## About the Book

"Microbial Biotechnology in Industry and Medicine" is an insightful exploration into how microorganisms are transforming industries and healthcare. This book delves into the practical applications of microbial biotechnology, illustrating how microbes are engineered and utilized in various sectors, including pharmaceuticals, agriculture, environmental management, and food production. It provides an in-depth look at how bacteria, fungi, and other microorganisms are harnessed for everything from producing antibiotics and biofuels to creating sustainable agricultural solutions and improving waste treatment processes. The book emphasizes both the science and the practical methodologies behind microbial biotechnology, covering topics like genetic engineering, fermentation technology, and metabolic pathway optimization. It discusses the role of microbes in producing valuable enzymes, vitamins, and therapeutic compounds, and explains how advances in biotechnology are enabling scientists to design microbes that can detoxify pollutants, enhance crop yields, and combat antibiotic-resistant pathogens. With contributions from experts in various fields, "Microbial Biotechnology in Industry and Medicine" provides readers with a comprehensive understanding of the tools, techniques, and innovations driving this dynamic area of research. It's an essential resource for students, researchers, and industry professionals interested in the applications of microbiology and biotechnology to solve pressing industrial and medical challenges.

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# Microbial Biotechnology in Industry and Medicine

Dr. Srijan Haldar

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# Microbial Biotechnology

## in

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# Contents

# Chapter - 1

## Biosynthesis of Indole-3-Acetic Acid by Microorganisms: A Short Review

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# Chapter - 1

## Biosynthesis of Indole-3-Acetic Acid by Microorganisms: A Short Review

**Shovana Pal, Keshab Ghosh and Aritri Laha**

**Abstract**

One of the primary signals involved in the interaction between the host and endophytes is the plant hormone indole-3-acetic acid (IAA). IAA can be synthesised by endophytes, which can affect plants' IAA homeostasis. Even though the production of IAA in microorganisms is well understood, the process by which IAA is produced by bacteria is still poorly understood. In the last ten or so years, several IAA production pathways in bacteria have been uncovered; nonetheless, numerous stages and molecular components are still present. In general Tryptophan (Trp)-dependent and Trp-independent routes have been identified for these. While the TAM pathway does not preclude other paths like those via IPyA or IAM, it is the most plausible Trp-dependent method. Numerous PGPB species linked with plants can supply many advantageous benefits to those plants that are part of the plant-rhizobacterial commune or the continuum of free-living or associative bacteria in the rhizosphere. Intending to enhance plant health, it is now feasible to metabolically modify a PGPB by adding new desirable functions, refining the advantageous functions, or inserting specific pathway genes.

**Keywords:** Tryptophan, IAA, PGPB, IPyA, IAM

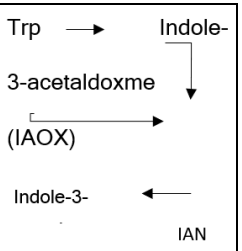
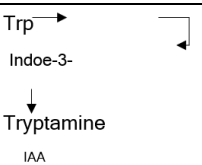
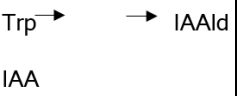
### Introduction

One of the essential phytohormones in plant growth is IAA, which regulates various physiology-related mechanisms such as cell division and expansion, differentiation and reactions of tissues to light and gravity (Zhao 2010). One of the well-characterized characteristics of many bacteria that promote plant development is their capacity to synthesise IAA. IAA can be produced by a vast range of microorganisms, including fungi and bacteria that interact with plants. IAA has a significant impact on microbial physiology in addition to its participation in interactions between bacteria and plants. IAA functions as a signalling molecule, for example. The vir regulon and chv genes

of *Agrobacterium tumefaciens* are repressed by IAA (Yuan *et al*; 2008). Depending on whether tryptophan (Trp) is employed as a precursor, the microbial production of IAA may be divided into tryptophan-dependent and tryptophan-independent routes. Specifically, L-Trp functions as an effective precursor for the production of IAA. Generally speaking, tryptophan is converted into IAA via the following primary routes. These processes produce IAA in a manner akin to those of plants (Patten *et al*; 1996). Tryptamine (TAM), indole-3-pyruvic acid (IPyA), indole-3-acetamide (IAM), and indole-3-acetaldoxime/indole-3-acetonitrile (IAOx–IAN) pathways are among the several potential pathways of the Trp-dependent pathway that are called the primary intermediate. These pathways may be integrated. Indole-3-acetaldehyde (IAD) functions as an intermediate in a few pathways. In most plants, the primary pathway is thought to operate through IPyA; however, things appear to be more complex because *A. thaliana* has both IAM and the IAOx-IAN pathway, which is linked to indole glucosinolates (Lehmann *et al*; 2010). Idole is probably employed as an IAA precursor in the Trp-independent pathways. When fungi interact with plants, they can release IAA, which promotes root development in the host plant or in the rhizosphere as an endophyte. IAA can potentially elicit disease symptoms or disrupt the plant defence mechanism when used by pathogenic fungi. As a result, plant pathogens especially fungi were frequently the focus (Chanclud and Morel *et al*; 2016).

**Table 1:** Microbial biosynthesis of IAA

| **A.** | **Tryptophan Dependent Pathway** | | | |
|---|---|---|---|---|
| | **Name of Pathway** | **Genes and Host** | **Responsible Enzymes** | **Mechanisms** |
| **1.** | Indole-3-Acetamide Pathway (IAM) | IaaM and IaaH *Pseudomonas savastanoi*, *Streptomyces* sp | Tryptophan monooxygenase and indole-3-acetamide hydrolase | Trp → IAM<br>IAA + $NH_3$<br>1. Oxidation<br>2. Hydrolyzation |
| **2.** | Indole-3-Pyruvic Acid Pathway (IPA/IPyA) | TyrR, IAD, IPDC *Azospirillum* sp, *Enterobacter cloacae, Bacillus thuringiensis* | Pyruvate decarboxylase, Trp amino transferase, alpha dehydrogenase | Trp → IPyA<br>Indole 3 acetaldehyde<br>IAA<br>1. Cytochrome p450<br>2. IAN nitrilase |

| | | | | |
|---|---|---|---|---|
| **3.** | Indole-3-Acetonitrile Pathway (IAN) | *NitA, IamA,* yhcX *Variovorax boronicumulans, Bacillus amyloliquefaciens* | IAA nitrilase, cytochrome P450 |  <br>1. Cytochrome p450<br>2. IAN nitrilase |
| **4.** | Tryptamine Pathway (TAM) | *B. cereus* | Trp decarboxylase, Amine oxidase, Acetaldehyde dehydrogenase |  <br>1. Trp decarboxylase<br>2. Amine oxidase<br>3. Aldehyde dehydrogenase |
| **5.** | Tryptophan Side-Chain Oxidase Pathway (TSO) | *Pseudomonas fluorescens* | Indole-3-acetaldehyde dehydrogenase |  |
| **B.** | **Tryptophan independent pathway** | | | |
| | | *Azospirillum brasilense, Saccharomyces cerevisiae* | | Indole as a precursor |

**Direct benefits of rhizobacterial IAA:** Growth enhancement has been attributed mostly to IAA rather than the N2-fixing ability of diazotrophic PGPB strains like Azospirillum. To synthesize IAA, most PGPBs adopt the IPyA route. A mutation in the *Pseudomonas putida* ipdC gene significantly reduced the main root growth of canola seedlings, as shown by Patten and Glick (2002a). IAA synthesis is host-specifically controlled in *Rhizobium meliloti* and *Rhizobium leguminosarum* improving the production of flavonoids that cause nods. The lack of N2 in an environment rich in $CO_2$ may have been caused by nodulation, which is thought to be a relatively recent phenomenon (about 60 million years) (Sprent, 2008). Thus, a significant developmental program would have needed to be manipulated with the help of bacteria to move from un nodulated to a nodulated root. In legumes, at the time of nodulation auxin accumulation produces determinate and indeterminate nodules that have been observed concerning the auxin-

responsive plant promoters GH3 and DR5. Rhizobia were observed to elicit a primary decline in GH3 activation surrounding the starting point of infection in a few hours in white clover, that is called to develop indeterminate nodules (Mathesius *et al*., 1998), followed by an increment that followed DR5 expression, seen *in Medicago truncatula*, according to Huo *et al*. (2006) to be sporadic, below the site where the nodule initiates. Still, it was stimulated in the place where the nodule forms.

In pathogenic bacterial species *P. savastanoi*, *Agrobacterium tumefaciens*, and *Pantoea agglomerans*, the capacity of produceing IAA has been found as a key pathogenicity determinant. Large amounts of IAA are typically synthesized by these bacteria via the IAM pathway. Numerous phytopathogens have provided information on the genes iaaM and iaaH, which are implicated in the pathway (Clark *et al*., 1993). Either plasmids or chromosomes may contain the genes. For example, in *A. tumefaciens*, these genes are found in the pTi plasmid's T-DNA region with the isopentenyl transferase (ipt) gene. Amino acids, organic acids, sugars, and other signals generated from plants activate the vir genes in *A. tumefaensis.* According to Escobar and Dandekar (2003), the main cause of cancer after T-DNA transfer into the genome of plant is the huge build-up of auxin and cytokinin caused by the actions of the iaaM, iaaH, and it coded enzymes.

**Regulation of IAA levels:** Microbes regulate their IAA levels by intricate and poorly understood methods. The issue is made worse by a variety of variables that interact to regulate the amount of IAA released by a microorganism, in addition to the quantity of IAA biosynthetic pathways present in a single bacterium. Bacteria's IAA biosynthesis and secretion are complex and multifaceted. It is evident that, in addition to genetic regulation, the expression of IAA biosynthesis genes must be adjusted to deal with a variety of sources. Variety of stressors related to the environment (Malhotra and Srivastava, 2008a, 2009; Spaepen *et al*., 2007b).

**Genetic regulatory mechanism of the IAA biosynthesis pathways:** The genes of IAM pathway are constitutively expressed in the majority of the microorganisms that have been gone through thus far. TMO (Tryptophan-2-monooxygenase) may be inhibited by both IAM and IAA, indicating that TMO may function in the IAA biosynthesis regulation (Hutcheson and Kosuge, 1985). The pathways are where involving IPyA and IAN are involved. *Rhizobium phaseoli, E. cloacae*, and supplementation significantly enhance the amount of IAA produced via the IPyA pathway. Plus various *A. brasilense* strains, However, in Agrobacterium and Rhizobium spp., IAM induces the production of nitrile hydratase (Kobayashi *et al*., 1995).

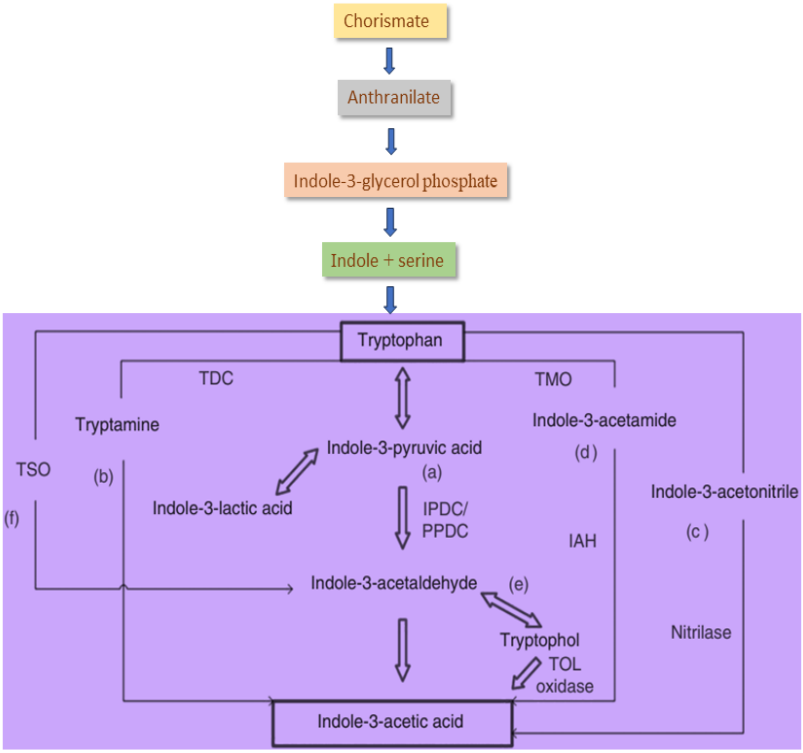



**Fig 1:** IAA biosynthesis pathways: (a) IPyA pathway, (b) TAM pathway, (c) IAN pathway (d) IAM pathway, (e) TOL pathway and (f) TSO pathway

## Conclusion

Numerous PGPB species that are linked with plants can supply several advantageous benefits to those plants that are part of the plant-rhizobacterial community or the group of free-living or associative bacteria in the rhizosphere. To enhance plant health, it is now feasible to metabolically establish a PGPB by adding new desirable activities, refining the advantageous ones, or introducing genes from particular pathways. The relationships with and among the bacterial PGRs remain unclear, despite significant progress in understanding the mechanisms behind auxin's cross-connection with other hormones and the interconnection of IAA signalling pathways in plants. But it's possible to assume that some plant auxin-signalling mechanisms would also be at work in the event of plant–bacteria interactions. It's crucial to remember that IAA plays a part in a variety of processes, including symbiosis, plant defence, root development stimulation, and pathogenesis. An improved comprehension. Understanding these mechanisms

will enable greater utilization of these functions in the direction of enhanced plant development and productivity. Investigations into the cis- and trans-acting PGR-regulating components and the potential modulatory function of plant root elements in the close relationship between bacteria and their plant partner.

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# Chapter - 2

## Sustainable Use of Waste Natural Products in Biomimetic Scaffolds & Tissue Engineering: A Concise Review


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# Chapter - 2

## Sustainable Use of Waste Natural Products in Biomimetic Scaffolds & Tissue Engineering: A Concise Review

**Pratiksha Bhowmik, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh**

**Abstract**

Tissue engineering is a prevalent form of biomedical engineering to regenerate new tissues, has gained considerable popularity as a possible substitute for organ or tissue transplantation. Apart from use of different chemicals as raw materials for synthetic tissue engineering, waste materials from plants, animals and microbes can be utilized to generate active ingredients popular nowadays as “bioceramics”. The eco-friendly attribute of plant-waste-derived biomaterials, as well as their striking resemblance to natural tissues, make these biomaterials appealing, as does the ability to extract hydroxyapatite, collagen, and chitin components, as well as marine debris for applications in the same.These constituents mimic the structure of natural bones and thus can be used as efficient tools for bioengineering. The choice of these smarter waste biomaterials render the artificial tissue to be safe to be used *in vivo* besides reducing the cost of manufacturing. This review thus hints to two way advantage of valorizing waste derived materials to prepare biomimetic scaffolds with ancillary advantage of contributing to environmental sustainability.

**Keywords:** Bioceramics, bioengineering, biomimetic scaffolds, tissue engineering, waste materials.

### Introduction

Artificial organs (including tissues) or organ transplantation are the first options for reconstructing the destroyed tissues or organs when they have been so severely diseased or lost due to cancer, congenital anomalies, or trauma that traditional pharmaceutical treatments are no longer applicable (Chapekar, M. S. 2000, Ikada, Y. 2006). Alternatives now in use, including mechanical devices or artificial prostheses, are not meant to blend in with the host tissue and do not restore organ function or tissue damage (Chapekar, M. S. 2000). Recently, tissue engineering has gained prominence as a possible substitute

for organ or tissue transplantation (Chapekar, M. S. 2000). Renovate a patient's own organs and tissues that are fully free of severe immunological rejection, restricted bio-functionality, and poor biocompatibility are the primary challenges connected with tissue engineering. Tissue engineering is frequently regarded as the ultimate perfect medical treatment because of its many benefits. Three fundamental techniques are used in biomedical engineering to regenerate new tissues: cells, scaffolds, and growth factors. (Hosseinkhani, H., & Hosseinkhani, M.2009).

Unwanted or useless items are considered as waste (or wastes). Natural waste products means substances that is produced by life or living organisms by a variety of procedures and actions involved in the manufacture, disposal, and harvesting of a wide range of goods, including textiles (Rossi *et al* 2017)., food, drinks (Pérez-Marroquín *et al* 2023)., cattle (Jayathilakan, K., *et al* 2016).Numerous environmental sustainability and waste management problems have arisen as a result of the large amount of organic waste that is routinely disposed of (Zamri, M. F. M. A. 2020). Waste from plants, animals, ships, marine organisms and industry use as scaffolds, dressings for wounds, bones, and other tissue engineering applications. Considering both socio-environmental and economic factors, the green and sustainable separation of natural products from agro-industrial waste is obviously appealing (Zuin, V. G., & Ramin, L. Z. 2018). Waste-based biomaterials have been investigated for tissue engineering applications in the dentistry (Abou Neel, E. A., J. C. 2014), skin (Kamel *et al* 2013), cardiology (Rabkin, E., & Schoen, F. J. 2002)., and orthopaedic domains (Deng, M., James, R., Laurencin, C. T., & Kumbar, S. G. 2012)., among other biomedical replacements and regeneration applications. The term "aquatic" describes life found in freshwater environments like lakes, dams, and marine waters (oceans and seas). Aquatic biomaterials, like fish and microorganisms, have drawn attention because of their adaptable qualities and range of uses (Tümerkan, E. A. 2023). Biomaterials can be extracted from seaweed (Gade, R., Tulasi, M. S., & Bhai, V. A. 2013), algae (Tsarpali *et al* 2021), aquatic bacteria (Kim, S. K. (Ed.). 2013), or aquatic sponges (Ehrlich, H., & Worch, H. 2007). Using a variety of extraction technique. The main issues with aquatic biomaterial uses include biocompatibility, local tissue reaction (Schmidt & Baier, 2000), and the toxicity of natural materials' breakdown products in various ways (Tümerkan, 2023). Various industries, including food and beverage, textiles, and power generating, have yielded waste material. These materials have drawn a lot of interest for use in several tissue engineering applications (Zamri *et al* 2021).

This review highlights different plant and animal derived waste materials and how they are been harnessed for the production of biomimetic scaffolds.

**Plant waste**

Biomaterials derived from plant waste have demonstrated exceptional potential as substitutes for synthetic materials in tissue engineering applications (Zamri *et al* 2021). These plant-based biomaterials are mostly obtained from sustainable sources and have characteristics that are comparable to those needed for tissue engineering, requiring little in the way of chemical processing (Iravani *et al* 2019). Plant waste components, such as the peels, cores, and leaves of processed plant products, contain a range of polysaccharide constituents that are removed to form plant cells that have been claimed to resemble human tissues. (Gershlak *et al* 2017).

**Spent leaves and flowers and extraction of resource materials**

Waste leaves are being used in tissue engineering for several reasons like natural scaffold structure, biocompatibility, sustainability, cost-effectiveness, abundance, non toxic degradation and wide renewable resources.

| Natural source of material | Fabrication type | References |
|---|---|---|
| 1. Spinach and parsley leaves | Decellurarized scaffold (cardiovascular) | . (Gershlak, J. R., Hernandez, S., Fontana, G., Perreault, L. R., Hansen, K. J., Larson, S. A., ... & Gaudette, G. R. 2017) |
| 2. Green tea leaf | Polyphenols fixing agent (bones) | (Shen, C. L., Wang, P., Guerrieri, J., Yeh, J. K., & Wang, J. S. 2008). |

**Spinach and parsley leaves:** The invention of cardiovascular de-cellularized Scaffold from the waste materials of Spinach and Parsley Leaves. The de-cellularized leaf scaffold's re-cellularization revealed striking similarities to human cells. When a tissue or organ is de-cellularized, the cellular material is removed, leaving behind an acellular scaffold made of extracellular matrix (ECM), the makeup of which varies depending on the tissue or organ from which it was produced, but the vascular network remains intact. (Gershlak *et al* 2017)

**Green tea leaf:** GTP, or green tea polyphenols, show promise as a treatment for osteoporosis in females. Results showing that GTP supplementation raised urinary GTP concentrations and bone mass through improvements in antioxidant capacity and/or reductions in oxidative stress damage point to a major role for GTP in women's bone health. (Shen *et al*2008).

**Corn silk extract:** Injectable hydrogels have demonstrated significant potential as scaffolds for bone and cartilage tissue creation. Combining β-

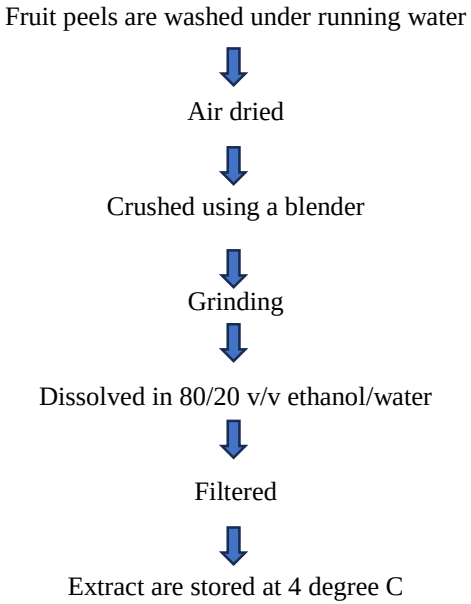
tricalcium phosphate, hyaluronic acid, and corn silk extract-nanosilver (CSE-Ag NPs) resulted in thermosensitive, injectable hydrogels suitable for bone tissue regeneration. The extracellular matrix (ECM) in mammalian connective tissues is primarily composed of HA, a naturally occurring polysaccharide that serves both physiological and biological functions. Moreover, HA regulates tissue remodelling, cell development, proliferation, and viscoelastic characteristics (Makvandi *et al* 2020).

**Fruit peels**

Fruit peels are the peelable outer covering that protects a fruit or vegetable. They are among the most nutrient-dense parts of plants since they are high in fibre, vitamins, minerals, and antioxidants. Fruit peel is incredibly rich in essential oils that give the fruit its distinct scent.

The majority of fruit peels are edible, but you should wash them well before consuming them.

**General mode of extraction**




**Jackfruit peels:** Hydroxy Apatite (HA) bio nano-composites were made using varying quantities of the natural pectin polymer that is extracted from jackfruit peels. One of the vital inorganic substances present in teeth and bones is apatite (HA). Due to its better cytocompatibility and bioactivity, hybrid organic-inorganic compounds are extremely appropriate for medicinal applications; they have found widespread use as drug delivery agents,

orthodontic, restorative, and orthodontic materials. In addition, bone is made of a composite substance made of a collagen framework supported by HA. (Govindaraj *et al* 2018).

**Lemon peels:** Several fruits and vegetables contain pectin, an anionic carbohydrate. The chitosan polymer is frequently mixed with other compounds to increase its biological or mechanical properties. Currently, scaffolds for the healing of cartilage, nerve, and bone tissue have been developed using gelatin-chitosan, collagen-chitosan, chitosan-hydroxyapatite, chitosan-chondroitin sulphate, and chitosan-hyaluronate-polyelectrolyte complexes. These research found that chitosan-based scaffolds stimulate cell growth and proliferation. Nanofibrous scaffolds composed of pectin, chitosan, and polyvinyl alcohol were developed for skin tissue engineering applications. The scaffolds' biocompatibility was proven using cell adhesion, proliferation, type I collagen synthesis, and cell morphology analysis. Lemon pectin has a high concentration, which allows for excellent swelling, degradability, and porosity. It maintains cell viability and is non-toxic (Demir *et al*. 2021).

**Flower extracts**

***Calendula officinalis* flower extract:** Biological skin coverings with improved wound healing efficacy were developed by coating acellular collagen I scaffolds with polymeric microparticles and then adding a hydroglycolic extract of Calendula officinalis flowers. Microparticles made from gelatin and collagen were created using a water-in-oil emulsion/cross-linking technique. The microparticles were then mixed with collagen suspensions in three different concentrations and lyophilized to create microparticle-loaded porous collagen scaffolds. Calendula officinalis extract inhibits enzymatic degradation of skin dressings *in vitro*. (Jiménez *et al*. 2015).

**Gardenia jasmine flower extract:** Genipin is a completely tested, non-cytotoxic crosslinking substance. Cartilage-derived matrix (CDM) | genipin is a contraction-free biomaterial designed for cartilage tissue engineering. Chitosan | genipin allows for dynamic compression of matrix formation and chondrocyte proliferation to regenerate cartilage. Chitosan genipin has the following key benefits in bone regeneration: increased mineral deposition; improved osteoblast adhesion, proliferation, and differentiation; increased expression of osteogenic differentiation markers; and greatly increased survivability of human adipose stem cells. (Muzzarelli, R. A., El Mehtedi, M., Bottegoni, C., & Gigante, A. 2016).

**Livestock waste**

Livestock waste components include calcium phosphate, hydroxyapatite (HAP), keratin, hyaluronic acid (HA), and collagen. Livestock resources are derived from common waste sources, including eggshells, bovine bones, feathers, and slaughterhouse waste. Eggshells, bones, feathers, and rooster combs provide excellent qualities for cell culture, membrane composites, scaffold composites, and hydrogel tissue applications. Livestock manure has beneficial biological and physical qualities, including cytotoxicity, hydrophilicity, and cell growth viability, making it a valuable resource for tissue engineering applications (Zhu *et al* 2016).

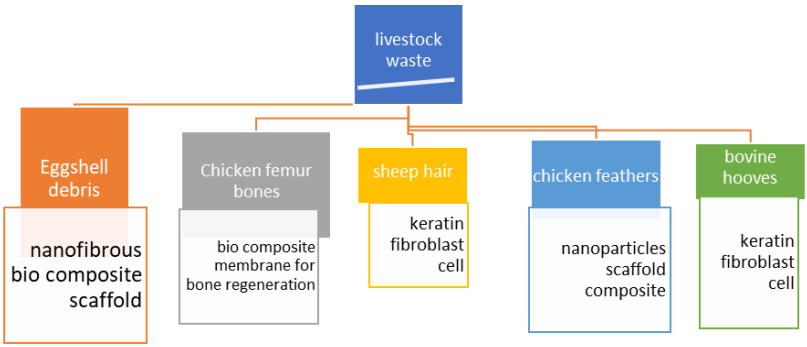



**Fig:** Recovery of materials from animal waste: Shells, Bones, hair, feathers and source materials from it

**Eggshell debris:** Eggshell waste was successfully used to synthesise hydroxyapatite. The eggshell-derived hydroxyapatite composite has increased heat stability, surface roughness, and swelling behaviour, promoting various cell activities. Furthermore, this bio composite material demonstrated favourable cytotoxicity for tissue engineering applications (Trakoolwannachai *et al* 2019). Eggshell-derived calcium phosphate waste is effective for bio scaffold proliferation and osteogenic synthesis in bone tissue

**Chicken femur bones:** Chicken bone-derived hydroxyapatite (HAP) improved the membrane biocomposite's bioactivity. Increasing HAP loading resulted in a significant increase in biodegradability, suggesting that chicken bone-derived HAP can be employed as an osteogenic filler. An *in vitro* bioactivity study found that the novel membrane has improved osteogenic characteristics. The presence of HAP lowered surface hydrophilicity, water absorption, and membrane breakdown rate (Bee *et al*. 2019).

**Sheep hair:** Keratin, an intermediate filament (IF), is present in the cytoskeleton of eukaryotes. Sheep hair keratin is a preferred choice for tissue

engineering because of its strong fibroblast cell proliferation capacity and low cost. The obtained keratin samples were rich in protein and thermally stable. Keratin also increased cell growth and survival while boosting cell adhesion and proliferation in cultured fibroblast cells (Ramya *et al*. 2020).

**Chicken feathers:** Keratin nanoparticles from chicken feathers interacted with the chitosan matrix, which retained its semi-crystalline structure. The introduction of keratin nanoparticles significantly increased the scaffolds' biodegradation and protein adsorption capabilities. Furthermore, the scaffolds were found to be non-cytotoxic to human osteoblastic cells (Saravanan *et al*. 2013).

**Bovine hooves:** Once the pulverised hooves were reduced to extract the keratin protein, which was found to be pure, the SDS-PAGE assay was used to identify two polypeptide chains with molecular weights between 45 and 60 KDa. The keratin obtained from the hoof of cows had a high denaturation temperature of 215 degrees Celsius, and it was still able to hold 50% of the sample's initial weight at 346 degrees Celsius. Over 90% of the cells in the keratin demonstrated biocompatibility (Kakkar *et al* 2014).

**Marine waste**

Natural products are made up of a variety of creatures, including marine waste. Numerous chemical components, including chitin, collagen, polysaccharides, biphasic calcium phosphate, aragonite (calcium carbonate), and hydroxyapatite (HAP), are present in these materials.

**Cockleshell:** Bioactive materials can induce particular tissue responses and come in a variety of forms based on ceramic, metallic, or polymeric materials. It has been demonstrated that chitosan-based materials are the best bioactive materials because of their exceptional qualities, which include their biocompatibility, biodegradability, and ability to be fabricated into a variety of shapes and bioactive materials (Islam *et al* 2020). From Cockleshells calcium carbonate (aragonite) nanoparticles is synthesized which is used in fractured bone. A simple biochemical reaction is required to easily convert powder into nanophase materials. One of the rarest biogenic polymorphs of calcium carbonate, argonite is widely utilised as a biomaterial for tissue scaffolding, sophisticated drug delivery systems, and bone restoration. (Islam *et al* 2013). Using a mechano-chemical, top-down technique, the powdered cockle shells, which were reduced in size to 75 µm, were converted into nanoparticles. The surfactant BS-12 (dodecyl dimethyl bataine) was added during the process. Applications for tissue-engineered scaffolds that can be utilised to treat load-bearing segmental bone defects (SBDs) are becoming

more and more necessary. In order to reconstruct SBDs, seven distinct configurations of three-dimensional (3D) unique nanocomposite porous structured scaffolds were created. This was accomplished by using an exceptional combination of gelatin, dextran, cockle shell ($CaCO_3$) nanoparticles (CCN), and dextrin to create the perfect bone scaffold (Mahmood *et al* 2017).

**Chitosan:** Widely utilised conductive polypyrrole (pPy) can induce an electrophysiological state in exogenous cardiomyocytes (CMs) because to its biocompatibility, conductivity, and ease of processing. With its multiscale, interconnected porous structure and appropriate stiffness,previously created flexible scaffold made of chitosan shell from mussels showed great promise as a tissue replacement for wound healing (Song, X., Mei, J., Ye, G., Wang, L., Ananth, A., Yu, L., & Qiu, X. 2019).

**Jellyfish collagen:** Refibrillated collagen from the jellyfish *Rhopilema esculentum* was used to create porous scaffolds that may be used in cartilage regeneration. Through measurements of turbidity and quantification of fibrillized collagen, the effects of temperature, salinity, and collagen content on the production of fibrils were assessed. A comparison of transmission electron microscopy and atomic force microscopy revealed that collagen fibrils were formed with a characteristic banding pattern. After refibrillizing jellyfish collagen under ideal circumstances, freeze-drying and chemical cross-linking were used to create porous scaffolds. There were no harmful effects of the substance found in cytotoxicity testing using human mesenchymal stem cells (hMSCs) (Hoyer *et al* 2014).

**Coral reef:** As a graft material, hydroxyapatite, a crystalline form of calcium phosphate found naturally in bone minerals, has shown considerable potential. Coral is an osteoconductive material used to extend bone transplants. These grafts contain viable cells derived from bone marrow osteoprogenitor cells, collagenous matrix, non-collagenous extracellular growth, and differentiation agents. Because autografts can perform osteogenesis, osteoinduction, and osteoconduction, they are the gold standard for bone regeneration. (Parizi *et al*. 2013).

**Industrial waste**

Nowadays, a variety of industrial waste types have been creatively transformed into different biomaterials for tissue engineering applications, such as porous scaffolds/sponges, fibres, and gels. Waste has been collected from a variety of industries, including the food and beverage, textile, and power generation sectors. Excellent biocompatibility is a result of waste-derived biomaterials' stable mechanical characteristics and osteoblast growth.

As a result, these materials have drawn a lot of interest in a variety of tissue engineering applications.

**Beer bagasse:** Waste material from the beverage production sector was recovered and used to create biomaterials that could serve as scaffolds for tissue engineering. At various fabrication temperatures, the bio minerals of the scaffold pellet derived from removed beer bagasse were prepared. The artificial samples had superior mechanical qualities, were cytocompatible, and had an osteoblastic cell growth tendency similar to that of bone. (Yates *et al* 2017).

**Soya bean:** Soy protein and β-chitin, two naturally occurring food industry byproducts, can be a great supply of biomaterials for creating 3D scaffolds using easier and more hygienic procedures. The combination of these two polymers results in sponge-like scaffolds (SLS) with excellent physicochemical characteristics. Excellent physicochemical characteristics of the created scaffold improved its ability to promote neovascularization. For cell delivery applications, the valorized scaffold made from industrial soybean waste demonstrated sufficient cell adhesion and proliferation. (Las *et al* 2020).

**Tanning Leather**: Applications for skin tissue may benefit from the collagen that is recovered from tanning waste in leather created biocomposite sponges, gels, and films using the biocollagen that was separated from tanning waste. In skin tissue applications, these polymers showed good swelling and gel strength. The network was tighter by the cross-linking alteration, reducing the amount of water that could get into the biocollagen components. This impact can be explained by the material's amazing resemblance to commercially accessible material as well as the stability of its mechanical properties (Catalina *et al* 2012).

**Fly ash:** Although fly ash is one of the most common solid wastes discharged into the atmosphere by power stations, its qualitative and quantitative uses for sustainability are unclear. The biggest issues with fly ash disposal are that it takes a large amount of land for landfills, which is damaging, and that the accumulation of heavy metals pollutes groundwater and soil. The enrichment of fly ash in silica, kaolin, iron, and alumina has been extensively documented in literature for its application in the production of nanosized particles, as well as its direct usage and disposal. In this context, one of the potential nano composites made from fly ash is recognised to be aluminosilicates. It has been demonstrated that fly ash geo-polymerization occurs naturally in an alkaline medium and forms aluminosilicates. Femur bone defects were actively repaired by collagen-incorporated rabbit bone

aluminosilicates generated from bio composites. The bio-composite of alumina silicate combined to produce human cell viability and good cell attachment (Ramanathan *et al* 2020).

**Conclusion and future aspects**

The bulk of biomaterials used in tissue engineering are derived from industrial, plant, marine, and livestock waste. Researchers are growing interested in commercialising waste-derived biomaterials (such as hydrogels, scaffolds, films, membranes, and nanofibers) for a variety of tissue engineering applications. The process of valuing waste for tissue engineering applications consists of several stages, including extraction, treatment and purification, chemical modification, process optimisation, and viability.

In compared to other waste-derived sources, plant-derived biomaterial is extremely abundant and represents economically viable tissue engineering techniques. The eco-friendliness of plant-waste-derived biomaterials, as well as their stunning resemblance to natural tissues, are the primary reasons for their appeal. When it comes to getting hydroxyapatite, collagen, and chitin components, marine debris is significantly more readily available than other waste items.

However, the applications of such waste have been focused on skin tissue and bone regeneration. This is because to the availability of HAP replacement compositions that closely resemble the structure of natural bones, as well as the decreased risk of human disease transmission from marine collagen compared to bovine and porcine collagen. As a result, extensive research has been conducted on the integration of marine garbage-derived materials for various tissue engineering applications. Compared to marine and plant-derived biomaterials, biomaterials derived from livestock and industrial waste have a more limited supply base. Different extraction and chemical modification processes have been utilised, depending on the waste source's qualities. However, due to the toxins emitted by the India is a riverine country. In Bengal area (West Bengal and Bangladesh) has lots of rivers, so river fish was/is easily available and people also like to eat fish very much. Bengali’s favourite food is fish. So fish waste like fish scale and bones are generated in huge amount. Although they are biodegradable but they take much more time to decompose in soil. Sometime river pollution causes huge amount of fish death. Fish scales are mostly composed of hydroxyapatite and fish bones contains calcium carbonate. These components are can have been used in tissue scaffolds, implants and tissue engineering process.

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# Chapter - 3

## Exploring the Microbial World of Lung Cancer: A Systematic Review of Metagenomic Studies

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# Chapter - 3

## Exploring the Microbial World of Lung Cancer: A Systematic Review of Metagenomic Studies

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**Abstract**

Our bodies host a variety of beneficial microorganisms that perform essential functions for our health. According to current research, a number of long-term lung disorders are associated with a variation within the population of microbes inside the lungs. Recently, Lung cancer has become the least frequent lung disease globally, causing over one million mortalities annually. Right now, compared with other cancer, pulmonary cancer had poor 5 years longevity rates, so revolutionary methods that cancer identification and evaluation must be used for improvement of the condition of patients. By choosing the bacteria that cause diverse forms of carcinomas, the area called metagenomics has grown. The percentage of cancers linked to infections caused by bacteria is approximately 16.1%. Metagenomics is a fair method for recognizing and analysing microbes in their natural surroundings. This method has completely changed how scientists discover, examine, & target the variety various microbes found in cancer patients' tissue biopsies. In this review, we present a summary on metagenomics approach of lung cancer. This review also discusses about lungs cancer, Metagenomics approaches for microbiome alteration & also briefly summarized the key challenges and opportunities regarding metagenomics impact on lung cancer disease.

**Keywords:** Lung cancer, metagenomics, metagenomics impact, microbiome alteration.

### Introduction

The microbiome is a group of microbes that colonize human bodies and provide communicates to them & might be beneficial, symbiotic, or harmful. Microorganisms, including bacteria, protozoa fungi & viruses, create organ-specific microbe populations. Different organs & tissues have different microbiomes, which differ by size and composition that are altered the environmental & patient conditions. These differences may give rise against diseases & our body's response against it.

It's common knowledge that illnesses that are infectious & humans' mortality are linked to species of microbes. Now more data than before to suggest that microorganisms etiopathogenesis plays a part to the growth of non- transmissible disorders like cancer. Imbalance of lung microbiome can also create cancer. Along with the potential roles of microbes imbalanced in respiratory and immune-mediated diseases, there's an increasing recognition of its possible effects on the growth of cancer (Round *et al*., 2018). For instance, inflammation has become accepted as a component of the characteristics of cancer, & the microbial community & its byproducts of metabolism impact its start & development. Research proves that inflammation may cause cancer by inducing instability in the genome and, if an abnormality is developed, by creating an atmosphere that is conducive for tumor growth (Demaria *et al*., 2010). There may be microbiological changes in the development of lung carcinoma. Significantly more *Streptococcus viridans* were found in the specimens of lung cancer patients (Cameron *et al*., 2017).

Globally, cancer of the lungs is the most prevalent cause of cancer-associated mortality. According to the International Agency for Research on Cancer's (IARC) GLOBOCAN 2020 projections of cancer incidence and mortality, lung cancer continues to be the primary cause of cancer-related death, accounting for an expected 1.8 million deaths (18%) in 2020 (Leiter *et al*., 2023). Cancer of the lungs percentages differ globally, as they do for other malignancies; the United States, the EU, & the nation of Australia have the highest rates, while Africa and Asia have the lowest rates. Lung cancer is now the most common illness in the world, with over one million new cases reported year. The great majority of these occur in men, with an estimated 50% occurring in developing countries. The reasons for this variation are not completely understood, and to some extent, this is still an active topic of research. So, early detection is needed to improve survival rates. The comprehension about the human cancer the microbiome, including the non-culturable living things, has greatly expanded with the growth of advanced methods employing genome sequencing, exposing its complexities (Souza *et al*., 2023). A reasonable technique for identifying and examining microorganisms in their natural habitat is metagenomics. This technique has fundamentally altered how researchers identify, investigate, and target the wide range of bacteria identified in samples of tissue from cancer patients. We give an overview of the metagenomics approach to lung cancer in this paper. This review also discusses about lungs cancer, Metagenomics approaches for microbiome alteration & also briefly summarized the key challenges and opportunities regarding metagenomics impact on lung cancer disease.

**Regarding lung cancer**

Lung Cancer (LC) is among the most prevalent form of cancer in the world. An estimated number of people have passed away from cancer of lungs. It has become the most prevalent cancer worldwide, affecting people of both sexes. The aberrant, uncontrollably growing cells in the lungs cause lung cancer. Compared with other cancers, lung cancer has an extremely poor five-year rate of survival thus, to enhance results for patients, new techniques for cancer detection and diagnosis are required.

The unchecked proliferation of dividing cells in the lung's tissues was the characteristic of cancer of the lungs, a malignant lung tumor. If treatment is not received, the tumor has a chance to metastasis to expand outside of the lungs & eventually into adjacent tissue as well as other organs in the body. The majority of lung growths which originate form epithelial cells in the lungs are called initial cancers of the lungs. The main types of lung cancer are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) (Araujo *et al*., 2020). Patients had an 80% incidence of NSCLC & 20% of SCLC. NSCLC is classified as mixes, massive cells carcinoma, squamous cell carcinoma, and adenocarcinoma (Araujo *et al*., 2020). Three subtypes in small cell lung cancer (SCLC) have been identified: mixed small cell and large cells carcinoma, small cell carcinoma, and small cell carcinoma (Araujo *et al*., 2020). Every kind has a unique treatment pattern & medical outcome.

Lung cancer's pathophysiology & risk elements are complicated yet not fully understood. A recent study found that smoking releases lipopolysaccharide (LPS), a bacterial endotoxin that can cause inflammation and change genetic composition (Mendez *et al*., 2019). These findings showed that elements in smoking that promote infection may induce early epigenetic alterations in the lung, that when combined with other variables (genetic, environmental, etc.) may culminate in lung cancer.

Research conducted in the past revealed that the risk of lung cancer is increased by prior lung conditions such as lung TB, pneumonia, emphysema, chronic bronchitis, and chronic obstructive pulmonary disease (COPD) (Brenner *et al*., 2011). Lung infections are caused by tumor necrosis factor (TNF), which is generated by Mycobacterium tuberculosis infections. Additionally, extracellular matrix (ECM) components are formed as a result of lung fibrosis brought on by M. tuberculosis infections. Consequently, lung damage and the epithelial cells might result in lung disease (Mao *et al*., 2018). According to epidemiological data, inflammation-induced tissue damage may either cause or accelerate the onset of lung cancer (Shi *et al*., 2015). The lungs

cancers samples collect from lung microbes, sputum, BAL, and lung biopsy are frequently employed. An investigation 16S rRNA gene sequencing that used to identify pulmonary microbiota similarities.

**The relationship between lung organisms & non-oncology lung is revealed by current research**

| Medical condition | *Microorganisms* | Specification of specimen | Detection methods | References |
|---|---|---|---|---|
| COPD (chronic obstructive pulmonary disease) | *Haemophilus and Pseudomonas* | BALF/lung tissue | 16S rRNA | (Erb-Downward JR *et al*., 2011) |
| | *Proteobacteria* | Bronchoscopy | 16S rRNA | (Hilty *et al*., 2010) |
| | *Firmicutes, particularly Lactobacillales* | Tissue of Lung | 16S rRNA | (Sze *et al*.,2012) |
| | *Streptococcus,Haemophilus, Veillonella, Moraxella & Prevotella* | Sputum | 16S rRNA | (Mayhew *et al*., 2018) |
| CF (cystic fibrosis) | *Stenotrophomonas, Haemophilus, Staphylococcus, Achromobacter & Pseudomonas* | BALF | 16S rRNA | (Laguna *et al*., 2016) |
| | *Stenotrophomonas, and Achromobacter* | Sputum | 16S rRNA | (Feigelman *et al*., 2017) |
| Asthma | *Sphingomonodaceae, Porphyromonas, Fusobacterium, Neisseria & Haemophilus* | Bronchoscopy | 16S rRNA | (Durack *et al*., 2017) |
| IPF (idiopathic pulmonary fibrosis) | *Staphylococcus and Streptococcus* | BALF | 16S rRNA | (Laguna *et al*., 2016) |

**Lungs microbiome and lung cancers**

The body's pulmonary tract's microbes might be broadly classified under three groups: the lung, upper respiratory, and oral/nasal microbiomes. Because both the nose and mouth have direct contact with the outside world, they are constantly changing, which has an impact on the microbiota in the upper part of the respiratory system. It has been demonstrated by scientists show the respiratory tract of healthy individuals are brimming with bacteria, which are further subdivided into the respiratory microbiota and pulmonary mycobiome. This has been made possible by the advancement of endoscopic procedures and genomics technologies (Ran *et al*., 2021). Pulmonary microbe colonization was demonstrated to be significantly influenced by oral "the microaspiration."

An overview of the relationship between carcinoma of the lung and the microbiota found in the lung. Most previous studies on lung microbiota employed specimens from oral, sputum, or bronchoscopy brushings. Such specimens are often regarded as maybe tainted by oral or upper pulmonary bacteria. According to certain research, collecting breath or oral pathogens could be linked to the microorganisms that cause lung cancer. On the other hand, studies conducted on 165 non-malignant lung cells among cancer victims revealed a pulmonary microbiota is distinct from the oral cavity and other body regions in certain ways. As a matter of fact, *protobacteria* rule it. Similar findings from further research demonstrate that *Proteobacteria* predominate in lung tissue specimens from people who have lung cancer (Marimón *et al*., 2023). Additionally, tissue from patients in advanced stages has a higher abundance of the genus *Thermus*, while those who have metastases have a higher abundance of Pneumonia (Marimón *et al*., 2023). In addition, smoking cigarettes and being exposed to air pollution alter the lung microbiome. However, the majority of research suggests the primary bacteria responsible for lung cancer may be *Streptococcus* and *Proteobacteria (*Wang *et al*., 2021).

**Immune reaction & pathways of lungs cancers**

Numerous studies conducted in the last few decades have demonstrated the critical role as long-lasting inflammation plays in the emergence of various cancers, including lung cancer. Tumor growth can be aided by imbalance in the gut microbiota, which under some circumstances can cause the inflammatory pathway to be activated and epithelial cells to proliferate and survive. TLR4 is an arrangement detecting transmitter that, starting the earliest stages of pathogen invasion, starts innate immunity (Fang *et al*., 2022). It's being researched at an increasing rate as evidence grows showing it's critical to the development of a tumor's surrounding Through the activation of the nuclear factor κB (NFκB) pathway, the release of infectious factors, and the activation of transcription 3 (STAT3), TLRs contribute to carcinogenic consequences (Herfs *et al*., 2009). Compared to para cancer tissue, lung cancer tissue expresses TLR4 more robustly. It has been shown that lung cancer cell proliferation was facilitated by exposure to smoke particles and non-typeable Haemophilus influenzae (NTHi). This was accomplished via the synthesis of TNF & IL-6, which further activated the the NFkB & the transcription factor STAT systems within the pulmonary tissue. Research demonstrated that IL-6 inhibition dramatically reduced blood vessel growth indicators, tumor cell proliferation, tumor cell intrinsic STAT3 activation, and lung cancer promotion (Lafferty *et al*., 2010).

Furthermore, it was found that Th17 cell-mediated inflammation is essential for the development of lung tumors. Furthermore, it shown that the tumor-promoting abilities of bacteria such as NTHi are affected by neutrophilic inflammatory responses and the epidermal cytokine IL-17C. Recently, the significance of NTHi in the pathophysiology of COPD has been revealed. Infections of the airways due to NTHi imitating COPD produces a situation that is conducive to lung tumor development and dissemination. NTHi may therefore serve as a link to lung cancer and COPD (Lafferty *et al*., 2010).

Furthermore, mounting data suggested that initial pulmonary cancer cell growth, surviving, & invasion of tissue were all significantly influenced by stimulation in the PI3K system. Characterised as an increase in the ERK & PI3K pathways in response to oral taxa (Veillonella and Streptococcus) that had been abundant in lung tumor patients' lower respiratory tracts. As Veillonella, Prevotella, and Streptococcus were introduced to airway epithelial cells *in vitro*, these same signaling pathways were likewise activated. These studies revealed, at least partially, the lung microorganisms use MAMPs (microbe-associated nucleotide recognition receptors) to raise the synthesis of cytokines & inflammatory compounds, and this in turn affects the progression of lung cancer.

**Metagenomic approaches in microbial alteration**

It is now established that microbe infections and the onset of cancer are linked. Lung cancer has COPD as a significant risk factor. One important aspect for COPD is ongoing inflammation. Typically, stable COPD patients also have infection of possibly toxic microorganisms. For those with COPD, inflammatory conditions, oxidative stress, immunology, and DNA damage may raise their chance of lung cancer (Lafferty *et al*., 2010). It suggests that long-term infections can increase the risk of cancer of the lungs by increasing the body's sensitivity with toxins. Bacterial infections have been shown to increase the risk of lung cancer. Additionally, H. pylori infection may promote the development and spread of lung cancer (Budisan *et al*., 2021). Through factors related to virulence, metabolism, immunological reactions, and inflammatory processes, microorganisms could contribute to cancer of the lungs more likely. Inflammatory factors, including as cyclooxygenase (COX)-2, interleukin (IL), and tumor necrosis factor (TNF)-a, are strongly linked to carcinogenesis (Herfs *et al*., 2009). By increasing the expression of the cathepsin protease within tumor-associated macrophages, IL-4 can stimulate the development of tumors. In addition, after receiving exposure to IL-1b, a cytokine that promotes inflammation and is frequently found in pulmonary

cancer and at-risk carcinogenic tiny environments, cells with NSCLC displayed an increasing epithelial-to-mesenchymal (EMT) phenotype. Chemical resistance, PD-L1 overexpression, including cell migration all have been connected to EMT and EMT-associated traits. Via inflammation-associated cytokine TNF-a may control Snail via the nuclear nuclear factor-kappa B (NF-kB) pathway, which is crucial for inflammation-induced EMT and cancer cell motility, attack, and metastases. The RhoA/Rho kinase pathway is stimulated by COX-2, which disrupts the development of cancer cell adhesion junctions and aids in the growth of tumors (Chang *et al*., 2009). Additionally increased in NSCLC tissue, COX-2 is associated with a poorer outcome. As a reminder, an infection of pathogenic microbes can trigger the production of cytokines associated with inflammation & increase the local inflammation, ultimately leading to the development and occurrence of malignancies.

The incidence of H. pylori (HP) positivity in patients with stomach cancer was substantially correlated with the existence or the lack of the macrophages from inhibitory factor (MIF). Mif transcription has a capacity to encourage tumor cell growth, metastatic potential, & alteration of cells. Furthermore, it was demonstrated that NSCLC increased Mif overexpression. Neutrophils and tumor cells that circulate (CTCs) interact in the bloodstream, causing cell cycle advancement and increases the risk of CTC tumor metastasis. The separation and characterisation of distinct CTC-associated white blood cells (WBCs) and matching cancer cells inside each CTC-associated WBC cluster served as evidence for this. Increased risk of pulmonary cancer in COPD patients (Lafferty *et al*., 2010). The risk of acute episodes of COPD may increase when non-typeable Haemophilus influenzae (NTHi) colonize an area. When IL-17C gene defective spreading cancer of the lung rats were exposed to NTHi, their tumor-associated neutrophil proliferation, growth, and entire number were much lower than in the air-exposed group. This suggests that IL-17C may play a role in the development of macrophage-mediated lung cancer.

The bacterium E. coli becomes extremely harmful after gaining virulence genes, including its protein poison cytotoxic necrosis-like factor 1 (CNF1), which boosts COX2 expression, produces the transcription factor NF-kB, and promotes cell-based motility. This ultimately leads to the development of tumors (Chang *et al*., 2009). CNF1-induced lung cancerous cells release VEGF, promoting sprouting in the cancer micro-environment. Treatment to cryolathe extending toxins results in a distinct cytotoxicity and a cell phase block that is reliant on the DNA injury reaction. Furthermore, individuals with precursory and malignant tumors had greater concentrations of a poisonous

mutation for Bacteroides fragilis, which is enterotoxigenic. In addition, the adverse consequences of bacterial fermenting might include the formation of chemicals including ammonia, amines, and phenols that are sulphides, and nitrosamines that are known to be carcinogenic. The enterohepatic hormonal movement, which affects the levels of circulatory and ejected hormone in addition to the chance of forming hormones-dependent malignancies, can be influenced by the microbiota due to its potential to deconjugate hormones. In general, it is understood that immunology and infection contribute to the development of lung tumors; however, the relationship between pulmonary microorganisms to lung tumors is currently unknown.

**The discovery of microbial modification in the case of lung cancer using the metagenomics approach**

| Sl. No. | Lungs types | Sample type | Detection methods | Significant outcome | Reference |
|---|---|---|---|---|---|
| 1. | LC+, LC− | Sputum | 16S rRNA sequencing | *Acinetobacter junii*, *Streptococcus viridans*, *Escherichia coli*, *Enterococcus sp*. and *Streptococcus intermedius* were among the bacteria that were found in higher concentrations. | Cameron *et al*. (91) |
| 2. | LC+ | BALF | 16S rRNA sequencing | developed of four taxa (*Veillonella*, *Megasphaera*, *Atopobium*, *and Selnomonas*) & two phyla (*Firmicutes* and *TM7*) | Lee *et al*. (92) |
| 3. | NSCLC healthy control | Saliva | 16S rRNA sequencing | Reduced the corresponding abundances of Prevotella, Bacteroides, Faecalibacterium, and Fusobacterium; increased the abundances of Veillonella and Streptococcus, two species of Firmicutes. | Zhang *et al*. (96) |
| 4. | Naver smoking | buccal /Sputum instances | 16S rRNA sequencing | Increased amounts of Granulicatella, Abiotrophia, & Streptococcus were found in sputum, along with increased concentrations of the Bacilli species Streptococcus infantis and Streptococcus anginosus. | (Hosgood *et al*., 2014) |

| | | | | | |
|---|---|---|---|---|---|
| 5, | LC+, management of illness and health | specimens from the inner lungs | 16S rRNA sequencing | An increase in Prevotella, Veillonella, & Streptococcus | (Tsay *et al*., 2018) |
| 6. | Normal Lungs | Tissue of lungs | 16S rRNA sequencing | Dominated by *proteobacteria*; patients in late stages had higher levels of the *genus Thermus*; those with developing metastases had higher levels of Legionella. | (Yu *et al*., 2016) |
| 7. | NSCLC | BALF/ Saliva/ faeces | 16S rRNA sequencing | The four main phyla that make up the gut & lung microbiota include Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. | (Bingula *et al*., 2018) |
| 8. | LUAD, LUSC adjacent normal samples | Tissue of lungs | 16S rRNA sequencing | Each of the four phylum that proliferated are Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria; phylum LUAD samples showed an increase in these bacteria. | (Apopa *et al*., 2018) |

**Lung microbiota involvement in cancer therapy trials**

An enhanced comprehension of the relationship between microorganisms, inflammation, and cancer has led to innovative approaches in the treatment of the disease. Meroterpenoids, derived from *Cystoseira usneoides*, a brown seaweed, have been found to have cytotoxic properties against lung cancer cells, notably lowering the generation of IL-6, IL-1b, & TNF-a, suppressing the activity of COX-2. By PI3K-AKT silencing, COX-2 can also increase gefitinib resistance and NSCLC metastasis (Deng *et al*., 2020). This is a unique therapeutic approach to treat NSCLC cells that are resistant to cancer. However, transplanting patients' dominant gastrointestinal bacteria into the intestinal wall of a cancerous pulmonary mouse model showed a therapeutic effect. When *cisplatin and Lactobacillus* bacteria were injected to lung cancer mice, the tumors shrank and their survival rate increased. Furthermore, anti-cytotoxic T-lymphocyte antigen (CTLA)-4 antibodies have been effectively employed in tumor immunotherapy; nevertheless, the anticancer efficacy of CTLA-4 is contingent upon the bacteria in the gut (Maynard *et al*., 2019). Research has shown that Bifidobacterium modifies dendrite cell growth and increases CD8+ T cell

activation, which in turn boosts the therapeutic benefits of PD-L1 and CTLA-4 (Aghamajidi *et al*., 2022). A recent study discovered that the cancerous tissues of the pancreatic duct adenocarcinoma (PDA) cells contain a large number of colonic fungus. Additionally, the removal of fungal may enhance the effects of chemotherapy and prevent tumor development in PDA mice. Microbiome-tumor antigen adverse reactions & the type & strength of the immune response might influence the microbiome's anticancer impacts. Furthermore, giving those who have early lung cancer a combination of *Enterococcus hirae* and *Barnesiella intestinihominis* together with chemotherapy may significantly boost effectiveness (Pizzo *et al*., 2022). Still, more research on the application of microbes in cancer treatment is required.

## Conclusions

We now have a far better understand of the connection between lung cancer and the gut microbes than we had a few years ago. Based on the aforementioned studies, we may conclude that individuals with lung cancer have altered microbiota and dramatically elevated levels of certain bacteria. At the opposite hand, little is understood regarding how changes to the composition and activity of the microbiome impact the development of cancer of the lungs. Because there aren't enough samples, the control of microbiota modification is still the major area of investigation for microbiota in lung cancer. Further study is needed to comprehend the mechanisms underpinning the microbiome's influence on the development and course of lung cancer as well as the relationships between the microbiome and lung cancer. If the growing quantity of microorganisms in lung cancer patients speeds up the disease's progression, early monitoring and intervention for both the detection and treatment of lung cancer patients become even more important. Additional comprehension of these pathways will provide insight into lung disease early detection, diagnosis, and therapy.

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# Chapter - 4

## Mitigating Adverse Effects of Heavy Metal through Biomineralization: A Metagenomic Perspective

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# Chapter - 4

## Mitigating Adverse Effects of Heavy Metal through Biomineralization: A Metagenomic Perspective

**Deeti Das and Pritha Pal**

**Abstract**

Alpha-proteobacteria, particularly magnetotactic bacteria, plays a key role in biomineralization by synthesizing diverse chemicals known as biominerals. These microbes adapt to harsh conditions, demonstrating heavy metal tolerance. Beyond alpha-proteobacteria, other organisms, like sea urchins and vertebrates, are involved. Metagenomic approaches extract non-culturable microorganisms' DNA from soil, aiding in gene exploration for bioremediation. This review delves into biomineralization's molecular mechanisms, emphasizing metagenomics' potential in understanding and applying bioremediation.

**Keywords:** Biomineralization, toxicity of heavy metals, metagenomics approach.

### Introduction

There are two distinct routes in the biomineralization process. One of which involves certain gene mediated pathways, where genes, diverse cell structures and various transcriptomes play integral roles in orchestrating the mineralization process (Dhami *et al*. 2013). Another aspect involves the biological process mediated mineralization, wherein minerals precipitate externally to organisms as a result of chemical changes in the microorganisms' environment. This phenomenon occurs when the chemical composition of the surroundings prompts mineral formation outside the organism's body (Görgen *et al*. 2021). According to Vargela and Pitroda (2019), both prokaryotes and eukaryotic organisms that secrete substantial amounts of minerals are involved in biomineralization. MICP, a biomineralization expansion, holds diverse applications across multiple fields in Table 1 (Yoshida *et al*. 2010).

**Table 1:** Application of Microbially Induced Carbonate Precipitation (MICP)

| MICP Application | |
|---|---|
| 1. Elimination of radioactive materials and heavy metals. | Heavy metals biomineralization |
| | Radionucleotide precipitation reduces groundwater contamination. |
| 2. Sequestration of atmospheric $CO_2$ | Chemical fixation of $CO_2$ into $MgCO_3$, $CaCO_3$, $CaMg(CO_3)_2$. |
| 3. Construction materials | Utilizing carbonate that has been biomineralized, building materials are produced. |
| 4. Polychlorinated biphenyls (PCBs) and calcium clearance | MICP facilitates the removal of unwanted calcium and PCBs. |
| 5. Diverse material production | Filler for plastics and rubber. |
| | Particles of fluorescence in writing ink |
| | Manufacturing of luminescent markers. |

Biomineralization is common in various organisms such as aquatic invertebrates, MTB, echinoderms and molluscs (Lefèvre and Bazylinski, 2013). Genes like Salmonella Enterica, PhoN, Urease, PhoK gene in *Sporosarcina pasteurii*, Isotig02195 and Isotig00817 in Pinctada fucata play a key role in biomineralization. Bioinformatics tools such as Blast, KEGG, Tremble, Clustalw, and Go annotations are used to analyse their sequences and protein transcriptomes. Go Annotations from Uniport facilitate the investigation of cell processes, molecular relationships, and subcellular location. Microscopic methods like as transmission electron microscopy and scanning electron microscopy have been used to continue researching the formation of $CaCO_3$ crystals in MTB (Liu *et al*. 2021). Various sequencing techniques, such as shotgun and next-generation methods, are employed for genome sequencing. Prokaryotes and eukaryotes generate diverse biominerals, each serving a specific purpose in the organism's molecular crystal structure in Table 2.

**Table 2:** List of various organic crystals along with their properties

| Biominerals name | Organisms and function of the crystal structure |
|---|---|
| 1. Aragonite | Fish/Gravity device. Mollusca/exoskeleton |
| 2. Calcite | Algae/part of exoskeletons and eye lens |
| 3. Calcium Oxalate | Plants/Ca store |
| 4. Gypsum, Celesite | Larvae of jellyfish and gravity gadget |

| | |
|---|---|
| | Support from cells and Acantharia |
| 5. $SiO_2$ | Algae/Exoskeletons |
| 6. Magnetite | Bacteria/magneto taxis |
| 7. Vaterite Amorphous | Ascidians/Spicules |
| 8. Hydroxyapatite | Vertebrates/Endoskeletons in the teeth. |

Arthropods and mollusks produce various compounds. Mollusk exoskeletons mainly consist of argonauts and calcite, providing strength. Arthropods, a major phylum, have a sturdy exoskeleton for protection in harsh environments, resisting desiccation (Colwell *et al*. 2005).

**Comprehensive grasp of metagenomics fundamentals**

In the dynamic realm of environmental biotechnology, specifically metabolomics, extracting DNA from non-culturable bacteria enables research into new genes, enzymes, healthcare applications, environmental biodegradation, metagenomic libraries, and biosurfactants. Pace and colleagues (1985) pioneered cloning DNA from environmental samples, leading to the recognition of the metagenome for discovering novel organisms and exploring diverse cellular and metabolic routes (Alves *et al*. 2018). Several studies indicate that the microorganisms in a metagenomic community has the potential to serve as a biomarker for detecting different types of pollution in air, water, and soil (Kisand *et al*. 2012). Certain environmental pollutants prompt the activation of microbes genes. For instance, cyclohexane accumulation induces the expression of cyclohexane-degrading enzymes. Various enzymes, including monooxygenases, esterases, laccases and phenol-degrading enzymes have been explored through metagenomics method. (Techtmann *et al*. 2016; Ufarte *et al*. 2015). Shotgun DNA sequencing combined with a metagenomic method and ambient DNA samples were used to study the clusters of genes and the structure of MTB bacteria.

1. The orderly procedures that must be followed while managing microbes that are not cultivable.
2. The direct choosing of metagenomic material through the setting with a variety of microorganisms.
3. The isolation of DNA from genomes.
4. Preparing the metagenomic library using a many short sequences of DNA.
5. Details from sequences via those libraries.
6. Determination of the metabolic processes route.

7. Using a comparative study to identify species.
8. Determining the latest coding patterns.
9. The annotation of Genomes.
10. Creation of a server and bioinformatics tool for keeping track of and examining those sequences.

The diagram illustrating several metagenomic methods for comprehending microbiological habitats are seen in Figure 1.

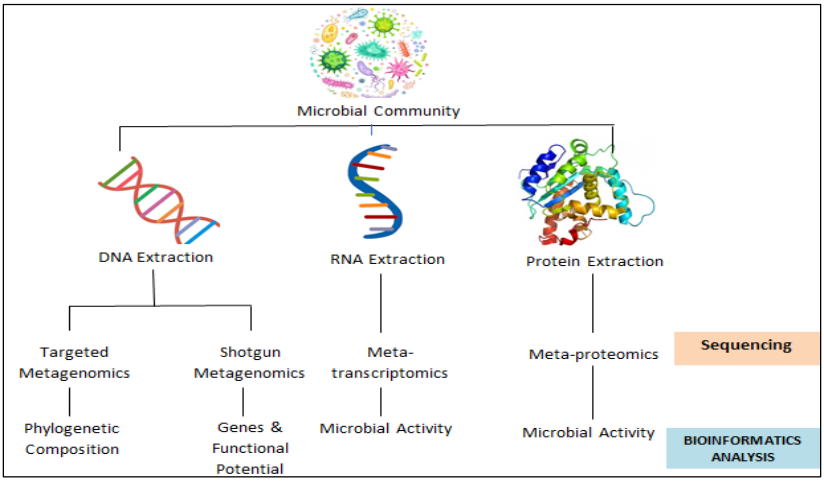


**Fig 1:** An Illustrative display of various metagenomics methods for understanding the microbes niche

**The idea of metagenomic library**

Numerous studies have explored aquatic ecosystems, plant microbiome and soil microbes investigating the importance of creating metagenomic libraries for screening specific genes or enzymes. Metagenomic DNA libraries from these sources reveal useful genes from unculturable microbes, paving the path for finding unique metabolites (Courtois *et al*. 2003).

**The link between metagenomics and biomineralisation**

Modern microbialites show the pivotal role of non-cyanobacterial lineage in precipitation of calcium carbonate. Metagenomic analysis using 16s and 18s rRNA genes reveals diverse microbes and eukaryotes, while Cyanobacteria (order Pleurocapsales) dominate deeper sections. Magnetotactic bacteria's metagenomic libraries highlight significant operons controlling magnetic properties, with recent studies indicating potential crystal loss upon mamAB operon deletion (Couradeau *et al*. 2013).

**Bioremediation via biomineralization-heavy metal toxicity**

Heavy metals pose health and environmental risks due to their widespread use in agriculture, industry and medical applications. While essential metals like iron, chromium, nickel, magnesium, copper, selenium play vital roles in metabolic pathways, exposure to harmful ones such as cadmium, arsenic and lead negatively impacts living organisms. Recognizing these dangers, effective measures like radionuclide-based groundwater remediation are crucial for removing toxic heavy metals (Silva *et al*. 2005).

**Metagenomic methods limitations in terms of biomineralization and bioremediation**

The metagenomic approach for gene and enzyme study has drawbacks, including fewer available genes in metagenomic DNA for enzyme synthesis. Enzymes may not function well in diverse environments, complicating their potential as pollution and for applications in bioremediation and biomineralization. Some enzymes may not express well in vectors like E. coli. (Alves *et al*. 2018).

**Conclusion and future perspectives**

Controlling industrial discharge and heavy metal pollution requires quick response. Microbial support in removal of metal from soil and water is successful, despite difficulties in using metagenomics and bioremediation. This provides a viable path for ecological preservation through methods such as metagenomics, metaproteomics and metatranscriptomics. Using genetic approaches, on-going research attempts to investigate the potential of microorganisms for converting pollutants, with the goal of scaling up these lab-scale activities to an industrial scale.

**Conflict of interest**

The authors declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships.

**Author contributions**

Deeti Das - Data collection and Writing-Original draft preparation; Pritha Pal. Formal reviewing and Supervision.

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# Chapter - 5

## The Current Approach to Bioartificial Pancreas

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# Chapter - 5

## The Current Approach to Bioartificial Pancreas

**Shreeya Das Mahapatra and Priyankar Pal**

**Abstract**

An apparatus with living cells that can produce insulin on the basis of glucose levels in the blood is called a bioartificial pancreas. It is intended to assist those who are unable to produce insulin on their own and have type 1 diabetes. The remote devices that are being watched, designed to resemble the natural organ's release of insulin, have been investigated. To reduce the need for immune-suppressive medication to fight the host, synthetic biocompatible semipermeable membranes have been used to separate host immune system cells, either autologous or xenogeneic, or ensembles of cells. A number of factors were addressed, including quantity of equipment implemented, the web page of implantation, the inclusion or exclusion of coimmobilized molecules or cells, the intrinsic properties of the barrier's breathable elastomer, and the media used for cell restraint or standstill. An insulin pump, a chamber containing the cells, and a continuous glucose monitor comprise a bioartificial pancreas. A semi-permeable membrane surrounds the chamber, letting nutrients and insulin in but keeping the immune system from attacking the cells. By encasing and shielding the pancreatic cells with a variety of materials and methods, scientists are striving to create and enhance bioartificial pancreas devices. One such is the convection-enhanced bioartificial pancreas, which improves nutrient transport and encapsulated cell survival by means of a fluid flow. This paper explains the idea and the majority of the worldwide research conducted over last few years to develop a bio-artificial pancreas device.

**Keywords:** Bio artificial pancreas, type 1 diabetes, Insulin pump.

### Introduction

Due to abundance, diabetes mellitus generates a grave risk to public health, illness related to hormones in developed nations (Kleinman *et al.*, 1988). Up to now, estimates are showing the fact that there are approximately 177 million cases of the disease worldwide, and by 2025, that number is

expected to have substantially doubled (Wongsawat, 2017). Type 1 diabetes, likewise referred to as diabetes mellitus is on increasingly prevalent disorder in which body's beta cells, which produce insulin are destroyed by an autoimmune reaction, leaving patients unable to produce the insulin required to process blood glucose.[4] Pancreatic islet transplantation was recently tried as an alternative therapy to maintain stable and long lasting diabetes mellitus reversal.[5] The body's built in resistance to foreign islets conveys one of the main obstacles to pancreatic islet transplantation.

**Molecular mechanism**

A complex interaction between genetic, environmental, and life style factors leads to diabetes. Insulin deficiency results from immune system attacks on the pancreatic beta cells that yield insulin in type 1 diabetes. Insulin resistance is an ailment relating to type 2 diabetes in which the pancreas is ceases to produce enough insulin to compensate for the cells futile adaptation to insulin (Shepherd *et al*., 1998). The insulin pump, glucose sensor, and computer that restricts the insulin delivery rate contribute to the mechanical artificial pancreas. The sensor could potentially require to be replaced on a regular basis after being implanted in the vena cava. While a mechanical artificial pancreas can control blood sugar levels, the fabrication of a biosensor sensing hyperglycemia has been hindered beyond the challenge of developing a steadfast, reliable and tactile transducer. An unacceptable possible side effect of an artificial pancreas is insulin overdosing, which could be dangerous and lead to life-threatening hypoglycemia due to a mistake in sensing, computing, or delivering the insulin (Pfeiffer, 1993). Additionally, it is very challenging to create a bionic pancreas that is purely biological reacts to fluctuations in haemoglobin concentrations levels fast enough (islets react in 10 minutes).

**Idea of a bio artificial organ**

Hormone deficiencies afflict an enormous number of people far and wide. Currently, patients may cope with many of these hormone deficient illnesses by consistently ingesting the nonexistent hormone (Kruse-Jarres *et al*., 1979). However, owing to the still exist significant swings in the levels of metabolites, verbal swapping dosage are likely to postpone the initial phase of the disease's barriers.

Currently, organ transplantation—either partial or whole—remains the only validated therapy for these illnesses, even the substantial hazards associated with the surgery. If a specific cell transplant is carried out rather than the removal of the entire organ, the surgical risk can be significantly decreased (Kerner *et al*., 1979). Strong reactions are always elicited whenever

allogeneic organs or cellular tissue migrate from one person to another, or from one species to another, as in xenotransplantation (Hunkeler, 2001). Researchers have been tackling development of devices that mitigate the need for antagonistic regimens of medication for the past 30 years. Cells or cell clusters within a synthetic biocompatible semipermeable membrane, which specimens the foreign tissue from the host's immune system, tend to accumulate in these so-called bio-artificial organs (Moussy, 2000). Six they are designed to perfectly replicate the appearance and capabilities of a functioning organ and can be implanted or imposed from within the circulated blood at any location at the centre of an entity retaining transmission aptitude. By shielding the cells from the assault of immune cells or antibodies, these devices are removing the need for immunosuppressive medications.

**Bio artificial pancreas**

Quite a few bio-artificial organ models, notably those of the pancreas, kidney, and liver, have already been studied (Langsch & Bader, 2001). The attributes and architecture of the membrane vary in accordance with the particular role of the organ that the bio-artificial device strives to reappear (Mackay *et al*., 1998). Any model has certain prerequisites that device must meet certain requirements in order to be approved as an alternative therapy, including: maintaining cell viability and functionality for extended periods of time (when cells are immobilised in the device), easy retrievability or biodegradability, and good diffusional properties of the membrane (Ohgawara *et al*., 1996). Theoretically, an improved approach to current IDDM therapies would be a bio-artificial pancreas with viable and functional islets of Langerhans shielded by a biocompatible semipermeable membrane (Tze *et al*., 1994). This would allow for more stringent blood glucose control without the need for immunosuppressive drug. Simply because of the pancreatic islets' inevitable physiological feedback, glucose intolerance can always be maintained, even during high-glucose stimulatory events (Kessler *et al*., 1997). As an instance, stimulatory substances are conveyed to beta cells, and afterwards enter the bloodstream as insulin quickly and effectively (Trivedi *et al*., 2001). As a result, a successful BAP model must prevent insulin from remaining close to the cells, which would harm anatomical feedback system, and need that guarantee rapid inducement and the span of time it typically requires for secretagogues to translates into a brief and potent dissipation prospective. A successful BAP model also requires that the material be completely biocompatible, not incite fibrosis around the device or activate inflammatory agents that would inevitably triggers the immune system.

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# Chapter - 6

## Microbiome-Based Biodiesel Production using Genetic Engineering

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# Chapter - 6

## Microbiome-Based Biodiesel Production using Genetic Engineering

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**Abstract**

As a kind of sustainable energy, biodiesel is a perfect replacement for diesel fuel derived from petroleum. It is typically produced by transesterifying triacylglycerides with alcohols. Numerous start-up biotechnology firms and research institutions are currently investigating microbial fermentation-based biodiesel production with the goal of establishing technologies for producing biodiesel that are more affordable, effective, and sustainable. The conversion of microorganisms with high production efficiency of biodiesel is dependent on genetic engineering. This study presents a concise overview of the primary microorganisms involved in the production of microbial biodiesel and the latest breakthroughs in metabolic engineering required for making the essential alterations. Recent reports have shown significant advancements in the use of bacteria, yeast, algae for the genetic engineering of fatty acid metabolic pathways to produce biodiesel feedstock sustainably. Relevant examples are used to highlight the removal or overexpression of the related enzymes for the de novo as well as ex novo production of biodiesel. Most of the time, nonetheless, it remains to be seen whether such engineering techniques for the creation of sustainable biodiesel will be successfully commercialized. This essay methodically outlines the limitations of the current state of research on genetic engineering techniques for biodiesel production using microbes, as well as a thorough analysis of the shortcomings of the traditional methods for producing biodiesel. This study also contains an overview of the technological obstacles that has to be conquered for genetic engineering technology to financially viable.

**Keywords:** Biodiesel. triacylglycerides. microbial fermentation, de novo and ex novo production, genetic engineering.

### Introduction

On a worldwide scale, a rising concern of petroleum crisis, depletion of non-renewable natural resources, and the impact of global warming at an

alarming stage have enforced the search for an alternative renewable source of energy for the sustainable future (Hegde *et al*. 2015). Life itself depends on energy, and the world economy and industry development rely on it as well. Because to the depletion of finite resources and the buildup of greenhouse gases in the atmosphere, the use of fossil fuels as energy sources is unsustainable (Lin *et al*. 2013). Based on the existing daily consumption of around 11.6 million tons of crude oil, it may be inferred that the available supplies will only be sufficient for a limited duration. The world economy will collapse dramatically due to skyrocketing oil costs, which will result from an ongoing increase in demand, if there are no suitable substitutes for crude oil (Kraisintu *et al*. 2010).

By transesterifying fatty acids with alcohol, monoalkyl (methyl, ethyl, or propyl) esters of fatty acids are produced chemically, which is what biodiesel is. In diesel engines, biodiesel works well when combined with fossil diesel fuel or when used pure (Magdouli *et al*. 2014). The establish of a competitive and sustainable alternative that is based on abundant and renewable feedstock, such as biomass or other regenerative sources, is imperative in order to break free from crude oil dependence and mitigate the environment's deterioration. Promoting research on sustainable and environmentally friendly biofuels is a response to growing environmental concerns and the depletion of oil sources. Because its chemical structure and energy content are identical to those of conventional diesel, biodiesel has garnered the greatest interest among all the biofuels. Furthermore, since biodiesel is compatible with current engine types and has been commercially blended with diesel as a transportation fuel in a number of countries, including Germany, Italy, and Malaysia, no modifications to the diesel engine are necessary (Lin *et al*. 2013). Utilizing microorganisms to their full potential in the production of biodiesel has enormous potential. Here, we provide a brief summary of the key microbes involved in the synthesis of biodiesel, significant metabolic pathways connected to this process, and prospective paths for building cost-effective and efficient systems for producing biodiesel. To meet the growing need for biodiesel feedstock production, the current analysis examines the shortcomings of traditional biodiesel production techniques as well as the advancements in genetic engineering technology. It also discusses the technological obstacles that must be overcome in order to build genetic engineering technology that is commercially viable and allows for the commercial introduction of biodiesel (Hegde *et al*. 2015).

**Materials and methods**

The essential information for this study was obtained by doing a

comprehensive search on PubMed, PubMed Central, Google, and published research papers and review articles from various countries, focusing on the microorganism and the generation of biodiesel by genetic engineering. We exclusively considered data that has been officially published, and we eliminated any conjectural assertions regarding exposure. The inclusion criteria encompass the utilization of data acquired from reputable publications pertaining to the topic matter. The study did not include languages other than English.

**Biodiesel**

Typically produced from triacylglycerides (TAG) through transesterification with alcohols, biodiesel is a form of sustainable energy that is a perfect replacement for petroleum-based diesel fuel. Research centers and start-up biotechnology firms are currently exploring the possibility of producing biodiesel using microbial fermentation as a more cost-effective and environmentally friendly method (Lin *et al*. 2013).

**Key microorganisms in the production of biodiesel**

Microbes, which are typically unicellular organisms, have the potential to serve as both a catalyst and a supply of substrates for biodiesel synthesis, including fatty acid sources and alcohols. Oleaginous microorganisms, commonly known as grease microorganisms, are widely used and researched in the present biodiesel production process as a source of fatty acids for transesterification. From a rich source of microorganisms, grease microbes can use or transform different agro-industrial resources (such as plant biomass) into cellular lipids (Lin *et al*. 2013).

**Bacterial species used in the production of biodiesel:** Bacterial species commonly accumulate polyhydroxyalkanoates (PHA), such as polyhydroxy-butyrate (PHB)36, for energy storage. On the other hand, most eukaryotic microorganisms exhibit TAG accumulation (lipid storage), with only a few prokaryotic microorganism groups appearing to be members of the *Actinomycetes* group, such as *Mycobacterium*, *Streptomyces*, *Nocordia*, *Gordonia* and *Rhodoccocus*. Fatty acids are produced during the exponential growth phase in order to support the manufacture of phospholipids, which are vital for both membrane composition and cell proliferation. Under restricted development conditions, actinomycetes accumulated intracellular PHA and TAGs and displayed a higher lipid content of up to 70% (w/w). In contrast, *Acinetobacter* was found to be the only species capable of accumulating free fatty acids, wax esters, mono-and di-acylgylcerols, and both intracellular and external TAGs during n-alkane cultivation for gram-negative strains

(Magdouli *et al*. 2014). It has been revealed by research that the regulatory mechanism and accumulation of lipids varies between gram-positive and gram-negative bacteria, with the former being thought to accumulate more lipids than the latter route. Therefore, more research is needed to validate these findings in bacterial populations and pinpoint any plausible interaction pathways (Lin *et al*. 2013).

**Fungal species employed in the synthesis of biodiesel:** Yeast and filamentous fungi are examples of fungi that fall within the general category of oleaginous microorganisms. It has been claimed that yeast, a type of unicellular fungal microbe, produces higher quantities of lipids than 20% CDW. They may be essential for the manufacture of biodiesel due to their short duplication time (less than an hour), less sensitivity to environmental conditions, and simplicity of cultivation as compared to microalgae. Just 25 of the 600 kinds of yeast that are known to exist are oleaginous. Furthermore, accumulated lipids have a high percentage of polyunsaturated fatty acids (PUFA) and a low percentage of SE, with up to 90% of TAGs present. Red yeast really produces very high levels of polyunsaturated fatty acids (PUFAs) more than 50% and are primarily found in the *Sporidiobolales* group and phylum *Basidiomycota* (Magdouli *et al*. 2014).

Moreover, there has been a growing interest in filamentous fungus. The wide spectrum of lipid accumulation in these organisms is attributed to the presence of potent extracellular lignolytic enzymes, such as lignin peroxidase, manganese-dependent peroxidase, laccase, invertase, and xylanase. Furthermore, these microorganisms have the ability to be grown on various substrates, such as residual lignocellulosic materials. Additionally, their production can be enhanced during the fermentation process to achieve greater quantities of lipid biomass. Fungi cultivated in growth mediums with high glucose concentrations and limited availability of nutrients like nitrogen, phosphorus, or sulphur can amass substantial amounts of TAG and SE. The majority of fungi have lipid buildup that exceeds 25% (w/w). The Zygomycetes group is one of the most frequently observed genera among them, and includes the following species: *Cunninghamella echinulata, M. vinacea, Trichosporan elegans, Rhizopus stolonifera, Candida boidinii, M. isabelline, Zygosaccharomyces rouxii. Culcherrima, Culverta, Pichia membranifaciens, Mortierella ramanniana* and *Oleophila.* (Lin *et al*. 2013) (Magdouli *et al*. 2014).

**Microalgae used in the production of biodiesel:** Microalgae have garnered significant attention as an economically feasible oil feedstock in recent times, owing to their distinct advantages such as ease of cultivation,

large group size, varied metabolic capacities, and elevated fatty acid composition. Numerous microalgae that can autotrophically such as *Chlorella vulgaris, Bradycoccus braunii, Navicula pelliculosa, Scenedsmus acutus, Cryptodinium cohnii, Dunaliella primolecta, Monallanthus salina, Neochloris oleoabundans, Phaeodactylum tricornutum,* and *Tetraselmis sueica* have been identified as sources of oil buildup (Lin *et al*. 2013).

**Table 1:** Key microorganisms used in biodiesel production (Lin *et al*. 2013) (Magdouli *et al*. 2014)

| Bacterial Sp. | Fungal Sp. | Microalgae |
|---|---|---|
| *Arthrobacter sp., Shewanella sp., Vibrio sp., Rhodococcus opacus, Bacillus alcalophilus, Alteromonas sp., Flexibacter sp., Mycobacterium, Streptomyces, Acinetobacter calcoaceticus* and *Rhodococcus opacuscan* | *Candida curvata, M. vinacea, Aspergillus oryzae, Rhodosporidium toruloides, Lipomyces sp., Cunninghamella echinulata, Rhizopus stolonifer, Mortierella ramanniana, M. isabellina, Trichosporan elegans, Oleophila,. Culcherrima, Culverta, Pichia membranifaciens* and *Zygosaccharomyces rouxii* | *Chlorella vulgaris, Monallanthus salina, Bradycoccus braunii, Scenedsmus acutus, Cryptodinium cohnii, Tetraselmis sueica, Neochloris oleoabundans, Phaeodactylum tricornutum, Navicula pelliculosa* and *Dunaliella primolecta* |

**Enhancing biodiesel production using genetically engineered microorganisms and their mechanisms:** The capacity of microbes to synthesize fatty acids relies on many dietary factors that have been previously discussed. Genetic engineering has been used as a supplemental technique to enhance the production of microbial diesel. This phenomenon has been observed in higher eukaryotes, fungi, and bacteria. Due to its renowned genetics, rapid growth rate, and suitability as a host cell for genetic manipulation, *E. coli* has been the primary focus of most research. The production of fatty acid butyl esters (FABEs) and wax esters, FAEEs, yielded positive outcomes. In addition, it has been noted that *S. cerevisiae* H1246, which carries the wax-ester synthase/diacylglycerol acyltransferase (WS/DGAT) gene from *A. baylyi* ADP1, generates fatty acid isoamyl esters (FAIEs) and fatty acid ethyl esters (FAEEs). The *Z. mobilis* genes pdc and adhB, responsible for encoding pyruvate decarboxylase and alcohol dehydrogenase, were inserted into *E. coli*. As a result, the levels of FAEES reached a maximum of 674 mg/L. Currently, modified *E. coli* is employed to synthesise FAEEs. In addition, the activation of genes responsible for the catalytic residue of *Clostridium stercorarium*'s endoxylanase and the xylanase of *Bacteroides ovatus* enhances the production of biodiesel from ethanol. The

concentration of biodiesel was able to reach 11.6 mg/L. Fatty acids, especially alkanes, are often considered to be very suitable fuel for internal combustion engines. The alkane operon from *Synechococcus elongatus* PCC7942 was included, leading to the creation of an *E. coli* strain capable of generating alkanes. The genetically altered Escherichia coli strain yielded around 0.3 g/L of a mixture of alkanes. *Vibrio furnissii* M1 is making significant progress in the synthesis of n-alkanes, as demonstrated by Magdouli *et al.* (2014). In order to obtain a suitable scale of biodiesel feedstock, extensive research and development efforts are required that go beyond the present conventional production processes. In subsequent sections, we will discuss how metabolic engineering and recombinant DNA technologies have been used to develop efficient techniques for enhancing oil production in different microorganisms and plants (Ansari *et al*., 2011). The use of fatty acid metabolism in *E. coli* has been growing in recent years due to several advantageous traits of the bacteria. These include its high rate of fatty acid biosynthesis (0.3 g/l per hour per gramme of dry cell weight), its ability to secrete the compounds and consume both five and six carbon sugars, and its ease of engineering. A unique approach has been devised to produce biodiesel in common bacterial hosts like *E. coli* by combining metabolic engineering and system biology in a multidisciplinary research method.

Microalgae have attracted significant attention as a feedstock for biodiesel synthesis in recent years due to their rapid growth, high lipid production, and ability to survive diverse environmental conditions. In broad terms, there are three categories of techniques employed to enhance lipid production in algae: transcription factor engineering, genetic engineering, and biochemical engineering. The major objective of the biochemical engineering technique is to boost lipid production in microalgae by optimising temperature, pH, salinity, and nutritional needs. This is achieved by shifting the metabolic flux generated in photobiosynthesis towards lipid biosynthesis (Magdouli *et al*., 2014). The TF technique has been demonstrated to affect several genes involved in diverse metabolic processes, resulting in a concurrent and integrated up- or down-regulation of these pathways. This has the capacity to greatly improve the production of advantageous metabolites. Although still in its early developmental phase, numerous transcription factors (TFs) have been discovered to possess the ability to regulate lipid production in both prokaryotes and eukaryotes. Transcriptional factors engineering can enhance biodiesel feedstock production in microalgae, establishing a solid platform for improvement. The TF technique has been proposed to affect several genes that are involved in multiple metabolic pathways, resulting in a

simultaneous integrated up or down regulation of these activities. This has the potential to greatly improve the production of advantageous metabolites (Ansari *et al*. 2011).

**Table 2:** Mechanisms involved in biodiesel production by microorganisms (Ansari *et al*. 2011)

| Microorganism | Mechanisms | Example |
|---|---|---|
| Bacterial sp. | Removal of fatty acyl-CoA synthetase gene and over expression of fatty acyl-ACP thioesterase (FAT) and ACC genes | *E. coli* |
| Fungal sp. | Deletion of the glycerol-3-phosphate dehydrogenase gene (GUT2) | *Y. lipolytica* |
| Algal sp. | *Cyclotella cryptica (alga) acetyl-CoA carboxylase 1 (Acc1) expression* | *Cyclotella cryptica* |

**De novo synthesis of biodiesel by microorganisms with recombinant DNA:** Metabolic engineering has been used in several papers to accomplish the de novo biosynthesis of biodiesel, this illustrates a typical process of creating biodiesel, such as FAEEs, by de novo biosynthesis in microorganisms. Numerous distinct routes, include the synthesis of ethanol, this route involves transesterification and the metabolism of fatty acids**.** Given its abundance of advanced genetic tools and the abundance of knowledge regarding its metabolism, *E. coli* is a great host for genetic engineering to manufacture high-value compounds. The simultaneous overexpression of the ethanol production genes from *Z. mobilis* and the wax ester synthase/acyl-CoA-diacylglycerol acyltransferase (WS/DGAT) gene from the *Acinetobacter baylyi* strain ADP1 results in the first report of the de novo biosynthesis pathway of biodiesel in the world in *E. coli* (Lin *et al*.2013).

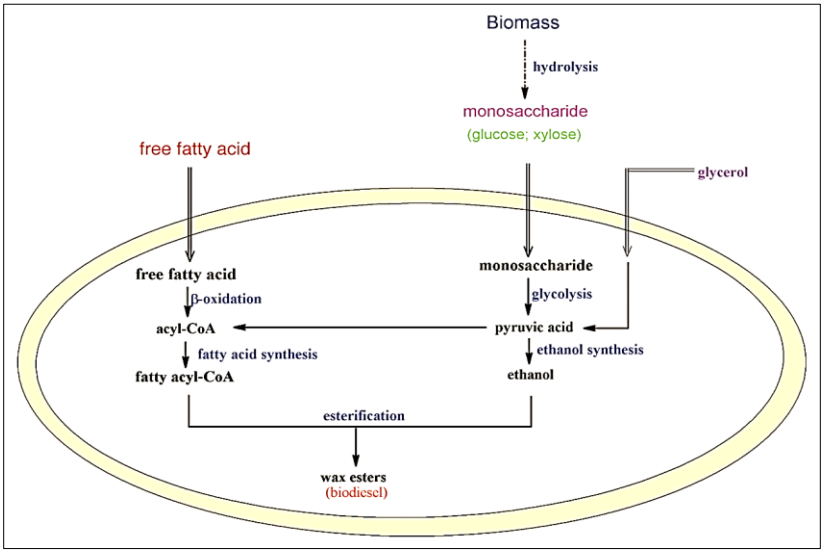


**Fig 1:** Microorganisms could have representative pathways created for the de novo biosynthesis of biodiesel (Lin *et al*. 2013)

**Ex novo synthesis of biodiesel by microorganisms with recombinant DNA:** Microorganisms use hydrophobic substrates like n-alkanes, fatty acids, and TAGs for the ex novo route. Fatty acids from the medium must undergo incorporation, transportation, and activation in order to generate thiolesters in order to take part in metabolic reactions. It is advised to perform lipase-catalyzed hydrolysis to produce free fatty acids if the substrate is present in TAG form. It is uncertain how free fatty acids are incorporated. However, it has been proven that lipolytic and proteolytic changes are necessary to carry out the availability of hydrophobic substrates. *Y. lipolytica* carries out these alterations by generating emulsifiers and surfactants in an effort to improve cell-substrate interaction and facilitate TAG hydrolysis (Magdouli *et al*. 2014).

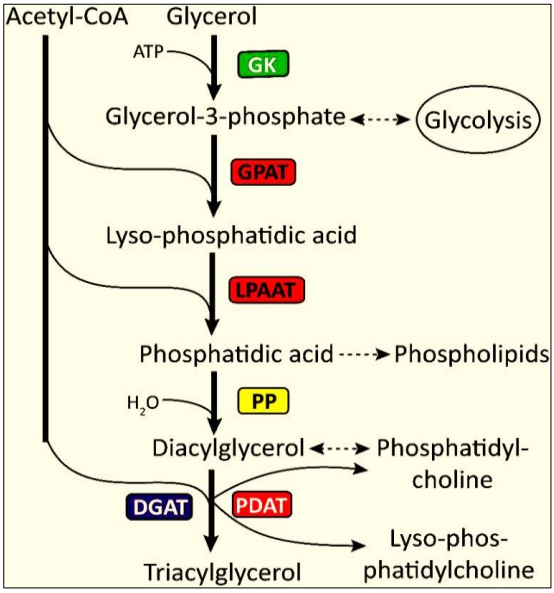


**Fig 2:** Pathway of Triacylglycerol production (Magdouli *et al*. 2014)

## Conclusion

Transesterification of fatty acid sources (such as TAGs) with short-chain alcohols results in the production of monoalkyl esters of long-chain fatty acids, such as FAMEs and FAEEs, which are then used to make biodiesel. To acknowledge microorganisms, need to be developed for this procedure. To facilitate the production of fatty acids and short-chain alcohols that are suitable for transesterification, as well as to have acyltransferases that are more active with respect to short-chain alcohols. Although acknowledged microorganisms are engaged in all of these functions, de novo synthesis is also involved. Naturally occurring microorganisms have not yet been found to produce fatty acid ester-based biodiesel (FAEEs). The possibility of creating biodiesel from scratch using a single microorganism can be achieved by modifying potential host organisms, as evidenced by experimental data and fundamental principles of the necessary metabolic processes. It is discovered that the most common host for this modification is *E. coli* strains. It is evident, therefore, that industrial biodiesel use is still a long way off from biodiesel generation in the existing modified strains. It is critical to develop microorganisms with increased efficiency and the ability to use free substrates in order to compete with fossil fuels. It was observed that numerous mechanisms would affect the synthesis of biodiesel. It's possible that overexpressing one or more particular enzyme genes won't result in significant facilitation.

## Future scope

The increasing amount of research demonstrating the substantial capacity of FAMEs and FAEEs for production has resulted in the determination that microorganisms are the most reliable and attractive source for the synthesis of biodiesel. This study provides an overview of the regulatory system governing the generation of fatty acids. Although genes involved in the biosynthesis pathways have been cloned and characterized, there is still limited knowledge about the precise details of lipid metabolism. There are numerous fundamental biological enigmas that have yet to be resolved. The majority of the queries concern the variations in fatty acid accumulation metabolic pathways seen in fungi, yeast, and other eukaryotic species as well as bacteria (both gram-positive and gram-negative). Furthermore, nothing is known about the possible connections between the ex-novo and de novo processes. For instance, the fact that yeast needs surfactants and extracellular matrix to function in the ex-novo route. Thus, new experimental strategies must be used to gain a deeper understanding. The efficient organisms that produce biodiesel will eventually result from advancements in genetic and metabolic pathways.

**Conflict of Interest:** The study does not include any potential conflicts of interest.

**Author contributions:** The data is acquired and interpreted by Akash Saha, Sagnik Sardar, Adityaa Sinha. This study was conceived, designed, and revised by Rupesh Dutta Banik.

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# Chapter - 7

## Functional Effect Regarding Specialised Metabolites Constructed Over Endophytic Fungi

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# Chapter - 7

## Functional Effect Regarding Specialised Metabolites Constructed Over Endophytic Fungi

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**Abstract**

Worldwide growth in populations and technological advances have resulted in a number of environmental issues, including the spread of crop diseases and human diseases as well as the development of bacteria, viruses, and fungi that have become resistant to multiple medications. Thus, there has been a significant change in favour of eco-friendly products in a number of important industries, including pharmacy, agriculture, and medical. Studying endophytic fungi and their biotechnological potential is crucial because they can be utilised for identifying an extensive variety of secondary metabolites in a cost-effective, ecologically friendly, and significant manner. The interesting host-associated fungal communities known as endophytic fungi (EF) penetrate the intracellular or intercellular gaps of their hosts' tissues. They inhabit the tissues of higher plants asymptomatically. The natural compounds, or secondary metabolites, that these endophytes produce include fungicides. Extensive variety regarding specialised metabolites are constructed over them along distinct structural properties due to their unique living conditions and these are recognised as an important source of biomolecules with potential medicinal applications and few of these metabolites have medicinal and ecological significance. They are an excellent source of important bioactive chemicals with cytotoxic, antibacterial, insecticidal, anticancer, and antioxidant properties that have been effectively identified. This review shows different types of naturally developed secondary metabolites like antimicrobial, anticancer, and antioxident compounds from plant associated endophytic fungi. This promotes the synthesis of a wide variety of biologically active specialised metabolites.

**Keywords:** Anticancer activity, antimicrobial activity, antioxidant activity, biological active compounds, endophytic fungi, secondary metabolites.

### Introduction

All organisms that, during a viable stage of their existence, colonise the

living interior tissues of their host are referred to as endophytes (Greek: endo = within + phyte = plant) (Zheng and Zhang *et al*., 2021). They develop within their plant hosts without displaying apparent signs of disease, and this type of growth requires continuous metabolic interactions between the fungus and the host (Schulz and Boyle *et al*., 2002). They live in the intercellular gaps, tissues, and organs of plants (Zheng and Zhang *et al*., 2021). Fungal endophytes can show symbiotic, mutualistic, commensalistic, and parasitic interactions with different hosts, according to the host genotype and environmental conditions. Fungal and bacterial populations that inhabit plant tissues and live there for all or part of their lives (Venieraki *et al*., 2017). They examined produced the catalyst required as invading together with colonising their own plant hosts (Schulz and Boyle *et al*., 2002). The inside cracks in roots serve as one of the primary entry points and endophytes can also live in particularly within the leaves (Venieraki *et al*., 2017).

After isolating the fungal endophyte via *Lolium persicum*, Freeman discovered it for the first time in 1904. Over three thousand different kinds of plants on the planet have to host one or more endophytes. Approximately 6,500 endophytic fungi were isolated (Schulz and Boyle *et al*., 2002). Their distinct physiological and metabolic processes allow them to both encode numerous bioactive substances and adapt to the particular environment found inside plants. Optimising the search for innovative bioactive secondary metabolites requires taking into account two important factors: (1) the secondary metabolites that a fungus produces can do match the ecological niche in which it lives, such as the mycotoxins produced by plant pathogens; and (2) metabolic interactions may promote the synthesis of secondary metabolites (Schulz and Boyle *et al*., 2002).

In comparison to fungal soil isolates and fungal diseases of plants, comparatively few endophytic fungal secondary metabolites have been isolated (Schulz and Boyle *et al*., 2002). These secondary metabolites are used as medicines. Plants can employ the bioactive compounds produced by endophytes to improve the growth, protect themselves from diseases, and potentially find new therapeutic targets known metabolites include phenols, phenolic acids, quinones, alkaloids, terpenoids, steroids, and peptides (Bano and Sharma *et al*., 2016).

Various endophytic fungi have produced substances like anticancer, antibacterial, anti-inflammatory, antiviral, antifungal, and have other properties. While some species have so far created materials that can be employed in the industrial sector, such solvents and enzymes, others have produced novel antimicrobials, such as the potent anti-cancer chemical taxol

from *Taxomyces andreanae* (Bano and Sharma *et al*., 2016). These enzymes include xylanase, phosphotases, amylases, proteinases, pectinases, cellulases, lipases, and laccase from the endophytic fungus *Monotospora* sp. (Patil and Sidhu *et al*., 2015).

**Endophytic fungi's significance**

The vast majority of plants live together symbiotically with endophytic fungi. They have the ability to protect their host plants from diseases and pests. By producing phytohormones, endophytes can promote growth without compromising with the host's capacity to absorb nutrients or speeding up its nutritional metabolism. Endophytes can reduce herbivory by producing alkaloids that are toxic to insects and other vertebrates. They enable host plants survive stresses from the environment including salt, heat, and other conditions. They serve an important role by lowering the degree of environmental degradation, loss of biodiversity, and deterioration of land and water brought on by excessive harmful organic pesticide, and dangerous gases. They can be employed as biocatalysts in the chemical transformation of medicines because of their ability to develop unique enzymes that aid in the production of substances of interest. They generate new bioactive metabolites that have latent sources of pharmacological leads and include antiviral, antibacterial, and anticancer chemicals. Endophytic fungi are the source of the drug taxol. Taxus, is used to treat ovarian and breast carcinoma among other cancers. In addition, it is utilised to treat a number of additional conditions that disseminate abnormal human cells. Certainly, endophytic fungi have developed the ability to produce bioactive substances that resemble those produced by the host plants, like hypericin, diosgenin, paclitaxel, podophyllotoxin, camptothecine, and vinblastine (Bano & Sharma *et al*., 2016). They are frequently employed in the production of vitamins, antibiotics, anticancer treatments, and pharmaceuticals that decrease cholesterol.

**Endophytic fungi and their relationships with the host plant**

Endophytic fungi are associated with plant microbiomes that reside in plant endospheric areas and generate a diverse range of chemically varied bioactive chemicals in response to environmental stimuli (Meena *et al*., 2019). Endophytes form symbiotic, mutualistic, or parasitic relationships with their host plant. Through seeds, endophytic fungi penetrate the growing plant and disperse throughout its various tissues (Patil *et al*., 2016). A mutualistic symbiosis is a common description of the endophyte-host relationship. In this case, certain endophytes acquire both food and protection from the host plant,

which in turn promotes plant growth and strengthens plant resistance to biotic (pesticides, pollutants, temperature etc.) and abiotic stress factors. Endophytes are thought to affect several important functions of the host plant, like encouraging the growth of plants, combating abiotic stressors and protecting against pathogen attacks. This Interactions can sometimes become dangerous. There are two recognised pathways by which an endophyte can develop into a phytopathogen. The first method is that the plant has wounds, which allow microbial pathogens to enter the plant without restriction. The second technique, known as plant become emaciated or senility is when an endophytic microbe changes from a favourable to a destructive state due to the production of toxins. This can occasionally lead to the death of the host plant (Slama *et al*., 2021).

**Potential biological activities of endophytic fungus**

Endophytic fungus produces a large number of specialized metabolites. A few illustrations of specialised metabolites Table 1 lists these plants along with their owners, which are generated by endophytic fungi and provides a wide range of possible biotic activities against other microorganisms and diseases.

**Table 1:** Endophytic fungi secondary metabolites with biological potential activities and their host plant

| Sl. No. | Plant host | Endophytic fungi | Secondary metabolites | Potential biological properties | References |
|---|---|---|---|---|---|
| 1. | *Plumeria acutifolia* | *Phomopsis sp.* | Terpenoid | Antibacterial activity | Kaul *et al*., 2012 |
| 2. | *Zea mays* | *Acremonium zeae* | -------- | Antibacterial and antifungal activity | Kaul *et al*., 2012 |
| 3. | *Tripterigium wiflordii* | *Cryptosporiopsis quercina* | Peptide | Antibacterial activity | Bano *et al*., 2016 |
| 4. | *Torreya taxifolia* | *Pestalotiopsis microsporum* | Quinone | Anticancer activity | Kaul *et al*., 2012 |
| 5. | *Catharanthus roseus* | *Mycelia sterilia* | Alkaloid | Anticancer activity | Kaul *et al*., 2012 |
| 6. | *Ginkgo biloba* | *Xylaria sp.* | Phenol and flavonoids | Oxidation inhibitor | Vasundhara *et al.* |
| 7. | *Terminalia morobensis* | *Pestalotiopsis microspora* | Pestacin and isopestacin | Oxidation inhibitor | Kaul *et al*., 2012 |

| | | | | | |
|---|---|---|---|---|---|
| 8. | *Trachelospermum jasminoides* | *Cephalosporium sp.* | Graphislactone A | Oxidation inhibitor | Kaul *et al*., 2012 |
| 9. | *Nerium oleander* | *Chaetomium sp.* | Flavonoids and phenolic acid | Oxidation inhibitor | Kaul *et al*., 2012 |

**Germicide pursuit of endophytic fungus**

Active organic natural compounds with a low molecular weight against other microorganisms very little amounts are known as antimicrobial agents (Kaul and Gupta *et al*., 2012). Numerous metabolites that endophytic fungi produced have been found to exhibit live antimicrobial activity and are found in a variety of chemical structural groups, including quinines, phenols, alkaloids, peptides, and flavonoids with antiviral, antifungal, and antibacterial properties. Antimicrobial agents that exhibit antifungal activity make up of pestaloside, ecomycins, pseudomycins, cryptocin, and pestalopyrone (Bano & Sharma *et al*., 2016).

Endophytic *Phoma* sp., which has been separated out of several therapeutic plants have the potential to contain antibacterial substances. In conjunction with cercosporamide, beta-sitosterol, and trichodermin, a novel alpha tetralone derivative (3S)-3,6,7-trihydroxy-alpha-tetralone stated to have been created by *Phoma* sp. Endophytic in *Arisaema erubescens*. These distinct substances demonstrated bactericidal and antifungal properties against two pathogenic fungi, including *Fusarium oxysporum*, *Rhizoctonia solani*, and *Magnaporthe oryzae*, as well as plant pathogenic bacteria, *Xanthomonas campestris* and *Xanthomonas oryzae*. The primary terpenoids that endophytic fungus produce are sesquiterpenes, diterpenoids, together with triterpenoids, all of which have antibacterial properties. An endophyte of *Plumeria acutifolia* Poiret, *Phomopsis sp*, yielded an ethyl acetate fraction a terpenoid substance having established antimicrobial properties. Endophytic *Chaetomium globosum* be isolated throu*gh G. biloba* and that it was the source of chaetoglobosins C, D, and A. The substances showed notable efficacy opposed to *Artemia salina* together with *Mucor miehei,* and they were derived from *Chlorinated azaphilone.* Considerable efficaciousness against *Aspergillus flavus* and *Fusarium verticollioides*, as well as antimicrobial efficacy opposed to the majority of drug-resistant forms of gram-positive bacteria, were demonstrated by *Acremonium zeae* endophytic in maize (Kaul and Gupta *et al*., 2012).

Steroids are naturally occurring chemicals that are widely produced by microbial communities as well as by plants and animals. The structure of

steroids, also known as sterane, is made up of four fused rings based on a carbon skeleton. Steroids are bio-lipid-based terpenoids. Additional pharmacological properties and physiological roles are attributed to endophytic steroids, including ergosterol, in their producers. Strong antibacterial properties have been observed in ergosterol and 5α,8α-epidioxyergosterol have been taken out of the endophytic fungus *Nodulisporium* sp. against variety of harmful microorganisms (Alam *et al*., 2021). The endophytic *Pestalotiopsisadusta* has yielded 2 novel medications pestalachloride A and B, which exhibit strong antifungal activity in the of *Verticillium aiboatrum, Gibberella zeae, and Fusarium culmorum*, three plant diseases (Bano & Sharma *et al*., 2016).

A novel peptide called cryptocandin was generated from the endophytic *Cryptosporiopsis quercina* after *Tripterigium wiflordii* demonstrated antimicrobial efficacy opposed to *Candida albicans*. Two novel antibacterial components, cyclo (Pro-Thr) and cyclo (Pro-Tyr), were generated by the mangrove plant-isolated endophytic fungus *Penicillium* sp. *Acrostichumaureurm* (Bano & Sharma *et al*., 2016).

**Anticancer activity of fungal endophytes**

It is known that fungi that are endophytes on plants are a significant a supply of later-stage metabolites that are biologically active. They have typically secrete substances that are cytotoxic in nature. The ability of fungal endophyte to generate significant plant-based anticarcinogenic medications have led to an increased focus on these sources of information (Vasundhara and Reddy *et al*., 2019). Currently, there are about 50 distinct fungal species known to produce 100 anticancer compounds. On 45 distinct endophytic fungal cell lines, nearly 19 distinct specialised metabolites' chemical classes have been determined.and shown to has anticarcinogenic qualities.. The quantity of anticancer compounds that have been extracted from endophytic fungus has continuously increased. Unchecked cell division, which encourages the spread of aberrant cells and tissue expansion is a characteristic of cancer. Representative examples include the plant-derived endophytic fungi that produce vinblastine, vincristine, podophyllotoxin, and paclitaxel (Taxol) derived from a number of *Pestalotiopsis* species and *Taxomyces* (Vasundhara and Reddy *et al*., 2019). The widely recognised and extensively modified tetracyclic diterpenoid bioactive substance paclitaxel, also known as taxol, was initially found in 1971 in the Taxus brevifolia woof. It has since been shown to have an effect on lung, ovarian, prostate, and breast cancers. The ability of the microtubules to become stable and disrupt their dynamic equilibrium is its main mode of action. Very small concentrations of paclitaxel

have been found in a variety of Taxus species' parts, including their needles, barks, as well as roots (Bano & Sharma *et al*., 2016).

A range of endophytic fungi were Identified and isolated from *Taxus chinensis* bark was examined by (Liu *et al*., 2009) 31 morphotypes have been assigned to the 115 endophytic fungal isolates that have been isolated. Cyclic dipeptides called diketopiperazines are known to inhibit the living thing cycle's G2/M phase. The group of compounds includes fumitremorgins, stephacidins, notoamides, tryprostatins, and many others that are generated by different strains of *Aspergillus fumigatus* and *Aspergillus fischer*. Human leukaemia P-388 has demonstrated efficacy against fumiremorgin C, while Prostate poison in humans PC-3 and human leukaemia U-937 have demonstrated antiproliferative activity against the associated substance 12,13-dihydroxyfumiremorgin C. Additionally, reports suggest that fumitremorgin C is an extremely effective a substance or process can damage cells or cause them to die opposed to colon and breast cancer that is resistant to multiple drugs. Gliotoxin's diketopiperazine ring has a disulfide bridge., a class of diketopiperazines with anticancer properties. This compound possesses antifungal, immunosuppressive, and antibacterial properties. It was extracted from *A. fumigatus* (Vasundhara and Reddy *et al*., 2019).

Torreyanic acid was produced as a quinone dimer by isolating Pestalotiopsis microsporum of *Torreya taxifolia*, an endophyte. It has been demonstrated that cytotoxic works five to 10 times as well in cell lines sensitive to protein kinase C agonists; it induces apoptosis in these cells. A new anticancer drug called ergoflavin was discovered in the *Micops elengi* leaf endophytes of the Indian medicinal plant. An alkaloid with cytotoxic properties, vincristine, was taken out of *Catharanthus roseus*'s endophytic *Mycelia sterilia*. This medication is primarily used as part of a chemotherapy regimen for nephroblastoma and acute lymphoblastic leukaemia (Kaul and Gupta *et al*., 2012).

**Antioxidant activity of endophytic fungi**

Antioxidants are compounds that have the potential to shield cells from the harm done by free radicals, which are unstable molecules (Kaul and Gupta *et al*., 2012). When it comes to preventing damage from reactive oxygen species (ROS) and oxygen-derived free radicals, antioxidant compounds are very effective. ROS can lead to carcinogenesis, cell degeneration, and damage to DNA, among other pathological consequences (Vasundhara and Reddy *et al*., 2019). Antioxidants have been demonstrated to be a promising medication for the management and avoidance of conditions like diabetes mellitus,

atherosclerosis, cancer, and heart disease, rheumatoid arthritis, neurodegenerative diseases, and ageing. Fungal endophytes linked to higher plants are emerging, and this presents a prospective source of new antioxidants. The compounds pestacin and isopestacin also exhibit antifungal and antimycotic properties (Bano and Sharma *et al*., 2016).

One derivative of naphthodianthrone is hypericin. It comes from a plant that is one of the main elements in the *Hypericum* genus and has a high therapeutic content. Pseudohypericin, protohypericin, protopseudophypericin, and the anthraquinone emodin, are their main constituents. Because of its ability to inhibit monoamine oxidase (MAO), hypericin has also been used as an antidepressant. Hypericin may also be used to improve wound healing, reduce inflammation, have antimicrobial and antioxidant properties, and treat seasonal affective disorder (Bano and Sharma *et al*., 2016).

Antioxidant properties among 49 endophytic fungi belonging to the family *Xylariaceae*, which are separated from Scapania verrucosa, a liverwort were investigated by Zeng *et al*. Two endophytic fungal isolates' ethyl acetate extracts were tested for their capacity to scavenge free radicals on hydroxyl and DPPH radicals. These studies suggested that endophytic fungi that were separated from *S. verrucosa* might offer a novel supply of antioxidants found in nature. Certain plant species that produce rhodiola have pharmacological effects that include antioxidant and antiaging qualities. From the *Ginkgo biloba* medicinal plant, Liu *et al*. identified endophytic *Xylaria* sp. And assessed its antioxidant capacity. Because the methanolic extract contained 41 identified compounds, including "phenolic" and "flavonoid" compounds, it demonstrated a strong antioxidant capacity (Vasundhara and Reddy *et al*., 2019).

Finding pestacin and isopestacin in *Terminalia morobensis*, a source of antioxidant chemicals from *Pestalotiopsis microspora,* antioxidant potential of this understudied group of fungi was investigated. Naturally occurring as a racemic mixture, pestacin, 1, 3-dihydro isobenzofuran functions by cleaving an uncommonly O-H abstraction and, to some degree, reactive C-H bonds, by O–H abstraction. Isopestacin, also known as isobenzofuranone, scavenges superoxide and hydroxyl free radicals, thereby acting as an antioxidant.

From an endophytic in *Trachelospermum jasminoides*, *Cephalosporium* sp., graphislactone A was isolated. *In vitro*, the compound demonstrated greater antioxidant activity when compared to the positive controls, butylated hydroxytoluene and ascorbic acid. The fungus *Chaetomium* sp. From *Nerium oleander* produces flavonoids and phenolic acid derivatives that have potent

antioxidant activity, it may be a promising antioxidant resource. (Kaul and Gupta *et al*., 2012).

## Conclusion

Healthy plants have endophytic fungi living inside of them without harming them and numerous plant species have been shown to harbour endophytic fungus. There may be uses for them in the food and medicine sectors and are of biotechnological interest and have demonstrated the ability to generate a broad variety of metabolites with biological functions. Their unique ability to form related compounds along with other substances that are bio-active and come from their owner plant has sparked interest in both applied and basic research fields. Researchers focused primarily on the variety of fungi that are endophytes, the interactions between the fungi with owner plants, and extraction of naturally occurring bio-active compounds from endophytic fungus. They are a valuable origin of numerous essential bio-active substances with microbes' inhibitor, insecticidal, cytotoxic, antioxidant, and anticancer properties. Numerous investigations demonstrate endophytes produce specialised metabolites on their own to protect themselves from pathogenic invasion. It also assembles the urgent need to find antibiotics that are not harmful to the environment, very effective, and do not interfere with the growth of resistant bacterial species. This review focused on the ways in which endophytic fungi and plants interact, as well as how these interactions affect the processes and mechanisms engaged in the process of producing specialised metabolites the fungal endophyte specialised metabolites that were separated. Fungal endophyte research has a lot of exciting discoveries in preserve for the future, which will be crucial for finding economical and environmentally responsible solutions to problems affecting people and the environment.

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# Chapter - 8

## Cystic Fibrosis: An Inherited Life-Threatened Genetic Disorder


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# Chapter - 8

## Cystic Fibrosis: An Inherited Life-Threatened Genetic Disorder

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### Abstract

Cystic fibrosis is a rare genetic disorder causing thick mucus buildup in organs like lungs, livers, and pancreas, caused by a mutated CFTR gene. Many complications are attached to this gene mutation, such as: Pneumothorax, and chronic infection in the respiratory system. Diabetes and DIO syndrome are associated with the digestive system. Fertility is reduced in a woman's body in the reproductive system. Some specific tests and therapies are too responsible for the diagnosis of this disease, including tests: in this test, blood samples are tested for the CFTR Genes that cause CF. The specialization of this test is to detect the levels of immunoreactive trypsinogen. For those with higher levels of immunoreactive trypsinogen in their blood, we can determine that cystic fibrosis is formed in their body. Medicaments: Some medicines that are important for the prevention of infections and also help the body get nutrients. Various medications are listed: Antibiotics can treat lung or pancreas infections. Different types of anti-inflammatory antibiotics try to prevent this disease such as: ibuprofen, and corticosteroids. CFTR Modulators: these are a class of drugs that can make the lungs work better and help to gain weight. DNA test:- this test is significant for detecting mutations in CFTR Genes. It is called a type of "genetics test". Sweat test: This test measures the amount of salt in the sweat. If a higher amount of salt is present in the sweat, it denotes cystic fibrosis disease.

**Keywords:** Autosomal recessive disease, cystic fibrosis, DNA test, genetic disorder, pneumothorax.

### Introduction

Cystic fibrosis is a life-shortening autosomal recessive disease affecting Northern Europeans due to a mutation in the cystic fibrosis transmembrane conductance regulator gene.

The disease affects various organs and kills 90% of patients, leading to lung infections and tissue degradation. It affects over 30,000 US and 70,000 global patients, with varying incidence rates globally. The median survival time for CF patients is 47.4 years, which is attributed to the CFTR protein, a chloride channel. That transports bicarbonate and chloride was determined in 1989. Variations in the CFTR gene that cause CFTR protein deficit or dysfunction determine the phenotype known as CF. The CFTR protein, with 27 exons and 1480 amino acids, is found in epithelial cells in various organs. Make up the CFTR protein. Mucous secretions thicken and become dehydrated due to aberrant fluid transfer caused by CFTR protein shortage or malfunction, ultimately compromising organ function. The prognosis for cystic fibrosis has significantly improved over the past 20 years due to therapeutic advancements and interdisciplinary healthcare provided by specialist centers. Adults with CF outnumber children in high-income nations, indicating that CF has evolved into an adult illness. However, due to its multisystem symptoms, cystic fibrosis remains linked to lower The CF National Research Strategy group limited access to expensive CFTR modulator treatments, despite their widespread availability to many CF patients the current state of CF and identifies obstacles, as not everyone has access to expensive medications and some individuals have non-responsive gene variation (Chen *et al*, 2021, Lu *et al*, 2021).

**Genetic mutation of CFTR**

CFTR gene mutations are classified into six categories, with classes I-III causing more severe disease. Clinical presentations may vary due to gene modifiers. CF genotype-phenotype associations vary between lung illness and pancreatic insufficiency. Therapeutic decisions are based on patient growth, lung function, and nutritional status (Zelenková *et al*, 2017).

**Characteristics of CFTR**

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a key protein in the ABC family. CFTR functions as a chloride channel, controlled by cAMP-dependent phosphokinases. Its structure includes six membrane-spanning motifs, two intracellular nucleotide-binding folds, and a highly charged 'R domain'. Phosphorylation of CFTR activates the chloride channel (Zelenková *et al*, 2017).

**Causes of cystic fibrosis**

The most frequent deadly hereditary condition is cystic fibrosis. Cystic fibrosis (CF) is an autosomal recessive disease caused by about 2000

mutations in the cystic fibrosis transmembrane conductance regulator protein gene is mutated in both copies, which results in cystic fibrosis. Those who own a single functional copy are carriers, everyone else is normal. The production of mucus, digestive juices, and perspiration is mediated by CFTR. Secretions that are typically then instead become bulky when CFTR is not functioning. Cystic fibrosis (CF) is caused by a mutation in the cystic fibrosis conductance regulator (CFTR) gene, causing 90% of cases and two-thirds of CF worldwide. Carriers of this mutation have a 1 in 4 chance of inheriting an aberrant copy from each parent. This results in abnormal salt and water movement, causing thick mucus and clogging ducts, causing recurrent infections, lung inflammation, and food malabsorption. Approximately 1500 distinct mutations have been identified to cause CF (Costello *et al*, 1988; Chen *et al*, 2021)

**Symptoms of cystic fibrosis**

Cystic fibrosis (CF) is a condition affecting the lungs, pancreas, liver, kidneys, and gut. It is typically diagnosed in infants and involves chronic sinusitis, persistent infection with common CF pathogens, recurrent pancreatitis, malnourishment, and male urogenital disorders. CF can lead to osteoporosis, sinus disease, and reproductive problems. Symptoms include lung illness, malnutrition, and gastrointestinal issues like dilated intestinal obstruction syndrome. In adults, CF can lead to liver enzyme levels, cirrhosis, and an increased risk of cholelithiasis and nephrolithiasis. CFRD is a distinct form of the disease, distinct from insulin-dependent and non-insulin-dependent diabetes.

Cystic fibrosis results from the pancreas's inability to produce enough insulin to respond to carbohydrate intake, leading to diabetes. Poorly managed hyperglycemia increases the risk of death and lung capacity loss. Osteopenia, osteoporosis, and low vitamin D levels are common endocrine consequences. Factors like male infertility, idiopathic pancreatitis, or nasal polyposis should be considered (Costello *et al*, 1988; Quint *et al*, 2021; Fan *et al*, 2017).

**Diagnose criteria of cystic fibrosis**

To diagnose CF43, a sibling with CF, positive neonatal screening, or clinical symptoms consistent with CF in at least one organ system and abnormal nasal potential difference (NDP) result are required.

**Difficulties with CF diagnosis**

It might be challenging to identify CF because of the vast range of clinical phenotype variations among persons with the disease. Chinese individuals

with CF experience fewer organ-related symptoms overall, and fewer digestive symptoms in particular than their caucasian counterparts, aside from the respiratory presentation. Approximately 10% - 15% of CF patients have minimal symptoms, healthy pancreas function, adequate nutrition, lung function deterioration, no family history, normal sweat test findings, and only one CFTR gene-associated mutation.

**Cystic fibrosis management & treatment**

Early intervention in cystic fibrosis involves assessing, refining, and implementing treatment regimens, with vigilant monitoring. Prospective patients should be hospitalized until test results confirm or rule out a diagnosis.

**Regimens for treating CF**

Antibiotics, airway clearance, and mucus thinner medication are all part of the treatment for CF lung disease. To thin mucus, hypertonic saline inhalation therapy is used to hydrate heavy mucus in the airways of CE patients. Water is extracted from airway epithelial cells by the solution's high osmotic pressure, which helps replace Cystic fibrosis (CF) patients who often require chest physiotherapy to remove purulent secretions, while long-term oral antibiotics are not recommended for infection control. Long-term azithromycin is recommended for CF patients due to its anti-inflammatory and antibacterial properties, while aerosolized antibiotics like tobramycin and aztreonam are beneficial for lung function. Significant neutrophil inflammation is the primary pathogenic characteristic associated with CF airways. Inflammation was once considered important for stopping the spread of illness, but mounting data indicates that excessive inflammation is often detrimental.

Therefore, regardless of the presence of *P. aeruginosa* infection, azithromycin is advised in clinical practice for any CF patients older than 6 who exhibit clinical indications of airway inflammation such as a persistent cough) or any decline in FEV1. Supplement diets with pancreatic enzymes, calories, and fat-soluble vitamins for CF patients' growth and nutrition. Novel drug types, like CFTR modulators, target mucociliary clearance and protein function. Several possible Trikafta, the third FDA-approved medication for CFTR protein deficiencies caused by F508del gene mutations, is effective in individuals with one copy of the F508del-CFTR mutation for at least 24 weeks. In end-stage patient treatment, lung transplantation may be a viable option.

**Management of cystic fibrosis**

Long-term follow-up and monitoring of CF patient status are crucial, including nutritional assessments, immunizations, and follow-up care. Psychological counseling is essential for optimal CF management, as chronicity can negatively impact mental health.

**Handling of cystic fibrosis**

Timely diagnosis, treatment, and long-term follow-up are crucial for cystic fibrosis patients. Nutritional assessments, immunizations, and monitoring are essential. Prompt assessment of exacerbations, treatment of serious infections, and follow-up care are crucial. The chronic nature of CF can negatively impact mental health, necessitating psychological counseling for treatment compliance and long-term prognosis (Michael *et al*, 2019).

**Conclusion**

Nowadays, the deadly hereditary CF disease is medicable but not curable. Approximately 90% of people with CF who are 2 years or older may benefit from a combination of CFTR potentiators such as: Ivacaftor, and Tezacaftor. We have to do more research dedicated to CF patients. Scientists continue to conduct research and make strides in the right way. Recently development of specific therapies that easily improve the basic pathophysiology. Until a cure is found treatments, proper diet, exercises, and CFTR potentiator medicines aid in giving people with CF disease a better quality of life.

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# Chapter - 9

## Green Synthesized Silver Nano: A Potential Hope for Environmental Remediation


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# Chapter - 9

## Green Synthesized Silver Nano: A Potential Hope for Environmental Remediation

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**Abstract**

Nanotechnology is regarded as the most significant innovation in science which deals with various promising aspects in the field of Health and Environment in the twenty-first century. In the present scenario, silver (Ag) nanoparticles (NPs) are of immense interest to researchers due to their remarkable outcomes, especially in environmental remediation. To fabricate a clean sustainable environment, a variety of emerging techniques have become the global focus to mitigate and reduce environmental pollution which is now a worldwide issue. Among them, the biogenic (plant-based) technique in the synthesis of NPs can be extensively explored. The green synthesized NPs can be widely employed for the treatment different dyes from industries through convenient and simple manner. The present study serves as a brief overview of recent trends in green synthesis, and application in dye removal of plant-extract-based AgNPs. In addition, we review the biogenic approaches in detail as promising methods for AgNPs synthesis due to their safety aspects. The function of AgNPs in environmental remediation is well addressed in this paper.

**Keywords:** Nanoparticles, green synthesis, AgNPs, Environmental remediation, Dye-removal.

### Introduction

Nanoscience is the innovation, handling, and application of molecules whose size is about 1 to 100 nm. According to Roy *et al*., (2022), there are several kinds of nanoparticles (NPs) like metallic, mineral, organic, polymer-based, and nanocomposite. The vital role of nanotechnology is identified in the field of optics, electronics, gene-mediated drug, mechanics, biomedical science, catalysis, optoelectronic devices, chemical industry (Singh *et al*., 2019).

Recently, the principle of sustainability has been totally ignored by numerous anthropogenic activities like industrial activities. Among various strategies, a new idea named nanotechnology can be adopted to restore a sustainable environment. Nanotechnology includes the synthesis and applications of nanomaterials at the nanoscale in various fields like chemistry, material science, physics etc. (Megarajan *et al*., 2022).

Immense interest was growing in generating new green approaches to the synthesis of NPs from biological molecules of living organisms due to its adverse applicability in various nanotechnology disciplines such as biosensing, electronics, catalysis, chemistry, medicine etc. Moreover, these green–based NPs are not involved in causing secondary pollution. Green synthesis of nanomaterials targets reducing the application of toxic chemicals and limiting the production of harmful by-products. In nanotechnology, cost-effective and non-hazardous plant- extracts have been used instead of detrimental reducing agents. The plant extracts have some special qualities like appropriate morphology, size dispersion, biological compatibility, and suitable surface functionalization. In addition, some biological molecules present in the plant extracts show vital antiviral, antibacterial, and cytotoxic effects (Pang *et al*., 2020; Rajkumar *et al*., 2021).

These plant-based engineered silver nanomaterials are highly applicable in nanoremediation to mitigate polluted media. This uniquely designed NP has drawn great attention to researchers due to some exceptional properties like catalytic activity (Jain *et al*., 2020). These are efficiently applicable in dye removal from industrial wastewater.

Nanostructures of silver are suitable as promising SERS in environmental chemical sensing and the detection of environmental disorders.

A little attention was paid on the contribution of silver NPs in environmental remediation and dye removal previously. It is also expected that this review will improve the biosynthesis process of AgNPs.

**Bio-synthesis of AgNPs**

Trees are considered as laboratory in environment that have significantly capacity in heavy metal absorption and detoxification by which environmental pollution can be mitigated. In Environmental Science, the biosynthesis of NPs regarded as “green chemistry” or “green synthesis” which is utilized for fabricating clean, economical and eco-friendly NPs using plants, fungi, bacteria etc. (Patel *et al*., 2023). There are some physical and chemical process in generating NPs that are advantageous for well – controlled size and shape NPs production at huge amounts. But the drawbacks of these processes are higher loss of energy and capital, using toxic chemicals, and production of

huge amounts bio – bio-waste. Moreover, toxicity, stability, and biocompatibility limit the application of NPs in biomedical fields. For example, the technique of water laser ablation can produce Ag NPs (20 – 50 nm) nano–spheroids. But the disadvantage is the insufficiency of the surface construction (Rather *et al*., 2021). Therefore, it is necessary to find out a biosafe and low – cost methods to fabricate nanomaterials. One of these methods is green technology which follows the principle of green chemistry. This process is environmentally – safe, non-toxic, low-risk, and low-cost because of using recyclable bioresources and converting them into more applicable, eco-friendly nanomaterials.

Green synthesis of NPs using plant extracts is regarded as the most reliable approach. The availability of green plants, easy handling, cheap reagents, etc makes this process advantageous (Y.T. Gebreslassieand H.G. Gebretnsae, 2021). There are some drawbacks also. It includes the change of color. Precursor salts change the color of the solution during plant incubation.

Leaves of the plants can be utilized for the bio-synthesis process of AgNPs preparation. At first, leaves are washed thoroughly. Then these are dried at shadow which follows the subsequent process of weighing and crushing. At the next step, Milli – Q water is mixed with crushing leaves. Afterward, this mixture is boiled and stirred continuously. Whatman filter paper is then used to filter the boiled solution. Finally, the deposited clear solution can be used for further experiments. (Keshari *et al*., 2020). Table 1 shows a list of the several plants that are employed in the literature to synthesize AgNPs for dye removal.

**Table 1:** List of different plants used in the synthesis of AgNPs for removal of dye as reported in the literature

| Plants | Size of AgNPs (nm) | Targeted Dyes | Ref. |
|---|---|---|---|
| *Polyalthia longifolia* | 5 to 20 | Methylene blue, Crystal violet Methyl Orange | Ghosh *et al*. 2020 |
| *Ruellia tuberosa* | 10 - 20 | Coomassie brilliant blue, | Seerangaraj *et al*., 2021 |
| *Plumbago auriculata* | 98 | Methylene blue | Bloch *et al*., 2022 |
| *Cestrum nocturnum L.* | 91.1 | 4 – nitroaniline, 4-nitrophenol, Congo red, Methylene Blue | Kumar *et al*., 2022 |
| *Punica granatum* | - | Brilliant Flavin Yellow – 40, Red - 227 | Rasool *et al*., 2024 |

**Role of plant extract in AgNPs green- synthesis**

During this process, a metal - salt is added to a phytochemical solution under various conditions. The metal-salt concentration, phytochemical type, pH and temperature of the solution etc. parameters govern the rate of production and stability of formed NPs. Flavones, terpenoids, carboxylic acids, sugars, ketones, polyphenols, phenolic acids, aldehydes etc. are some metabolites present in plant extract that play a vital role in stabilizing and reducing of NPs (Chand *et al*., 2020).

Terpenoids are organic polymers that originated from five-carbon isoprene units in the plant extract. It is reported that terpenoids have strong antioxidant properties and convert silver ions into NPs. Flavonoids including anthocyanins, isoflavonoids, flavones, flavanones, and chalcones classes contain different functional groups. A tautomeric conversion takes place in flavonoids and a reactive hydrogen atom is released which reduces metal ions to NPs (Adeyemi *et al*., 2022).

Green synthesis techniques of NP generation include three (Figure 1) stages (Shukla and Iravani 2018; Vanlalveni *et al*., 2021):

a) Reduction Phase: At reduction stage, $Ag^{+}$ ions are converted to zero – valent Ag atoms by phytoactives present within plant extracts through electron transfer process.

b) Growth Phase: In the growth Phase, growing of zero-valent metal ions occur by aggregation of NPs in different shapes such as linear, rod-shaped, triangular, hexagonal etc.

c) Termination stage: In the termination stage, NPs are stabilized and capped by phytochemicals.

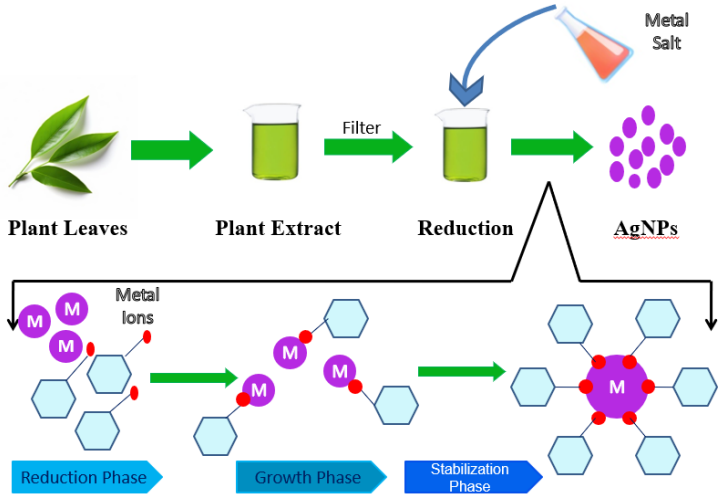


**Fig 1:** Schematic of bio-synthesis process of AgNPs

**Application of AgNPs for dye degradation**

Green synthesized AgNPs can degrade hazardous dyes like Methyl Blue, Methyl Orange, Methyl Red, Safranine O, etc. due to their catalytic activity. To make a stock solution, at first, 1L of distilled water is added to 10 mg of dye. Thereafter, silver NPs are mixed with stock ultrasonically. Finally, this stock can be utilized through UV-visible spectroscopy to determine dye absorbance maxima.

Bio-synthesized silver NPs are applicable for purifying industrial effluents and waste water-containing organic dyes due to their catalyzation power and stability (Seku *et al*., 2021). Isa and Lockman, 2019 investigated the degradation of Methyl Blue by AgNPs produced from plant extracts with the help of $NaBH_4$ which is a strong reducing agent. $NaBH_4$ acts as an electron donor and a hydrogen supplier during this process. Due to its high negative potential, it serves as a medium to exchange the electrons between $BH_4^-$ ion and dye. Electron and hydrogen continuously transport between $NaBH_4$ and absorbed dye molecule. The molecular formula of Methylene Blue is $C_{16}H_{18}ClN_3S$.

Methyl Red, one of the potential dyes becomes a threat to the environment recently. AgNPs as photocatalysts can degrade it significantly. The degradation value of Methyl Red by silver NPs is $1.03\times10^{-3}$ $min^{-1}$. Safranine O, a derivative of phenazine, is a heterocyclic azine group of dyes. UV-visible spectrophotometer monitors the degradation of Safranine O by AgNPs where the foremost absorption peak is at 519 nm (Jyoti and Singh, 2016).

## Conclusion

The convergence of green chemistry and nanotechnology has randomized the way for the development of nontoxic, large-scale nanomaterial synthesis methods. This review paper provides a current overview of the potential application of these plant-mediated NPs in environmental remediation especially in dye removal. The plant extract derived from renewable plant-based resources enhances the controlled synthesis method which makes size uniformity and stability of NPs with catalytic capacity to dye removal. Despite the significant progress in this field, the exponential accumulation of NPs in the environment needs to be focused, on so that harmful effects can be minimized and inspire us to utilize these green synthesized NPs as pioneers of environmental remediation.

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# Chapter - 10

## Invisible Foes, Audible Impact: Unmasking Indoor Air Pollution - A Silent Threat to Human Health


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# Chapter - 10

## Invisible Foes, Audible Impact: Unmasking Indoor Air Pollution - A Silent Threat to Human Health

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**Abstract**

Millions of people die each year as a result of indoor air pollution (IAP), which poses a major risk to human health. Numerous contaminants have the potential to cause indoor air pollution (IAP), thus it's critical to pinpoint their primary sources and concentrations as well as develop control and improvement plans for Indoor air quality (IAQ). The main sources of major pollutant emissions, their health impacts, and concerns about IAP-based disorders, such as sick building syndrome (SBS) and building-related illness (BRI), are reviewed and evaluated critically here. Furthermore, the methods and techniques for regulating and lowering the concentrations of pollutants are highlighted, and the latest developments in the endeavours to address and enhance IAQ, along with their corresponding benefits and possibilities, are outlined.

**Keywords:** Indoor air pollution, indoor air quality, health impact, indoor air quality, aerosol.

### Introduction

Over the past twenty years, an increasing amount of scientific research has demonstrated that interior air pollution can be significantly higher than outdoor pollution (Leech J.A., *et al.* 2002). Since most people spend their time indoors in their homes, offices, or other working locations, public facilities, or when commuting, the majority of human exposure to contaminated air takes place indoors. Nonetheless, regulations and criteria for air quality have been set mostly for outdoor air. The health impacts of indoor air pollution, both indoor and outdoor, are particularly relevant, especially among susceptible population groups, given the longer exposure times in various interior microenvironments.

The World Health Organization (WHO) estimates that 3.8 million (https://www.who.int/en/news-room/fact-sheets/detail/household-air-

pollution-and-health) fatalities per year are caused by indoor air pollution, or Indoor Air Pollution. IAP can be produced by residents' activities inside of houses or buildings, including cooking, smoking, using electronics, using consumer goods, and emitting emissions from building materials-aerosol, Particular matter (PM), Carbon monoxide ($CO_2$) volatile organic compounds (VOCs), biological contaminants, and others are among the harmful pollutants found inside building (Kumar P. *et al*, 2013)

IAP usually consists of a complex mixture of particulate particles and other gaseous components. IAP compositions differ significantly depending on sources, ventilation rates, and emission rates. Thus, for effective IAQ treatment, locating the sources of air pollution is crucial. (Hamanaka R.B. *et al* 2018). Additionally, it is believed to be critical to set up monitoring systems to ascertain the concentrations of indoor pollutants in addition to critical IAQ management and improvement initiatives.

**Indoor Air Quality (IAQ) and Indoor Air Pollution (IAP)**

The EPA defines IAQ as the air quality within and surrounding buildings and structures, with an emphasis on how it impacts the health and comfort of building occupants. In contrast, indoor air quality (IAP) refers to the presence of pollutants in high concentrations found in non-industrial buildings' indoor air, including biological factors, particulate matter (PM), volatile organic compounds (VOCs), inorganic compounds, physical chemicals, and others that may have detrimental effects on human health. There are two main reasons why thermal conditions are important in indoor air quality:

**Many problems associated with low IAQ can be readily addressed by adjusting temperature and relative humidity**

**Involvement of Building construction materials**

Through ventilation, contaminants from outside the building are brought into when their concentrations rise. As a result, in addition to the lifetimes and mixing ratios of these contaminants, the ventilation rate has a significant influence on the link between outdoor air pollution and IAQ. IAP is typically caused by waste gases, tobacco smoke, pesticides, solvents, cleaning supplies, particles, dust, mold, fibers, and allergens released during daily activities. Additionally, millions of mold, fungi, pollen, spores, germs, viruses, and insects like roaches and dust mites thrive because of the favorable conditions that humans produce. Particulate matter (PM), carbon dioxide ($CO_2$), sulfur dioxide ($SO_2$), carbon monoxide (CO), and nitrogen dioxide ($NO_2$) are among the gases released into indoor air environments by combustion sources and cooking actions.

Crucially, ventilation system operation and design also have a big impact on indoor air quality. Ventilation produces appropriate IAQ and a healthy interior environment by replacing the stale indoor air with fresh outdoor air. The operation of ventilation in a building has various advantages such as: (i) supplying fresh air and oxygen for human respiration; (ii) diluting smells and vapors, as well as indoor air pollutants, to short-term exposure thresholds of hazardous chemicals; (iii) controlling aerosols inside buildings by using outdoor air with low aerosol concentration; (iv) regulating internal humidity; and (v) establishing appropriate air distribution and fostering a healthy and comfortable environment.

In some buildings or climates, natural ventilation systems are inadequate, even though the residents may readily embrace them. The widespread usage of mechanical ventilation systems in buildings these days dramatically raises energy consumption. In order to reduce energy consumption and promote the use of sustainable technologies, hybrid ventilation systems are made to benefit from both mechanical and natural ventilation systems. The drawbacks of natural ventilation will be made up for in hybrid ventilation systems by mechanical components. In conclusion, ventilation is crucial to achieving appropriate indoor air quality (IAQ) in buildings' HVAC systems, but it also contributes to energy use.

**Table 1:** Probable indoor air pollutants and relative health hazards

| Pollutant | Sources | Health Effects | ref |
|---|---|---|---|
| 1. Particulate Matter | Tobacco smoke, cooking, cleaning agents | Respiratory irritation, cardiovascular problems | |
| 2. Volatile Organic Compounds (VOCs) | Paints, solvents, cleaning products | Eye, nose, and throat irritation, long-term health issues | |
| 3. Formaldehyde | Building materials, furniture, tobacco smoke | Respiratory irritation, potential carcinogen | |
| 4. Carbon Monoxide (CO) | Combustion appliances (e.g., stoves, heaters) | Headaches, dizziness, nausea, potentially lethal | |
| 5. Nitrogen Dioxide ($NO_2$) | Combustion processes (e.g., gas stoves, heaters) | Respiratory irritation, increased asthma symptoms | |
| 6. Radon | Soil, building materials | Lung cancer (particularly in | |

| | | | |
|---|---|---|---|
| | | combination with smoking) | |
| 7. Biological pollutants | Mold, pollen, pet dander, dust mites | Allergic reactions, respiratory issues | |
| 8. Lead particles | Lead-based paints, dust, soil | Neurological and developmental issues in children | |
| 9. Asbestos | Building materials, insulation | Respiratory issues, lung cancer | |
| 10. Ozone ($O_3$) | Indoor air reactions, electronic devices | Respiratory issues, aggravation of existing conditions | |

**IAQ guidelines and standards**

It is clear that even at low air pollutant concentrations, prolonged exposure to indoor anthropogenic activities can degrade IAQ and pose serious health hazards to people. The scientific community and pertinent organizations have tried to create and implement IAQ standards and recommendations in order to address these IAQ issues. The international community finally established IAQ standards and guidelines based on an integrated building strategy after much effort (Avgelis A., *et al*. 2004). The aim is to remove, or at least reduce, potential dangers to human populations because the WHO (World Health Organization) and USEPA (United States Environmental Protection Agency) state that the purpose of IAQ guidelines is to provide a crucial database as a reference for the prevention of detrimental repercussions of IAP and protection of public health **(**WHO report, 2010.)

The WHO and USEPA standards often specify the highest values for particular times periods (e.g., one hour, twenty-four hours, or a year) (Becher R., *et al.* 2000). Furthermore, as the WHO and USEPA's IAQ guidelines are often applied for the control of IAQ within homes, schools, hospitals, public buildings, and workplaces, it appears that they are not applicable to occupational sectors. Each nation will also create unique criteria or norms that are appropriate for their own unique set of circumstances

**The effects of indoor air pollution to human health**

**Building-associated illness**

Decreased indoor air quality (IAQ) in homes and buildings has been connected to a number of symptoms and diseases in recent decades. Even at low concentrations, indoor exposure to inorganic, chemical, physical, and

biological pollutants is frequent, pervasive, and prolonged. Consequently, there has always been a lot of concern and attention focused on the negative impacts of IAP on human health. The World Health Organization defines "building-associated illness" as any illness brought on by indoor environmental variables, which are typically separated into two categories: Building-related illnesses (BRI) and sick building syndrome (SBS).

**Sick Building Syndrome (SBS):** Acute health and comfort effects of SBS will appear when patients spend a certain amount or duration of time in a building, but they and their causes are difficult to clearly identify.

Four classes have been established by the WHO to categorize SBS symptoms caused by IAP: Asthma and asthma-like symptoms, such wheezing and chest tightness; (ii) neurotoxic effects, like headaches, irritability, and fatigue; (iv) dryness and irritation of the skin; gastrointestinal problems (like diarrhea), and others.

**Building-Related Illness (BRI):** BRI recognizes illnesses and symptoms that have a proven causal agent and are brought on by exposure to indoor air quality issues. Although biological agents are more prevalent, chemicals such as formaldehyde, xylene, pesticides, and benzene are acknowledged as causative contributors. Common sources of indoor biological pollutant emissions include buildings with cooling towers, humidification systems, filters, drain pans, moist surfaces, and water-damaged building components. BRI symptoms, which include fever, chills, tightness in the chest, muscle aches, and coughing, have been connected to the flu. Significant lung and respiratory problems are also likely to occur. Legionnaires' disease, hypersensitivity pneumonitis, and humidifier fever are common BRI ailments. According to reports, there are four main ways that indoor environmental contaminants might result in BRI symptoms; (i) Immunologic, (ii) infectious, (iii) toxic, and (iv) irritant**.**

**Acute respiratory infection:** Because pollutants enter the human body by breathing, the respiratory system is frequently where IAP effects are most prominent. Acute respiratory infections (ARIs) can be divided into acute lower respiratory infections (ALRIs) and upper respiratory infections based on the part of the respiratory tract that is affected. URIs, or upper respiratory infections, are typically minor conditions brought on by biological contaminants and manifest as symptoms including cough, sinusitis, and otitis media.

**Pulmonary diseases:** Allergy and pulmonary conditions such atopic dermatitis, allergic rhinitis, and asthma are linked to inhaled air pollution.

Furthermore, One of the primary risk factors for the development of chronic inflammatory lung diseases is thought to be smoking,such as lung cancer, asthma, and chronic obstructive pulmonary disease (COPD). Exposure to IAP can have a substantial impact on the maximum amount of lung function obtained; as a result, lung function declines. PM and CO are two examples of harmful particles that might affect lung development early in pregnancy.

Exposure to indoor air pollution has the potential to exacerbate asthma attacks or produce asthma symptoms. Acute exposure to combustion smoke has been shown to increase bronchial reactivity, cause inflammation and irritation, and is thought to be the primary cause of asthma exacerbations, particularly in youngsters. Research has shown that children aged 5 to 14 who reside in homes that burn coal, wood, or kerosene have a 1.6 relative risk of experiencing an asthma attack.

**Cardiovascular Diseases (CVDs):** Solid fuel consumption in homes can release a number of pollutants, including as heavy metals, PM, CO, PAHs, and other organic pollutants, that are connected to CVDs. Certain acute cardiovascular diseases (CVDs), including ischemic stroke, myocardial infarction, cardiac arrhythmia, heart failure, and atrial fibrillation, are more common in people exposed to PM2.5. According to reports, PM can lead to oxidative stress, systemic inflammation, elevated blood coagulability, and imbalances in the autonomic and vascular systems, which can result in CVDs. Significant increases in fibrinogen, platelet activity, plasma viscosity, and the release of endothelins - a family of powerful vasoconstrictor molecules are also primarily caused by PM.

**Conclusion**

To minimize indoor air pollution, several practical recommendations can be implemented. First and foremost, adequate ventilation is crucial. Proper ventilation helps to exchange indoor air with fresh outdoor air, reducing the concentration of pollutants. Regular maintenance of heating, ventilation, and air conditioning (HVAC) systems is essential to ensure their efficiency and prevent the circulation of pollutants. Additionally, the use of air purifiers with HEPA filters can help trap and remove particles, allergens, and pollutants. Avoiding smoking indoors and using non-toxic cleaning products also significantly contributes to reducing indoor air pollution. Finally, promoting the use of indoor plants, such as spider plants or snake plants, can act as natural air purifiers by absorbing pollutants and enhancing overall indoor air quality. Implementing these recommendations fosters a healthier indoor environment, safeguarding the well-being of occupants.

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# Chapter - 11

## Navigating Neurotechnological Frontiers: Prospects and Trajectories of Neuralink's Brain-Machine Interface


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# Chapter - 11

## Navigating Neurotechnological Frontiers: Prospects and Trajectories of Neuralink's Brain-Machine Interface

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### Abstract

Neuralink, a pioneering force in neurotechnology, aims to revolutionize brain-machine interface (BMI) technology, eliciting widespread anticipation regarding its potential applications and forthcoming advancements. This abstract navigates through the anticipated uses and trajectories of Neuralink's BMI system, envisioning transformative impacts across medical, communicative, and augmentative realms. Neuralink's BMI harbors promise in reshaping healthcare by offering innovative interventions for neurological conditions like Parkinson's disease and spinal cord injuries, employing neural stimulation and control. Furthermore, it presents opportunities for augmenting communication and autonomy for individuals with disabilities through direct brain-to-computer interfacing. Looking ahead, Neuralink's journey is marked by progress in miniaturization, wireless connectivity, and neural decoding algorithms. Miniaturization endeavors to refine implantable devices, prioritizing less invasive solutions for enhanced usability. Innovations in wireless connectivity aim to seamlessly integrate neural implants with external devices, facilitating real-time data exchange. Additionally, advancements in decoding algorithms seek to improve the precision and efficiency of translating neural signals into actionable commands, broadening the functionality of Neuralink's BMI system.

**Keywords:** Brain-machine interface, future directions, medical applications, neuralink, neurotechnology.

### Introduction

In recent years, the union of neuroscience and technology has paved the way for modern and ground-breaking options poised to revolutionize our understanding and interaction with the human brain. Among these ground breaking advancements stands Neuralink, a pioneering initiative led by Elon

Musk, the visionary entrepreneur renowned for his ventures such as Tesla and SpaceX. Established in 2016, Neuralink remained relatively obscure until July 16, 2019, when it unveiled a seminal white paper elucidating its ambitious mission and transformative projects. At its core, Neuralink seeks to unravel the complexities of the brain, with a multifaceted goal encompassing the comprehension and treatment of neurological disorders, enhancement of cognitive abilities, and establishment of symbiosis between humanity and artificial intelligence. Through the development of state-of-the-art brain-machine interfaces (BMIs), Neuralink endeavors to transcend the constraints of conventional medical paradigms and chart new territories in the realm of neuroscientific inquiry.

By delving into its neurobiological foundations, engineering breakthroughs, and early testing endeavors, this paper aims to elucidate the profound implications of Neuralink's initiatives in reshaping the trajectory of neuroscience and neurosurgical interventions. As we embark on this odyssey, it becomes apparent that Neuralink represents not merely a scientific pursuit, but a paradigmatic shift poised to redefine our comprehension of the human mind and unlock its boundless potentials (Fiani *et al*., 2021).

**Background**

Among the most promising frontiers in this domain is the development of brain-machine interfaces (BMIs), which offer the potential to restore sensory and motor function, treat neurological disorders, and augment human capabilities. The origins of BMIs can be traced back to pioneering experiments conducted decades ago, where researchers first demonstrated the ability to translate neural activity into actionable commands for external devices. These early investigations laid the groundwork for a field poised to transform the landscape of human-machine interaction and deepen our understanding of brain function. In the intervening years, BMIs have evolved from rudimentary prototypes to sophisticated systems capable of decoding complex neural signals with increasing accuracy. This progress has been propelled by interdisciplinary collaboration, drawing on expertise from neuroscience, engineering, computer science, and clinical medicine. Milestones in BMI research include the development of microelectrode arrays for recording neural activity, the refinement of signal processing algorithms for decoding neural signals, and the integration of BMIs with prosthetic devices to reinstate the motor functioning in paralysed individuals.

Despite these advancements, significant challenges remain in the field of BMI research. One major hurdle is the need to enhance the channel count of

neural recording devices to capture the activity of large neuronal populations at the single-cell level. Traditional electrode arrays, while effective, are often limited in scalability and biocompatibility, hindering their widespread adoption in clinical settings. Moreover, the surgical implantation of electrode arrays poses risks such as tissue damage, inflammation, and infection, necessitating novel approaches to electrode design, implantation techniques, and long-term biocompatibility. In this context, Neuralink emerges as a pioneering force in BMI technology, leveraging cutting-edge innovations in materials science, robotics, and electronics to push the boundaries of what is achievable. By developing ultra-fine electrode "threads" and a state-of-the-art neurosurgical robot capable of precise, high-throughput implantation, Neuralink aims to overcome longstanding barriers to high-channel-count neural recording. Furthermore, Neuralink's emphasis on miniaturized, low-power electronics holds the promise of revolutionizing neural signal processing and transmission, paving the way for fully implantable, wireless BMI systems with unprecedented performance and reliability (Musk E, 2019).

**Imperative for advancing brain-machine**

Brain machine could be utilized and applied in the following areas (Pisarchik *et al*., 2019):

1. **Enhancing sensory and motor functions:** Neuralink's goal is to enhance sensory and motor functions in individuals facing disabilities or neurological disorders. Through establishing direct brain-to-device communication, Neuralink has the potential to empower paralyzed individuals, allowing them precise control over prosthetic limbs and ultimately improving their quality of life.
2. **Treatment of neurological disorders:** Neuralink's innovative technology holds promise for treating a wide range of neurological conditions, including Parkinson's disease, epilepsy, and depression. By modulating neural activity through targeted stimulation or inhibition, Neuralink could alleviate symptoms and improve outcomes for patients suffering from these debilitating disorders.
3. **Advancement of basic neuroscience research:** Neuralink's high-channel-count neural recording capabilities offer the potential to deepen our understanding of brain function and dynamics. By enabling researchers to simultaneously monitor the activity of large neuronal populations at single-cell resolution, Neuralink could uncover new insights into brain circuits, plasticity, and information processing, fostering transformative discoveries in neuroscience.

4. **Enhancement of human capabilities:** In addition to therapeutic applications, Neuralink has the potential to augment human capabilities and cognition. By integrating advanced neural interfaces with external devices or artificial intelligence systems, Neuralink could empower individuals to enhance their sensory perception, memory, and decision-making abilities, opening up new frontiers in human enhancement and augmentation.
5. **Facilitation of human-AI collaboration:** Neuralink's development aligns with the increasing convergence of human intelligence and artificial intelligence (AI). By establishing seamless communication between the human brain and AI systems, Neuralink could facilitate synergistic collaboration between humans and machines, leading to more efficient problem-solving, creativity, and innovation across diverse domains.
6. **Advancement of biomedical engineering:** Neuralink's interdisciplinary approach brings together expertise from neuroscience, engineering, computer science, and medicine, driving innovation and advancement in biomedical engineering. By pushing the boundaries of materials science, robotics, and electronics, Neuralink is catalyzing breakthroughs in implantable medical devices, surgical techniques, and bio-computing platforms with far-reaching implications for healthcare and beyond.

**Neural probe**

The Musk E team (2019) has developed a specialized process to create neural probes that minimize tissue displacement. These probes use various biocompatible thin film materials and feature a polyimide substrate and dielectric, enclosing a gold thin film trace. Each array includes a "thread" section with electrode contacts and traces, and a "sensor" area where the thin film interfaces with custom chips for signal amplification and acquisition. High-throughput production is achieved via wafer-level microfabrication, with each wafer accommodating ten thin film devices, each equipped with 3072 electrode contacts. These arrays consist of either 48 or 96 threads, each hosting 32 independent electrodes. Integrated chips are flip-chip bonded to the contacts on the sensor area of the thin film, ensuring a compact design and high channel count. To minimize tissue displacement while maintaining high channel counts, stepper lithography and other microfabrication techniques are employed to form the metal film at submicron resolution. The research team has developed an array of over 20 distinct threads, featuring various electrode designs incorporating reference electrodes either on separate threads or

alongside recording electrodes ("on-probe references"). These threads exhibit widths ranging from 5 µm to 50 µm and a nominal thickness of 4-6 µm, comprising multiple layers of insulation and conductor materials. Parylene-c deposition is employed to facilitate the manipulation of these thin, elongated threads prior to insertion, with each thread culminating in a loop to aid in needle threading. To enhance electrode performance, surface modifications such as PEDOT:PSS and IrOx coatings are applied to reduce impedance and enhance charge-carrying capacity. Benchtop testing reveals impedances of 36.97 kΩ (SD 4.68) and 56.46 kΩ (SD 7.10) for PEDOT:PSS and IrOx, respectively, with particularly promising outcomes observed for PEDOT:PSS. In order to achieve compact integration with electronics, an innovative alignment and flip-chip bonding technique is employed. Gold stud bumps on the printed circuit board (PCB) serve as alignment markers and temporary supports for the thin film, while a custom shuttle enables precise positioning. Integrated chips are subsequently flip-chip bonded directly to contacts on the thin film and pads on the PCB. This approach facilitates the creation of a compact package containing 3072 channels within a footprint of 23×18.5 $mm^2$.

**Neural Encoding**

Neural encoding and decoding are crucial concepts in neuroscience, shedding light on how the brain processes external stimuli. These processes have evolved significantly since Edgar Adrian's pioneering work on recording neural spikes. Neural encoding involves translating external stimuli into patterns of neuronal activity, incorporating properties like firing rates, spike timing, and population coding. For instance, neurons in the visual cortex exhibit selectivity to features such as orientation and motion, reflecting the brain's representation of visual information. Conversely, neural decoding aims to extract meaningful information from observed neural activity patterns. Using computational algorithms and statistical models like multivariate pattern analysis and machine learning, researchers can infer characteristics of external stimuli or cognitive states from recorded neuronal signals. The relationship between encoding and decoding is often framed within Bayesian inference, which probabilistically relates observed neural activity to potential stimuli or cognitive states. Neural encoding and decoding are foundational concepts in neuroscience, providing insights into perception, cognition, and behavior. By understanding how the brain processes external inputs, researchers can develop applications in brain-computer interfaces, neuroprosthetics, and cognitive neuroscience (Zhao *et al.*, 2023).

**Variants of Neural Implants**

Different types of neural implants serve various functions and address specific neurological conditions. Here are several common types (Ionescu *et al*., 2024):

**Deep Brain Stimulation (DBS) Implants:** DBS implants involve electrodes implanted into deep brain regions, like the thalamus or basal ganglia. These electrodes connect to a pulse generator placed under the skin, near the collarbone. It treats disorders related to movement by sending electrical stimulus to modulate abnormal brain activity.

**Cortical implants:** Also called intracortical electrodes, cortical implants are directly implanted into the cerebral cortex to interface with specific brain areas. Researchers use these implants for studies or experimental therapies to record neural activity, stimulate neural circuits, or restore lost sensory or motor functions.

**Cochlear implants:** These prosthetic devices restore hearing in severe hearing loss or deafness cases. They include electrodes surgically implanted into the cochlea, stimulating auditory nerve fibers to transmit signals to the brain, bypassing damaged hair cells.

**Retinal implants:** For individuals with retinal degenerative diseases like retinitis pigmentosa or age-related macular degeneration, retinal implants or artificial retinas aim to restore vision. These implants have microelectrode arrays inserted into the retina, stimulating remaining retinal cells to transmit visual information to the brain.

**Spinal cord stimulators:** Implanted to alleviate chronic pain or restore motor function in spinal cord injury cases, these devices provide electrical stimulation to the spinal cord or peripheral nerves. They modulate pain signals or activate motor pathways, reducing pain or enhancing motor control.

**Brain-Computer Interfaces (BCIs):** BCIs establish direct communication between the brain and external devices like computers or prosthetic limbs. They can be invasive, with implanted electrodes, or non-invasive, using scalp-mounted electrodes or other sensors to detect neural activity.

**Neuroprosthetic limbs:** These advanced prosthetic limbs, also known as bionic limbs, are controlled by neural signals. They restore lost limb function by utilizing neural signals from the brain or peripheral nerves to control the movement of robotic or bionic limbs.

These various types of neural implants serve diverse purposes and offer hope for improving the lives of individuals with neurological conditions or disabilities. Each type has unique designs, applications, and therapeutic potentials, contributing to advancements in neuroscience and neuroengineering.

**Brain implant and neural connection**

Brain implants, also referred to as neural implants or neuroprosthetics, possess an extraordinary capacity to foster neural connections within the brain, thereby enabling a spectrum of functions ranging from the restoration of lost sensory or motor functions to the augmentation of cognitive abilities. These implants interact directly with the nervous system, comprising neurons and synapses, to establish or modulate neural connections. One method through which brain implants facilitate neural connections is via microelectrode arrays (MEAs). These arrays consist of miniature electrodes implanted into specific brain regions. By detecting and/or stimulating neural activity, MEAs can promote the formation of new neural connections or reinforce existing ones. For example, in individuals with neurological disorders like Parkinson's disease, MEAs can stimulate precise brain regions to alleviate symptoms by restoring normal neural communication patterns.

Additionally, brain implants can utilize sophisticated techniques such as optogenetics, involving the genetic modification of neurons to respond to light stimulation. Optogenetic implants allow for precise control over neural activity with exceptional spatial and temporal resolution, enabling researchers to induce or inhibit neural connections with remarkable precision. This method holds promise for treating various neurological conditions by modulating aberrant neural circuits. Furthermore, brain-computer interfaces (BCIs) represent another avenue through which brain implants promote neural connections. BCIs translate neural activity directly into commands capable of controlling external devices, such as prosthetic limbs or computer interfaces. Through training and adaptation, users can learn to modulate their neural activity, leading to the establishment of new neural pathways that facilitate effective communication between the brain and external devices.

In addition to restoring lost function, brain implants have the potential to enhance cognitive abilities by augmenting neural connections associated with learning and memory. For instance, researchers explore neurostimulation techniques like transcranial magnetic stimulation (TMS) or direct current stimulation (tDCS) to modulate neural activity in regions linked to learning and memory processes. By promoting synaptic plasticity, these techniques

may foster the formation of new neural connections and enhance cognitive function (Dabbour *et al*., 2021).

**Deep Recurrent Neural Networks (RNNs) & Convolutional Neural Networks (CNNs)**

Deep recurrent neural networks (RNNs) represent a specialized type of artificial neural network designed specifically to handle sequential data, such as time series or natural language. Unlike conventional feedforward neural networks, which process input data in a single pass, RNNs feature loops within their architecture, enabling them to retain memory of previous inputs. This memory capability empowers RNNs to capture temporal dependencies within sequential data and make predictions based on contextual information. In deep RNNs, multiple layers of recurrent units are stacked on top of each other, allowing for the extraction of more intricate hierarchical representations from sequential data. Each layer within a deep RNN comprises recurrent units, such as Long Short-Term Memory (LSTM) cells or Gated Recurrent Units (GRUs), which are adept at learning long-range dependencies and mitigating issues like the vanishing gradient problem often encountered in training deep networks.

Deep RNNs have demonstrated efficacy across various domains, including speech recognition, language modeling, machine translation, and time series prediction. Their capacity to model temporal dynamics and capture intricate patterns within sequential data renders them indispensable across numerous applications.

Convolutional neural networks (CNNs) constitute a category of deep neural networks predominantly utilized for image processing and computer vision tasks, represented in Fig 1. Inspired by the organization of the animal visual cortex, CNNs are engineered to autonomously and adaptively acquire spatial hierarchies of features from input images. CNNs encompass multiple layers, including convolutional layers, pooling layers, and fully connected layers. Convolutional layers apply filters (kernels) to input images, extracting features through convolutions. Subsequently, pooling layers down sample the feature maps derived from convolutional layers, reducing spatial dimensions while retaining vital information. Fully connected layers are tasked with making predictions based on the extracted features. A noteworthy advantage of CNNs lies in their ability to autonomously learn hierarchical representations of visual data, commencing from rudimentary features like edges and textures and progressively advancing to more intricate patterns and objects. This hierarchical feature acquisition renders CNNs highly adept at tasks such as image classification, object detection, and image segmentation.

In recent years, CNNs have been extended to non-image data, including text and time series, through techniques such as one-dimensional convolutions and temporal convolutions. This adaptability has significantly broadened the applicability of CNNs to diverse domains beyond computer vision. Deep recurrent neural networks (RNNs) and convolutional neural networks (CNNs) stand as two formidable architectures within the domain of deep learning, each possessing distinct strengths and applications. While RNNs excel at modeling sequential data with temporal dependencies, CNNs are highly proficient in processing spatial data like images. Furthermore, the amalgamation of these two architectures, often referred to as hybrid models, has shown promising outcomes across various tasks necessitating simultaneous processing of spatial and temporal information (Toh *et al*., 2019).

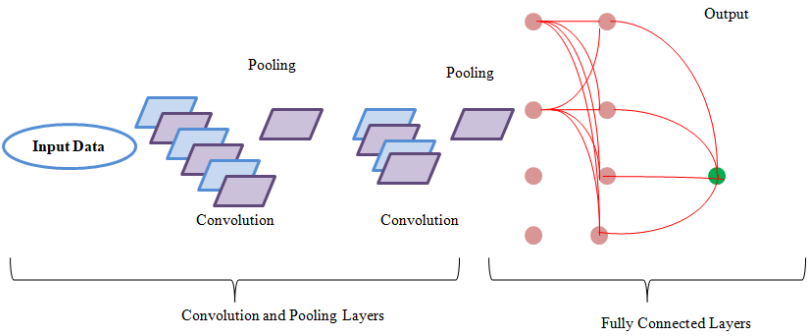


**Fig 1:** Convolutional neural networks (CNNs)

## Current status

The current landscape of neural implants showcases ongoing progress and evolving applications across multiple domains, including neuroscience, medicine, and technology. Here are some key aspects of the present situation:

**Clinical utilization:** Neural implants remain a cornerstone in clinical practice for treating neurological disorders and restoring sensory or motor functions. Deep brain stimulation (DBS) implants are commonly deployed to manage movement disorders like Parkinson's disease, while cochlear implants and retinal implants offer solutions for hearing and vision impairments. Additionally, neuroprosthetic devices and brain-computer interfaces (BCIs) are increasingly employed to restore limb function and improve communication in individuals with paralysis or limb loss.

**Technological progress:** Advances in microfabrication, materials science, and biocompatibility have led to the development of more sophisticated neural implant technologies. Miniaturization and wireless

connectivity have enhanced the safety, efficacy, and usability of implants, enabling precise targeting of neural circuits and reducing risks such as infection or tissue damage.

**Neural interface innovations:** Research in neural interface technologies, including BCIs and neurostimulation devices, is rapidly expanding. BCIs are being explored for various applications, from restoring motor function in paralysis to facilitating communication for individuals with locked-in syndrome. Non-invasive BCIs, such as those based on scalp-mounted electrodes or wearable devices, offer potential solutions for broader adoption and accessibility.

**Understanding neuroplasticity and rehabilitation:** Insights into neuroplasticity, the brain's adaptive capacity, have influenced the design of neural implants and rehabilitation approaches. Combined strategies, including neurostimulation, sensory feedback, and intensive therapy, leverage neuroplasticity to promote recovery and functional improvement in individuals with neurological conditions.

**Research and development:** Ongoing research endeavors focus on advancing neural implant technologies to enhance their efficacy, safety, and long-term reliability. Innovative approaches, such as optogenetics, neural tissue engineering, and closed-loop neurostimulation systems, hold promise for expanding therapeutic possibilities and addressing existing limitations in neural implant design and implementation.

**Ethical and societal considerations:** As neural implant technologies become more integrated into healthcare and daily life, ethical and societal considerations gain importance. Issues concerning patient autonomy, privacy, informed consent, and equitable access to neurotechnologies necessitate careful attention to ensure responsible development and deployment of neural implants.

**Future prospect**

The future prospects for neural implants hold immense potential to revolutionize healthcare, scientific inquiry, and human capabilities. Envisioned advancements include refinements in targeting specific neural circuits and seamless integration with the nervous system, facilitated by breakthroughs in materials science and biocompatibility. The ongoing trend towards miniaturization and wireless connectivity is expected to persist, enabling less invasive procedures and increased patient mobility. Anticipated closed-loop systems will optimize therapeutic outcomes by dynamically adjusting stimulation parameters in response to real-time neural activity.

Brain-machine interfaces (BMIs) are poised to establish direct brain-device communication, facilitating prosthetic control, computer interaction, and cognitive augmentation. Furthermore, emerging regenerative therapies may complement neural implants, fostering neural repair and functional recovery. Artificial intelligence and machine learning algorithms are anticipated to play a pivotal role in optimizing neural implant functionality and tailoring therapies to individual patients. Ethical considerations surrounding privacy, consent, and equitable access will remain critical as neural implant technologies progress. In summary, interdisciplinary collaboration, ethical vigilance, and continued research and development efforts will be essential to unlock the transformative potential of neural implants in the coming years.

## Conclusion

The future of neural implants holds immense promise for advancing healthcare, scientific inquiry, and human capabilities. Anticipated advancements include precision targeting of neural circuits and seamless integration with the nervous system, facilitated by breakthroughs in materials science and biocompatibility. The ongoing trend towards miniaturization and wireless connectivity is expected to continue, enabling less invasive procedures and increased patient mobility. Closed-loop systems are anticipated to optimize therapeutic outcomes by dynamically adjusting stimulation parameters in response to real-time neural activity. Brain-machine interfaces (BMIs) are poised to establish direct brain-device communication, facilitating prosthetic control, computer interaction, and cognitive augmentation. Furthermore, emerging regenerative therapies may complement neural implants, fostering neural repair and functional recovery. Artificial intelligence and machine learning algorithms are expected to play a pivotal role in optimizing neural implant functionality and tailoring therapies to individual patients. Ethical considerations surrounding privacy, consent, and equitable access will remain paramount as neural implant technologies progress. In conclusion, interdisciplinary collaboration, ethical vigilance, and continued research and development efforts will be essential to unlock the transformative potential of neural implants in the coming years.

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# Chapter - 12

## Mycobacteriophages: A Bacteriophage Therapeutic Approach for the Treatment of *Mycobacterium tuberculosis* Infections: A Review


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# Chapter - 12

## Mycobacteriophages: A Bacteriophage Therapeutic Approach for the Treatment of *Mycobacterium Tuberculosis* Infections: A Review

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**Abstract**

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), is a highly infectious disease and a global health concern. According to the WHO TB report, 9 million new active cases of tuberculosis infections are reported each year, resulting in 2 million deaths. The recent rise of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains has resulted in increased morbidity and death, emphasizing the need for novel therapeutic approaches. Among the different emerging antimicrobial approaches, phage therapy is seen as a precise and promising alternative. Long before the discovery of antibiotics, phage formulations served as anti-infective agents. Phages have developed with their hosts and have all of the capabilities necessary to infect and kill bacteria, regardless of antibiotic resistance. Mycobacteriophages, viruses that infect bacteria such as *Mycobacterium* spp., including *M. tuberculosis*, represent a promising alternative to antibiotic resistance. Since each bacteriophage has a specific host range, mycobacteriophages' bacteriolysis characteristics make them more appealing for treating infectious diseases. Additionally, phage cocktails can widen the spectrum of bacterial lysis action. Recent studies have also demonstrated that combining antibiotics with phages is an efficient way to combat infective bacteria. Phage treatment has certain limits and issues. For example, the human immunological response to phage treatment, the transmission of antibiotic resistance genes, growing phage resistance, and safety concerns. This study discusses the general characteristics of mycobacteriophages, their mechanisms of killing *M. tuberculosis*, and both their advantages and disadvantages as therapeutic and prophylactic agents against drug-resistant *M. tuberculosis* isolates.

**Keywords:** *Mycobacterium tuberculosis*, drug-resistance, mycobacteriophages, bacteriolysis, phage therapy.

## Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), is a highly infectious disease and a global health concern (Zeynali Kelishomi *et al*., 2022). Until the coronavirus pandemic, tuberculosis was the leading cause of mortality worldwide, even exceeding HIV/AIDS. Ten million cases of tuberculosis were reported globally in 2021; of these, 1.6 million fatalities occurred among HIV-negative individuals and an additional 187,000 among HIV-positive individuals (Ouyang *et al*., 2023). Although treatable, tuberculosis is gradually becoming almost incurable due to the increasing and widespread development of resistance to first-line anti-TB drug therapies like isoniazid and rifampicin (Ouyang *et al*., 2023; Seung *et al*., 2015). Multidrug-resistant (MDR) MTB isolates are resistant to both of these antibiotics. When traditional first-line medicines are used, the existence of these drug-resistant strains may result in treatment failure. Despite the availability of second-line drugs, extensively drug resistant tuberculosis strains (XDR-TB) are resistant to all first- and second-line drugs (Ouyang *et al*., 2023; Zeynali Kelishomi *et al*., 2022). The last 20 years have seen the emergence of multi-drug resistant (MDR), extensively drug resistant (XDR), extremely drug-resistant (XXDR), and total drug resistant (TDR) strains of *Mycobacterium tuberculosis* as a global threat. (Zeynali Kelishomi *et al*., 2022). Antibiotics are additionally known to cause various side effects, including hypersensitivity, photodermatitis, fever, nausea, and vomiting. (Castells *et al*., 2019; Ouyang *et al*., 2023). A number of therapeutic strategies, such as phage therapy, which has demonstrated remarkable potential against a variety of bacterial pathogens, have been proposed in order to combat the rapidly increasing rate of antibiotic resistance in the disease and its side effects, particularly the emergence of extensively drug-resistant (XDR-TB) and multidrug-resistant (MDR-TB) strains of the infection. (Kortright *et al*., 2019; Zeynali Kelishomi *et al*., 2022). Phage-mediated lysis of infectious disease-causing bacteria is usually not a new anti-infection therapeutic approach, despite the increased attention given to the issue of antibiotic resistance. (Abedon *et al*., 2011; Ouyang *et al*., 2023).

## Mycobacteriophages: Phage therapy in the treatment of drug-resistant tuberculosis

Mycobacteriophages comprise a diverse group of bacteriophages that use mycobacteria as their hosts for infection. (Ouyang *et al*., 2023). They are

globally widespread organisms, and soil, sewage, water and any other habitat that mycobacteria have invaded are among the most common places on the planet to find these organisms (McNerney & Traore, 2005; Ouyang *et al*., 2023). Since their discovery in 1946 in soil and leaf mould, (Gardner & Weiser, 1947), mycobacteriophages have been found and categorized into over 12,000 different species. (Ouyang *et al*., 2023).

The 1990s saw the sequencing of the first mycobacteriophage genome (Hatfult & Sarkis, 1993; Ouyang *et al*., 2023), with over 2,000 strains sequenced by 2023 (Ouyang *et al*., 2023).Since the late 1940s, mycobacteriophages have been identified and studied because of their increasing significance of treating mycobacterial infections (Hatfull, 2010; Ouyang *et al*., 2023). The early work focused on characterizing novel phages and using them as typing tools to identify clinical mycobacterial isolates. This persisted into the 1970s. However, in the subsequent two decades, the enormous efficacy of antibiotics hampered mycobacteriophage research (Hatfull, 2012; Ouyang *et al*., 2023). This scenario altered in response to the advancement of molecular genetics and the increasing prevalence of antibiotic resistance. The revival of mycobacteriophage research began in the late 1980s (Jacobs *et al*., 1989; Ouyang *et al*., 2023), as interest in their molecular biology evolved, as their use in genetic modification tools for mycobacterium (Bardarov *et al*., 2002; Hatfull, 2018; Jacobs *et al*., 1987, p. 19; Lazraq *et al*., 1991; Marinelli *et al*., 2008; McNerney, 1999; Murphy *et al*., 2018; Ouyang *et al*., 2023; Tufariello *et al*., 2014; Wetzel *et al*., 2021).

Mycobacteriophages had previously been studied as prospective treatment alternatives for TB *in vitro* (Azimi *et al*., 2019) and in *M. tuberculosis*-infected guinea pigs (Diacon *et al*., 2022; Sula *et al*., 1981; Zemskova & Dorozhkova, 1991), but not in human beings. Although these early trials were considered encouraging, they were unable to produce a cure. The reason provided for this was that the phages used were temperate and did not cause lysis of cells, they failed to penetrate tuberculous granulomas, or neutralize host immune responses to huge numbers of phages treated repeatedly (Diacon *et al*., 2022; McNerney, 1999). It has been proposed to use mycobacteriophages prophylactically to prevent *M. tuberculosis* infection at least since 2012 (Diacon *et al*., 2022; Hatfull, 2012, 2014; Hatfull & Vehring, 2016). After researching the ideal nebulizer type for phage inhalation, Carrigy *et al*. (Carrigy *et al*., 2017, 2019) exposed mice to an aerosol of mycobacteriophage D29 inhalation, followed 30 minutes later by an aerosol of *M. tuberculosis* H37Rv (Diacon *et al*., 2022). Bacteriophage aerosol pre-treatment had a preventive effect, considerably reducing the *M. tuberculosis*

load in mice lungs 24 hours and 3 weeks following the challenge. This shows that just one dosage of nebulized mycobacteriophage aerosol may result in protection against *M. tuberculosis* infection following exposure to significantly lower number of bacteria than those used in this experiment (Carrigy *et al*., 2019; Diacon *et al*., 2022).

A successful phage cocktail for TB therapy must meet certain essential conditions. Since only a small percentage of bacterial cells survive the infection and because lytic phages self-eradicate when there are no more bacterial hosts available, they are attractive (Diacon *et al*., 2022). In addition to producing well-developed and surviving lysogens, temperate phages have the ability to transmit genes to bacteria, which may increase antibiotic resistance or host pathogenicity. To make temperate phages suitable for therapeutic use, they are genetically altered to lose their ability to chromosomally integrate or turn off lytic genes (Diacon *et al*., 2022). Therefore, it is essential to develop phage cocktails that are both diverse enough to prevent the emergence of phage resistance variants and capable of infecting and eliminating a wide variety of clinical isolates (Diacon *et al*., 2022; Guerrero-Bustamante *et al*., 2021).

Phage treatment is the direct delivery of live pathogenic phages to a patient to lyse the pathogen responsible for a relevant infection (Ouyang *et al*., 2023). By using its receptor binding proteins (RBPs), which are found at the end of the phage tail, the lytic phage identifies the particular surface receptor when it approaches the surface of a host cell (Kortright *et al*., 2019; Ouyang *et al*., 2023; Stone *et al*., 2019). When a specific adhesion takes place, the nucleic acid of the phage enters the host cell, initiating the process of phage genome replication (Ouyang *et al*., 2023). The host cell will then be lysed by the newly generated phage particles, which will then spread throughout the surrounding area and infect more bacterial cells. The very last phase of the expression of phage genes results in the production of the peptidoglycan-degrading enzyme phage-encoded endolysin, inducing bacterial lysis. By slipping through the tiny membrane holes that the protein holing creates, endolysins can penetrate their substrates and break down the cell wall from within (Ouyang *et al*., 2023; Stone *et al*., 2019).

**Table 1:** Applications of mycobacteriophages in TB

| Applications | Descriptions |
|---|---|
| Typing of clinical Mycobacterium isolates | Identifying new *M. tuberculosis* isolates by applying pre-determined host-specific mycobacteriophage groups. |
| TB diagnostics | Reporter phages (e.g., LRP, Φ2DRMs) are used to detect active illness, drug susceptibility, and treatment efficacy of |

| | |
|---|---|
| | M. tuberculosis, including identification of persister bacilli. |
| Mycobacterial genomic tools | To develop *M. tuberculosis* mutants, using gene exchange, allelic exchange, chromosomal marker recombination and transduction, point mutations, transposons, and insertions.<br>To introduce immune-based selection markers (superinfection immunity) in mycobacterial hosts. |
| Prophylaxis and therapeutics | To lyse and eradicate mycobacterial hosts efficiently, using lytic enzymes derived from mycobacteriophages or the whole phages.<br>Synergistic therapy combining mycobacteriophages and endolysins with anti-TB medicines. |

*Source*: Adapted and modified from (Allué-Guardia *et al*., 2021)

Mycobacteriophages are potential therapeutic agents against drug-resistant tuberculosis due to several general phage characteristics (Allué-Guardia *et al*., 2021; Gordillo Altamirano & Barr, 2019; Principi *et al*., 2019): (a) Phages are able to lyse and kill their hosts at the site of infection. By nature, they only infect and proliferate inside their bacterial hosts, causing no harm to human eukaryotic cells (Allué-Guardia *et al*., 2021; Ooi *et al*., 2019); (b) One of the primary benefits of phage therapy over medication use is that its host range is primarily limited to *Mycobacterium* spp. This enables the development of customized treatments for particular *M. tuberculosis* strains with little apparent collateral damage to the human microbiota (Allué-Guardia *et al*., 2021; Bogovazova *et al*., 1991); (c) According to estimates, there are 1031 virus particles in the entire phage population, making them the most common species in the biological community (Allué-Guardia *et al*., 2021; Aziz *et al*., 2015; Hatfull, 2008). They may be isolated from several different environments using standard and well-established microbiology procedures (Lima-Junior *et al*., 2016), and are simple to identify using molecular biology tools (Endersen *et al*., 2013). Recent efforts to sequence and characterize new mycobacteriophages (Bajpai *et al*., 2018), have revealed a high diversity of phages (Caratenuto *et al*., 2019), suggesting the possibility of discovering novel methods to destroy the M. tuberculosis cell envelope (Allué-Guardia *et al*., 2021); (d) It is simple to modify phages to have the right characteristics for phage therapy; (e) Compared to medication therapies, phage treatments require fewer dosages because they can reproduce inside the pathogen by exploiting the machinery of the bacterial cells to produce additional viral particles (Allué-Guardia *et al*., 2021; Bogovazova *et al*., 1991); and finally, (f) In comparison to pharmaceuticals, phages are easily propagated on a broad scale in an *in vitro* setting, greatly reducing production costs (Allué-Guardia *et al*., 2021; Principi *et al*., 2019).

However, there remain challenges to overcome before

mycobacteriophages might be extensively employed to treat MDR-, XDR-, and XXDR-TB (Allué-Guardia *et al*., 2021; Young & Gill, 2015). Table 2 below provides an overview of these.

**Table 2:** Phage therapy challenges and their possible solutions in drug-resistant TB treatment

| Challenges and Limitations | Possible Solutions |
|---|---|
| Host specificity | Screening the global phage database.<br>Phage host range extension by guided phage evolution and genetic engineering.<br>Designing and developing screening bioinformatics tools to identify specific *M. tb* host virulence factor epitopes (such as the efflux pump). |
| Human ALF's unknown effect on the *M. tb* cell membrane | Determine the ways in which the *M.tb* cell membrane undergoes modifications or adaptations to the diverse habitats that it encounters during infection [such as interaction with ALF, within the phagosome, extracellular space, inside granulomas or cavities, or during transmission (in sputum)]. |
| Phage's entry into intracellular *M. tb* | New approaches for delivering phages [such as *M. smegmatis*, the Trojan horse theory and phage microencapsulation].<br>Using phage bioengineering, distinct macrophage receptors (the mannose receptor, or MR) can be identified. |
| Phage resistance in *M.tb* | Using various phage cocktails.<br>Phage-drug synergistic therapy along with the mammalian host immune system.<br>Sequential therapy for phages.<br>Personalized phage therapy. |
| The risk of anaphylaxis and overactivation of the mammalian host immune system | Optimize the pathways for phage delivery.<br>Determine phage dose and frequency.<br>Enhance the phage-host immune system synergy in mammals. |
| Lack of phage therapy regulations | Standardize worldwide phage manufacturing rules (under GMP guidelines). |
| Phage cytotoxicity to the human host | Using highly lytic phages that resist genome integration with *M.tb*.<br>Using phage genetic engineering, possible phage pathogenic factors within the mammalian host can be eliminated.<br>Explain the role of unidentified phage genes. |

*Source*: Adapted and modified from (Allué-Guardia *et al*., 2021)

## Combination therapy

Public health and medical research are facing significant issues due to the emergence of drug-resistant bacterial infections including MDR-TB and XDR-TB (Zeynali Kelishomi *et al*., 2022). In the lack of effective treatment techniques for MDR-TB and XDR-TB isolates, inventive and novel strategies are required. The extended treatment duration, adverse effects, and high cost in developing nations have resulted in unfavorable agreements over the use of treatment techniques, further surgeries, and the emergence of drug-resistant

strains (Zeynali Kelishomi *et al*., 2022). Novel antimicrobial medicines, like bedaquiline, have been progressing; yet, the demand for new treatment regimens is unavoidable (Catalão *et al*., 2019; Zeynali Kelishomi *et al*., 2022). Phage therapy by itself may never be able to completely replace the need for conventional drugs; however, long-term combinations of mycobacteriophage-drug treatment approaches that cooperate to boost the host's immune response may turn out to be the most successful for a number of reasons (Allué-Guardia *et al*., 2021). It makes evolutionary sense in the first place since it keeps bacteria from developing a resistance to mycobacteriophages. Second, it's been stated that the combined use of phages and drug therapies is more effective in controlling pathogenic microbes than either treatment alone. Regardless of whether the pathogen is drug-resistant or not, this combined effect has been observed in numerous studies (Torres-Barceló & Hochberg, 2016), which, in tuberculosis, could result in a shorter duration of treatment or a lower dosage of drugs (Allué-Guardia *et al*., 2021). As a result, this may prevent the future progression of drug resistance (Allué-Guardia *et al*., 2021). The small peptide AK15, derived from mycobacteriophages, and its corresponding isomer AK15-6 have significant anti-*M. tuberculosis* characteristics. Through membrane disruption, AK15 and AK15-6 both inhibited *M. tuberculosis* simultaneously (Zeynali Kelishomi *et al*., 2022). Additionally, they exhibited cell selectivity and beneficial outcomes in combination with rifamcin. They were successful in lowering the number of mycobacteria in mice with *M. tuberculosis* infection (Yang *et al*., 2019; Zeynali Kelishomi *et al*., 2022).

Carlos *et al*. developed a cocktail of five phages that decreases phage resistance and cross-resistance while also effectively killing *M. tuberculosis* strains (Guerrero-Bustamante *et al*., 2021; Zeynali Kelishomi *et al*., 2022). Furthermore, these phages have no antibiotic antagonistic effects and infect both isoniazid-resistant and isoniazid-sensitive strains (Wetzel *et al*., 2021; Zeynali Kelishomi *et al*., 2022). Yeswanth *et al*. examined the impact of phage cocktails on mycobacterium proliferation. Isolates of *M. tuberculosis* were found to be susceptible to infection by two of their five phages cocktails (D29 and TM4). These two phages, as well as DS6A, were developed in the host *M. tuberculosis* (H37Ra) (Zeynali Kelishomi *et al*., 2022). Mycobacteriophages synergized with antimicrobial drugs such as rifampicin and isoniazid (Zeynali Kelishomi *et al*., 2022). Finally, mycobacteriophages were found to successfully suppress *M. tuberculosis* for several weeks during both the lag and log phase. These results have substantial implications for the development of phage treatment for *Mycobacterium* spp. (Kalapala *et al*., 2020; Zeynali

Kelishomi *et al*., 2022). As a direct advantage, the systemic application of combination treatments in MDR patients globally may reduce the conversion rate and, consequently, the number of XDR and XXDR-TB cases, which arise from MDR cases failing their therapy (Allué-Guardia *et al*., 2021).

**Conclusion**

This narrative review has provided an overview of the current usage of bacteriophages to diagnose and treat mycobacterial infections (Shield *et al*., 2021). Different bacteriophage-based diagnostics have been created, and they have enormous potential since they are quick, inexpensive, and simple to use. Phages have the potential quickly diagnose, treat, and manage tuberculosis and NTM if they are successfully implemented (Shield *et al*., 2021). Phage therapy has been found to be safe in the few case reports and brief series that have had some positive outcomes. However, the procedure, from identifying active phages to receiving regulatory approval, is far from straightforward, and it is currently restricted to certain cases (Shield *et al*., 2021). Extensive clinical studies are required to determine the clinical benefits. *M. tuberculosis* drug resistance emerged soon after streptomycin, para-aminosalicylic acid, and isoniazid were introduced as first-line therapies (Shield *et al*., 2021). Given the increasing prevalence of phage treatment and phage diagnosis, their concurrent usage must carefully examine the potential of resistance and how to handle it in order to maintain their value for mycobacterial infections (Shield *et al*., 2021). In order to learn more about how neutralizing antibodies are made and how they relate to the phage structure or any inherited human traits, additional research is also necessary (Shield *et al*., 2021).

**Future perspectives**

Bacteriophages use complex processes to lyse bacterial hosts, which have received little attention to far. Thus, further research is required to fully comprehend their enzymatic machinery, regulatory mechanisms, and biochemical characteristics (Zeynali Kelishomi *et al*., 2022). Mycobacteria are distinguished by their complex cell envelope, which is necessary for their survival within cells. So, suppression of its development might be a beneficial approach to treat TB (Zeynali Kelishomi *et al*., 2022). A possible substitute would be to use mycobacteriophages prophylactically rather than for therapeutic purposes. For example, family members or coworkers of patients newly diagnosed with pulmonary TB can swallow aspirated phages to restrict the transmission and acquisition of the infection (Eniyan *et al*., 2020; Pohane *et al*., 2014; Zeynali Kelishomi *et al*., 2022). Despite its various benefits in the treatment of infections, phage therapy has several challenges.

As an example, we may note the lack of regulation for this procedure and the absence of adequate scientific data (Pelfrene *et al*., 2016; Zeynali Kelishomi *et al*., 2022). A greater understanding of mycobacteriophage treatment, and the relationship between the phage and the immune system of the host could be gained from the increasing number of *in vitro* and *in vivo* studies (Zeynali Kelishomi *et al*., 2022). Mycobacteriophages can be used in combination with antibiotics to treat infections where antibiotics alone are ineffective. Mycobacteriophage therapy may be personalized by thoroughly examining its structure and enzymes (Luong *et al*., 2020), and the use of antibiotics together and customized phage therapy may prove to be an effective treatment for drug-resistant TB (Zeynali Kelishomi *et al*., 2022). These potential approaches may be implemented in the near future, with the employment of mycobacteriophages to help in the resolution of drug-resistant TB cases becoming a viable alternative when drugs fail to do so (Allué-Guardia *et al*., 2021). However, before this, selective, effective, safe, and standardized phage formulations will need to be developed (Allué-Guardia *et al*., 2021; Luong *et al*., 2020), which, when combined with drugs and the host immune response, could make it possible to treat otherwise untreatable TB (Allué-Guardia *et al*., 2021).

## Conflict of interest

The authors declare no conflict of interest.


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