitamin E supports immune regulation, modulates inflammation, and helps maintain cardiovascular and neurological health.

c) Vitamin E Deficiency

Although vitamin E insufficiency is not frequent in healthy people, it can happen in diseases like fat malabsorption, chronic liver illness, genetic disorders that impact the transport of vitamin E, premature birth, or severe nutritional deficiencies. Tissues that are particularly vulnerable to oxidative stress, such as the neurological system and red blood cells, are the main targets of deficiency (32).

i. ***Neuromuscular Disorders:*** Vitamin E deficiency causes progressive neuromuscular dysfunction, including peripheral neuropathy, muscle weakness, ataxia, impaired balance, and reduced reflexes due to oxidative damage to nerves and muscles (33).

ii. ***Hemolytic Anemia:*** Hemolytic anemia is a significant haematological sign of vitamin E deficiency, especially in preterm newborns and people with elevated oxidative stress. Erythrocyte membranes are shielded by vitamin E (34).

d). Vitamin E toxicity: Vitamin E toxicity is very uncommon but may occur with excessive supplementation, leading to impaired vitamin K–dependent coagulation and an increased risk of bleeding, including hemorrhagic stroke. Other reported adverse effects include gastrointestinal disturbances, fatigue, headache, and muscle weakness (35)

D) Vitamin K (Phylloquinone/ Menaquinone)

a) Sources: Green leafy vegetables, Gut Microbiota.

b) Biochemical Functions of Vitamin K: Vitamin K is a fat-soluble vitamin essential for blood coagulation, bone metabolism, and calcium regulation by acting as a cofactor for the γ-carboxylation of vitamin K-dependent proteins (36).

i. ***Vitamin K as a Cofactor for Gamma-Carboxylation:***Vitamin K acts as a cofactor for γ-glutamyl carboxylase, enabling γ-carboxylation of glutamate residues to form calcium-binding proteins essential for blood coagulation and bone metabolism (37).

ii. ***Activation of Prothrombin (Coagulation Factor II):*** Vitamin K is essential for activation of prothrombin (factor II), enabling calcium binding and thrombin formation for fibrin clot formation and normal blood coagulation (38).

iii. ***Activation of Coagulation Factors VII, IX, and X:*** Vitamin K activates coagulation factors VII, IX, and X through γ-carboxylation, enabling normal thrombin generation, clot formation, and prevention of excessive bleeding (38).

iv. ***Role of Vitamin K in Blood Coagulation and Bone Metabolism:*** Vitamin K supports hemostasis by activating clotting proteins and promotes bone health by activating osteocalcin and other calcium-regulating proteins, enhancing bone mineralization while preventing abnormal calcium deposition in soft tissues and blood vessels (39).

c) Vitamin K Deficiency: Vitamin K deficiency may result from poor intake, malabsorption, antibiotics, liver disease, neonatal deficiency, or reduced gut synthesis, primarily affecting coagulation and potentially bone health

i. ***Hemorrhagic Disorders and Bleeding Tendency:*** Vitamin K deficiency impairs activation of coagulation factors II, VII, IX, and X, causing defective clot formation and bleeding manifestations such as bruising, prolonged bleeding, and haemorrhage (40).

ii. ***Prolonged Prothrombin Time (PT):*** Prothrombin time (PT) extension brought on by decreased activity of vitamin K-dependent clotting factors, especially factor VII, is one of the first test results indicating vitamin K insufficiency. PT evaluation is frequently used to monitor anticoagulant medication, test coagulation state, and detect vitamin K insufficiency (41).

iii. ***Vitamin K Deficiency Bleeding (VKDB) in Newborns:*** Newborns are prone to vitamin K deficiency due to low stores and immature gut flora, leading to vitamin K deficiency bleeding (VKDB). Routine vitamin K administration after birth prevents this condition (42) .

iv. ***Effects on Bone Health:*** Vitamin K supports bone health by activating osteocalcin, promoting calcium incorporation into bone tissue, maintaining bone mineralization, remodelling, and skeletal strength. Deficiency is associated with reduced bone density and increased fracture risk (43) .

d) Vitamin K Toxicity: Vitamin K toxicity is rare with natural forms (phylloquinone and menaquinone) because excess amounts are rapidly metabolized and excreted. However, high doses of the synthetic form (menadione, vitamin K_3) can cause hemolytic anemia, hyperbilirubinemia, and liver toxicity, particularly in infants (35).

Table 1: Biologically active coenzyme (or active form) and major deficiency disorders of vitamins

Vitamins	Biologically Active Coenzyme / Active Form	Major Deficiency Disorder(s)
Fat Soluble Vitamins		
Vitamin A (Retinoids)	Retinal (11-cis-retinal), Retinoic acid	Night blindness, Xerophthalmia, Keratomalacia, Impaired immunity
Vitamin D (Calciferol)	Calcitriol [$1,25(OH)_2D$]	Rickets, Osteomalacia, Osteoporosis, Muscle weakness
Vitamin E (Tocopherol)	α-Tocopherol (functions as antioxidant; no coenzyme form).	Hemolytic anemia, Peripheral neuropathy, Ataxia, Myopathy.
Vitamin K	Reduced Vitamin K (Vitamin K hydroquinone; cofactor for γ-carboxylation)	Hemorrhagic disease, Prolonged PT, Vitamin K deficiency bleeding (VKDB), Increased fracture risk.

Water Soluble Vitamins		
Vitamin B1 (Thiamine)	Thiamine Pyrophosphate (TPP)	Beriberi (dry & wet), Wernicke–Korsakoff syndrome
Vitamin B2 (Riboflavin)	Flavin Mononucleotide (FMN), Flavin Adenine Dinucleotide (FAD)	Ariboflavinosis, Angular cheilitis, Glossitis, Seborrheic dermatitis
Vitamin B3 (Niacin)	Nicotinamide Adenine Dinucleotide (NAD^+), Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$)	Pellagra (Dermatitis, Diarrhea, Dementia).
Vitamin B5 (Pantothenic Acid)	Coenzyme A (CoA), Acyl Carrier Protein (ACP)	Burning feet syndrome, Fatigue, Paresthesia (rare)
Vitamin B6 (Pyridoxine)	Pyridoxal Phosphate (PLP)	Sideroblastic anemia, Peripheral neuropathy, Dermatitis, Seizures
Vitamin B7 (Biotin)	Biotin (coenzyme for carboxylases)	Dermatitis, Alopecia, Neurological symptoms
Vitamin B9 (Folate)	Tetrahydrofolate (THF)	Megaloblastic anemia, Neural tube defects, Hyperhomocysteinemia
Vitamin B12 (Cobalamin)	Methylcobalamin, Adenosylcobalamin	Megaloblastic anemia, Peripheral neuropathy, Subacute combined degeneration

3. Water-Soluble Vitamins

Water-soluble vitamins, including B-complex vitamins and vitamin C, require regular intake as they are minimally stored and excess amounts are excreted. They function mainly as coenzymes in metabolism, energy production, DNA synthesis, and cellular maintenance. Vitamin C also supports collagen formation, immunity, iron absorption, and antioxidant defense. Deficiency can cause metabolic, neurological, hematological, and connective tissue disorders (44).

A. Vitamin B1 (Thiamine)

a) Sources: Whole grains, fortified cereals, pork, liver, legumes, nuts, seeds, yeast.

b) Biochemical Active form: Thiamine pyrophosphate (TPP)

c) Biochemical Functions of Vitamin B1: Thiamine (vitamin B1) is a water-soluble vitamin that functions primarily as thiamine pyrophosphate (TPP), a coenzyme essential for carbohydrate metabolism and cellular energy production (45).

i. ***Oxidative Decarboxylation:*** TPP acts as a coenzyme for pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain α-keto acid dehydrogenase**,** facilitating oxidative decarboxylation of α-keto acids for ATP production (46).

ii. ***Role in the Krebs Cycle:*** Thiamine is required for the activity of α-ketoglutarate dehydrogenase in the Krebs cycle, promoting efficient ATP generation through aerobic metabolism, particularly in the brain and heart (46).

iii. ***Role in Carbohydrate Metabolism:*** Thiamine has a central role in carbohydrate metabolism because carbohydrates must be converted into usable cellular energy.

iv. ***Pentose Phosphate Pathway and Neural Function:*** As a coenzyme for transketolase, thiamine supports the pentose phosphate pathway, contributing to NADPH and nucleotide synthesis. It is also essential for normal nervous system function by maintaining efficient glucose metabolism in neural tissues (46).

d) Deficiency of Vitamin B1: Deficiency manifestations include: Beriberi: Dry beriberi: Peripheral neuropathy, muscle weakness, sensory disturbances.Wet beriberi: Cardiovascular dysfunction, edema, tachycardia, and heart failure.Wernicke–Korsakoff syndrome: Neurological disorder characterized by confusion, ataxia, ophthalmoplegia, memory impairment, and cognitive dysfunction. Metabolic impairment: Reduced carbohydrate utilization and accumulation of pyruvate and lactate due to impaired oxidative decarboxylation.

B. Vitamin B2 (Riboflavin)

a) Sources: Milk, eggs, liver, meat, fish, green leafy vegetables, fortified cereals.

b) Biochemical Functions Forms: FAD & FMN.

c) Biochemical Functions of Vitamin B2 (Riboflavin)

Vitamin B2 (riboflavin) is essential for growth, cellular metabolism, and energy production. It acts as a precursor for FMN and FAD, coenzymes involved in oxidation–reduction reactions (47).

i) Energy Production and Oxidation–Reduction Reactions

Converted to FMN and FAD, riboflavin functions as a coenzyme in energy production, carbohydrate, fat, and protein metabolism, electron transport chain, antioxidant defense (glutathione regeneration), activation of vitamins B6 and folate, niacin synthesis, and maintenance of healthy skin, eyes, oral mucosa, and nervous system (47) (48).

c) Deficiency of Vitamin B2

Riboflavin Deficiency (Ariboflavinosis): Deficiency usually occurs due to Poor dietary intake, Malabsorption disorders, Chronic alcoholism, Increased requirements (pregnancy, lactation). Clinical Manifestations: Angular cheilitis, cracks at corners of mouth, Cheilosis - dry, fissured lips, Glossitis (i:e) magenta-colored tongue, Stomatitis, Seborrheic dermatitis, Conjunctivitis, Photophobia, Corneal vascularization, Fatigue and weakness. Effect on Hematological Health. Riboflavin deficiency may impair iron metabolism and contribute to anemia. Neurological and Growth Effects. May lead to impaired growth in children and can affect normal nervous system functioning (49).

C) Vitamin B3 (Niacin)

a) Sources: Meat, fish, poultry, peanuts, whole grains, legumes.

b) Biochemical Functions Forms: NAD and NADP (50).

c) Biochemical Functions of Vitamin B3 (Niacin)

i. Role in Energy Metabolism: Niacin forms NAD^+ and $NADP^+$, which act as electron carriers in metabolic pathways. These coenzymes transfer hydrogen and electrons during oxidation reactions to produce ATP (energy).
ii. Carbohydrate Metabolism: Participates in glycolysis, citric acid (Krebs) cycle, and oxidative phosphorylation. Essential for conversion of glucose into usable cellular energy.
iii. Lipid Metabolism: NADPH is required for fatty acid synthesis, cholesterol synthesis, triglyceride metabolism and supports normal lipid homeostasis.
iv. Protein and Amino Acid Metabolism: Functions as a coenzyme in oxidation and degradation of amino acids. It helps in converting dietary proteins into energy and cellular components.
v. Cellular Oxidation–Reduction Reactions: NAD/NADP participate in several dehydrogenase enzyme systems and helps in maintaining cellular redox balance.
vi. DNA Synthesis and Repair: Niacin contributes to DNA repair mechanisms, cell signalling pathways and maintenance of genomic stability.
vii. Antioxidant Function: NADPH supports regeneration of reduced glutathione, helping protect cells against oxidative stress (51).

d) Deficiency of Vitamin B3

i. Deficiency of niacin causes Pellagra, classically characterized by the "3 Ds":
Dermatitis – photosensitive skin lesions; Diarrhea – gastrointestinal disturbances, Dementia – neurological impairment, if untreated, a fourth D may occur (i:e) Death.
ii. Neurological Manifestations: Deficiency may lead to Irritability, Depression, Memory impairment, Confusion.
iii. Gastrointestinal Manifestations include Glossitis (inflamed tongue), Stomatitis, Nausea and abdominal discomfort. Niacin is used in management of dyslipidemia because it decreases LDL cholesterol, decreases triglycerides, increases HDL cholesterol, historically used as a lipid lowering agent (52).

e) Toxicity (Excess Intake): High doses may cause skin flushing and itching, hepatotoxicity, hyperglycemia, hyperuricemia, and gastrointestinal upset (53).

D). Vitamin B5 (Pantothenic Acid)

a) Sources: Liver, meat, eggs, mushrooms, whole grains, legumes.

b) Biochemical Form: Component of Coenzyme A (54) .

c) Biochemical Functions of Vitamin B5 (Pantothenic Acid)

Pantothenic acid (vitamin B5) is a component of coenzyme A and acyl carrier protein, making it essential for carbohydrate, fat, and protein metabolism. It supports energy production, fatty acid synthesis and oxidation, steroid hormone and acetylcholine synthesis, and the formation of phospholipids and other vital cellular components (55).

d) Deficiency of Vitamin B5

Pantothenic acid deficiency is uncommon because it is widely distributed in foods; however, deficiency may occur in severe malnutrition. i). Symptoms of deficiency include fatigue, weakness, irritability, headache, gastrointestinal disturbances, muscle cramps, numbness and tingling sensations ii). Burning Feet Syndrome: A characteristic manifestation associated with severe deficiency. Patients experience: Burning pain in feet, Tingling and numbness, Sensory discomfort iii). Effects on Energy Production: Deficiency of vitamin B5 reduces CoA availability, impairing ATP production, Fatty acid metabolism and overall cellular energy generation (56).

E) Vitamin B6 (Pyridoxine)

a) Sources: Meat, fish, poultry, bananas, potatoes, legumes, whole grains.

b) Biochemical Form: Vitamin B6 (Pyridoxine) exists in three major forms: Pyridoxine, Pyridoxal and Pyridoxamine.Its active coenzyme form is Pyridoxal Phosphate (PLP) (57).

c) Biochemical Functions of Vitamin B6 (Pyridoxine): Vitamin B6 (pyridoxine) acts as a coenzyme in amino acid metabolism, neurotransmitter and heme synthesis, glycogen breakdown, lipid metabolism, and homocysteine regulation. It is essential for normal nervous system function, hemoglobin production, and energy metabolism (58)

d) Deficiency of Vitamin B6: Vitamin B6 deficiency may occur due to malnutrition, alcoholism malabsorption disorders and long-term use of drugs (e.g., isoniazid). i) Hematological Manifestations: Causes microcytic hypochromic (sideroblastic) anemia due to impaired heme synthesis. ii) Neurological Manifestations: Deficiency may produce Peripheral neuropathy, Irritability, Depression, Seizures, Confusion iii) Dermatological Manifestations include Dermatitis, Glossitis, Cheilosis and Seborrheic skin changes. iv) Elevated Homocysteine Levels: Deficiency of vitamin B6 may increase homocysteine concentration and contribute to cardiovascular risk.

F) Vitamin B7 (Biotin/Vitamin H)

a) Sources: Eggs, liver, nuts, seeds, legumes, whole grains.

b) Biochemical Form: Coenzyme for Carboxylation reactions.

c) Biochemical Functions of Vitamin B7 (Biotin): Biotin acts as a coenzyme for carboxylation reactions involved in carbohydrate, fat, and amino acid metabolism. It supports gluconeogenesis, fatty acid synthesis, energy production, and also contributes to gene regulation, cell growth, and normal metabolic function (59).

d) Deficiency of Vitamin B7: Biotin deficiency is rare but may occur with prolonged raw egg white intake, malabsorption, long-term antibiotic use, or inherited disorders. It causes dermatitis, alopecia, neurological symptoms, and impaired fatty acid, glucose, and amino acid metabolism. Untreated inherited biotin metabolism defects can result in severe neurological and metabolic complications(60).

G. Vitamin B9 (Folate)

a) Sources: Green leafy vegetables, legumes, citrus fruits, liver, fortified cereals.

b) Biochemical Form: The biologically active form is Tetrahydrofolate (THF), which functions as a carrier of one-carbon units in several metabolic reactions (61).

c) Biochemical Functions of Vitamin B9 (Folic Acid) (62): Folate is essential for one-carbon transfer reactions, DNA and nucleotide synthesis, amino acid metabolism, red blood cell formation, and normal cell growth. It also supports foetal development and regulates gene expression through methylation reactions.

d) Deficiency of Vitamin B9: Folate deficiency causes megaloblastic anemia, fatigue, glossitis, and elevated homocysteine levels. During pregnancy, it increases the risk of neural tube defects, while excessive folic acid may mask vitamin B12 deficiency (63).

H. Vitamin B12 (Cobalamin)

- **Source:** Eat, Fish, Eggs, Dairy
- **Biochemical form:** The active coenzyme forms are Methylcobalamin and Adenosylcobalamin (Deoxyadenosylcobalamin)(64).
- **Biochemical Functions of Vitamin B12 :**Vitamin B12 is essential for DNA synthesis, red blood cell formation, and one-carbon metabolism with folate. It supports methionine synthesis, fatty acid and amino acid metabolism, normal nerve function, and homocysteine regulation(65) .
- **Deficiency of Vitamin B12 (Cobalamin):** Vitamin B12 deficiency causes megaloblastic anemia, neurological dysfunction, peripheral neuropathy, and, in severe cases, spinal cord degeneration. It is characterized by elevated homocysteine and methylmalonic acid levels (66)

I. Vitamin C (Ascorbic Acid)

a) Sources: Citrus fruits, Tomatoes and Berries

b) Biochemical Functions of Vitamin C (Ascorbic Acid)

Vitamin C Essential for collagen synthesis, antioxidant defense, iron absorption, carnitine and neurotransmitter synthesis, immune function, wound healing, tissue repair, and hydroxylation reactions in metabolism (67).

c) Deficiency of Vitamin C: Causes scurvy, characterized by defective collagen synthesis, bleeding gums, easy bruising, petechiae, poor wound healing, bone pain, fatigue, fragile

connective tissue, reduced iron absorption (iron deficiency anemia), and increased requirements during pregnancy, lactation, smoking, infections, and wound healing.

d) Toxicity: High doses may cause gastrointestinal discomfort, diarrhea, increased risk of kidney stone formation in susceptible individuals (68).

5. Vitamins and Disease Prevention

Vitamins are essential for disease prevention by supporting immune function, antioxidant defense, metabolism, and tissue repair.Adequate vitamin intake helps maintain bone, cardiovascular, and neurological health while reducing the risk of anemia, osteoporosis, infections, and other chronic diseases.A balanced diet is therefore crucial for long-term health and disease prevention (69).

6. Clinical Assessment of Vitamin Status

Clinical assessment of vitamin status combines dietary history, physical examination, and laboratory tests to identify deficiencies or excesses. Accurate diagnosis requires integrating clinical findings with biochemical markers while considering individual health and physiological factors (70).

Future Perspectives

Advances in nutrigenomics and precision nutrition are enabling personalized vitamin recommendations based on genetic, metabolic, and lifestyle factors. These approaches have the potential to optimize health, prevent chronic diseases, and improve clinical nutrition through individualized dietary interventions.

Conclusion

Vitamins are indispensable micronutrients that regulate essential biochemical pathways involved in metabolism, growth, immunity, antioxidant defense, and cellular function. Both deficiency and excessive intake can lead to significant clinical disorders, highlighting the importance of maintaining optimal vitamin status. A thorough understanding of vitamin biochemistry and clinical applications is fundamental for the prevention, diagnosis, and management of nutritional and metabolic diseases. Advances in nutrigenomics and precision nutrition are further expanding the role of vitamins in personalized healthcare. Collectively, vitamins remain central to promoting health, preventing disease, and improving clinical outcomes.

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CURCUMIN: A VERSATILE LEAD MOLECULE IN MEDICINAL CHEMISTRY

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Abstract

Curcumin, the bioactive molecule isolated from the rhizomes of *Curcuma longa* (turmeric), has attracted considerable attention in medicinal era owing to its diverse pharmacological activities and satisfactory safety profile. From centuries, it has been used in traditional, while contemporary research has established its therapeutic effect against anticancer, anti-inflammatory, antimicrobial, cardiovascular diseases, diabetes, neurodegenerative etc. The broad-spectrum activity of curcumin is primarily attributed to its ability to regulate multiple molecular targets and signaling pathways involved in oxidative stress, inflammation, apoptosis, angiogenesis, and cell proliferation. These characteristics make curcumin an effective lead molecule for the design and development of novel therapeutic agents in medicinal era.

Despite this, the importance of curcumin is deteriorated by poor aqueous solubility, low bioavailability, and rapid metabolism. To overcome this, extensive research has focused on structural conversions, synthesis of curcumin and their derivatives, and the development of advanced drug delivery systems, including nanoparticles, liposomes, and nanocarriers.

This chapter comprehensively reviews chemistry, curcumin moiety-based drug discovery, and future perspectives, highlighting its significance as a versatile lead bioactive molecule for developing safer and more effective therapeutic agents in medicinal era.

Keywords: Curcumin, *Curcuma longa*, Medicinal Chemistry, Lead Molecule, Drug Discovery, Pharmacological Activities.

Introduction

Heterocyclic compounds are good pharmacophore and associated with promising biological activity. They are ring-shaped molecular frameworks containing one or more heteroatoms, such as nitrogen (Devi Diksha, Singh Raman, 2026), oxygen, or sulphur, and represent one of the most important structural motifs in medicinal chemistry. The remarkable success of these active scaffolds, in the development of clinically approved drugs highlights the importance of biologically active lead molecules. In a similar context, the broad pharmacological profile of curcumin has established it as a valuable natural lead compound for the rational design and development of novel therapeutic agents.

Turmeric is recognized to contain a large amount of curcumin, a yellow pigment that is a type of polyphenol and has long been used as a spice and coloring compound. Turmeric holds a good position as a herb in the AMS (Ayurvedic medicine system) and CMS (Chinese medicine

systems). It holds medical properties which provide overall well-being to the body. Curcuma longa (belonging to the same family as ginger), whose roots have a characteristic orange or intense yellow colour. The Rhizome part of the Turmeric plant is used in the medical and cosmetic world. It is found in East Indies region and grows naturally in the wide range of tropical and subtropical hot and humid regions in Asia, Africa, and Central and South America. The use of turmeric is very ancient. It is also mentioned in the Ayurvedic medicine system which is around 500 BCE. The use of turmeric gets famous day by day.

Latin Name: Curcuma is the Latin name for turmeric or Haldi.
Family Member: Turmeric belongs to the Zingiberaceae family, the same as the ginger family.
Common Name: Haldi is the common form of turmeric in Hindi. In Sanskrit, it is known as Harida. Manjal is also famous in South India.
Habitat: Turmeric is a tropical plant, that required humid warm weather with a lot of rainfall
Season: Turmeric is harvested in the early winter season.

Nutritional Composition

Turmeric is full of nutritional value. It contains fibre, protein, niacin, vitamins C, E and K, Na, K, Ca, Cr, Fe, Mg, and Zn. But, since it is a seasoning to give flavour and aroma, the amount consumed is very little. It is a powerful medicinal plant used in various biological activities like antioxidant, anti-inflammatory, antibacterial, anti-microbes, Improving Liver Function, Improving Digestion, Natural Pain Relief, Appetite-Enhancing Effect, Reduces Arthritis, Immune-enhancing effect, Reduce Irritable bowel syndrome, Reverse Alzheimer's Disease, improvement in Skin Health etc. The consumption of turmeric or curcumin as a nutritional supplement is considered safe when administered orally or topically but still there are side effects of turmeric like nausea, diarrhoea, or stomach upset in some people. Turmeric contains Vitamin C, vitamin B6.

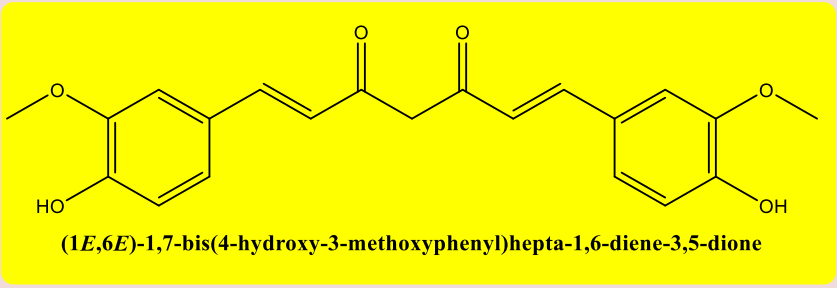
Biological Importance and Chemistry of Curcumin:

- Curcumin's structure is crucial for its biological activities.
- It is a polyphenol, specifically a diarylheptanoid, which is a type of phenolic pigment.
- This Phenolic Pigment is responsible for the yellow color of turmeric.
- Curcumin's structure includes a seven-carbon linker and three major functional groups: an α, β-unsaturated β-diketone moiety and an aromatic O-methoxy-phenolic group.
- These functional groups are responsible for its various biological activities, including anti bacterial, anti-inflammatory, and antioxidant properties.

Curcumin's structure is a great example of how a molecule's biological activity traces directly to its architecture — each functional group plays a distinct role. The phenolic groups are antioxidants, the diketone chelates metal ions, and the conjugated unsaturation extends its

reactivity. On each side there is aromatic groups — each carries a methoxy (–OCH_3) and a hydroxyl (–OH). α,β-unsaturated portion of the linker: two conjugated C=C double bonds sitting right next to the carbonyls, which extends the molecule's conjugation and gives curcumin its yellow color. There is also β-diketone - two ketone (C=O) groups separated by a single CH_2, which is the site that lets curcumin chelate metal ions and tautomerize into its enol form. Together, the seven carbons between the two rings (the two vinyl carbons on each end plus the three-carbon diketone core) make up the "seven-carbon linker".

Curcumin



(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

Curcumin; Diferuloylmethane; Natural yellow; Kachi haldi; MF: $C_{21}H_{20}O_6$, MW: 368.4 g/mol, IUPAC Name: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione. Found in 2 form keto and enol.

Chemical Structure: Curcumin is a beta-diketone with two hydrogens substituted by feruloyl groups.

Literature was sourced through a PubMed search using relevant terms such as curcumin, and found that curcumin gives promising research outcomes (El-Rakabawy *et al.,* 2025; LeBlanc *et al.,* 2026; Zafar *et al.,* 2025) as bioactive molecule .

Further in view of exploding pharmacological importance literature survey revealed that, curcumin is a naturally occurring polyphenol molecule (El-Saadony *et al.,* 2025) and active component of turmeric which is responsible for good biological activities and better nutritional aspects. It is lipophilic and has demonstrated *in vitro* and *in vivo* therapeutic effects through multiple pathways.

Curcumin modulated key routes including PI3K/Akt/mTOR (8 studies), NF-κB (7), AR signaling, and apoptosis-related regulators. Valuable outcomes included apoptosis, necroptosis, cell cycle arrest, suppression of migration and angiogenesis. Clinical trials evaluating optimized curcumin delivery systems in well-defined PCa populations are greatly proposed by author (Esmaeli & Dehghanpour Dehabadi, 2025).

Complementing these findings, the next study reveals that curcumin polyphenol (Jiao *et al.,* 2025) (derived from *Curcuma longa)*, has secured significant consideration for its neuroprotective properties, particularly through epigenetic mechanisms. Author's research explores the multifaceted role of curcumin in molecular key-pathways involved in neuroplasticity, including histone modifications, DNA methylation, and non-coding RNA

regulation. Additionally, it also influences neurogenesis, synaptic remodeling, and mitochondrial biogenesis, which are important for maintaining and upholding brain function in aging and neurodegenerative conditions such as Alzheimer's and Parkinson's disease.

In a parallel analysis, the authors (Yu *et al.,* 2025) further illustrate cognitive aging is a rising public health concern, and curcumin, a bioactive molecule , has been proposed as a promising intervention due to its anti-inflammatory and antioxidant properties. Finally, they concluded that curcumin enhances cognitive function in AD models.

Building a bridge to the earlier work, the later article confirms that parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons, aggregation of α-synuclein (α-Syn), lysosomal dysfunction, and mitochondrial impairment and curcumin (Li *et al.,* 2026) has demonstrated neuroprotective effects against PD pathology; however, its poor bioavailability, rapid systemic clearance, and limited blood-brain barrier permeability remain significant challenges to be overcome.

In contrast to the prior work, further Mohammed and coauthor emphasizes that there is no significant differences were observed between the two groups at the 2-, 4-, and 12-week follow-ups. Additionally, curcumin (Al-Maweri *et al.,* 2025) and corticosteroids demonstrated comparable efficacy in clinical improvement of lesions across different time points.

Aligning with the earlier conclusions, the later research shows, that curcumin as a therapeutic agent in liver cancer such as anti-proliferative, pro-apoptotic, anti-inflammatory, or anti-metastatic roles. Curcumin notably induced apoptosis in liver cancer cells, with fourteen studies reporting dose-dependent increases in apoptosis markers such as caspase-3 and Bax expression. Anti-inflammatory activities were evident in five studies, with curcumin inhibiting NF-κB activation, a key pathway in liver cancer progression. Additionally, antioxidant and anti-angiogenic properties were observed, as curcumin reduced lipid peroxidation and decreased VEGF expression. Two studies highlighted curcumin's potential in suppressing metastasis, with dose-dependent inhibition of liver cancer cell migration and invasion. Curcumin demonstrates promising anti-cancer effects (Ma *et al.,* 2025), including apoptosis induction, inflammation modulation, and metastasis inhibition.

Integrating these insights, the researcher Zisheng and co-workers (Yan *et al.,* 2025) proposed a unified interpretation that curcumin is very useful for the treatment of pituitary adenomas. Pituitary adenomas (PAs), accounting for 10 to 15% of intracranial tumors, affect significant morbidity through endocrine dysfunction and mass effects. Their work also synthesizes evidence on curcumin's dual roles: suppressing tumor progression and ameliorating hormone-driven metabolic disorders. It is concluded that, Curcumin bioactive molecule is able to concurrently target tumor growth, hormone hypersecretion, and metabolic complications positions it as a unique "one drug, multiple effects" candidate.

Moving further, the subsequent paper explores ginger-derived vesicle-like nanoparticles loaded with curcumin to alleviate ionizing radiation-induced intestinal damage via gut microbiota regulation.

Engineered biomimetic nanovesicles (GDN-Cur) exhibited promising stability in the gastrointestinal tract, thereby notably enhancing the oral bioavailability of Cur. Consequently, the intrinsic antioxidative, anti-inflammatory, and anti-apoptotic properties of GDNs and Cur granted this nanosystem exceptional protective effect against IR-induced injuries, especially in mitigating intestinal damage. Particularly, the dysbacteriosis triggered by IR could be counteracted through the oral administration of GDN-Cur (X. Zhang *et al.,* 2025), resulting in gut microbiota regulation-mediated syndrome mitigation. Furthermore, elevated abundances of *Akkermansia muciniphila* (*A. muciniphila*), a bacterial strain of *Akkermansia* taxa responsive to GDN-Cur, especially their supernatants, were associated with post-radiation protection of intestinal function. This beneficial effect was attributed to the identified radioprotective metabolites secreted by *A. muciniphila*, such as tanespimycin (17-AAG), which was demonstrated to deactivate AKT/NF-κB signaling pathway. These findings reveal the impact of plant products on radioprotective microbes and metabolites to target host processes and alleviate IR-induced intestinal damage, shedding light on new insights in the development of novel radioprotectants.

Curcumin and Curcuma longa extract have limited and inconsistent effects on inflammatory biomarkers in patients with Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). Larger, well-designed Randomized controlled trials (RCTs) are needed to clarify their clinical utility as adjunct therapies

The next study regarding atherosclerosis, a chronic inflammatory disease defined by plaque formation in artery walls, is still a significant cause of cardiovascular morbidity and mortality globally. Despite advancements in conventional medicines, the search for natural substances with several therapeutic applications continues. Curcumin(Yadav *et al.,* 2025), a polyphenol produced from Curcuma longa, has emerged as a promising anti-atherosclerosis treatment due to its powerful anti-inflammatory, antioxidant, lipid-modulating, and endothelial-protective characteristics.

Curcumin/piperine (Y. Zhu *et al.,* 2025) enhances renal and intestinal integrity and modulates sodium butyrate (NaB) levels to alleviate mitigates diabetic kidney disease (DKD) through the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) (Nrf2/HO-1) signaling pathway. Curcumin/piperine significantly improved renal injury and intestinal structural integrity, upregulated the expression of macrophage antigen CD68 and other inflammatory markers in the kidneys, and increased NaB levels in feces ($p < .05$). High glucose conditions enhanced inflammation and oxidative stress in HK-2 cells, whereas C/P and NaB pretreatment significantly inhibited these effects, reducing interleukin-6, IL-1β and other markers

($p < .05$), and increasing Nrf2 and HO-1 expression. Nrf2 inhibitor ML385 reduces the expression of Nrf2 and HO-1 and weakens the beneficial effects of C/P and NaB on HK-2 cells.

Curcumin (R. Zhang *et al.,* 2025) controls adipose tissue-derived mesenchymal stem cells (ADSCs) senescence via the FoxO3-mediated signaling pathway, and FoxO3 directly regulates Becnl to induce autophagy. Intra-articular injection of curcumin-pretreated ADSCs enhances joint homeostasis for Osteoarthritis (OA) treatment. This study provides evidence for the potential clinical application of curcumin-pretreated ADSCs in enhancing the therapeutic effects of OA treatment.

In addition to its well-established biological activities, curcumin (Liu *et al.,* 2025) also ameliorates benzalkonium chloride-induced dry eye disease in mice. Dry eye disease (DED) is worsened by benzalkonium chloride (BAC), a preservative in ophthalmic formulations, which induces ocular damage via inflammation, apoptosis, and oxidative stress. Using network toxicology, molecular docking, dynamics simulations, and murine models, they also elucidated BAC's mechanisms and evaluated curcumin (CUR) as a therapeutic agent

Beyond its traditional benefits evidence, the study retrieved that turmeric/curcumin (Moradi Baniasadi *et al.,* 2025) has a therapeutic effect on some obesity indicators, which could contribute to weight management in individuals with prediabetes and type 2 diabetes mellitus (T2DM).

Following this discussion, consideration is directed toward neuroprotective Potential of Curcumin (Islam *et al.,* 2025) in Neurodegenerative Diseases. Clinical research indicates that curcumin can penetrate the blood-brain barrier, making it an effective treatment option. The regulation of these pathways reduces neuronal damage and improves neurons' survival and functionality. In addition, curcumin's anti-inflammatory properties and low toxicity make it a promising long-term treatment option for NDs

In continuation of the above discussion, further studies emphasized the significance of curcumin in medicinal chemistry. Curcumin (Fan *et al.,* 2025) significantly enhanced m6A methylation of PPARα mRNA by inhibiting FTO, thereby activating the PPARα/CPT1α signaling pathway, reducing lipid accumulation, and slowing the progression of Non-alcoholic fatty liver disease (NAFLD). In conclusion, we revealed for the first time that curcumin alleviates hepatic cell lipid accumulation by regulating.

Another noteworthy aspect extensively discussed in the literature is, by regulating Apelin expression, curcumin (Akca *et al.,* 2025) may serve as a potential adjunct in cardioprotective approaches where apelin acts as an important mediator in Dox cardiotoxicity and may be used as a target for treatment of certain cardiomyopathies.

Several studies reports that structural diversity of curcumin and its derivatives is for biological activity. Their findings indicate that a structural modification of the keto form of curcumin (Molski, 2025), (involving the attachment of two 4-hydroxy-3-methoxyphenyl-prop-1-en-2-one

moieties to the methylene group), referred to as di-curcumin, exhibits enhanced chemical reactivity and increased anti-radical potential.

In this regard, the existing literature also provides substantial evidence supporting the broad therapeutic spectrum of curcumin in Ulcerative colitis (UC), chronic inflammatory bowel disease. Natural products, such as curcumin (Ning *et al.,* 2025) , have emerged as potential effective drugs for UC treatment due to their multi-target and multi-mechanism therapeutic advantages

This comprehensive analysis presented by Budan F and Coauthors(23) revealed that, curcumin intake impacts postmenopausal symptoms (e.g., improving symptoms of osteoporosis) through its antioxidant and anti-inflammatory effects but its different forms and doses, combinations, and durations of interventions may influence outcomes

In Continuation, the extensive research conducted on curcumin (J. Zhu *et al.,* 2025) explored that it may exert anti-CRC (Colorectal cancer) effects by inducing caspase-1-mediated pyroptosis, emphasizing its pharmacological activity. Further findings also suggest that curcumin could be integrated with current CRC treatment strategies, particularly in targeting pyroptosis to enhance tumor suppression.

Complementing the previous findings, reports have explored the protective effect of curcumin (Wang *et al.,* 2025) against sepsis-associated acute kidney injury (SA-AKI) in vitro and in vivo, and clarify the role of ferroptosis in this protection. Curcumin attenuates SA-AKI, likely by suppressing inflammation and ferroptosis via the ACSL4/GPX4 signaling pathway.

Conclusion

Curcumin, a natural polyphenolic compound obtained from *Curcuma longa*, continues to attract considerable attention in medicinal chemistry because of its broad therapeutic potential and good structure activity relationship. The studies reviewed in this article indicate that curcumin shows valuable effects against several diseases, including cancer, diabetes, cardiovascular disorders, microbial infections, inflammatory conditions, and neurodegenerative diseases etc. Although its clinical application is restricted by poo

r solubility and low bioavailability, recent progress in the synthesis of curcumin analogues and advanced drug delivery systems has shown positive results. Overall, curcumin remains a beneficial lead molecule for future drug discovery, and further preclinical and clinical investigations are expected to support its development into safe and effective therapeutic agents.

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MICROBIOME ENGINEERING AND MULTI-OMICS TECHNOLOGIES IN PRECISION ANIMAL NUTRITION: MOLECULAR MECHANISMS AND BIOTECHNOLOGICAL INNOVATIONS

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Abstract

The gastrointestinal microbiome act as key regulator of nutrient metabolism and immune function in precision animal nutrition. This chapter highlights recent advances technologies in microbiome engineering and multi-omics for modulation of host–microbiome interaction. It discusses innovative strategies, including probiotics, prebiotics, postbiotics, engineered microorganisms, bacteriophage therapy and CRISPR-based genome editing along with genomics, metagenomics, transcriptomics, proteomics and metabolomics for functional analysis of dietary response. The integration of artificial intelligence and digital twin technologies provide predictive nutritional modelling and precision feeding for sustainable improvement in animal health, feed efficiency, productivity and environmental issues.

Keywords: Gut Microbiome, Multi-Omics, Artificial Intelligence, Precision Nutrition, Livestock

1. Introduction

Animal nutrition has shifted from conventional nutrient-based feeding strategies toward precision nutrition, where dietary interventions are specifically to improve animal health, production and sustainability of livestock (Khan *et al.,* 2024; Nath, 2026). Additionally, the gut microbiome play role on nutrient digestion, energy production, immune regulation, intestinal barrier integrity and host metabolism (Mohammad *et al.,* 2025). Therefore, gut microbes involving in molecular interactions with the host through microbial metabolites, signalling as well as immune pathways, thereby shaping the animal phenotype and overall performance (Rowland *et al.,* 2017). Recent advances in molecular biology demonstrated that dietary nutrients govern many physiological effect on host indirectly through microbial metabolism. They regulate nutrient sensing, epithelial homeostasis, inflammatory responses and systemic metabolism through various pathways (Jyoti & Dey, 2025; Li *et al.,* 2026). Consequently, the host and its microbiome are considered as the integrated biological system.

These recent research studies have led to the emerging of microbiome engineering, a rapidly evolving discipline that aims to manipulate microbial community composition or function to

improve host health and nutritional efficiency (Duhan *et al.,* 2025). Simultaneously, the rapid advancement of multi-omics technologies, which transformed microbiome research from descriptive microbial profiling to mechanistic functional analysis and provide characteristics of microbial composition, gene expression, protein activity, metabolic pathways and host responses (Yang *et al.,* 2025). Therefore, integration of microbiome engineering with multi-omics which important for modern livestock production, which faces more challenges associated with antimicrobial resistance (AMR), climate change, greenhouse gas emissions, feed resource scarcity and the growing global demand for animal-derived foods (Bunny *et al.,* 2026; Hossein-Zadeh, 2026). Microbiome-targeted dietary approaches have the potential to enhance feed conversion efficiency, reduce enteric methane production, improve nitrogen utilization without any negative impact on productivity (Khan *et al.,* 2024; Ogwu *et al.,* 2026).

Despite these much advancement, still there is various challenges remaining in microbiome engineering. The microbiome is a dynamic ecosystem conditions which population is affected by host genetics, age, diet, management practices and environmental conditions (Ma *et al.,* 2023; Lin *et al.,* 2026). Additional challenges including inter-individual variability among the different host, functionality diversity among microbial taxa, lack of standardized analytical techniques and difficulties in integration of large multi-omics (Yang *et al.,* 2025). For these limitations, require of interdisciplinary approaches which combine microbiology, biotechnology, nutrition, computational biology and artificial intelligence for sustainable development of livestock. Therefore, this chapter highlights the molecular basis of host–microbiome interactions, recent advances in microbiome engineering and multi-omics technologies and their integration into precision animal nutrition.

2. The Gastrointestinal Microbiome as a Central Regulator

The gastrointestinal (GI) microbiome (functional metabolic organ) extends the functional capacity of the host beyond its own genome (Carroll *et al.,* 2009). It also often referred to as the second genome or accessory genome of the host. This microbial gene pool encodes of various enzymes that are absent in mammalian genomes, which do the degradation of indigestible dietary components, biosynthesis of essential nutrients, detoxification of harmful compounds, and production of bioactive metabolites that influence on physiology of host (Khan *et al.,* 2024).

As a systems biology point of view, the gastrointestinal microbiome functions as a complex biochemical reactor in which dietary substrates are converted into thousands of metabolites that participate in host cellular signalling. With these signals, it interacts with molecular and provide communication network linking microbial metabolism with host physiological processes. Functional integration of diet, gut microbiota, and host molecular signalling in precision animal nutrition is illustrated in Figure 1.

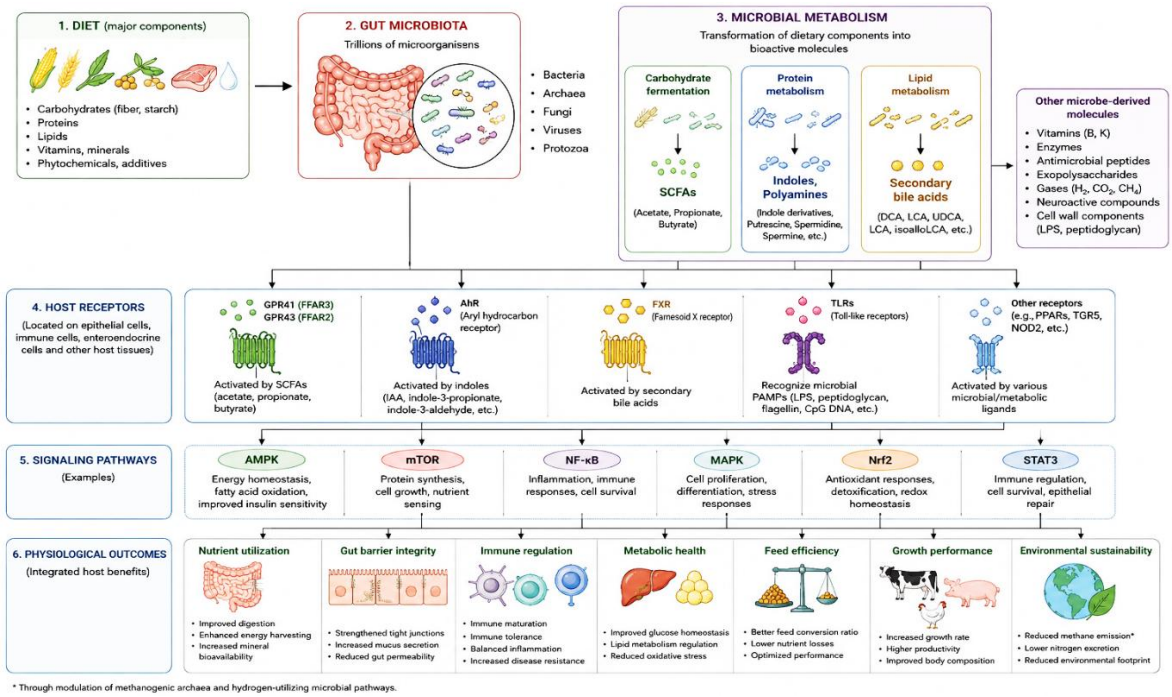

Figure 1: Conceptual Framework of the Gut Microbiome as a Functional Integration of Diet, Gut Microbiota, and Host Molecular Signalling

In ruminants, the microbiome plays important role by fermentation of structural carbohydrates into volatile fatty acids (VFAs), which provide approximately 60–80% of the metabolizable energy requirement of host (Park *et al.,* 2024). Specific microbial populations also synthesize microbial protein and B-complex vitamin via participating in nitrogen recycling (Jiang *et al.,* 2022; De Oliveira *et al.,* 2025). Conversely, methanogenic archaea which convert hydrogen into methane, which as energy loss to the animal and a significant source of greenhouse gas emissions. Engineering of these microbial ecosystems provide opportunities to enhance feed efficiency and reduce environmental impacts. In monogastric (poultry and pigs), microbiome improve carbohydrate fermentation, bile acid regulation, immunity and maintain epithelial integrity in host (Pickard *et al.,* 2017; Jia & Dong, 2024; Mohammad *et al.,* 2025). Beneficial gut microbes compete with pathogen for nutrients and adhesion sites with produce antimicrobial compounds and modulating mucosal immune responses which inhibit pathogenic colonization (Pickard *et al.,* 2017).

In addition to this, gut microbiome is recognized as a regulator of endocrine and neuroimmune signalling through the gut–brain and gut–liver axes (Mohammad *et al.,* 2025). Microbial metabolites regulate glucagon-like peptide-1 (GLP-1), peptide YY (PYY), serotonin and other hormones that play important role in appetite, energy balance, insulin sensitivity and stress responses (De Silva & Bloom, 2012). Microbial-derived molecules interact with immune receptors, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors which regulates inflammatory signaling pathways such as nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK) and the Janus kinase–signal

transducer and activator of transcription (JAK–STAT) (Zhou *et al.,* 2019; Wicherska-Pawłowska *et al.,* 2021; Mohammad *et al.,* 2025). These interactions provide nutritional responses not arise from dietary composition alone but from coordinated molecular communication among diet, microbiota and host tissues. Collectively, these all finding suggested the gastrointestinal microbiome as a central regulator of precision animal nutrition.

3. Microbiome Engineering: Innovative Strategies for Precision Animal Nutrition

Recently, Microbiome engineering play very important role in precision animal nutrition. It alters the structure and function of gastrointestinal microbial communities lead to improve in host health, nutrient utilization and production (Khan *et al.,* 2024; Duhan *et al.,* 2025). Microbiome engineering has specific targeted strategies (ecological, molecular and biotechnological) for reshaping microbial ecosystem and their functional potential for improving gut health (Moon, 2024; Obi, 2026). Nutritional based strategies modulating the gut microbiota mainly through antibiotics, probiotics, prebiotics, organic acids, fiber sources etc, (Duhan *et al.,* 2025; Kango & Nath, 2024; Ravanal *et al.,* 2025). Although these interventions have not varied much successful, their effects are non-specific manner and not consistent and fail to account for inter-individual microbial variability. Globally, restriction of antibiotic as a growth promoters, along with advances in microbial ecology and high-throughput sequencing technologies, has increased the transition toward precision microbiome engineering, where interventions are targeted based on microbial functions (Moon, 2024; Duhan *et al.,* 2025).

Modern microbiome engineering is mainly focus on the principle that microbial communities have ecological characteristics. Consequently, effective manipulation of the microbiome requires an understanding of microbial interactions with their host. Thus, Engineering strategies focus on modification of microbial metabolic networks, beneficial functional improvement, reduce pathogenic populations and improvement in physiological outcomes (Khan *et al.,* 2024; Mohammad *et al.,* 2025). This systemic level perspective, separate microbiome engineering from traditional microbial supplementation and possess greater goals towards precision nutrition.

Advances in molecular biotechnology have expanded the microbiome engineering technologies for improving health of animals. Recent advanced approaches include next-generation probiotics, designer prebiotics, synbiotics, postbiotics, bacteriophage therapy, CRISPR-based genome editing and synthetic biology (Ma *et al.,* 2022; Ali *et al.,* 2024; Chaudhari & Ranjan, 2024; Kango & Nath, 2024; Xiang *et al.,* 2025). These technologies improve selective modulation of microbial communities with greater specificity and reproducibility. Major microbiome engineering strategies with its mechanism, application in animal nutrition is presented in Table 1.

In livestock production, microbiome engineering has opportunities to address various present challenges. Modulation of rumen environment led to improve fiber degradation, microbial protein synthesis, reduce methane emissions and increase feed conversion efficiency in ruminants (Park *et al.,* 2024; De Oliveira *et al.,* 2025; Zhao *et al.,* 2025; Hossein-Zadeh, 2026).

Table 1: Major Microbiome Engineering Strategies for Precision Animal Nutrition

Strategy	Mechanism of Action	Representative Examples	Applications in Animal Nutrition	References
Probiotics	Competitive exclusion of pathogens, SCFA production, immune modulation, enhancement of intestinal barrier	*Lactobacillus*, *Bifidobacterium*, *Bacillus subtilis*, *Enterococcus faecium*	Improved feed efficiency, gut health, growth performance, reduced enteric diseases	Kango & Nath, 2024; Moon, 2024
Prebiotics	Selective stimulation of beneficial microorganisms and SCFA production	Inulin, FOS, GOS, MOS, resistant starch	Enhanced nutrient digestibility, improved immunity and intestinal health	Ravanal *et al.,* 2025
Synbiotics	Synergistic combination of probiotics and prebiotics	*Bacillus* + FOS; *Lactobacillus* + Inulin	Better gut health, growth and disease resistance	Yadav *et al.,* 2022
Postbiotics	Delivery of microbial metabolites or inactivated microbial cells that modulate immunity and epithelial integrity	Butyrate, exopolysaccharides, microbial peptides, cell-wall fragments	Improved intestinal barrier function, reduced inflammation	Prasad *et al.,* 2025
Engineered Probiotics / Synthetic Biology	Genetically modified microorganisms produce therapeutic molecules, enzymes or vitamins	Engineered *Lactococcus lactis*, *E. coli* Nissle 1917	Precision nutrient delivery, pathogen inhibition, bio-sensing	Ma *et al.,* 2022; Xiang *et al.,* 2025
CRISPR-Based Microbiome Editing	Precise editing of microbial genomes to modify metabolic pathways or eliminate undesirable traits	CRISPR-Cas9, Cas12, Cas13	Methane mitigation, antimicrobial resistance control, Improve fermentation	Ali *et al.,* 2024; Chaudhari & Ranjan, 2024
Bacteriophage Therapy	Selective lysis of pathogenic bacteria without disturbing beneficial microbiota	Phages targeting *Salmonella*, *E. coli*, *Clostridium perfringens*	Reduction of enteric pathogens, lower antibiotic use	Zhao *et al.,* 2025; Rahman *et al.,* 2026
AI-Guided Microbiome Engineering	Integration of multi-omics, machine learning and network biology to predict microbiome responses	Machine learning, Digital Twin models, systems biology	Personalized nutrition, biomarker discovery, precision feeding	Kinkpe *et al.,* 2025

Despite these advancements, microbiome engineering remains as an early stage of translational application. Therefore, successful nutritional engineering requires precise characterization of microbial function, uses of advance computational modelling, standardized analytical methods and long-term validation under livestock production (Choudhary *et al.,* 2024; Yu *et al.,* 2024; Yang *et al.,* 2025). Overcoming from these challenges, will be essential for translating of laboratory technologies into practical precision nutrition strategies under production system.

4. Multi-Omics Technologies for Precision Animal Nutrition

The rapid advancement of high-throughput sequencing and analytical technologies has transformed animal nutrition from focused on only nutrient requirements into a data-driven science knowing the complex host–microbiome interactions at multiple biological levels (Choudhary *et al.,* 2024; Yu *et al.,* 2024). These multi-omics technologies provide characteristics of the genome, transcriptome, proteome, metabolome and phenome which provide information regarding relationship between diet, microbial function and animal performance (Ramos-Lopez *et al.,* 2022; Wanapat *et al.,* 2024).

The concept of multi-omics which provide the integrative analysis of multiple biological datasets which is generated through various omics platform. Multi-omic technologies create biological networks through integration of various genes, transcripts, proteins, metabolites, microbial communities and phenotypic traits (Kinkpe *et al.,* 2025; Choudhary *et al.,* 2024). This integrated approach becomes the cornerstone in precision animal nutrition.

Within the gastrointestinal ecosystem, each omics technology provides different but complementary biological information of the host. Genomics analyses genetic variation associated with nutrient utilization and disease susceptibility in host while metagenomics involves the characterization of the taxonomic composition and functional potential of different species of microbes (Yu *et al.,* 2024; Choudhary *et al.,* 2024). Transcriptomics and metatranscriptomics are involved in the active expression of host and microbial genes under specific dietary conditions, while proteomics provides complete identification of functional proteins that are responsible for enzymatic activity and cellular signalling (Wanapat *et al.,* 2024; Yang *et al.,* 2025). Additionally, Metabolomics analyses small molecules that directly mediate through host–microbiome interactions (Jia & Dong, 2024; Li *et al.,* 2026). Integration of these datasets provides a complete understanding of how dietary components affect the microbial metabolism and subsequently their effect on the host. Various multi-omics technologies with its application is presented in Table 2.

In livestock production, multi-omics has emerged as an essential tool for the identification of various biomarkers associated with feed efficiency, methane emission, nutrient digestibility, immunity, reproductive performance and disease resistance (Yu *et al.,* 2024; Park *et al.,* 2024; Ogwu *et al.,* 2026). Unlike conventional nutritional biomarkers, which mainly depend on blood metabolites and production traits, multi-omics which identification of molecular components

that predict animal performance before phenotypic changes occur (Choudhary *et al.,* 2024; Kinkpe *et al.,* 2025). Such capabilities of these technologies support the development of individualized feeding strategies and improve genetic and nutritional programs. These technologies provide identification of key regulatory pathways, microbial biomarkers and nutrient–gene interactions which are remain undetected using single-omics analysis. Consequently, multi-omics has become the next-generation precision nutrition, allowing designing of dietary interventions based on molecular phenotypes (Ramos-Lopez *et al.,* 2022; Nath, 2026).

Table 2: Major Functional Multi-Omics Technologies and Their Applications in Precision Animal Nutrition

Omics Technology	Major Analytical Technologies	Key Biological Information provided	Representative Biomarkers / Outputs	Applications in Precision Animal Nutrition
Genomics	Whole-genome sequencing (WGS), SNP genotyping, Genome-wide association studies (GWAS), Long-read sequencing	Host genetic variation associated with nutrient utilization, feed efficiency, disease resistance	SNPs, QTLs, copy number variants (CNVs), structural variants	Nutrigenomics, genomic selection, marker-assisted breeding, identification of nutrition-responsive genes
Epigenomics	Whole-genome bisulfite sequencing (WGBS), Reduced representation bisulfite sequencing (RRBS), ChIP-seq, ATAC-seq	Epigenetic regulation of gene expression influenced by diet and environment	DNA methylation, histone modifications, chromatin accessibility	Nutritional programming, fetal programming, nutriepigenomics, long-term dietary responses
Transcriptomics	RNA sequencing (RNA-Seq), Single-cell RNA sequencing (scRNA-Seq), Microarray	Host gene expression and transcriptional responses to dietary interventions	mRNA, miRNA, lncRNA	Identification of nutrient response pathways, immune regulation, intestinal physiology
Metagenomics	Shotgun metagenomic sequencing	Taxonomic composition and functional potential of microbial communities	Metagenome-assembled genomes (MAGs), carbohydrate-active enzymes (CAZymes)	Characterization of gut microbiota, functional microbiome analysis

Metatranscriptomics	Total microbial RNA sequencing	Actively expressed microbial genes under specific dietary or physiological conditions	Differentially expressed microbial genes, active metabolic pathways	Functional analysis of microbial activity, fiber degradation, fermentation pathways, methane metabolism
Proteomics	Liquid chromatography–mass spectrometry (LC–MS), MALDI-TOF MS	Host protein abundance, post-translational modifications and signaling proteins	Digestive enzymes, transport proteins, cytokines, signaling proteins	Identification of nutritional biomarkers, nutrient transport mechanisms, immune regulation
Metaproteomics	LC–MS coupled with shotgun protein identification	Proteins expressed by the entire microbial community and their functional activities	Microbial enzymes, carbohydrate-active enzymes, methanogenesis proteins, transporter proteins	Functional characterization of rumen and intestinal microbiota, microbial metabolism, host–microbiome interactions
Metabolomics	LC-MS, Gas chromatography–mass spectrometry (GC-MS), Nuclear magnetic resonance (NMR)	Global metabolite profiles reflecting host and microbial metabolism	Short-chain fatty acids (SCFAs), bile acids, amino acids, indoles, polyamines, organic acids	Biomarker discovery, metabolic phenotyping, evaluation of nutritional interventions, gut health assessment
Integrated Multi-Omics	Artificial intelligence, machine learning, network biology, systems biology platforms	Integration of genomic, transcriptomic, proteomic, metabolomic and phenotypic datasets to identify biological networks	Multi-omics approaches, predictive biomarkers, regulatory networks	Precision nutrition, biomarker discovery, digital twins, predictive livestock management, systems-level understanding of host–microbiome interactions

(Information collected from Ramos-Lopez et al., 2022; Choudhary et al., 2024; Wanapat et al., 2024; Yu et al., 2024)

Despite these advances, each omics possesses varying strengths and limitations. Individual technologies provide only a partial view of a biological process. Therefore, integrated analysis is essential for accurate interpretation (Kinkpe *et al.,* 2025; Yang *et al.,* 2025). Standardization of sampling procedures, analytical techniques, reference databases and computational methodologies which remain a major priority for translating multi-omics research into practical livestock nutrition (Yu *et al.,* 2024; Choudhary *et al.,* 2024). Continuous improvement in data integration, systemic biological modelling and AI-driven analytics are expected to increase the implementation of precision animal nutrition at research as well as commercial levels (Kinkpe *et al.,* 2025; Nath, 2026; Lin *et al.,* 2026).

5. Emerging Technologies in AI-Driven Precision Animal Nutrition

In addition to, integrating biological datasets, AI provide identification of complex non-linear relationship among host genetics, microbial functions, environmental factors and dietary components which are very difficult to detect using conventional statistical methods (Kinkpe *et al.,* 2025; Ramos-Lopez *et al.,* 2022; Yang *et al.,* 2025). Machine learning and deep learning algorithms both facilitate the biomarker discovery, prediction of feed efficiency, methane emissions, disease susceptibility and nutrient requirements at the individual animal level (Choudhary *et al.,* 2024; Park *et al.,* 2024; Kinkpe *et al.,* 2025). Additionally, digital twin technology is recently emerging era of technologies which virtual representation of individual animals with continuously updated using real-time sensor data and multi-omics information to provide physiological responses (Brown-Brandl & Tao, 2025). Recent advances in explainable AI (XAI), federated learning, cloud–edge computing and Internet of Things (IoT)-enabled precision livestock farming are expected to improve model transparency, data security, and real-time decision-making (Tedeschi *et al.,* 2025; Vardhan *et al.,* 2025). However, challenges related to standardized data integration, high infrastructure expenditure, biological validation and regulation of its uses remain significant barriers. Future research will focus on integration of multi-omics with real-time phenotypic and environmental data to develop interpretable and accurate precision nutrition systems which enhance productivity, animal welfare and environmental sustainability (Bunny *et al.,* 2026; Lin *et al.,* 2026; Nath, 2026).

6. Integration of Microbiome Engineering, Multi-Omics and Artificial Intelligence

The integration of microbiome engineering, multi-omics and artificial intelligence (AI) which provide the complete framework from nutritional management to predictive and adaptive animal response for improving life of individual and it represented as Figure 2. Various feed ingredients, including conventional feed ingredients and other functional feed additives which impact on the population and metabolic activity of the gut microbes through microbiome engineering strategies such as probiotics, prebiotics, synbiotics, postbiotics, bacteriophage therapy and engineered microorganisms (Ma *et al.,* 2022; Kango & Nath, 2024; Prasad *et al.,* 2025; Rahman *et al.,* 2026).

Afterward, host–microbiome interactions are analysed by using multi-omics technologies (genomics, metagenomics, transcriptomics, proteomics, and metabolomics) which collectively provide mechanistic interactional view of host genetics, microbial diversity, gene expression, protein function and metabolic pathways (Ramos-Lopez *et al.,* 2022; Choudhary *et al.,* 2024; Wanapat *et al.,* 2024; Yu *et al.,* 2024). These multidimensional datasets which futher integrated with artificial intelligence (AI), machine learning (ML) and digital twin technologies to predict nutritional responses and provide precision feeding strategies (Brown-Brandl & Tao, 2025). Ultimately, this integrated approach increase nutrient utilization, improves gut health, increases feed efficiency and animal productivity, reduces enteric methane emissions and antibiotic uses, and promotes environmentally sustainable and economically efficient livestock production through evidence-based precision nutrition (Bunny *et al.,* 2026; Lin *et al.,* 2026; Nath, 2026).

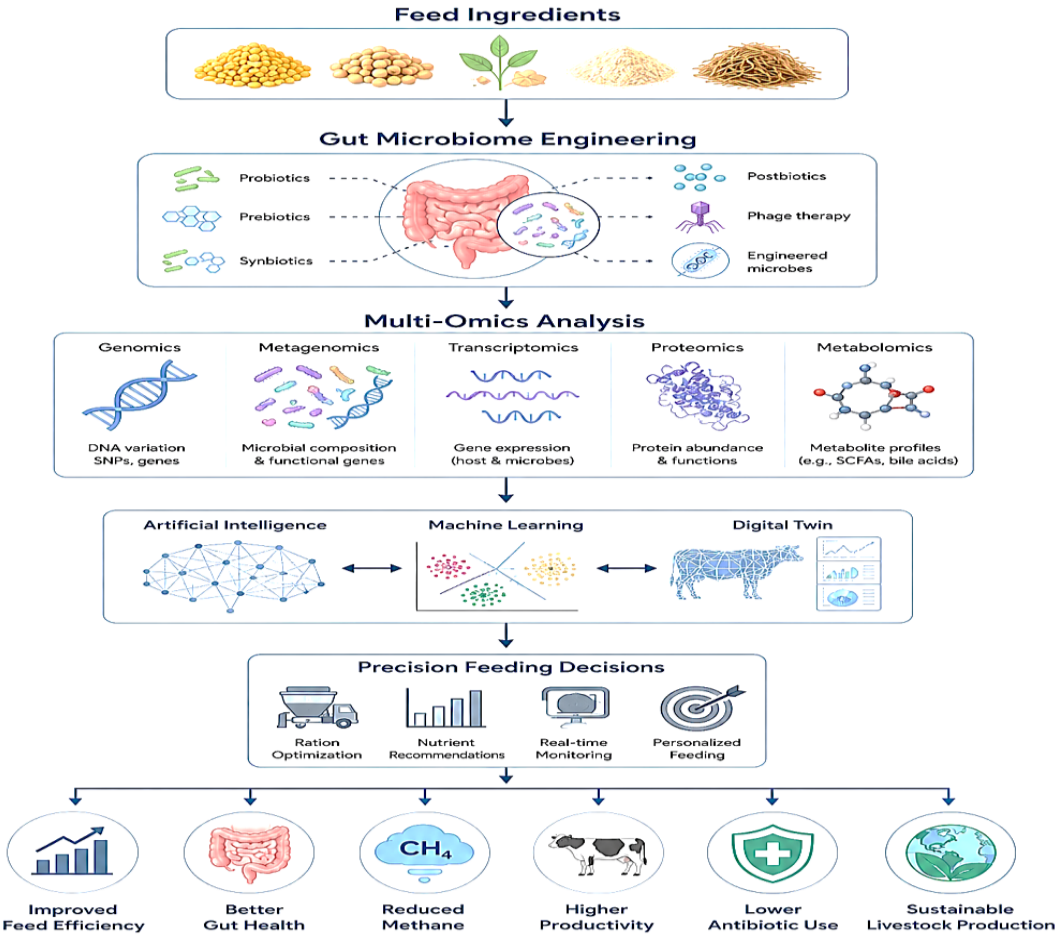

Figure 2: Integrated Framework of Microbiome Engineering, Multi-Omics and Artificial Intelligence for Precision Animal Nutrition

Conclusion

The integration of microbiome engineering, multi-omics technologies and artificial intelligence is transformed the precision animal nutrition from conventional feeding toward mechanism-based nutritional interventions. Advances in molecular microbiology, biotechnology, and systemic biology provide targeted modulation of host–microbiome interactions to improve

nutrient utilization, animal health, productivity and environmental sustainability. Collectively, these interdisciplinary approaches represent as a major frontier for next-generation livestock production and precision animal nutrition.

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INDUSTRIAL ENZYMES: MOLECULAR DESIGN AND BIOTECHNOLOGICAL APPLICATIONS

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Abstract
Enzymes are biological catalysts that accelerate biochemical reactions with high specificity and efficiency under mild conditions, making them indispensable in industrial biotechnology. Industrial enzymes are widely applied in the food, detergent, textile, pharmaceutical, paper, biofuel, and environmental sectors because they improve process efficiency while reducing energy consumption and environmental pollution. Although enzymes are obtained from plants, animals, and microorganisms, microbial enzymes dominate commercial production due to their rapid growth, high productivity, ease of genetic manipulation, and cost-effectiveness. Industrial enzymes are classified into seven Enzyme Commission (EC) classes based on the reactions they catalyze, including oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and translocases. Recent advances in recombinant DNA technology, protein engineering, CRISPR-Cas genome editing, enzyme immobilization, and synthetic biology have significantly enhanced enzyme production, catalytic efficiency, stability and industrial applicability. These innovations have expanded the use of enzymes as sustainable biocatalysts for green manufacturing and environmental protection. Overall, enzyme biotechnology continues to play a vital role in the development of efficient, eco-friendly and economically viable industrial processes.

Keywords: Industrial Enzymes, Microbial Enzymes, Enzyme Biotechnology, Recombinant Enzymes, Protein Engineering, Enzyme Immobilization.

1. Introduction

All forms of life rely on enzymes and also produce them. Enzymes are macromolecules that function as organic catalysts, facilitating most of an organism's biochemical reactions essential for maintaining and supporting life activities. Enzymes can also be extracted from cells and utilized to catalyze a wide variety of commercially significant processes. They represent a remarkable advancement in the field of bioprocess technology. These biocatalysts are universally present in plants, animals, and microbial cells. Most functional enzymes are proteins, such as trypsin and papain. Small subsets of enzymes, composed of RNA, are known as ribozymes. Enzymes function by lowering the activation energy required to initiate a reaction, thereby accelerating the process. Microorganisms that produce specific enzymes, or purified enzymes themselves, have a wide range of applications, including ethanol production (e.g.,

Saccharomyces cerevisiae and *Zymomonas mobilis*), forensic science (e.g., restriction enzymes), and polymerase chain reaction (e.g., DNA polymerases). Enzymes are widely utilized across various industries due to their catalytic properties. In the food industry, they play a significant role in processes such as baking, starch conversion, dairy product production, and beverage processing. In the textile industry, enzymes contribute to achieving an attractive texture in the final product. Many other industries, including healthcare, pharmaceuticals, and chemical manufacturing, leverage enzymes to enhance efficiency and output. Additionally, enzymes are crucial in paper and pulp production, as well as in the detergent industry. They are also extensively employed in waste management for purifying polluted water and treating solid waste (Nelson & Cox, 2021).

Enzymes are protein molecules that function as biological catalysts, accelerating the rate of chemical and metabolic reactions by lowering the activation energy without affecting the reaction's equilibrium. Every metabolic process within a cell relies on enzyme catalysis to efficiently drive metabolic pathways. These natural tools play a crucial role in providing everyday products in an environmentally sustainable manner. Over the years, enzymes have become integral to industrial and biochemical processes due to their remarkable efficiency and versatility. Their specificity in substrate binding and ability to catalyze reactions under diverse environmental conditions make them eco-friendly and pollution-free alternatives to traditional methods. Enzymes deliver comparable outcomes under mild conditions, offering significant advantages in sustainability. Most industrial enzymes exhibit peak performance within specific pH and temperature ranges, ensuring optimal activity. Microbial enzymes, in particular, have become invaluable in biochemical and industrial applications, thanks to their extraordinary properties and adaptability (Srivastava *et al.,* 2023).

2. Classification of Industrial Enzymes

Industrial enzymes are biological catalysts that accelerate specific biochemical reactions under mild operating conditions. They are extensively employed in sectors such as food processing, pharmaceuticals, detergents, textiles, leather, pulp and paper, biofuels, agriculture, and environmental biotechnology. Based on the type of reaction they catalyze, the International Union of Biochemistry and Molecular Biology (IUBMB) classifies enzymes into seven major classes according to the Enzyme Commission (EC) numbering system. This standardized classification facilitates enzyme identification and supports their industrial application and commercialization (IUBMB, 2024).

2.1 Oxidoreductases (EC 1)

Oxidoreductases catalyze oxidation-reduction (redox) reactions by transferring electrons or hydrogen atoms between donor and acceptor molecules. These enzymes play essential roles in cellular respiration, detoxification, and biosynthetic pathways. Industrially, oxidoreductases are widely used in biosensors, clinical diagnostics, wastewater treatment, textile bleaching, food

preservation, and pharmaceutical manufacturing. Representative enzymes include glucose oxidase, catalase, laccase, alcohol dehydrogenase, and peroxidase. Laccases and peroxidases are particularly valuable in environmentally friendly bleaching and bioremediation processes because they reduce the need for harsh chemical oxidants (Bilal *et al.,* 2021; IUBMB, 2024).

2.2 Transferases (EC 2)

Transferases catalyze the transfer of functional groups such as methyl, amino, glycosyl, acyl, or phosphate groups from one molecule to another. These enzymes are fundamental to metabolic regulation and biosynthetic pathways. In industrial biotechnology, transferases are used in the synthesis of pharmaceuticals, nucleotides, specialty carbohydrates, and fine chemicals. Kinases, aminotransferases, glycosyltransferases, and methyltransferases are among the most important members of this enzyme class. Their high substrate specificity enables the production of complex biomolecules with minimal by-product formation (Sheldon & Brady, 2022).

2.3 Hydrolases (EC 3)

Hydrolases catalyze the cleavage of chemical bonds through the addition of water and represent the largest group of commercially exploited industrial enzymes. They account for the majority of the global enzyme market because of their broad applications in food processing, detergents, leather treatment, textile processing, pulp and paper manufacturing, and biofuel production. Common hydrolases include proteases, amylases, lipases, cellulases, xylanases, pectinases, and chitinases. Proteases dominate the detergent industry by degrading protein-based stains, whereas cellulases and xylanases facilitate lignocellulosic biomass conversion into fermentable sugars for bioethanol production (Singhania *et al.,* 2020; Bilal *et al.,* 2021).

2.4 Lyases (EC 4)

Lyases catalyze the cleavage or formation of chemical bonds without involving hydrolysis or oxidation. These enzymes remove or add functional groups to produce compounds containing double bonds or cyclic structures. Lyases have important applications in pharmaceutical synthesis, amino acid production, and food processing. Examples include fumarase, pyruvate decarboxylase, pectate lyase, and carbonic anhydrase. Pectate lyases are widely employed in fruit juice clarification and textile processing because they efficiently degrade plant cell wall pectins (Bilal *et al.,* 2021).

2.5 Isomerases (EC 5)

Isomerases catalyze intramolecular rearrangements that convert one isomer into another without altering the molecular formula. These enzymes are indispensable in carbohydrate metabolism and industrial sugar processing. Glucose isomerase is among the most economically significant industrial enzymes because it converts glucose into fructose during the manufacture of high-fructose corn syrup (HFCS). Other examples include racemases, epimerases, and mutases, which are increasingly used in pharmaceutical synthesis and biocatalysis (McDonald & Tipton, 2023).

2.6 Ligases (EC 6)

Ligases catalyze the joining of two molecules by forming covalent bonds using energy derived from ATP or other nucleotide triphosphates. These enzymes are indispensable in molecular biology, recombinant DNA technology, synthetic biology, and genome engineering. DNA ligase is one of the most widely used enzymes in biotechnology laboratories, where it joins DNA fragments during cloning, sequencing, and genetic engineering. Aminoacyl-tRNA synthetases and carboxylases are additional examples with important biological and industrial functions (IUBMB, 2024).

2.7 Translocases (EC 7)

Translocases constitute the newest enzyme class recognized by the IUBMB and catalyze the movement of ions or molecules across biological membranes. These enzymes are essential for maintaining cellular homeostasis and energy metabolism by transporting protons, metal ions, amino acids, and other metabolites. ATP synthase and various membrane transport ATPases are representative translocases. Although their industrial applications are still emerging, they are increasingly important in membrane biotechnology, synthetic biology, bioenergetics research, and the development of next-generation bioprocesses (McDonald & Tipton, 2023; IUBMB, 2024).

3. Sources of industrial enzymes

Industrial enzymes are obtained from three principal biological sources: plants, animals, and microorganisms. Although enzymes from all three sources have been utilized for centuries, microorganisms have emerged as the dominant source for commercial enzyme production due to their rapid growth, ease of genetic manipulation, scalability, high productivity, and cost-effectiveness. Advances in fermentation technology, recombinant DNA technology, protein engineering, and synthetic biology have further expanded the industrial production of enzymes from microbial systems. Today, more than 90% of commercially available industrial enzymes are produced using microorganisms because they offer superior catalytic efficiency, process flexibility, and environmental sustainability.

3.1 Plant-Derived Enzymes

Plants represent one of the earliest sources of industrial enzymes and continue to contribute significantly to the food, pharmaceutical, and cosmetic industries. Plant enzymes are generally extracted from fruits, seeds, latex, leaves, stems, and roots. These enzymes are biodegradable, non-toxic, and are often recognized as safe for food and pharmaceutical applications. Proteolytic enzymes such as papain from *Carica papaya*, bromelain from *Ananas comosus*, and ficin from *Ficus* species are widely used in meat tenderization, brewing, food processing, wound debridement, digestive formulations, and anti-inflammatory therapies. Plant-derived amylases, pectinases, and polyphenol oxidases also find applications in fruit juice clarification, starch processing, and beverage production. Despite their advantages, large-scale utilization of plant

enzymes is constrained by seasonal availability, variations in enzyme yield due to environmental conditions, limited extraction efficiency, and relatively high production costs. Consequently, plant enzymes are primarily employed in specialized industrial applications where their unique catalytic properties provide distinct advantages.

Examples of Plant Enzymes

Enzyme	Source	Industrial Applications
Papain	*Carica papaya*	Meat tenderization, pharmaceuticals, cosmetics
Bromelain	*Ananas comosus*	Food processing, anti-inflammatory preparations
Ficin	*Ficus carica*	Cheese production, protein hydrolysis
Malt amylase	Germinating barley	Brewing, baking, starch hydrolysis

3.2 Animal-Derived Enzymes

Animal tissues have traditionally served as important sources of digestive and coagulating enzymes. These enzymes are commonly isolated from the pancreas, stomach, liver, and intestinal tissues of cattle, pigs, poultry, and marine organisms. Animal-derived enzymes are particularly valued in pharmaceutical formulations, dairy processing, clinical diagnostics, and biomedical research. Pepsin, trypsin, chymotrypsin, pancreatic lipase, and chymosin (rennin) are among the most commercially important animal enzymes. Chymosin is extensively used in cheese manufacturing due to its ability to coagulate milk proteins with high specificity. Similarly, trypsin and chymotrypsin are widely employed in cell culture, protein digestion, peptide sequencing, and pharmaceutical manufacturing. However, industrial dependence on animal-derived enzymes has declined because of ethical concerns, zoonotic disease risks, religious restrictions, variability in enzyme quality, and limited raw material availability. Recombinant enzyme production has largely replaced many animal-derived enzymes in industrial applications.

Examples of Animal Enzymes

Enzyme	Source	Industrial Applications
Pepsin	Gastric mucosa	Protein digestion, pharmaceuticals
Trypsin	Pancreas	Cell culture, biotechnology, proteomics
Chymotrypsin	Pancreas	Pharmaceutical formulations
Chymosin (Rennin)	Calf stomach	Cheese manufacturing
Pancreatic lipase	Pancreas	Lipid digestion, diagnostics

3.3 Microbial Enzymes

Microorganisms have become the most important source of industrial enzymes because they can be cultivated rapidly under controlled fermentation conditions using inexpensive substrates. Bacteria, fungi, yeasts, and actinomycetes produce a wide range of extracellular and intracellular enzymes with excellent catalytic properties suitable for industrial-scale production. Microbial enzymes offer several advantages over plant and animal enzymes, including high production yields, shorter production cycles, ease of downstream processing, greater enzyme stability, broad

substrate specificity, and the ability to function under extreme environmental conditions such as high temperatures, alkaline pH, acidic environments, and elevated salinity. Furthermore, microbial strains can be genetically engineered to improve enzyme activity, thermostability, substrate specificity, and overall production efficiency (Singh *et al.,* 2023).

Examples of Microbial Enzymes

Microorganism	**Major Enzymes Produced**	**Industrial Applications**
Bacillus subtilis	Amylase, Protease, Cellulase	Detergents, food processing, textiles
Bacillus licheniformis	Alkaline protease	Detergents, leather processing
Aspergillus niger	Glucoamylase, Pectinase, Phytase	Food processing, fruit juice clarification
Trichoderma reesei	Cellulase	Bioethanol, pulp and paper industries
Saccharomyces cerevisiae	Invertase	Baking, brewing, ethanol production
Kluyveromyces lactis	Lactase	Dairy industry

4. Recombinant and Genetically Engineered Enzymes

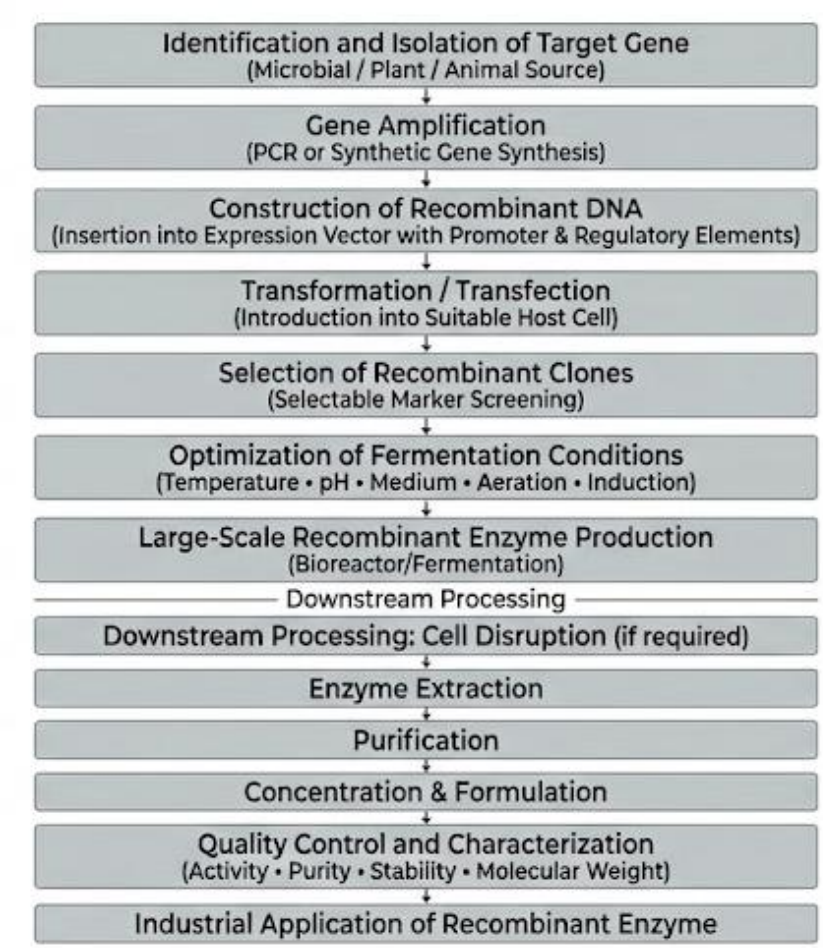
Modern industrial biotechnology increasingly relies on recombinant microorganisms for enzyme production. Genes encoding enzymes of commercial importance are cloned into high-expression hosts such as *Escherichia coli*, *Bacillus subtilis*, *Pichia pastoris*, and *Saccharomyces cerevisiae*. Recombinant DNA technology enables high-yield enzyme production while reducing dependence on natural biological sources. Protein engineering and directed evolution further improve enzyme performance by enhancing catalytic efficiency, thermostability, solvent tolerance, pH stability, and substrate specificity. These engineered enzymes are increasingly applied in pharmaceuticals, biofuels, food processing, environmental biotechnology, and fine chemical synthesis. Emerging genome-editing tools such as CRISPR-Cas systems continue to accelerate the development of next-generation industrial biocatalysts with tailored properties (Nielsen & Keasling, 2022).

Recombinant enzyme technology has revolutionized industrial biotechnology by enabling the large-scale production of highly efficient enzymes through genetic engineering. Unlike conventional enzyme production, which relies on naturally occurring microorganisms, plants, or animals, recombinant technology involves the insertion of genes encoding desired enzymes into suitable host organisms for controlled expression. This approach ensures consistent enzyme quality, higher production yields, improved catalytic properties, and reduced manufacturing costs. Recombinant enzymes have become indispensable in industries including pharmaceuticals, food processing, detergents, textiles, biofuels, diagnostics, agriculture, and environmental biotechnology (Nielsen & Keasling, 2022).

4.1 Principle of Recombinant Enzyme Technology

Recombinant enzyme technology is based on recombinant DNA (rDNA) techniques, where the gene encoding a target enzyme is isolated, cloned into an appropriate expression vector, and introduced into a host organism. The transformed host expresses the recombinant protein under optimized fermentation conditions, after which the enzyme is purified and formulated for industrial use. This technology enables the production of enzymes with enhanced stability, substrate specificity, catalytic efficiency, and resistance to extreme environmental conditions (Nielsen & Keasling, 2022).

Production Process of Recombinant Enzymes



4.2 Expression Systems for Recombinant Enzyme Production

The selection of an expression host depends on the enzyme's structural complexity, post-translational modification requirements, production cost, and intended industrial application.

4.2.1 Bacterial Expression Systems

Bacteria are the most widely used hosts for recombinant enzyme production because of their rapid growth, simple genetics, inexpensive cultivation, and high productivity (Singh *et al.,* 2023. Common bacterial hosts include: *Escherichia coli*, *Bacillus subtilis* and *Bacillus licheniformis*. These organisms are extensively employed for producing amylases, proteases, cellulases, lipases, DNA polymerases, and numerous industrial biocatalysts.

4.2.2 Yeast Expression Systems

Yeasts combine the advantages of microbial cultivation with the ability to perform certain eukaryotic post-translational modifications. Common hosts include: *Saccharomyces cerevisiae, Pichia pastoris* (*Komagataella phaffii*) and *Kluyveromyces lactis.* Yeast expression systems are widely used for producing recombinant lipases, phytases, lactases, and pharmaceutical enzymes.

4.2.3 Filamentous Fungal Expression Systems

Filamentous fungi naturally secrete large quantities of extracellular enzymes, making downstream purification relatively simple. Important fungal hosts include: *Aspergillus niger*, *Aspergillus oryzae and Trichoderma reesei*. These fungi are major producers of cellulases, xylanases, pectinases, glucoamylases, and phytases.

4.2.4 Mammalian Cell Expression Systems

Mammalian cells are primarily used when enzymes require complex glycosylation or other post-translational modifications essential for therapeutic activity (Nielsen & Keasling, 2022). Common mammalian hosts include: Chinese Hamster Ovary (CHO) cells and Human Embryonic Kidney (HEK293) cells. These systems are mainly employed in the production of therapeutic enzymes and biopharmaceutical proteins.

4.3 Advantages of Recombinant Enzyme Technology

Recombinant enzyme technology offers numerous advantages over conventional enzyme production:

- High enzyme yield and productivity
- Consistent product quality
- Reduced production costs
- Improved enzyme purity
- Enhanced catalytic efficiency
- Increased thermal and pH stability
- Improved solvent tolerance
- Tailored substrate specificity
- Reduced dependence on natural biological resources
- Environmentally sustainable manufacturing
- Scalable industrial production

5. Purification of Enzymes

Enzymes are typically found in complex mixtures with other substances such as proteins, nucleic acids, polysaccharides, and lipids. To assess the degree of enzyme purity, the specific activity is measured, which refers to the enzyme activity relative to the total protein content. Several methods can be employed to purify the enzyme and increase its specific activity, depending on the intended use and the required purity (Chapman *et al.,* 2021; Gupta & Rathi, 2021). For enzymes used as diagnostic reagents or in clinical therapeutics, achieving a high degree of purity

is essential. These enzymes are often prepared to ensure a high level of specificity in the reaction they catalyze. Some common purification methods include:

- **Precipitation:** Using salts or organic solvents to precipitate proteins and separate them from the desired enzyme.
- **Chromatography:** Techniques such as ion-exchange, affinity, or size-exclusion chromatography can help isolate specific enzymes based on charge, affinity, or size.
- **Ultrafiltration:** Membrane filtration to concentrate the enzyme and remove smaller contaminants.
- **Electrophoresis:** To separate proteins based on their size and charge, although this method is more often used for analysis than large-scale purification.
- **Filtration:** Using a filter to physically remove the precipitate from the liquid.
- **Centrifugation:** Spinning the mixture at high speeds to force the precipitate to the bottom of the container, allowing the supernatant to be decanted or separated. Salting out is a relatively simple and cost-effective technique to begin the purification process, especially when aiming to concentrate the protein or remove unwanted contaminants before more refined purification steps are applied.

6. Economic Importance of Microbial Enzymes

Microbial enzymes have become a cornerstone of several industrial applications**,** playing a crucial role in a variety of sectors due to their efficiency**,** specificity**,** and environmental benefits. One of the first and most widely recognized uses of microbial enzymes on a large scale was in the detergent industry. The enzymes derived from microorganisms have revolutionized the way laundry detergents and other cleaning products are formulated, making them more effective and environmentally friendly. Below are some of the key applications of microbial enzymes, particularly in detergents:

6.1 Detergents

The detergent industry was one of the first to harness the power of microbial enzymes, and enzymes like bacterial proteinases**,** amylases, and cellulases are now widely used to improve cleaning performance.

- **Proteinases**: Bacterial proteinases are the most widely used detergent enzymes, breaking down protein-based stains like food, blood, and sweat. Proteinases, which are mainly derived from bacteria such as *Bacillus* species, are engineered to be stable under the harsh conditions encountered in washing machines, such as high pH**,** oxidizing agents**,** and anionic detergents.
- **Amylases**: These enzymes break down starch-based stains (e.g., from food, sauces, or greasy materials). Amylases hydrolyze gelatinized starch, which tends to bind other stain components and stick to fabric fibers. By breaking down the starch, amylases help remove the stain and restore the fabric's appearance.

- **Cellulases**: These enzymes are used to degrade cellulose fibers and help in the color brightening and softening of textiles. The use of cellulases in laundry detergents has been particularly beneficial for cotton fabrics. Cellulases act on the microfibrils of cotton fibers, which are produced during washing, and reduce pilling, improving the fabric's overall texture and appearance.

7. Industrial Applications of Recombinant Enzymes

Recombinant enzymes have transformed numerous industrial sectors.

7.1 Food Industry

Recombinant amylases, glucoamylases, lactases, pectinases, and lipases are widely employed in baking, brewing, dairy processing, fruit juice clarification, starch conversion, and production of high-fructose syrups.

7.2 Detergent Industry

Recombinant alkaline proteases, lipases, amylases, cellulases, and mannanases improve stain removal while reducing washing temperatures and detergent consumption.

7.3 Textile Industry

Cellulases, pectinases, and catalases are used in fabric biopolishing, denim finishing, desizing, and eco-friendly textile processing.

7.4 Pulp and Paper Industry

Recombinant xylanases and laccases facilitate pulp bleaching, reduce chlorine usage, and improve paper quality.

7.5 Biofuel Industry

Cellulases, hemicellulases, and β-glucosidases hydrolyze lignocellulosic biomass into fermentable sugars for sustainable bioethanol production.

7.6 Pharmaceutical Industry

Therapeutic enzymes such as recombinant asparaginase, uricase, tissue plasminogen activator, and glucocerebrosidase are widely used for treating leukemia, gout, thrombotic disorders, and lysosomal storage diseases.

7.7 Environmental Biotechnology

Recombinant oxidoreductases, laccases, peroxidases, and dehalogenases are applied in wastewater treatment, pollutant degradation, heavy metal remediation, and biosensor development.

8. Recent Advances in Enzyme Biotechnology

Enzyme biotechnology has experienced remarkable progress over the past two decades, driven by advances in molecular biology, genomics, bioinformatics, protein engineering, and synthetic biology. Modern biotechnological approaches have significantly enhanced enzyme discovery, production, stability, and catalytic performance, enabling their widespread application in industrial manufacturing, healthcare, agriculture, environmental remediation, and renewable

energy. The integration of computational tools, high-throughput screening, and genome-editing technologies has transformed enzymes into highly efficient and sustainable biocatalysts capable of replacing conventional chemical catalysts in numerous industrial processes (Chaplin & Bucke, 2021).

8.1 Protein Engineering and Directed Evolution

Protein engineering has emerged as one of the most powerful strategies for improving enzyme properties. Rational protein design utilizes structural and biochemical knowledge to introduce targeted amino acid substitutions that enhance catalytic efficiency, substrate specificity, thermostability, solvent tolerance, and pH stability. In contrast, directed evolution mimics natural selection through repeated cycles of random mutagenesis and high-throughput screening to generate enzymes with superior industrial performance. These approaches have produced robust variants of proteases, cellulases, lipases, and amylases that function efficiently under harsh industrial conditions, thereby improving productivity while reducing manufacturing costs (BRENDA, 2024).

8.2 Synthetic Biology

Recombinant DNA technology has enabled the large-scale production of industrial enzymes using genetically engineered microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Pichia pastoris*, and *Aspergillus niger*. Synthetic biology has further expanded these capabilities by designing artificial metabolic pathways, optimizing gene expression systems, and constructing microbial cell factories capable of producing enzymes with enhanced catalytic properties. These advances have facilitated the commercial production of high-value enzymes for pharmaceuticals, food processing, biofuels, and fine chemical synthesis.

8.3 CRISPR-Cas Genome Editing

The development of CRISPR-Cas genome-editing technology has revolutionized enzyme biotechnology by enabling precise modification of microbial genomes. CRISPR-based approaches allow targeted insertion, deletion, or replacement of genes involved in enzyme biosynthesis, thereby improving enzyme yield, catalytic activity, and metabolic efficiency. Genome editing has also accelerated the development of industrial microbial strains with enhanced stress tolerance and improved substrate utilization, contributing to more efficient and sustainable bioprocesses (Grand View Research, 2024; MarketsandMarkets, 2024).

8.4 Metagenomics and Enzyme Discovery

Metagenomics has greatly expanded the repertoire of industrial enzymes by enabling the identification of novel biocatalysts from uncultivable microorganisms present in diverse environments such as soil, marine ecosystems, hot springs, and deep-sea sediments. Functional and sequence-based metagenomic approaches have led to the discovery of numerous thermostable, alkaliphilic, acidophilic, halophilic, and solvent-tolerant enzymes with exceptional

catalytic properties. These extremozymes have become valuable resources for industries requiring enzymes capable of functioning under challenging process conditions.

8.5 Immobilized Enzyme Technology

Recent innovations in enzyme immobilization have substantially improved enzyme stability, reusability, and operational efficiency. Advanced immobilization supports, including nanomaterials, magnetic nanoparticles, metal-organic frameworks, hydrogels, and biodegradable polymers, provide enhanced surface area and improved mass transfer characteristics. Immobilized enzymes exhibit prolonged catalytic activity, simplified product recovery, and lower operational costs, making them highly attractive for continuous industrial bioprocessing and bioreactor applications.

8.6 Nanobiotechnology and Nanozymes

Nanotechnology has introduced novel opportunities for enzyme stabilization and catalytic enhancement. Nanomaterials serve as effective supports for enzyme immobilization, improving enzyme activity and resistance to thermal and chemical denaturation. In addition, nanozymes—nanomaterials possessing enzyme-like catalytic properties—have emerged as promising alternatives to natural enzymes due to their remarkable stability, low production cost, and resistance to environmental degradation. Nanozymes are increasingly applied in biosensors, diagnostics, environmental remediation, and biomedical research.

8.7 Artificial Intelligence and Machine Learning

Artificial intelligence (AI) and machine learning (ML) have become valuable tools for enzyme discovery, structure prediction, and functional optimization. AI-driven computational platforms enable rapid prediction of enzyme structures, substrate specificity, catalytic mechanisms, and protein stability. Machine learning algorithms facilitate enzyme engineering by identifying beneficial mutations and predicting enzyme performance under various industrial conditions. These computational approaches significantly reduce experimental time and accelerate the development of next-generation industrial biocatalysts.

8.8 Multi-omics Technologies

The integration of genomics, transcriptomics, proteomics, metabolomics, and fluxomics has provided comprehensive insights into enzyme regulation and cellular metabolism. Multi-omics approaches facilitate the identification of novel biosynthetic pathways, regulatory networks, and metabolic bottlenecks, enabling rational optimization of microbial strains for enhanced enzyme production. These technologies are increasingly employed in systems biotechnology to improve industrial fermentation processes and develop highly productive microbial cell factories.

8.9 Green Enzyme Biotechnology

The growing emphasis on sustainable industrial development has accelerated the adoption of green enzyme biotechnology. Enzymes enable environmentally friendly manufacturing by replacing harsh chemical catalysts, reducing energy consumption, minimizing toxic waste

generation, and operating under mild reaction conditions. Enzyme-assisted bioprocesses support circular bioeconomy initiatives through efficient biomass conversion, biodegradable product synthesis, and renewable resource utilization. Consequently, enzyme biotechnology plays a central role in achieving sustainable industrial development and environmental conservation (Gupta *et al.,* 2022).

9. Challenges and Future Prospectus

Despite remarkable advances, several challenges continue to limit the large-scale application of industrial enzymes. Many enzymes exhibit poor stability under extreme industrial conditions, such as high temperatures, extreme pH, and organic solvents, resulting in reduced catalytic efficiency. High production and downstream processing costs remain major economic constraints. Recombinant expression systems often encounter issues related to protein folding, low expression levels, and post-translational modifications. Additionally, regulatory approval, biosafety concerns, and the need for novel enzymes with improved substrate specificity and operational stability continue to present significant challenges for industrial enzyme biotechnology.

The future of enzyme biotechnology is highly promising due to advances in protein engineering, synthetic biology, CRISPR-based genome editing, artificial intelligence, and metagenomics. These technologies will facilitate the development of highly stable, efficient, and tailor-made enzymes for diverse industrial applications. The discovery of novel extremozymes, improved enzyme immobilization techniques, and AI-assisted enzyme design are expected to enhance industrial productivity while reducing production costs. Furthermore, enzyme biotechnology will play an increasingly important role in green manufacturing, renewable energy production, environmental remediation, and the development of a sustainable circular bioeconomy.

Conclusion

Although several technical and economic challenges remain, continuous advances in recombinant DNA technology, synthetic biology, artificial intelligence, protein engineering, and systems biotechnology are rapidly overcoming existing limitations. The next generation of industrial enzymes will be more efficient, robust, and environmentally sustainable, enabling greener manufacturing processes and expanding the applications of enzyme biotechnology across multiple industrial sectors. These developments will position enzymes as indispensable biocatalysts in the future of industrial biotechnology and sustainable global development.

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APPLICATION OF MOLECULAR BIOLOGY IN THE MANAGEMENT OF PHYTOPATHOGENIC FUNGI

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Abstract

Phytopathogenic fungi are among the most destructive plant pathogens, causing severe losses in crop yield and quality worldwide. Conventional disease management strategies, including chemical fungicides, resistant cultivars, and cultural practices, are increasingly challenged by fungicide resistance, pathogen evolution, environmental concerns, and climate change. The integration of molecular biology into plant pathology has revolutionized the diagnosis, monitoring, and management of fungal diseases. Molecular tools such as polymerase chain reaction (PCR), quantitative PCR (qPCR), DNA sequencing, next-generation sequencing (NGS), transcriptomics, proteomics, RNA interference (RNAi), CRISPR/Cas genome editing, and comparative genomics have significantly improved pathogen identification, understanding of host-pathogen interactions, and the development of sustainable disease management strategies. This review discusses the present status of molecular biology applications in managing phytopathogenic fungi and highlights future prospects.

Introduction

Plant pathogenic fungi account for approximately 70–80% of infectious diseases affecting economically important crops. Diseases such as rusts, smuts, powdery mildew, Fusarium wilt, blast, anthracnose, and late blight significantly reduce agricultural productivity worldwide. Traditional disease management relies on Fungicide application, Resistant varieties, Crop rotation, Sanitation and Biological control. However, these approaches often suffer from Emergence of fungicide-resistant strains, Difficulty in early diagnosis, Environmental contamination and non-specific disease control. Molecular biology provides highly sensitive, rapid, and specific techniques that enable precise diagnosis, monitoring, and targeted management of fungal pathogens (Hariharan and Prasannath, 2021; Ansari *et al.,* 2026).

Molecular Biology in Managing Phytopathogenic Fungi

A. Molecular Diagnosis of Fungal Pathogens (Aslam *et al.,* 2017; Hariharan and Prasannath, 2021)

Early and accurate diagnosis is the foundation of disease management. Molecular diagnosis of fungal pathogens refers to the use of nucleic acid–based techniques to detect and identify fungi directly from diseased samples. These methods offer rapid, sensitive, and specific identification compared with conventional microscopy and culture, making them valuable for the early

diagnosis and management of fungal infections. Molecular diagnostic methods detect fungal DNA or RNA using amplification, hybridization, or sequencing technologies. These techniques identify fungal species based on unique genetic markers such as Internal Transcribed Spacer (ITS) regions, 18S and 28S ribosomal RNA genes, LSU rDNA, SSU rDNA β-tubulin gene, Calmodulin gene, Translation Elongation Factor 1-α (TEF1-α), etc. Major techniques employed include Conventional PCR, Nested PCR, Multiplex PCR, Real-time PCR (qPCR). Loop-mediated Isothermal Amplification (LAMP), DNA Barcoding, ITS sequencing, Whole Genome Sequencing (WGS), etc.

Advantages of Molecular Diagnosis

- Rapid turnaround time
- High sensitivity and specificity
- Early detection before culture positivity
- Accurate species identification
- Detection of mixed infections
- Useful for unculturable or slow-growing fungi

Limitations

- High cost
- Need for specialized laboratory infrastructure
- Requirement for trained personnel
- Possibility of false-positive results due to contamination
- Lack of standardized protocols for some fungi
- Limited availability in resource-constrained settings

Future Perspectives

Advances in molecular diagnostics are improving the speed and accuracy of fungal detection. Emerging technologies include:

- Next-Generation Sequencing (NGS)
- Whole Genome Sequencing (WGS)
- Metagenomic sequencing
- CRISPR-based diagnostic assays
- Point-of-care molecular testing
- Artificial intelligence-assisted interpretation of molecular data

These methods have greatly improved identification of *Fusarium*, *Colletotrichum*, *Alternaria*, *Rhizoctonia*, *Phytophthora*, and other pathogens. Molecular diagnostic techniques have transformed the diagnosis of fungal infections by providing rapid, sensitive, and accurate detection of fungal pathogens. PCR-based assays, sequencing, and other advanced molecular methods enable early diagnosis, precise species identification, and improved patient

management. Despite challenges related to cost and infrastructure, these technologies are becoming increasingly important in plant pathology and are expected to play an even greater role in the diagnosis of invasive and emerging fungal diseases.

B. Genomics and Comparative Genomics in Phytopathogenic Fungi Management (Cools and Hammond-Kosack, 2013)

Genomics is the study of the complete genetic material (genome) of an organism, including its structure, function, evolution, and interactions. In plant pathology, genomics helps identify genes involved in plant defense, pathogen virulence, and host–pathogen interactions.

Comparative genomics involves comparing the genomes of different plant species, pathogen strains, or both to identify similarities and differences in gene content, organization, and evolution. These comparisons provide insights into disease mechanisms and support the development of disease-resistant crops. Comparative genomics also aids in understanding host specificity and emergence of new races.

Whole genome sequencing has enabled researchers to identify Virulence genes, Pathogenicity islands, Effector proteins, Secreted enzymes, Secondary metabolite genes, Evolutionary relationships, etc.

Objectives

- Identify genes responsible for disease resistance in plants.
- Understand pathogen virulence and evolution.
- Study host–pathogen interactions.
- Develop resistant crop varieties.
- Improve disease diagnosis and surveillance.
- Support sustainable disease management strategies.

Advantages

- Early identification of disease resistance genes.
- Improved understanding of pathogen evolution.
- Faster development of resistant crop varieties.
- Enhanced disease diagnosis and surveillance.
- Reduced pesticide use.
- Increased crop productivity and sustainability.
- Supports precision breeding and biotechnology.

Limitations

- High cost of sequencing and analysis.
- Requirement for advanced laboratory facilities.
- Need for skilled bioinformatics expertise.
- Large and complex genomic datasets.

- Ethical and regulatory concerns regarding genetically edited crops.

Future Perspectives

- AI-assisted genomic data analysis.
- Pan-genome studies for crop improvement.
- Metagenomics for plant microbiome research.
- CRISPR-based precision breeding.
- Portable sequencing technologies for field diagnosis.
- Integration of genomics with precision agriculture and digital farming.

Genomics and comparative genomics have revolutionized plant disease management by enabling the identification of resistance genes, understanding pathogen biology, and improving host–pathogen interaction studies. These approaches support rapid disease diagnosis, marker-assisted breeding, genome editing, and sustainable crop protection. As sequencing technologies become more affordable and bioinformatics tools continue to advance, genomics will play an increasingly important role in developing disease-resistant crops and ensuring global food security.

C. Transcriptomics in Phytopathogenic Fungi Management (Weiliang Zuo *et al.,* 2019)

Transcriptomics is the study of the complete set of RNA transcripts (the transcriptome) produced by a cell, tissue, or organism under specific conditions. In plant disease management, transcriptomics helps understand how plants and pathogens respond to infection by analyzing changes in gene expression. It provides valuable insights into host defense mechanisms, pathogen virulence, and host–pathogen interactions. RNA sequencing (RNA-Seq) reveals genes expressed during infection.

Transcriptomics involves - Isolation of RNA from plant or pathogen tissues, Conversion of RNA into complementary DNA (cDNA), Analysis of gene expression using sequencing or hybridization techniques and Comparison of transcript profiles between healthy and infected samples.

Objectives of Transcriptomics in Plant Disease Management

- Identify genes involved in plant defense responses.
- Understand pathogen infection mechanisms.
- Study host–pathogen interactions.
- Discover biomarkers for early disease diagnosis.
- Identify candidate genes for developing disease-resistant crops.
- Support breeding and genetic engineering programs.

Advantages

- Genome-wide analysis of gene expression.
- High sensitivity and accuracy.

- Detection of novel genes and transcripts.
- Improved understanding of plant immunity.
- Supports breeding for disease resistance.
- Facilitates biomarker discovery.
- Enhances disease diagnosis and management.

Limitations

- High cost of sequencing and data analysis.
- Requires high-quality RNA samples.
- Large datasets require advanced bioinformatics tools.
- Gene expression does not always correlate with protein levels.
- Requires validation using additional techniques.

Future Perspectives

- Single-cell transcriptomics to study gene expression at the cellular level.
- Dual RNA-Seq for simultaneous analysis of plant and pathogen transcripts.
- Integration of transcriptomics with genomics, proteomics, and metabolomics.
- AI and machine learning for transcriptome data analysis.
- Precision breeding using transcriptomic biomarkers.
- Development of rapid diagnostic tools based on gene expression signatures.

Transcriptomic analyses have identified numerous fungal effectors that suppress plant immunity. Transcriptomics is a powerful approach for understanding gene expression changes during plant–pathogen interactions. It provides insights into plant defense mechanisms, pathogen virulence, and molecular responses to infection. Techniques such as RNA-Seq, DNA microarrays, and qRT-PCR have greatly advanced plant disease research, enabling early disease diagnosis, identification of resistance genes, and development of disease-resistant crop varieties. As sequencing technologies and bioinformatics continue to improve, transcriptomics will remain a key tool in sustainable plant disease management and crop improvement.

D. Proteomics in Phytopathogenic Fungi Management (Wende Liu *et al.,* 2021; Corrêa *et al.,* 2025)

Proteomics is the large-scale study of the complete set of proteins (proteome) produced by a cell, tissue, or organism under specific conditions. Since proteins are the functional molecules that carry out most biological processes, proteomics helps in understanding plant responses to pathogen infection, pathogen virulence, and host–pathogen interactions. It is an important tool for developing disease-resistant crops and improving plant disease management.

Proteomics involves - Extraction of proteins from plant or pathogen tissues; Separation of proteins using electrophoresis or chromatography; Identification and characterization of proteins using mass spectrometry; Analysis of protein expression, structure, interactions, and post-

translational modifications. Techniques used in proteomics are Two-Dimensional Gel Electrophoresis (2D-GE), Mass Spectrometry (MS), Protein Microarrays and Western Blotting. Proteomics identifies proteins involved in infection, colonization, host recognition, signal transduction and enzyme secretion by the pathogen. Proteomic studies have revealed numerous fungal enzymes like cell wall degrading enzymes such as cutinases, pectinases, cellulases, ligninases, etc. These proteins contribute directly to pathogenicity. Proteomics also identifies proteins involved in plant defense, such as pathogenesis-related (pr) proteins, peroxidases, defensins, etc. These proteins help plants resist pathogen infection.

Objectives of Proteomics in Plant Disease Management

- Identify proteins involved in plant defense.
- Understand host–pathogen interactions.
- Discover pathogen virulence proteins and effectors.
- Identify protein biomarkers for early disease diagnosis.
- Develop disease-resistant crop varieties.
- Improve strategies for disease control and crop protection.

Advantages

- Provides direct information on functional molecules.
- Identifies proteins involved in disease resistance.
- Detects post-translational modifications.
- Improves understanding of host–pathogen interactions.
- Supports biomarker discovery for disease diagnosis.
- Aids in developing disease-resistant crop varieties.

Limitations

- Protein extraction can be technically challenging.
- Proteins are less stable than DNA and RNA.
- High cost of advanced instruments.
- Requires skilled personnel and bioinformatics expertise.
- Low-abundance proteins may be difficult to detect.

Future Perspectives

- Quantitative proteomics for precise protein measurement.
- Single-cell proteomics to study disease responses at the cellular level.
- Integration with genomics, transcriptomics, and metabolomics (multi-omics).
- AI-based analysis of proteomic datasets.
- Discovery of novel protein biomarkers for rapid disease diagnosis.
- Precision breeding using proteomic information.

Proteomics is a powerful tool for understanding the molecular basis of plant diseases by analyzing proteins involved in plant defense and pathogen infection. It complements genomics and transcriptomics by providing direct insights into the functional molecules responsible for disease resistance and susceptibility. Proteomic approaches contribute to early disease diagnosis, identification of resistance-related proteins, development of disease-resistant crops, and sustainable plant disease management. As proteomic technologies continue to advance, they will play an increasingly important role in crop improvement and global food security.

E. Molecular Marker-Assisted Breeding in Phytopathogenic Fungi Management (Jiang, 2013; Manjot Kaur *et al.,* 2025)

Molecular Marker-Assisted Breeding (MAB), also known as Marker-Assisted Selection (MAS)**,** is a plant breeding approach that uses DNA-based molecular markers linked to desirable genes or quantitative trait loci (QTLs) to select plants with traits such as disease resistance. Unlike conventional breeding, MAS allows breeders to identify resistant plants at the seedling stage without exposing them to the pathogen, making breeding faster, more accurate, and more efficient. Marker-assisted selection (MAS) accelerates breeding for disease resistance.

Common types of molecular markers employed are RFLP (Restriction Fragment Length Polymorphism); RAPD (Random Amplified Polymorphic DNA); AFLP (Amplified Fragment Length Polymorphism); SSR (Simple Sequence Repeat or Microsatellite); SNP (Single Nucleotide Polymorphism) – the most widely used marker due to its abundance and high accuracy; SCAR (Sequence Characterized Amplified Region)**.**

Steps in Marker-Assisted Breeding - Identification of resistance genes through genetic mapping; Marker development for genes or QTLs associated with resistance; DNA extraction from breeding materials; Marker analysis using PCR or sequencing; Selection of resistant plants based on marker results; Field evaluation to confirm resistance and agronomic performance; Release of improved disease-resistant varieties.

Objectives of Marker-Assisted Breeding

- Develop disease-resistant crop varieties.
- Accelerate plant breeding programs.
- Identify resistance genes at an early stage.
- Improve breeding accuracy and efficiency.
- Reduce dependence on pesticides.
- Enhance crop yield and quality.

Advantages

- Faster than conventional breeding.
- High selection accuracy.
- Selection is independent of environmental conditions.
- Detects resistance genes at any developmental stage.

- Reduces time, labor, and cost of breeding.
- Enables pyramiding of multiple resistance genes.
- Reduces pesticide use and supports sustainable agriculture.

Limitations

- High initial cost for marker development.
- Requires advanced laboratory facilities and skilled personnel.
- Effective markers are not available for every trait.
- Recombination between markers and target genes may reduce selection accuracy.
- Less effective for traits controlled by many genes unless genomic selection is used.

Future Perspectives

- Integration of MAS with genomics, transcriptomics, proteomics, and phenomics.
- Wider use of genomic selection (GS) for complex disease resistance.
- CRISPR-Cas genome editing combined with marker-assisted breeding.
- High-throughput SNP genotyping and automated breeding platforms.
- AI and machine learning for marker discovery and breeding decisions.
- Climate-resilient and disease-resistant crop development.

Molecular Marker-Assisted Breeding is a powerful and efficient approach for improving disease resistance in crops. MAS has been widely applied in breeding resistance against rust, blast, wilt, and powdery mildew diseases. By using DNA markers linked to resistance genes, breeders can rapidly identify and select desirable plants, shorten breeding cycles, and develop durable disease-resistant varieties. The integration of MAS with modern genomic technologies and genome editing is transforming plant disease management and contributing to sustainable agriculture and global food security.

F. Host–Pathogen Interaction Studies in Phytopathogenic Fungi Management (Weiliang Zuo *et al.,* 2019)

Host–pathogen interaction refers to the biological, molecular, and biochemical interactions between a plant (host) and a disease-causing organism (pathogen) such as fungi, bacteria, viruses, nematodes, or phytoplasmas. Studying these interactions helps scientists understand how pathogens infect plants and how plants defend themselves, leading to improved disease management and the development of resistant crop varieties.

Molecular biology has greatly enhanced understanding of effector proteins, plant immune receptors, gene-for-gene interactions, signal transduction pathways, hormonal regulation, systemic acquired resistance, etc. These studies facilitate the development of resistant cultivars and novel disease control strategies.

Objectives of Host–Pathogen Interaction Studies

- Understand the mechanisms of pathogen infection.

- Identify plant defense responses.
- Discover resistance (R) genes and pathogen avirulence (Avr) genes.
- Develop disease-resistant crop varieties.
- Improve disease diagnosis and control strategies.
- Reduce crop losses and pesticide use.

Advantages

- Better understanding of disease mechanisms.
- Supports development of resistant crop varieties.
- Enables early disease detection.
- Reduces dependence on chemical pesticides.
- Promotes sustainable agriculture.
- Improves crop productivity and food security.

Limitations

- Host–pathogen interactions are highly complex.
- High cost of advanced molecular techniques.
- Requires sophisticated laboratory facilities.
- Large datasets require bioinformatics expertise.
- Pathogens evolve rapidly, potentially overcoming plant resistance.

Future Perspectives

- Multi-omics integration (genomics, transcriptomics, proteomics, and metabolomics).
- Artificial intelligence and machine learning for predicting host–pathogen interactions.
- Single-cell omics for detailed analysis of infection processes.
- CRISPR-based improvement of plant immunity.
- Precision breeding for durable disease resistance.
- Real-time monitoring of pathogen evolution using genomic surveillance.

Host–pathogen interaction studies provide essential insights into the molecular and cellular events that occur during plant infection. Understanding how pathogens invade plants and how plants activate defense mechanisms enables researchers to identify resistance genes, improve disease diagnosis, and develop resistant crop varieties. The integration of molecular biology, genomics, transcriptomics, proteomics, and genome editing has greatly advanced plant disease management, contributing to sustainable agriculture and enhanced global food security.

G. RNA Interference (RNAi) in Phytopathogenic Fungi Management (Ansari *et al.,* 2026)

RNA Interference (RNAi) is a natural gene-silencing mechanism in which double-stranded RNA (dsRNA) suppresses the expression of specific genes by degrading or blocking the corresponding messenger RNA (mRNA). In plant disease management, RNAi is used to silence essential genes of plant pathogens (fungi, viruses, bacteria, nematodes, and insects) or plant susceptibility genes,

thereby reducing disease development and enhancing crop resistance. RNAi is based on sequence-specific gene silencing. When double-stranded RNA corresponding to a target gene enters the cell, it is processed into small interfering RNAs (siRNAs). These siRNAs guide the degradation of complementary mRNA, preventing protein synthesis and thereby silencing the target gene.

Mechanism of RNAi

- dsRNA is introduced into plant cells through genetic transformation or by external application.
- The enzyme Dicer cuts dsRNA into small interfering RNAs (siRNAs) of about 21–24 nucleotides.
- The siRNAs are incorporated into the RNA-Induced Silencing Complex (RISC).
- The guide strand of siRNA directs RISC to complementary mRNA molecules.
- RISC cleaves the target mRNA, preventing translation into protein.
- As a result, the target gene is silenced.

Two major approaches:

- Host-Induced Gene Silencing (HIGS): Plants are genetically engineered to produce dsRNA targeting fungal genes. The pathogen takes up the RNA during infection, leading to silencing of essential genes.
- Spray-Induced Gene Silencing (SIGS): dsRNA molecules are sprayed directly onto crops. The pathogen absorbs the RNA, leading to gene silencing without genetic modification of the plant.

Objectives of RNAi in Plant Disease Management

- Develop disease-resistant crop varieties.
- Control plant viruses, fungi, nematodes, and insect pests.
- Silence pathogen virulence genes.
- Improve plant immunity.
- Reduce the use of chemical pesticides.
- Promote sustainable agriculture.

Advantages

- Highly specific and targeted gene silencing.
- Eco-friendly with minimal effects on non-target organisms.
- Reduces dependence on chemical pesticides.
- Effective against viruses, fungi, nematodes, and insect pests.
- Can provide durable disease resistance.
- Supports sustainable crop protection.

Limitations

- High cost of dsRNA production and delivery.
- Variable stability of RNA molecules under field conditions.
- Efficient delivery into target organisms can be challenging.
- Potential off-target effects if sequences are not carefully designed.
- Regulatory and public acceptance issues for genetically engineered RNAi crops.

Future Perspectives

- Development of stable and cost-effective dsRNA formulations.
- Wider adoption of Spray-Induced Gene Silencing (SIGS).
- Integration of RNAi with CRISPR-based genome editing.
- Nanotechnology-based RNA delivery systems.
- Combined use of RNAi with conventional breeding and marker-assisted selection.
- AI-assisted design of highly specific RNAi targets.

RNAi has shown promise against many pathogens including *Fusarium, Botrytis*, *Sclerotinia*, *Magnaporthe.* RNA Interference (RNAi) is a powerful and precise biotechnology for plant disease management. By selectively silencing genes involved in pathogen virulence, virus replication, nematode development, or plant susceptibility, RNAi provides an effective and environmentally friendly alternative to chemical pesticides. Approaches such as Host-Induced Gene Silencing (HIGS) and Spray-Induced Gene Silencing (SIGS) have demonstrated significant potential for developing disease-resistant crops. As delivery methods improve and production costs decrease, RNAi is expected to play an increasingly important role in sustainable agriculture and integrated plant disease management.

H. CRISPR/Cas Genome Editing in Phytopathogenic Fungi Management (Keykhasaber *et al.,* 2026)

CRISPR/Cas genome editing is a modern biotechnology tool that enables precise modification of plant genomes by introducing targeted changes in specific DNA sequences. It is based on a natural defense mechanism found in bacteria and has been adapted for crop improvement. In plant disease management, CRISPR/Cas technology is used to develop disease-resistant crops by modifying resistance genes, eliminating susceptibility factors, and improving plant immune responses. CRISPR/Cas9 has become an important tool for functional genomics, gene knockout, pathogenicity studies, development of resistant crops and identification of virulence genes

Workflow of CRISPR/Cas-Based Disease Resistance Development

- Identification of target gene associated with disease susceptibility or resistance.
- Design of guide RNA.
- Introduction of CRISPR/Cas components into plant cells.
- Genome editing and DNA repair.

- Screening of edited plants.
- Disease resistance testing.
- Development of improved crop varieties.

Objectives of CRISPR/Cas Technology in Plant Disease Management

- Develop crops with improved resistance to pathogens.
- Modify plant susceptibility (S) genes.
- Introduce or enhance disease resistance traits.
- Study gene functions involved in plant immunity.
- Reduce dependence on chemical pesticides.
- Accelerate crop improvement programs.

Advantages

- Highly precise and targeted genome modification.
- Faster than conventional breeding.
- Can modify multiple genes simultaneously.
- Useful for both basic research and crop improvement.
- Reduces pesticide dependency.
- Can develop durable disease resistance.
- Some edited crops may not contain foreign DNA.

Limitations

- Possible off-target mutations.
- Delivery of CRISPR components can be challenging in some crops.
- Regulatory differences among countries.
- Resistance may be overcome by rapidly evolving pathogens.
- Requires advanced molecular biology facilities.

Future Perspectives

- Development of multiplex CRISPR editing for multiple resistance traits.
- Combination with genomics, transcriptomics, and proteomics.
- AI-assisted guide RNA design.
- Gene editing for climate-resilient and disease-resistant crops.
- Development of non-transgenic genome-edited varieties.
- Precision agriculture integrating genome editing with disease forecasting.

CRISPR/Cas genome editing has emerged as a powerful tool for modern plant disease management. By precisely modifying resistance genes, susceptibility genes, and immune pathways, CRISPR enables the development of crops with enhanced and durable disease resistance. Integration of CRISPR technology with molecular breeding, genomics, and other

advanced approaches offers promising solutions for sustainable crop protection and global food security.

I. Molecular Detection of Fungicide Resistance (Brent and Hollomon, 2005; Zhonghua *et al.,* 2005)

Fungicide resistance is the ability of a fungal pathogen to survive and reproduce despite exposure to a fungicide that previously controlled it. It develops mainly due to genetic changes, such as mutations, gene overexpression, or alterations in metabolic pathways. Molecular detection of fungicide resistance involves identifying these genetic changes using DNA- and RNA-based techniques, allowing rapid diagnosis and effective disease management. Molecular markers detect mutations responsible for fungicide resistance such as CYP51 mutations, β-tubulin mutations, Cytochrome b mutations etc.

Objectives of Molecular Detection

- Early detection of fungicide-resistant pathogen populations.
- Identification of resistance-causing mutations.
- Selection of effective fungicides for disease control.
- Monitoring development and spread of resistance.
- Supporting integrated disease management strategies.

Advantages

- Rapid and accurate detection.
- Identifies resistance before disease control failure.
- Requires small amounts of pathogen material.
- Helps reduce fungicide misuse.
- Enables precision disease management.
- Supports epidemiological studies.

Limitations

- Requires knowledge of known resistance mutations.
- New resistance mechanisms may remain undetected.
- Requires specialized equipment and expertise.
- Initial development costs can be high.
- Genetic resistance does not always directly predict field performance.

Future Perspectives

- Development of portable molecular diagnostic devices.
- Integration of NGS with resistance surveillance.
- AI-based prediction of fungicide resistance evolution.
- CRISPR-based detection systems for rapid diagnosis.
- Real-time monitoring of pathogen populations in agricultural fields.

- Integration with precision agriculture technologies.

Molecular detection of fungicide resistance is an important component of modern plant disease management. Techniques such as PCR, qPCR, sequencing, and NGS enable rapid identification of resistance mechanisms and support informed fungicide use. By monitoring resistant pathogen populations and guiding integrated disease management strategies, molecular diagnostics help prolong fungicide effectiveness, reduce crop losses, and promote sustainable agriculture.

Conclusion

Molecular biology has transformed the management of phytopathogenic fungi by enabling rapid diagnosis, detailed characterization of pathogens, understanding of host–pathogen interactions, and the development of targeted, environmentally sustainable management strategies. Technologies such as PCR, next-generation sequencing, comparative genomics, transcriptomics, RNA interference, CRISPR/Cas systems, and molecular marker-assisted breeding are now central to modern plant disease management. Future integration of molecular biology with artificial intelligence, nanotechnology, precision agriculture, and multi-omics approaches is expected to shift disease management from reactive to predictive and precision-based systems, improving crop productivity while reducing reliance on chemical fungicides.

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INTEGRATING BIOTECHNOLOGY AND MUTATION BREEDING TO ACCELERATE CROP IMPROVEMENT FOR SUSTAINABLE AGRICULTURE

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Abstract
Innovative breeding techniques are needed to maintain production in the face of enormous challenges to global agriculture, including soil degradation, increasing pests and diseases, and climate change. Among other methods, the combination of biotechnological techniques and induced mutation breeding provides a quick way to produce new genetic variation for crop development. This chapter highlights molecular characterization platforms like MutMap, TILLING and genotyping-by sequencing for quick mutant detection; compares mutation breeding with cross breeding, highlighting their complementary roles in generating and combining useful alleles; discusses integration of mutation breeding with modern genome editing systems like CRISPR/Cas9 for precision trait improvement; summarizes the significant advancements made through physical and chemical mutagenesis in the development of superior crop varieties; describes how worldwide mutant breeding operations are supported by international initiatives like the IAEA Mutant Varieties Database. Faster production of robust, high-yielding cultivars with improved adaptability is made possible by the convergence of biotechnology and mutagenesis. A framework for sustainable food security in the face of shifting environmental conditions is offered by this integrated breeding program.

1. Introduction

In order to improve productivity and sustainability, precision farming systems and modern breeding technologies are progressively influencing the current agricultural sector [Dias, 2020]. However, a number of complicated problems, including severe temperatures, drought, salinity, heavy metal toxicity, and pest and pathogen infestations, still affect crop production worldwide. Plant growth and yield potential are severely restricted by these biotic and abiotic factors [Anwar and Kim 2020]. Plant breeders are concentrating on combining traditional breeding with contemporary biotechnological and molecular techniques to create high-yielding, stress-tolerant, and quality-improved cultivars appropriate for a variety of situations in order to get around these constraints [Krishna *et al.* 2016]. One of the most important methods for creating new genetic variation is mutation breeding. This method, which was developed in the early 20th century, creates heritable mutations by using chemical mutagens like sodium azide and ethyl methanesulfonate as well as physical mutagens like gamma rays, X-rays, and ion beams. Thousands of enhanced crop varieties for cereals, legumes, fruits, and ornamentals have been

released as a result of these induced genetic changes. Mutation breeding generates completely new allelic variants, hence increasing the available gene pool, in contrast to cross breeding, which rearranges existing alleles among parental lines. It is especially useful; there is little natural variation in autogamous or genetically homogeneous crops. The framework of mutant breeding has changed into a precision-driven field with the development of molecular biology and genomics. Targeted modifications in certain loci are now possible because to site-directed mutagenesis and genome editing technologies like CRISPR/Cas systems, Zinc Finger Nucleases (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs) [Dheer *et al.* 2020]. Among these, CRISPR/Cas9 is notable for its ease of use, high specificity, and adaptability, allowing for the direct modification or repair of genes linked to desirable traits. However, prior molecular characterisation and thorough understanding of gene activities are necessary for its successful implementation. The rapid multiplication and screening of mutant lines under controlled conditions made possible by the integration of in vitro mutagenesis with tissue culture methods has further expedited the breeding pipeline.

In these systems, molecular markers help identify desired genotypes, tissue culture methods facilitate multiplication and stability evaluation and induced mutagenesis produces genetic variety [Wilde, 2016]. In addition to shortening breeding cycles, this combination enables early-stage selection for characteristics like increased yield, stress tolerance and disease resistance.

Although mutation breeding is an effective method for creating new genetic variety, its accuracy and efficiency are significantly increased when it is combined with biotechnology. Recombinant DNA technology, RNA interference (RNAi), chromosome engineering, marker-assisted recurrent selection (MARS) and genomic selection (GS) are only a few of the many techniques used in modern biotechnology to speed up variety development [Mishra *et al.* 2017].

By facilitating targeted gene manipulation, controlled introgression of advantageous alleles, and early trait prediction, these techniques have transformed conventional breeding. High-throughput genotyping platforms have made it possible to quickly identify induced mutations and map the causative genes using techniques like MutMap, TILLING and whole-genome sequencing.

The use of genetic variation has changed with the shift from mutant breeding to integrated molecular breeding techniques. This was time-consuming and frequently constrained by the lack of knowledge about the underlying genetic alterations. On the other side, a transparent, data-driven framework where genomic, transcriptomic and phenotypic data are examined together to choose the best variants is provided by the present integration of mutagenesis with molecular tools. Breeders can now utilize focused editing methods to replicate or improve these mutations in different backgrounds thanks to this shift, which has also made it easier to identify individual nucleotide changes responsible for desirable traits.

Each strategy has intrinsic advantages and disadvantages, despite impressive advancements. Although it is comparatively random, mutation breeding is nonetheless economical and useful

for many kinds of crops. Although genome editing and biotechnology offer high precision, they are limited by intellectual property concerns, complicated regulations, and the need for sophisticated laboratory infrastructure. Therefore, their synergistic integration—where the precision of molecular and genomic techniques is complemented by the exploratory potential of mutagenesis.

A potent, complementary, and innovative approach to sustainable crop development is the combination of biotechnology and mutant breeding. The creation of novel alleles by induced mutations, their validation and improvement using molecular tools, and their deployment through sophisticated selection methods are all made possible by this unified framework. Researchers can expedite the development of resilient, high-yielding, and climate-smart cultivars to address the global concerns of food security and agricultural sustainability by bridging traditional and modern breeding paradigms.

2. Crop Improvement and Productivity Through Mutation Breeding

Nowadays, most people agree that one of the most potent and useful tools available to plant breeders is mutation breeding. It has been widely used to enhance particular features in a variety of crops, especially in autogamous species (self-pollinating crops) with low natural variability and a narrow genetic base. By altering one or two crucial agronomic traits that restrict production or quality, breeders might improve well-adapted, elite cultivars without compromising the desired qualities of the original genotype [Mbindav& Masaki, 2021]. Three main forms of mutagenesis are commonly used in mutation breeding. (i) Induced mutagenesis, in which random changes in the genome are produced by applying chemical or physical mutagens (such as sodium azide, EMS, or X-rays, gamma rays, or ion beams). (ii) Site-directed (or site-specific) mutagenesis, a more accurate method that use synthetic oligonucleotides to make targeted modifications at certain loci utilizing tools like CRISPR/Cas9, TALENs, or ZFNs.

(iii) Insertional mutagenesis, which is frequently employed for gene tagging and functional analysis, is the process of inserting exogenous DNA sequences (such transposons or T-DNA) into the genome to alter or disrupt gene function [Bado, *et al.* 2015]. Due to its affordability and shown ability to produce meaningful variety, induced mutagenesis continues to be the most used method in mutation breeding. Genetic variation produced by induced mutation can be used as a useful genetic resource in hybridization programs to introduce desired alleles or directly lead to the introduction of novel cultivars [Raina *et al.* 2018].

A mutagen is used as the agent that modifies the DNA sequence of plant cells through nucleotide substitution, deletion, insertion, or rearrangement in a conventional mutation breeding scheme. In addition to practical factors like availability, cost, convenience of handling, and the necessary mutation spectrum, the choice of mutagen is crucial and is typically influenced by previous experimental findings for the target species.

In breeding programs, physical and chemical mutagens are frequently employed to cause heritable alterations in the genome, ultimately improving crop quality, yield, and stress tolerance. Mutation breeding becomes an even more potent tactic for hastening the creation of superior crop varieties when combined with contemporary selection methods and molecular technologies.

Physical mutagens, especially ionizing radiation like gamma rays, have been used more frequently than chemical mutagens in the production of officially released crop varieties throughout the history of plant mutation breeding. Its high penetrative ability, reproducibility, and suitability for treating large seed populations, gamma irradiation accounts for the largest share of registered mutant cultivars worldwide, according to data compiled in the FAO/IAEA Mutant Variety Database [IAEA, 2022]. Over two-thirds of released mutant varieties in major cereals, such as rice and wheat, were produced using radiation-based techniques, according to reviews of mutation breeding programs. This is due to early establishment of irradiation facilities, strong institutional support, and long-standing breeder familiarity with physical mutagens [Melsen & Wouw, 2021]. Similar patterns may be seen in cotton, where agronomically beneficial diversity, such as early maturity, altered plant architecture, stress tolerance, and yield-related traits, has historically been mostly produced by radiation-induced mutagenesis [Patel, 2011]. Physical mutagens are preferred in variety development because of their wide mutation spectrum and consistent capacity to produce phenotypically visible variation that is appropriate for traditional selection procedures.

On the other hand, although they contribute fewer types to official release statistics, chemical mutagens, especially sodium azide and ethyl methanesulfonate (EMS), are becoming more common in research-driven mutant breeding and functional genomics. Chemical mutagens are preferred because they are simple to use, need little infrastructure, and produce a high frequency of point mutations, which are particularly useful for analyzing gene function and generating allelic series for quantitative traits [Rana *et al.* 2020; Henikoff *et al.* 2004]. Chemical mutagenesis has been widely employed in cotton to create TILLING populations and produce modest changes in stress response genes, herbicide tolerance, and fiber quality, which supplement rather than replace radiation-based methods. Chemical mutagens are becoming even more useful in precision breeding and pre-breeding programs thanks to developments in next-generation sequencing, TILLING, and other high-throughput mutation detection techniques. Overall, chemical mutagens currently predominate in experimental mutation breeding and gene discovery activities, suggesting that their relative utilization is very context-dependent rather than mutually exclusive, even though physical mutagens are still more common in the creation of released mutant cultivars.

2.1 Physical Mutagens

Based on the type of radiation they release, physical mutagens can be roughly divided into two groups. First, Subatomic particles like electrons, protons, neutrons, alpha particles, beta particles,

fast neutrons and ion beams make up ionizing atomic particles. These particles possess enough energy to directly interact with atomic structures in biological tissues, resulting in DNA alterations and ionization and another, Ionizing electromagnetic radiation, such as cosmic, gamma, and X-rays. Due to their high intensity and ability to profoundly permeate plant tissues, these radiation types can cause chromosomal abnormalities, base changes and breakage in DNA strands.

Over 70% of officially released mutant crop types worldwide are the result of physical mutagens, which have been important in mutation breeding operations. Physical mutagenesis has a major influence on modern crop improvement, as seen by the widespread adoption of these varieties due to their improved features, such as increased yield, stress tolerance, and superior quality qualities. Their high penetration rate and reliable mutagenic effectiveness, gamma rays are the most popular physical mutagen in plant mutation breeding. These high-energy electromagnetic radiations are frequently produced by nuclear processes or radioisotopes like cobalt-60 (60Co) and cesium-137 (137Cs) [Oladosu *et al.* 2016]. Interestingly, gamma irradiation has been used to create more than 92% of officially released mutant rice cultivars. Gamma rays have been effectively used to produce genetic variety in crops other than rice, such as coriander, tomato, anthurium, mung bean, and potato. For instance, in vitro potato seedlings exposed to gamma irradiation at dosages of 20 and 40 Gy produced genotypes resistant to late blight, while 40 Gy treatments also produced heat-tolerant lines. In Solanum tuberosum, even modest doses like 2.5 Gy greatly increased the production of microtubers.

A more recent class of physical mutagens with clear benefits is ion beams. Ion beams are produced by particle accelerators utilizing charged particles such 20Ne, 12C, 7Li, 40Ar, and 56Fe. Ion beams cause complicated and localized DNA damage, such as significant deletions and chromosomal rearrangements, because of their high linear energy transfer (LET), which allows for effective targeted mutagenesis. Their efficacy has been shown in rice, Arabidopsis, and chrysanthemum, leading to characteristics including improved pigment accumulation, altered flower color, and UV-B resistance [Yamaguchi *et al.* 2003].

Lewis Stadler initially used X-rays to induce mutations in maize and barley, and they are still a crucial mutagenesis technique. Drought-tolerant barley and soybean cultivars like Yubian 31 and Anni have been developed as a result of X-ray irradiation at 100 Gy, while disease-resistant types like AC-Albright have been produced through hybridization of X-ray-derived mutants [Muller *et al.* 1954]. Neutrons are quite successful at causing significant genetic changes, while being utilized less frequently due to safety concerns and limited penetration. Kiren and NIAB-IRRI-9 are two salt-tolerant rice and chickpea types that have been produced using rapid neutron treatments (15 Gy). Despite its limited penetration, ultraviolet (UV) light causes mutations through the creation of pyrimidine dimers and has been exploited to create drought-tolerant,

early-maturing maize mutants. Lack (1954) was the first to establish the mutagenic potential of UV radiation.

Table 1: List of promising mutant varieties with improved traits where physical mutagens were used

Mutant Variety	Crop (Latin Name)	Physical Mutagen	Dose	Improved Trait(s)
Akichikara	*Oryza sativa* L.	Gamma rays	200 Gy	Short and stiff culm; high yield
M-102	*Oryza sativa* L.	Gamma rays	250 Gy	Very early maturity, semi-dwarfness, medium grain size
Kiren	*Cicer arietinum* L.	Neutrons	–	Salt tolerance
Changwei 19	*Triticum aestivum* L.	Gamma rays	350 Gy	Salt and alkali tolerance
Anni	*Hordeum vulgare* L.	X-rays	100 Gy	Drought tolerance
NIAB Karishma	*Gossypium* spp.	Gamma rays	300 Gy	High-temperature and salt tolerance
ART/OB/98/SW1	*Zea mays* L.	UV light	–	Early maturity and drought tolerance
ART/OB/98/SW6	*Zea mays* L.	UV light	–	High yield and drought tolerance
GMO77	*Oryza sativa* L.	Gamma rays	50–350 Gy	High starch content and improved industrial quality
GMO45	*Oryza sativa* L.	Gamma rays	–	Improved industrial quality and abiotic stress tolerance
Kharchia 65	*Triticum aestivum* L.	Gamma rays	40 kR	Salt tolerance
Bakhtawar-92	*Triticum aestivum* L.	Gamma rays	20 kR	High germination percentage (96.47%)
Alpina	*Hordeum vulgare* L.	Gamma rays	240 Gy	Semi-dwarfness

2.2 Chemical Mutagens

Because chemical mutagens can cause precise and heritable genetic changes, especially point mutations, they are frequently used in mutation breeding. Ethyl methane sulphonate (EMS), sodium azide (NaN3), polyethylene glycol (PEG), colchicine, acridine orange, and 2,4-dichlorophenoxyacetic acid (2,4-D) are examples of chemical mutagens that are often utilized. Among these, EMS is one of the most popular and successful mutagens used in plant breeding. It

works by alkylating DNA's guanine base, which causes mispairing during replication-more precisely, alkylated guanine pairs with thymine rather than cytosine. Because of the high frequency of G/C to A/T transitions that follows, EMS is a dependable method for creating specific genetic variation. Wheat and rice are two of the key crops in which EMS has been effectively utilized to induce mutations.

Table 2: List of promising mutant varieties with improved traits where chemical mutagens were used

S. No.	Mutant variety	Chemical Mutagen	Mode of action/Doses	Improved traits
1	Taichung 65 (rice)	N-methyl-N-nitrosourea (MNU)	Nucleotide substitutions in the fragments of three genes, *OsAHP1*, *OsSAD1* and *PLA1*.	Create chimeric plants with a high frequency. showed higher seed fertility of above 90%
2	MGE12 and MGE13 (rice)	N-methyl-N-nitrosourea (MNU)	Changes in the causal gene *Os07g0603700*	Breeding for high oil content
3	Co86032 (sugarcane)	Ethyl methane sulphonate (EMS) & Sodium azide (SA)	Somaclonal variation. Dose at 1.0 lM L-1, 0.8 µM L-1 & 1.5 mg LP-1	Yellow leaf disease (YLD) resistance
5	SA-11-1, SA-11-2 (Common bean)	Sodium azide (NaN3)	Mutations in color or enhancing genes.	Antioxidant activity increases
6	18-599M (maize)	Sodium azide (NaN3) & Gamma radiation 1.0 mmol/L of NaN3 & 20Gy gamma ray.	Mutation in GST-1 gene	Highly drought-tolerant varieties

Nevertheless, EMS's mutagenic effectiveness varies with dose. It can significantly impede seedling growth at higher concentrations. According to Olaolorun *et al.,* for instance, the shoot length of seedlings of sesame (*Sesamum indicum*) treated with 1.8% EMS was 46% shorter than that of seedlings treated with 0.4% EMS. Polyethylene glycol (PEG), which is frequently used in conjunction with EMS, has also been utilized to increase sugarcane's resistance to drought. By replicating drought conditions and acting as an osmotic stress agent, PEG makes it possible to select for stress-tolerant mutants. By preventing microtubule polymerization, colchicine, another strong chemical mutagen, interferes with the production of spindle fibers during mitosis [de

Moura & Van Houten, 2010]. Colchicine is a common chemical used to induce polyploidy in plants because it causes chromosomal doubling. Colchicine is frequently employed to develop plants with increased vigor, larger organs, and changed fertility because it is a mitotic toxin that causes significant genetic diversity.

Traditionally employed as a pesticide and bactericide, sodium azide (NaN3) is also a potent mutagen in both plants and animals. It is converted into azidoalanine in plant cells, a mutagenic substance that enters the nucleus and interacts with DNA to cause point mutations. Sodium azide is one of the most powerful chemical mutagens for plants and functions similarly to alkylating agents. Its use is effective, reasonably priced, and appropriate for large-scale mutagenesis.

Numerous variables, including as mutagen concentration, treatment duration, solution pH, temperature, and seed soaking time, affect effective chemical mutagens. Mutation frequency, germination rate, pollen fertility, lethality, and other physiological and morphological traits are all greatly impacted by these factors [Olaolorun *et al.* 2019]. The potential of chemical mutagens to produce useful allelic variations without depending on exotic or wild germplasm, which may contain harmful alleles or undesired associated characteristics, is one of its main advantages. However, as high dosages might result in fatal mutations or irreversible genetic harm, careful adjustment of therapy settings is crucial. To increase mutation efficiency while limiting side effects, it is therefore essential to comprehend how dose, duration, and environmental factors interact.

3. Using Genetic Engineering and Biotechnology to Improve Agricultural Crops

Agricultural biotechnology, also known as agritech, is a subfield of agricultural science that uses contemporary scientific methods and instruments, such as genetic engineering, molecular diagnostics, genetic fingerprinting, and tissue and cell culture, to modify and enhance living things, including microorganisms, plants, and animals, for agricultural uses. Crop biotechnology is a fast developing sub-discipline within this science that makes it possible to precisely transfer desirable features from one plant species to another, such as increased nutritional content, pest resistance, and stress tolerance. By providing alternatives to conventional breeding methods, this has transformed crop improvement by enabling the creation of high-yielding, disease-resistant, and climate-resilient cultivars.

Herbicide tolerance, insect resistance, abiotic stress tolerance, disease resistance, and enhanced nutritional quality are just a few of the agriculturally significant traits that can be precisely introduced, modified, or silenced through genetic engineering, a potent molecular breeding technique. This method overcomes the genetic constraints imposed by traditional breeding by enabling the transfer of genes both within and across taxonomic boundaries. By giving direct access to well-characterized genes and regulatory elements, genetic engineering has revolutionized DNA research and plant breeding since its inception in the 1970s, resulting in previously unheard-of control over crop trait development.

Global agriculture has been significantly impacted by the commercialization of transgenic crops created through genetic engineering. Insect-resistant and herbicide-tolerant genetically modified crops have greatly boosted crop productivity while lowering yield losses from weeds and pests. Additionally, the use of chemical herbicides and insecticides has significantly decreased as a result of their acceptance, which has reduced greenhouse gas emissions, environmental contamination, and total production costs related to intensive chemical inputs. By enabling decreased tillage systems, these advantages have also aided conservation agriculture methods.

The field of plant biotechnology has evolved recently due to the development of genome editing tools, especially CRISPR/Cas9, which allow for extremely accurate, efficient, and predictable alterations of plant genomes. Genome editing, in contrast to conventional transgenic techniques, enables precise insertions, deletions, or base substitutions at certain genomic regions, frequently without the introduction of foreign DNA. While reducing unwanted genetic alterations and off-target consequences, this precision speeds up the development of superior crop types.

When taken as a whole, these biotechnological developments have significantly advanced plant trait validation, functional genomics, and gene identification. In order to meet future agricultural demands, the integration of genetic engineering and genome editing has improved our understanding of the intricate gene networks governing important agronomic traits and has produced novel tools for creating high-yielding, stress-resilient, and nutritionally enhanced crops.

The exact, targeted insertion, deletion, or replacement of particular DNA sequences within an organism's genome is made possible by a collection of cutting-edge molecular technologies known as "genome editing." Genome editing offers unprecedented control over gene function and trait expression by enabling deliberate and site-specific genetic alterations, in contrast to traditional breeding and random mutagenesis. Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas systems are examples of designed nucleases that produce double-strand breaks at specific genomic locations. The cell's natural DNA repair mechanisms, such as homology-directed repair (HDR) or non-homologous end joining (NHEJ), fix the resultant breaks, producing predictable genetic results. It allows for precise and targeted crop improvement while drastically reducing breeding cycles, genome editing has become a game-changing technology for sustainable agriculture. Genome editing circumvents many of the drawbacks of conventional and mutation-based breeding, including linkage drag and drawn-out selection procedures, by specifically targeting genes linked to important agronomic qualities. This accuracy increases breeding efficiency and accuracy by enabling the quick generation of superior crop varieties with few unintentional genetic changes.

Novel crop varieties with increased production potential, higher resistance to insect pests and plant diseases, tolerance to herbicides and abiotic stresses, and superior nutritional quality have already been produced as a result of the use of genome editing. Examples include targeted

changes in metabolic pathways to improve micronutrient content, disrupted susceptibility genes for long-lasting disease resistance, and edited crops with altered plant architecture for increased productivity. All things considered, genome editing is a paradigm change in contemporary plant breeding, offering a strong and adaptable foundation for creating crops that are nutritionally enhanced, resource-efficient, and climate resilient in order to satisfy future needs for global food security.

By permitting extremely accurate, effective, and programmable genetic alterations across a wide range of crop species, the CRISPR/Cas9 system-a widely used third-generation genome editing technology-has revolutionized plant biotechnology. Compared to previous genome editing tools, CRISPR/Cas9 has been successfully applied to the majority of crop genomes due to its ease of use, adaptability, and versatility, greatly expediting trait development and functional genomics research [89]. The Cas9 endonuclease in the system is directed by a single-guide RNA (sgRNA) to a complementary target DNA sequence next to a protospacer adjacent motif (PAM), where site-specific cleavage takes place [Kumar *et al.* 2013].

The main way that CRISPR/Cas9 works is by creating double-strand DNA breaks (DSBs) at certain genomic locations. The cell's natural DNA repair mechanisms then fix these breaks, usually by error-prone non-homologous end joining (NHEJ), which typically leads in tiny insertions or deletions (indels) that cause gene disruption or knockout. Alternatively, homology-directed repair (HDR) can enable targeted gene insertion or precise sequence substitution when a homologous donor template is available, albeit HDR efficiency is still rather low in the majority of plant systems. CRISPR/Cas9 has become a key component of contemporary plant genomics and breeding because of its remarkable effectiveness in producing loss-of-function mutations through NHEJ-mediated repair. It is widely utilized for multiplex genome editing, functional gene validation, gene transformation, and the quick generation of knockout lines for important agronomic features [Jaganathan *et al.* 2018]. Additionally, its application across a variety of crop species has increased due to advancements in delivery technologies such as ribonucleoprotein (RNP) delivery, particle bombardment, and Agrobacterium-mediated transformation. When taken as a whole, CRISPR/Cas9 is a fundamental tool in modern crop improvement techniques, allowing for precise genetic manipulations that promote accelerated and sustainable breeding operations.

4. Integrated Methods for Enhanced Plant Breeding Using Biotechnology and Mutant Breeding

The depletion of natural resources, population expansion, and climate change continue to pose increasing problems to global food security. Crop production must be significantly increased using creative and integrative breeding techniques that mix traditional and molecular methods in order to meet the growing demand for food. It is necessary to simultaneously incorporate several

genes that impart tolerance and production stability into crop plants in order to address complicated restrictions including drought, salinity, and new pests and diseases.

A comprehensive understanding of trait development at the molecular level is crucial for current mutant breeding efforts. The detection and application of mutagenesis-induced variants has been completely transformed by recent developments in high-throughput sequencing and mutation mapping techniques. Rapid identification of causal single-nucleotide variants is made possible by tools like MutMap, MutChromSeq, and other sequencing-based pipelines, especially in crops like tomato, rice, and wheat. This speeds up the finding of useful alleles that improve yield and quality. Similar to this, integrated breeding pipelines have been successfully applied in maize, where the combination of transgenic selection, induced mutagenesis, and molecular breeding has produced hybrids with enhanced yield stability, drought tolerance, and water use efficiency appropriate for African agro ecosystems.

Another illustration is the Hor-TILLUS population of spring barley, which was created by TILLING (Targeting Induced Local Lesions in Genomes) with chemical mutagens like N-methyl-N-nitrosourea (MNU) and sodium azide (NaN3). Functional genomics and pre-breeding have benefited greatly from this resource, which has made it possible to identify alleles linked to grain quality and resistance to abiotic stress [Wang *et al.* 2024].

The effectiveness of integrating CRISPR/Cas-mediated genome editing with mutant breeding has been further demonstrated by recent investigations. CRISPR/Cas9 has been used to duplicate beneficial mutations that were first found in chemically mutagenized tomato and maize populations, resulting in non-transgenic, precisely edited lines with better fruit firmness and kernel properties [Fisher *et al.* 2013]. Beneficial mutations become more diverse, frequent, and heritable when these technologies are integrated. Although genetic engineering is still a crucial tactic, its widespread use is still restricted by laws and public worries over genetically modified organisms (GMOs). Therefore, a non-transgenic but effective method for crop improvement is to combine biotechnological methods like tissue culture, genome sequencing, and molecular marker-assisted selection with mutant breeding [Giller *et al.* 2021].

In many breeding projects, integrated techniques are still neglected despite notable advancements. Molecular biologists, data scientists, and plant breeders must work together more closely to expand their use. To fully realize the potential of integrated mutation-biotechnology pipelines, issues including polygenic trait control, genotype-by-environment interaction, and regulatory approval must be addressed. In the end, these multidisciplinary methods are the most promising way to create crop types that are both nutritionally superior and climate resilient, which will support the world's food production in the ensuing decades.

Conclusion

Although mutation breeding has proven to be very beneficial in solving modern agricultural problems, there are still a number of unanswered questions and information gaps that prevent it

from reaching its full potential. The genetic basis of many mutation-derived features is still poorly understood, and the connection between induced mutations and complicated agronomic phenotypes is frequently not fully recognized, despite the enormous number of mutant varieties that have been released globally. Additionally, unpredictability, low selection efficiency, and the time-consuming nature of phenotype-based screening limit traditional mutation breeding, underscoring the need for more accurate and predictive methods. By enabling targeted gene manipulation, quick trait validation, and increased selection accuracy, the integration of contemporary biotechnological tools like genetic engineering, genome editing, high-throughput phenotyping, and omics-based analyses presents promising opportunities to get around these restrictions.

However, issues with public acceptance, legal frameworks, off-target impacts, and the scarcity of transformation and editing techniques for numerous agricultural species continue to be major obstacles to widespread adoption. Coordinated efforts to enhance genome annotation, create effective delivery methods, and set up standardized workflows that integrate mutation induction with molecular characterization and functional validation will be necessary to address these problems. In order to supplement traditional plant breeding, this review emphasizes the necessity of a comprehensive and integrative breeding approach that strategically aligns mutant breeding with cutting-edge biotechnologies. The development of high-performing crop varieties with optimum yield, quality, and tolerance to biotic and abiotic challenges is anticipated to be accelerated by such synergistic approaches, supporting sustainable agricultural production and global food security.

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METAGENOMIC AND BIOPROSPECTING STRATEGIES FOR DISCOVERING NOVEL PHARMACEUTICAL SECONDARY METABOLITES

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1. Introduction

Natural products have been immensely valuable in drug development, with approximately 24% of approved drugs derived from natural sources or their derivatives (Newman *et al.,* 2021). Unlike the limited medicinal resources from plants and animals, microbes found in soil, air, oceans, and even as endophytes offer a promising alternative for drug lead discovery. This is due to their remarkable diversity and distinctive biosynthetic pathways that enable them to produce bioactive compounds. The genetic ability of organisms to synthesize specialized small molecules is encoded in their genomes. However, the vast potential of microbial secondary metabolites remains largely unexplored. Using bacteria in bioprospecting offers several advantages, such as their suitability for large-scale fermentation and the possibility of genetic engineering to boost metabolite production.

Traditionally, the discovery of bioactive compounds has depended on the cultivation of organisms under controlled laboratory conditions. Most environmental microorganisms cannot be cultured using standard techniques. The emergence of high-throughput sequencing and genome mining has transformed this field, allowing researchers to explore microbial biosynthetic capabilities more effectively. These advanced approaches have enabled a more targeted and efficient search for novel bioactive secondary metabolites (Anita *et al.,* 2024).

Microorganisms such as *Pseudomonas*, *Bacillus*, *Streptomyces*, and fungi are prolific producers of secondary metabolites with important pharmaceutical and industrial applications. These metabolites include peptides, phenazines, bacteriocins, lactones, anthracyclines, quinolones, aminoglycosides, macrolides, siderophores, and other bioactive compounds. Although not essential for the basic growth and survival of microbes, secondary metabolites play crucial ecological roles and provide valuable compounds, such as antibiotics, anticancer agents, pigments, growth-promoting substances, and immunosuppressants (Table 1). Among bacteria, *Pseudomonas* is known to produce metabolites such as pyocyanin and rhamnolipids, which have medical, cosmetic, and environmental applications. *Bacillus* species are important sources of antimicrobial peptides with antibacterial, antiviral, and antitumor activities (Eltokhy *et al.,* 2024). Actinomycetes, especially *Streptomyces*, remain the most important group for antibiotic discovery, producing a wide range of clinically useful compounds and biosynthetic gene clusters that continue to attract attention for novel drug discovery (Veilumuthu and Christopher, 2022).

Fungal secondary metabolites, such as polyketides, terpenoids, peptides, and phenolic compounds, also contribute significantly to the food, nutraceutical, and pharmaceutical industries (Han *et al.,* 2024).

Table 1: Major microbial groups and their secondary metabolites.

Microbial Group	Important Secondary Metabolites	Major Significance
Pseudomonas	Pyocyanin, rhamnolipids, phenazines, quinolones	Antimicrobial, anticancer, cosmetic, environmental applications
Bacillus	Lipopeptides, bacteriocins, antimicrobial peptides	Antibacterial, antiviral, antitumor activity
Actinomycetes / *Streptomyces*	Antibiotics, macrolides, aminoglycosides, glycopeptides, polyketides	Major source of clinically important antibiotics
Fungi	Polyketides, terpenoids, peptides, phenolic compounds	Food, nutraceutical, and pharmaceutical uses

Metagenomics and bioprospecting have emerged as powerful approaches for unlocking the hidden potential of microbial communities, particularly marine microorganisms, as sources of novel pharmaceutical and industrial metabolites. By directly accessing genetic material from environmental samples, these methods overcome the limitations of traditional culture-based techniques and have enabled the discovery of bioactive compounds, enzymes, and novel genetic systems with broad utility. In bioprospecting, microbial resources are explored for valuable molecules and microorganisms with desirable properties, supporting applications in drug discovery, food safety, fermentation, and industrial bioprocessing (Priscilla *et al.,* 2025). Metagenomic screening is commonly performed using sequence-based and function-based strategies, each contributing to the identification of previously unknown biosynthetic genes and metabolites. With the support of high-throughput sequencing, artificial intelligence, and machine learning, metagenomic bioprospecting is expanding the chemical diversity available for natural product discovery and offers new opportunities for the development of next-generation therapeutics (Chaachouay & Zidane, 2024).

2. Importance Of Secondary Metabolites

Secondary metabolites produced by microorganisms are highly important because they possess a wide range of biologically active properties and have transformed medicine, agriculture, and the industry. Although they are not essential for the basic growth or survival of microbes, these compounds provide valuable functions, such as antimicrobial defense, anticancer activity, immunosuppression, cholesterol reduction, and enzyme inhibition (Table 2). Many clinically useful drugs, including antibiotics, antifungals, and anticancer agents, have been derived from microbial secondary metabolites, particularly from actinomycetes and fungi. Their structural

diversity also makes them excellent lead compounds for developing of new pharmaceuticals and other bioactive products.

Table 2: Major types of microbial secondary metabolites, their sources, and their biological significance

Type of Secondary Metabolite	Major Microbial Source(S)	Important Activities / Examples
Alkaloids	Marine bacteria, actinomycetes, fungi, sponges-associated microbes	Antibacterial, anticancer, anti-inflammatory, antifungal, anti-HIV; examples include indole alkaloids, lodopyridone, vincristine-like compounds
Glutarimides	*Streptomyces* spp.	Antibacterial, antifungal, and anticancer activity; example: streptoglutarimides
Polyketides	Actinobacteria, fungi, plants, protists, insects, molluscs	Antibacterial, antifungal, anticancer, antiviral, immunosuppressive, cholesterol-lowering, anti-inflammatory; examples include rapamycin, oleandomycin, actinorhodin, daunorubicin
Terpenoids	Bacteria, fungi, plants, marine organisms	Vitamins, hormones, pigments, flavors, fragrances, and drugs such as taxol and artemisinin; also, antimicrobial and signaling roles
Non-ribosomal peptides (NRPs)	Fungi, bacteria	Antibiotic, antifungal, immunosuppressive, and cytotoxic activities; examples include penicillins, cephalosporins, echinocandins, cyclosporine

Researchers can efficiently explore metagenomic data by combining advanced bioinformatics tools with high-throughput screening to identify new natural product pathways and bioactive compounds. In microorganisms, especially actinobacteria such as *Streptomyces*, secondary metabolites are encoded by conserved biosynthetic gene clusters that include adjacent genes for enzymes involved in the production of metabolites (Mehrezi *et al.,* 2020). *Streptomyces* species are particularly important because their large, GC-rich genomes contain many clusters that produce structurally diverse compounds such as antibiotics, anticancer agents, antifungals, and immunosuppressants (Javad *et al.,* 2024). Although many clinically important drugs have already been discovered from these bacteria, a large number of biosynthetic clusters remain silent under standard laboratory conditions, making actinomycetes a major untapped source for future drug discovery (Abdelmohsen, & Ahmed., 2022).

3. Metagenomic and Bioprospectiong Approaches

Metagenomics is the study of the total genetic material present in a microbial community in a natural environment, including bacteria, fungi, and other uncultured microorganisms. It is based

on the direct extraction and analysis of DNA from environmental samples without the need for cultivation, allowing researchers to explore microbial diversity, community structure, and hidden biosynthetic potential. This approach has become an important tool for accessing unculturable microbes and discovering novel genes, functions, and bioactive products from diverse ecological niches such as soil, oceans, plants, and animals (Neelakanta and Sultana, 2013; Bhupender *et al.,* 2020).

Bioprospecting is the systematic exploration of biological resources to identify useful compounds for societal benefit, and microbial bioprospecting focuses on the vast diversity of microorganisms found in both ordinary and extreme environments (Angelin *et al.,* 2025; Fernado *et al.,* 2023). Although the term is relatively recent, humans have long used microbes for applications such as fermentation and medicine. Metagenomic and next-generation sequencing approaches have greatly expanded its scope by enabling access to unculturable microbes and their biosynthetic potential. In particular, metagenome-based mining allows the identification of biosynthetic gene clusters responsible for secondary metabolite production, and tools such as antiSMASH, PRISM, CLUSEAN, NP. searcher, and NRPminer support the detection of these clusters in genomic and metagenomic data (Olga *et al.,* 2019; Shohreh *et al.,* 2024).

3.1 Extraction of Environmental DNA and Metagenomic Library Construction

Conventionally, microorganisms are cultured before DNA extraction; however, approximately 99% of environmental microorganisms remain uncultured in laboratory settings. The initial and crucial step in constructing a metagenomic library involves obtaining high-concentration, large-fragment DNA. Two essential considerations for metagenomic DNA extraction are: first, capturing the genetic material of all microorganisms present in the sample, and second, maintaining the integrity and purity of the target DNA fragments while minimizing contamination from environmental sources. A novel adaptation of the direct saline DNA extraction method is introduced, involving multiple steps. First, the genetic material is isolated and purified, and a metagenomic library is constructed. The library is made by cloning DNA fragments into specific vectors, which are then put into host cells. Subsequently, the cells are screened for the desired genes or functions (Robert *et al.,* 2020). The criteria for choosing vectors in DNA cloning, focus on how they help amplify target DNA fragments and make it easier to screen after transformation and expression. It lists plasmids, cosmids, fosmids, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), and phages as common vectors for DNA cloning (Fig 1).

3.2 Types of Metagenomic Approaches and Their Screenings

Metagenomic approaches for metabolite identification are generally divided into function-based and sequence-based screening. Function-based screening searches for a measurable phenotype or bioactivity directly in metagenomic libraries, whereas sequence-based screening uses known DNA sequences, primers, or probes to detect target genes using PCR or hybridization. As noted

by Devika *et al.,* (2023), function-based screening detects desired biological activity, whereas sequence-based screening is limited to genes with known sequence information.

In function-based metagenomics, environmental DNA is cloned into suitable vectors and expressed in host cells, allowing for direct testing of bioactive products so that bioactive products. This approach is useful for discovering antimicrobial, antiviral, antioxidant, anticancer, and enzyme activities, including cellulase and agarase-related functions of these proteins. This is especially valuable when the target phenotype is unknown; however, success depends on host-vector compatibility, insert size, gene abundance, screening method, and expression efficiency in the surrogate host (Ramyasree *et al.,* 2022).

Figure 1: Simplified workflow for environmental DNA extraction, purification, and metagenomic library construction from environmental samples

In sequence-based analysis, screening relies on conserved genes and known motifs, such as polyketide synthase or cellulase genes, using PCR amplification or probe hybridization techniques. This method is flexible because it does not require the cloned genes to be expressed in the host; however it cannot identify completely novel sequences that do not match existing references. Lu *et al.,* (2021) emphasize that this strategy is best suited for known targets and conserved families.

High-throughput metagenomic sequencing expands these approaches by analyzing large environmental DNA datasets using NGS platforms such as Illumina, Ion Torrent, Ion Proton, 454, and SOLiD. These data can support both sequence-based and function-based discovery, and functional tools such as SIGEX, FACS, and METREX help identify bioactive compounds more efficiently. Trindade *et al.,* (2015) described this as a way to reduce redundancy compared with traditional culture-based methods, while Devika *et al.,* (2023) highlighted SIGEX and related functional screening methods.

For biosynthetic gene cluster analysis, bioinformatic methods help identify silent or weakly expressed BGCs that may encode secondary metabolites. Researchers often combine metabolomics with NMR, MS, PCA, PLS-DA, and GNPS molecular networking to dereplicate known compounds and prioritize the most promising strains. Tilmann *et al.,* (2018) note that these methods strengthen rapid compound identification, while recent genomic approaches aim to activate silent clusters and enable heterologous expression for natural product discovery.

3.3 Bioprospecting Approaches

Culture-independent bioprospecting allows the discovery of new secondary metabolites without culturing microbes in the laboratory. A common strategy is to build metagenomic clone libraries from environmental DNA and express them in heterologous hosts, where clones can be screened for traits such as color, bioactivity, and compound production. Advanced analytical methods, including HPLC-MS, have helped identify novel metabolites from environmental DNA libraries. For example, turbomycin was discovered from melanin-like colonies in a metagenomic soil library, while palmitoyl putrescine was identified through bioactivity screening of bromeliad tank water eDNA. Another important culture-independent strategy is PCR- and sequence-guided screening of metagenomic libraries. Conserved biosynthetic markers such as acetylation and KS domains can be targeted to identify promising clones, as demonstrated by the discovery of malacidins from soil samples using amplicon sequencing, phylogenetic analysis, and eSNaPD-guided cloning into *Streptomyces albus*.

A similar sequence-based approach led to the discovery of metharithmycin, an analog of mithramycin. Degenerate primers targeting KSα genes helped identify the BGC, which was then cloned into *S. albus* for bioassay-guided fractionation and pathway analysis. This shows how eDNA mining can reveal novel anti-cancer compounds and expand access to hidden biosynthetic diversity (Aileen *et al.,* 2021).

In function-based bioprospecting, environmental DNA is introduced into host organisms such as *E. coli* and screened for desired activities, including substrate breakdown or metabolite production. Positive clones are then sequenced to identify the responsible genes, but the method is labor-intensive and may fail when the DNA is poorly expressed in the host or when multiple genes are required for the function.

Sequence-based screening is faster and more efficient because it uses known protein or gene sequences, often supported by curated databases and machine-learning models, to identify potential enzymes or biosynthetic genes. This approach reduces time and cost compared with traditional enzyme discovery and improves the odds of finding useful biocatalysts.

4. Pharmaceutical Relevance of Metagenomic and Bioprospecting Approaches

Antibiotic resistance is a major pharmaceutical problem because multidrug-resistant bacteria are becoming more common in hospitals and the community. Key threats include *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. These bacteria resist drugs through horizontal gene transfer, reduced membrane permeability, efflux pumps, drug-inactivating enzymes, and target modification (Macro *et al.,* 2021). The spread of resistance is worsened by antibiotic overuse, globalization, and weak stewardship, making the development of new antimicrobials urgently needed.

Natural products remain a major source of antibiotics and other drugs. Microorganisms, especially actinobacteria and *Streptomyces*, produce thousands of bioactive compounds, and many approved drugs are derived from natural products or their derivatives (Table 4). MRSA and resistant *Neisseria gonorrhoeae* show why these matters. MRSA carries the mecA gene and can cause severe, hard-to-treat infections, while *N. gonorrhoeae* has developed resistance to several antibiotic classes and remains a high-priority pathogen (Roberto *et al.,* 2019).

Table 3: List of natural products (secondary metabolites) produced by bacteria and fungi.

Secondary Metabolite	Biosynthetic Origin	Major Use	Producing Organism	Type of Organism
Acarbose	Glycoside	Antidiabetic (HM)	*Actinoplanes sp.*	Actinomycete
Actinomycin	*NRPS*	Antitumor (HM)	*Streptomyces anulatus*	Actinomycete
Actinorhodin	*PKS II*	Antibacterial (RT)	*Streptomyces coelicolor*	Actinomycete
Adriamycin	*PKS II*	Antitumor (HM)	*Streptomyces peucetius*	Actinomycete
Albomycin	Peptidyl-nucleoside	Antibacterial	*Streptomyces sp. ATCC*	Actinomycete
Amphomycin	*NRPS*	Antibacterial	*Streptomyces canus*	Actinomycete
Amphotericin B	*PKS I*	Antifungal (HM)	*Streptomyces nodosus*	Actinomycete
Antimycin	*NRPS-PKS I*	Research tool; Piscicide	*Streptomyces (multiple sp.)*	Actinomycete

Apramycin	Aminoglycoside	Antibacterial (AH, RT)	*Streptoalloteicus hindustanus*	Actinomycete
Vancomycin	*NRPS-PKS I*	Immunomodulator (HM)	*Streptomyces hygroscopicus*	Actinomycete
Avermectin (Ivermectin)	*PKS I*	Anthelmintic (HM, AH)	*Streptomyces avermitilis*	Actinomycete
Alphas	*NRPS*	Herbicide (CP)	*Streptomyces viridochromogenes*	Actinomycete
Clavulanic acid (augmentin)	Other	Antibacterial (HM)	*Streptomyces clavuligerus*	Actinomycete
Compactin	*PKS*	Cardiovascular (HM)	*Penicillium brevicompactum*	Fungus
Coumermycin	Aminocoumarin	Antibacterial (RT)	*Streptomyces rishiriensis*	Actinomycete
Cyclosporin A	*NRPS*	Immunomodulator (HM)	*Tolypocladium inflatum*	Fungus
D-Cycloserine	Other	Antitubercular (HM)	*Streptomyces lavendulae*	Actinomycete
Daptomycin	*NRPS*	Antibacterial (HM)	*Streptomyces roseosporus*	Actinomycete
Dalbavancin	*NRPS*	Antibacterial (HM)	*Nonomuraea sp.*	Actinomycete
Daunorubicin	*PKS II*	Antitumor (HM)	*Streptomyces peucetius*	Actinomycete
Echinocandin	*NRPS*	Antifungal (HM)	*Aspergillus nidulans*	Fungus
Fusidic acid	Terpine	Antibacterial (HM)	*Fusidium coccineus*	Fungus
Geldanamycin	*PKS I*	Antitumor (S)	*Streptomyces hygroscopicus*	Actinomycete
Gentamicin	Aminoglycoside	Antibacterial (HM)	*Micromonospora purpurea*	Actinomycete
Gramicidin S	*NRPS*	Antibacterial (RT)	*Bacillus subtilis*	Bacillales
Hygromycin	Aminoglycoside	Antibacterial (AH)	*Streptomyces hygroscopicus*	Actinomycete

Josamycin	*PKS I*	Antibacterial (HM)	*Streptomyces narbonensis*	Actinomycete
Kanamycin (amikacin)	Aminoglycoside	Antibacterial (HM)	*Streptomyces kanamyceticus*	Actinomycete
Kirromycin	*NRPS-PKS I*	Antibacterial (RT)	*Streptomyces collinus*	Actinomycete
Lasalocid	*PKS I*	Coccidiostat (AH)	*Streptomyces lasaliensis*	Actinomycete
Lincomycin (clindamycin)	Other	Antibacterial (HM)	*Streptomyces lincolnensis*	Actinomycete

5. Challenges and Future Perspective

- Metagenomic bioprospecting is promising, but a major challenge is extraction of high-quality DNA from environmental samples because inhibitors can affect PCR and sequencing, and the DNA is often fragmented and low in yield.
- Another challenge is assembly and annotation of metagenomic data, as environmental samples contain highly diverse and mostly unculturable microorganisms, making the datasets complex and difficult to interpret.
- Despite these issues, the future is promising because advances in genomics, synthetic biology, and metagenomics will accelerate the discovery of new bioactive compounds, especially from marine and other untapped microbial sources.
- Uncultured microorganisms are especially important because they represent most microbial diversity and may lead to the discovery of new antibiotics, antiviral agents, immunosuppressants, protease inhibitors, terpenes, polysaccharides, polyketides, and peptides.
- Improved culturing methods and microbial engineering are expected to expand drug discovery and help address antimicrobial resistance, transplantation medicine, sustainable agriculture, and global health.

Conclusion

Metagenomics and bioprospecting have opened a new frontier in the search for life-saving medicines. By allowing us to explore the genetic wealth hidden in uncultured microbes from diverse environments, these approaches have made it possible to discover novel secondary metabolites that were previously inaccessible. These compounds hold great promise in addressing urgent medical challenges, such as antibiotic resistance and cancer. As tools like high-throughput sequencing, and functional screening continue to evolve, our ability to find and harness these bioactive molecules becomes faster, smarter, and more efficient. The combination of cutting-edge technology and nature's hidden diversity is paving the way for the next generation of pharmaceuticals—ones that could offer new hope where existing treatments fall

short. In essence, metagenomic and bioprospecting strategies are not just scientific advancements; they represent a powerful step toward a healthier future, where nature and innovation work hand-in-hand to solve some of our toughest medical problems.

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