## About the Book

"Articles from Different Disciplines that Serve as a Link Between Research and Development" is a comprehensive anthology that brings together key scholarly articles spanning various fields, each chosen for its role in bridging the gap between theoretical research and practical application. This book emphasizes the interdisciplinary nature of innovation, showcasing how advances in one field often inspire breakthroughs in another. Readers are introduced to a range of topics, from biomedical research and engineering to environmental science, information technology, and social sciences, with each article illustrating the process of transforming ideas into actionable, real-world solutions.

The collection is curated to demonstrate how theoretical insights lead to developments that benefit industries, address societal challenges, or spur new technologies. In addition to the articles, each section includes commentary that provides context and explains the importance of interdisciplinary collaboration in advancing research outcomes. This book is ideal for researchers, professionals, and students seeking a deeper understanding of the processes that link scientific inquiry with tangible innovations and the dynamic role that interdisciplinary approaches play in accelerating progress across sectors. Through its diverse selection, the book encourages readers to appreciate how collaborative research can lead to groundbreaking developments and drive meaningful change in the modern world.

**Published by**
**AkiNik Publications®**
**169, C-11, Sector - 3, Rohini,**
**Delhi - 110085, India**
**Toll Free (India): 18001234070**
**Email: akinikbooks@gmail.com**

ISBN 9789361354069
9 789361 354069
₹ 729 US$ 16

Articles from Different Disciplines that Serve as a Link between Research and Development

AkiNik Publications

# Articles from Different Disciplines that Serve as a Link between Research and Development

Dr. Subhasis Sarkar

# Articles from Different Disciplines that Serve as a Link between Research and Development

**Editor**

**Dr. Subhasis Sarkar**
Associate Professor, Swami Vivekananda University, Kolkata, West Bengal, India

**AkiNik Publications ®**
**New Delhi**

***Published By:*** *AkiNik Publications*

*AkiNik Publications*
*169, C-11, Sector - 3,*
*Rohini, Delhi-110085, India*
*Toll Free (India) – 18001234070*
*Phone No.: 9711224068, 9911215212*
*Website: www.akinik.com*
*Email: akinikbooks@gmail.com*

***Editor:*** *Dr. Subhasis Sarkar*



***Publication Year:*** *2024*

***Edition:*** *1st*

***Pages:*** *147*

***ISBN:*** *978-93-6135-406-9*

***Book DOI:*** *https://doi.org/10.22271/ed.book.2993*

***Price:*** *₹729/-*

***Registration Details***

- *Printing Press License No.: F.1 (A-4) press 2016*
- *Trade Mark Registered Under*
  - *Class 16 (Regd. No.: 5070429)*
  - *Class 35 (Regd. No.: 5070426)*
  - *Class 41 (Regd. No.: 5070427)*
  - *Class 42 (Regd. No.: 5070428)*

# List of Authors


1. Dr. Aritri Laha, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
2. Dr. Bidisha Ghosh, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
3. Dr. Debjit De, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
4. Dr. Pritha Pal, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
5. Dr. Priyankar Pal, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
6. Dr. Semanti Ghosh, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
7. Dr. Sabyasachi Ghosh, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
8. Dr. Srijan Haldar, Associate Professor, Swami Vivekananda University, Kolkata, 700121, India
9. Ms. Suranjana Sarkar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India
10. Dr. Subhasis Sarkar, Assistant Professor, Swami Vivekananda University, Kolkata, 700121, India

# Contents

# Chapter - 1

## Diversity of Arsenic Resistance Genes

**Authors**

**Rakhi Hira**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Aritri Laha**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 1

## Diversity of Arsenic Resistance Genes

**Rakhi Hira and Aritri Laha**

**Abstract**

Phylogenetic research revealed that ACR3(1) and ACR3(2) were primarily found in acting bacteria and Alpha proteobacteria, though arsB was more common in Firmicutes and Gamma proteobacteria. This work revealed an exclusive set of bacteria with noteworthy resistance traits and the capacity to breakdown astatine, and it repeated the utilization the degenerate primers for the identification of the substance transportation genes across different bacteria. The article illustrates the constant genetic stress that arsenic has caused throughout Earth's history and puts forth a hypothesis that this biological group's poisoning resistance mechanisms have a different ancestral genesis and retention. In brief, the research offers significant knowledge of environmental adapting, detoxification, and the comparative genes of eukaryotic arsenic metabolic.

**Keywords:** Diversity of arsenic, arsenic resistance, resistance genes arsenic.

### Introduction

Arsenic, a toxic metalloid, is released into the environment through natural processes and human activities like mining. It exists in two common forms, arsenate (As(V)) and arsenite (As(III)), causing distinct cellular damage. Arsenate mimics phosphate and disrupts metabolic reactions, while arsenite binds to thiol groups in proteins, impairing their function. Microorganisms have developed resistance strategies, including oxidation or methylation of arsenic and active extrusion from cells. The arsenite detoxification machinery (ars genes) is widespread in bacteria and archaea, typically comprising three (arsRBC) or five (arsRDABC) genes. This includes ArsB, an integral membrane protein, ArsA, an associated ATPase subunit, ArsC, an arsenate reductase, ArsR, a repressor, and ArsD, a second repressor, contributing to the regulation of ars gene expression.

The atmosphere is exposed to harmful metalloid arsenic as a result of both biological reactions and human actions like mining. It causes different types

of cellular harm in its two typical forms, arsenic (As(V)) and arsenite (As(III)). While arsenite attaches to protein thiol groups and impairs protein activity, aspartate mimics phosphate and disturbs metabolic processes. Microorganisms have evolved resistance mechanisms, such as active cell expulsion and the oxidation or degradation of arsenic. Bacteria and archaea are known to possess the ars genes, which are responsible for detoxifying arsenite. These genes usually consist for three (arsRBC) or five (arsRDABC). Helping to the control of ars gene expression are ArsB, an essential membrane protein; ArsA, a related ATPase component; ArsC, an arsenate oxidase; ArsR, a repressor; and ArsD, a second repressor. Living things are involved in and essential to the activities of the worldwide arsenic geocycle (Mukhopadhyay *et al*., 2002; Zhu *et al*., 2014).living things are involved in and essential to the activities of the worldwide arsenic geocycle (Mukhopadhyay *et al*., 2002; Zhu *et al*., 2014).The two major forms of arsenic are the trivalent form of (AsO2−) and a pentavalent form of arsenate (AsO43−), which naturally interconvert under the action of microbes. More poisonous, arsenite binds lightly to thiol bonds in molecules such as glutathione but well to groups of sulfhydryl in proteins. When arsenate and phosphate compete, energetics and transport processes are disrupted, leading to toxicity. Like their methyl derivatives, organic arsenic molecules are found in nature and usually less toxic than their inorganic counterparts. Arsenic mobility and accessibility in the environment are greatly impacted by these steps (Mukhopadhyay *et al*., 2002; Oremland and Stolz, 2003; Stolz *et al*., 2006).

**The ARS operons**

Toxin exposure in the environment drives evolutionary changes in organisms, promoting the synthesis or transfer of resistance genes. Similar to other dangerous compounds, bacteria have developed genetic defense mechanisms against the toxicity of arsenic. Ars operons, which are common in bacteria and archaeal species, are one of the most important of these. Ars operons are far more common in bacterial genomes than tryptophan biosynthesis genes. This emphasizes how important it is for microbial evolution to have adaptive responses to harmful metalloids and heavy metals. (Silver and Phung, 2005).

It was found that Staphylococcus aureus's pI258 plasmid confers resistance to heavy metal compounds, arsenate, arsenite, and antibiotics. Eventually, an Escherichia coli strain suffering a patient suffering from a urinary tract infection was revealed to carry the R773 plasmid, which included genes for arsenic resistance (Hedges and Baumberg, 1973). According to Busenlehner *et al*., (2003), the ArsR gene codes for the metalloregulatory

protein ArsR, which is a member of the SmtB/ArsR family. By bind to the promoters of the ars operon, ArsR performs the function of a trans-acting transcriptional repressor. ArsR releases from DNA in response to interactions with arsenite, enabling operon translation.

Together with ArsB, the ATPase protein ArsA produces an arsenite secretion pump that is powered by Hydrolysis of ATP (Yang *et al*., 2012). It has been proposed that ArsA forms primary arsenite transporters by interacting with several membrane proteins in addition to ArsB (Castillo and Saier, 2010). Integral membrane protein ArsB uses two energy coupling strategies to remove arsenite from the cell plasma and prevent its accumulation: either the potential through the membrane in arsRBC operons, which uses the protein motive force, or ArsA's hydrolysis of ATP in complex operons (Yang *et al*., 2012).

**Bacterial strains and growth conditions**

Growing at 30 °C in Tris-buffered minimum media containing 0.2% sodium gluconate, *Alcaligenes eutrophus* AE126 is now known as *Cupriavidus metallidurans*. *Cenibacterium arsenoxidans* ULPAs1 was grown in CDM medium at a temperature of 25 °C. Luria-Bertani medium was used to cultivate additional reference strains, such as *C. glutamicum*, Shewanella spp., Mycobacterium smegmatis, and Escherichia coli variations, at either 30 °C or 37 °C.

To test for arsenic and antimonite resistance, solid medium (LB agar) was used. In stationary phase, isolates or samples from reference strains were calibrated to an OD600 of 0.3. These colonies were spotted in five microliters on LB plates supplemented with different amounts of potassium antimonyl tartrate (0.1–12.8 mM), sodium arsenite (1.75–112 mM), or sodium arsenate (20–640 mM). The lowest metalloid concentration that inhibited growth after 72 hours at 25 °C (for isolates) or 30/37 °C (for reference strains) was found to be the minimum inhibitory concentrations, or MICs. Using the AgNO3 technique, arsenite oxidase action was detected in CDM agar plates 48 hours at 25 °C. A positive control, *C. arsenoxidans* ULPAs1, was used.

**Distribution ARS prokaryotic genes**

Organisms including E. coli, *Yersinia* spp., *Acidiphilium multivorans* AIU 301, *Serrate marcescens*, *Halobacterium sp*. NRC-1, along with *Sinorhizobium sp*. M14, the paragraph describes a variety of plasmids and transposons harboring arsenic resistance genes, such as arsRBC or arsRDABC clusters. These genetic components, which have been found in bacteria such as *Leptospirillum ferriphilum*, *Acidithiobacillus caldus*, and *Bacillus subtilis*,

allow organisms to share arsenic resistance features through a process known as horizontal gene transfer.

Except for the arsA gene in the former the arsBC gene pair is widely distributed in Gram-positive and Gram-negative bacterial chromosomes and plasmids. Certain bacteria possess independent arsC genes, such as *P. aeruginosa*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. There is even a second arsC genes in *P. aeruginosa*. The small size of ArsR-ArsC proteins suggests an evolutionary benefit for arsenite sensing/detoxification, as evidenced by the fusions of these proteins found in the genomes of *L. ferriphilum*, *Microbacterium*, and *Sinorhizobium*. (Wu *et al*., 2010; Mukhopadhyay *et al*., 2002)

**Additional arsenic resistance genes**

It was later discovered that the B. subtilis arsRBC operon, which was previously believed to store a typical ArsB protein found in membranes (Sato and Kobayashi, 1998), actually encodes a novel a carrier with Acr3 homology, similar to yeast and a variety of bacteria, including *Bacteroides vulgatus* ATCC 8482, *R. palustris*, *H. arsenicoxydans*, *O. tritici*, *C. jejuni*, and *Microbacterium sp*. Additionally, homologs of Acr3 were discovered in the pHZ227 linear plasmid obtained from Streptomyces FR-008. It is noteworthy that Acr3 and ArsC in *Mycobacterium tuberculosis* are combined into a single polypeptide (Mukhopadhyay *et al*., 2002). The main arsenite outflow pump in the arsenic-resistant Burkholderiales family, Acr3, was discovered by Li *et al*., (2014). Achour *et al*., (2007) demonstrated that Actinobacteria and Alpha bacteria dominant using Acr3, whereas Firmicutes and Gamma proteobacteria dominated arsB by a different PCR technique. In a PCR investigation of 58 arsenic-resistant microbial isolates of polluted soils, Cai *et al*., (2009) verified the preponderance of the Acr3 gene over arsB.

**Resistance to organoarsenicals**

For many years, the microbial alteration of organoarsenicals, namely the methylation/demethylation of arsenic by bacteria, has been known (Bentley and Chasteen, 2002). The importance of this mechanism in the worldwide arsenic geocycle has been highlighted by the recent emergence of its molecular details (Mukhopadhyay *et al*., 2002; Oremland and Stolz, 2003). Arsenic methylation/demethylation cycles affect the availability and toxicity of arsenic, which affects the environment (Zhu *et al*., 2014). Not all methylated derivatives of arsenic are less harmful than their inorganic counterparts, despite the fact that methylation of arsenic is typically thought of as a detoxifying process (Petrick *et al*., 2000; Bentley and Chasteen, 2002; Stolz *et al*., 2006).

## Conclusion and future perspective

Since the beginning of time, microorganisms have evolved genetic defense mechanisms such as ars operons to fend against exposure to arsenic. These systems, which detoxify both organic and inorganic arsenic, are present in prokaryotic genomes and are essential for the worldwide arsenic cycle. Because ars genes are so common among bacteria and are frequently found on plasmids and mutations called transposons horizontal gene transfer is important for the spread of genes. This indicates the previous origin of ars genes.

## Acknowledgement

I would like to express my special thanks of gratitude to the higher dignitaries of Swami Vivekananda University for allowing me to carry out the study.

## References

**1.** Busenlehner, L. S., Pennella, M. A., and Giedroc, D. P. (2003). The SmtB/ArsR family of metalloregulatory transcriptional repressors: structural insights into prokaryotic metal resistance. FEMS Microbiol. Rev. 27, 131–143. doi: 10.1016/S0168-6445(03)00054-8

2. Achour, A. R., Bauda, P., and Billard, P. (2007). Diversity of arsenite transporter genes from arsenic-resistant soil bacteria. Res. Microbiol. 158, 128–137. doi: 10.1016/j.resmic.2006.11.006

3. Bentley, R., and Chasteen, T. G. (2002). Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol. Mol. Biol. Rev. 66, 250–271. doi: 10.1128/Mmbr.66.2.250-271.2002

4. Castillo, R., and Saier, M. H. (2010). Functional promiscuity of homologues of the bacterial ArsA ATPases. Int. J. Microbiol. 2010:187373. doi: 10.1155/2010/187373

5. Cai, L., Liu, G., Rensing, C., and Wang, G. (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiol. 9:4. doi: 10.1186/1471-2180-9-4

6. Ghosh, M., Shen, J., and Rosen, B. P. (1999). Pathways of As(III) detoxification in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. U.S.A. 96, 5001–5006. doi: 10.1073/pnas.96.9.5001

7. Mukhopadhyay, R., Rosen, B. P., Phung, L. T., and Silver, S. (2002). Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. 26, 311–325. doi: 10.1016/S0168-6445(02)00112-2

8. Li, R., Haile, J. D., and Kennelly, P. J. (2003). An arsenate reductase from Synechocystis sp. Strain PCC 6803 exhibits a novel combination of catalytic characteristics. J. Bacteriol. 185, 6780–6789. doi 10.1128/JB.185.23.6780-6789.2

9. Li, X., Zhang, L., and Wang, G. (2014). Genomic evidence reveals the extreme diversity and wide distribution of the arsenic-related genes in Burkholderiales. PLoS One 9:e92236. doi 10.1371/journal.pone.0092236

10. Mukhopadhyay, R., Rosen, B. P., Phung, L. T., and Silver, S. (2002). Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. 26, 311–325.doi 10.1016/S0168-6445(02)00112-2

11. Mukhopadhyay, R., Rosen, B. P., Phung, L. T., and Silver, S. (2002). Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. 26, 311–325. doi: 10.1016/S0168-6445(02)00112

12. Silver, S., and Phung, L. T. (2005b). A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. J. Ind. Microbiol. Biotechnol. 32, 587–605. doi: 10.1007/s10295-005-0019-6

13. Hedges, R. W., and Baumberg, S. (1973). Resistance to arsenic compounds conferred by a plasmid transmissible between strains of Escherichia coli. J. Bacteriol. 115, 459–460.

14. Rosen, B. P. (1999). Families of arsenic transporters. Trends Microbiol. 7, 207–212. doi: 10.1016/S0966-842X(99)01494-8

15. Sato, T., and Kobayashi, Y. (1998). The ars operon in the skin element of Bacillus subtilis confers resistance to arsenate and arsenite. J. Bacteriol. 180, 1655–1661.

16. Qin, J., Rosen, B. P., Zhang, Y., Wang, G., Franke, S., and Rensing, C. (2006). Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. Proc. Natl. Acad. Sci. U.S.A. 103, 2075–2080. doi: 10.1073/pnas.0506836103

17. Petrick, J. S., Ayala-Fierro, F., Cullen, W. R., Carter, D. E., and Vasken Aposhian, H. (2000). Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. Toxicol. Appl. Pharmacol. 163, 203–207. doi: 10.1006/taap.1999.8872

18. Zhu, Y. G., Yoshinaga, M., Zhao, F. J., and Rosen, B. P. (2014). Earth abides arsenic biotransformations. Annu. Rev. Earth Planet Sci. 42, 443–467. doi: 10.1146/annurev-earth-060313-054942

19. Zhu, Y. G., Yoshinaga, M., Zhao, F. J., B. P. (2014). Earth abides arsenic

biotransformations. Annu. Rev. Earth Planet Sci. 42, 443–467.doi: 10.1146/annurev-earth-060313-054942

20. Wu, B., Song, J., and Beitz, E. (2010). Novel channel enzyme fusion proteins confer arsenate resistance. J. Biol. Chem. 285, 40081–40087. doi : 10.1074/jbc.M110.184457

21. Zhu, Y. G., Yoshinaga, M., Zhao, F. J., and Rosen, B. P. (2014). Earth abides arsenic biotransformations. Annu. Rev. Earth Planet Sci. 42, 443–467. doi: 10.1146/annurev-earth-060313-054942

22. Oremland, R. S., and Stolz, J. F. (2003). The ecology of arsenic. Science 300, 939–944. doi: 10.1126/science.1081903

23. Hedges, R. W., and Baumberg, S. (1973). Resistance to arsenic compounds conferred by a plasmid transmissible between strains of Escherichia coli. J. Bacteriol. 115, 459–460.

# Chapter - 2

## A Systematic Review of Treatment of Antibiotics and Water-Borne Pathogens by Piezo-Catalytic Nanomaterials


**Authors**

**Shrestha Mukherjee**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

**Semanti Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

**Subhasis Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

**Suranjana Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

**Bidisha Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Telinipara, Barasat- Barrackpore Rd, Bara Kanthalia, West Bengal, India

# Chapter - 2

## A Systematic Review of Treatment of Antibiotics and Water-Borne Pathogens by Piezo-Catalytic Nanomaterials

**Shrestha Mukherjee, Semanti Ghosh, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh**

**Abstract**

Water is crucial for ecosystem survival and is circulated through land, rivers, lakes, oceans, and the atmosphere. However due to constant infringement of potentially toxic contaminants like pharmaceuticals, dyes, insecticides, and petroleum into aqueous medium, water pollution leads to a global concern for access to clean potable water. Concomitant increased microbial populations in water systems, including bacteria, viruses, and protozoa, can cause waterborne diseases through ingestion, airborne transmission, or contact with contaminated water. The indiscriminate use of antibiotics has augmented the growing abundance of antimicrobial-resistant infections and lower susceptibility to these water-borne pathogens. Antibiotic residues in the environment are thus a result of various antibiotic usage patterns, primarily for combating bacterial infections and improving livestock farming. The increasing prevalence of antimicrobial-resistant infections and reduced susceptibility to water-borne pathogens is causing significant health impacts. Clean drinking water is crucial, and early detection is vital for antibiotic removal. Nanotechnology's rapid development in various fields is paving the way for water and wastewater treatment, while piezoelectric materials offer advanced catalytic techniques. The non-centrosymmetric structures of piezoelectric materials, which contain electric dipoles, generate an external electric field when they undergo mechanical deformation. This technology has the potential to reduce aquatic pathogens and antibiotics in various fields, including water and wastewater treatment. This review describes nano-sensors based on piezoelectric materials for the removal of pathogens and antibiotics in waterbodies.

**Keywords:** Antimicrobial resistant, antibiotics, ecosystem survival, piezoelectric material, water-borne pathogens.

## Introduction

The hazard of antibiotic contamination to the environment is increasing. Antibiotics are typically found in water, which leads to drug resistance in microorganisms. Wastewater from factories, farms, and hospitals is the main source of contamination. With the discovery of penicillin in 1929, many more antimicrobial agents have been invented to treat microbial infection. The environmental impact of antibiotics, which are used in over 100,000 tons annually worldwide, is a developing concern because freshwaters contain considerable levels of these compounds due to their extensive presence in the environment [1]. Antibiotic parent chemicals and metabolites can escape water treatment systems and enter the ecosystem, causing significant environmental effects on ecosystems and human health. The concentration of antibiotics in aquatic systems may interact with native species, altering their genetic makeup and structure [2]. Water pollution due to leaching from wastewater treatment plants, pharmaceutical industry, agricultural activities are the key sources for antibiotic-resistant bacteria, resistance genes [3]. Antibiotics, or antimicrobials, are commonly used to treat microbial illnesses. Nowadays, these antibiotic residues are collaboratively work with water borne pathogens and affecting the human population. However, repeated exposure to the water pathogens can lead to the development of resistance, forming resistant water borne pathogens colonies. These pathogens somehow colonize to the human body and which is major threat to us. World Health Organization (WHO) has identified Antimicrobial Resistance (AMR) as one of the top 10 global public health threats due to its increasing number of cases [4]. AMR poses a significant threat to global development and public health, causing 4.95 million fatalities and 1.27 million deaths in 2019 [5]. Therefore, WHO established the Global Antimicrobial Resistance and Use Surveillance System (GLASS) and the Global Action Plan for managing AMR to achieve its objectives [6]. Also, there are various pathogens to be aware of in India. They are *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Shigella sp* and *Escherichia coli* etc. [7]. Therefore, to protect the human population, this environmental pollution treatment is essential. With the help of piezo electric nanomaterial, nano-science have shown a promising future in this field. Some piezo electric materials such as quartz, topaz, schorl tourmaline etc. having capability to generate electrical energy when they are subjected to mechanical stress. This catalysis starts a redox process by converting mechanical energy into electrical energy through the acceptance and donation of electrons [8]. Piezo-catalysis is a technique that immediately breaks down contaminants. Under external bias and mechanical stress, it improves the electrocatalytic reaction. The removal of several

contaminants, such as pharmaceutical residues, dyes, pesticides, herbicides, and radionuclides, is accomplished by the synergistic impact of piezoelectric potential and photocatalytic reaction, or piezo-photocatalysis. Ultrasonic is commonly used as the mechanical source in lab-scale research of piezo-catalysis for contaminants removal in water and wastewater because it mimics physiological oscillation.

**Pollution mediated through the action of antibiotics and water-borne pathogen**

From some recent studies it has been observed that aquatic habitats are optimal for the acquisition and spread of antimicrobial resistance. Antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARBs) are rapidly proliferating in farmed aquatic animals and ecosystems because the aquaculture sector primarily depends on antibiotics to manage diseases. Antibiotics like tetracycline, aminoglycosides, and macrolides are essential for human treatment [9]. Through culture-independent research, it has been demonstrated that ARGs have been indirectly transferred from fish origin to human diseases. Aquaculture, animal husbandry, wastewater treatment plants, and other sources are constant supplies of ARG and ARB for freshwater bodies such as rivers, streams, and lakes [10]. Some of the pathogens and their resistance to the antibiotics are shown in the following Table 1.

**Table 1:** Studies on antibiotics and their source and effects

| Class of Antibiotics | Antibiotics | Source of the contamination | % of Antibiotics in Water | Effects | References |
|---|---|---|---|---|---|
| Beta-lactams | Ampicilin Cefoperazone Cefotaxime | Effluents of Waste water Treatment plant to the Lake water. | 42-70 | Resistance is heightened by gene mutations and horizontal gene transfer. | [11, 12] |
| Tetracyclines | Tetracycline, Doxycycline | Due to regular contact to antibiotic residues from veterinary, clinical, aquaculture, and industrial facilities. | 67-85 | AMR is starting to pose a global health threat. | [13] |
| Fluoroquino-lones | Norfloxacin, Ciprofloxacin | Effluents of hospitals, pharmaceutical companies, aquaculture, livestock farm. | 35-47 | The threat of antimicrobial resistance to global health is beginning to manifest. | [14] |

| | | | | | |
|---|---|---|---|---|---|
| Macrolides | Erythromycin, Clarithromycin, Roxithromycin | Used in healthcare industry | 57-80 | Antibiotic resistance is a serious therapeutic problem. | [14, 15] |

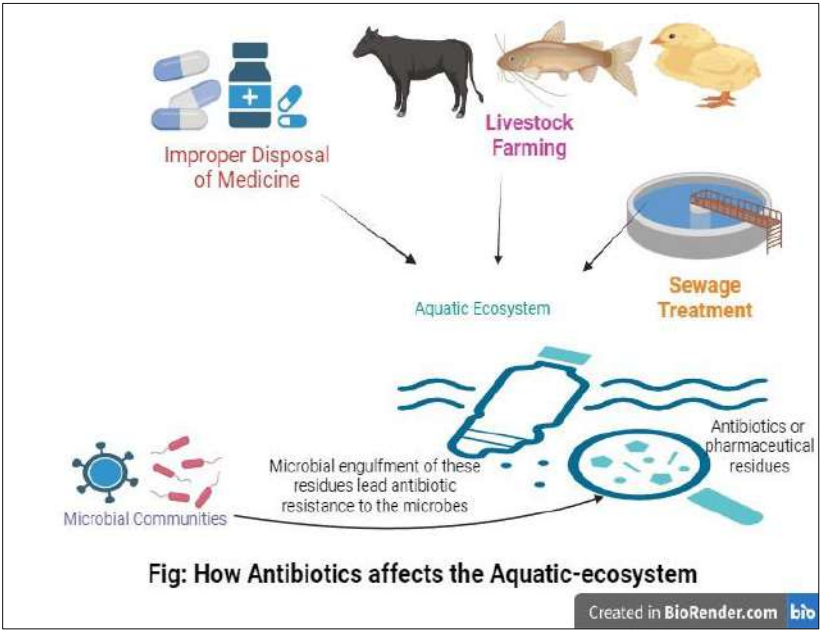


**Fig: How Antibiotics affects the Aquatic-ecosystem**

From some existing studies it also been observed that risks to public health arise from entero-pathogen contamination of water supplies, which includes land spreading, agriculture, and the discharge of animal waste [16]. Aquatic body's bacterial burden is heightened by the effluent from hospitals, industries, livestock farms, and fecal matter disposal sites. Among the coliform groups that have historically been used to evaluate water microbiological contamination and public health security, *Escherichia coli* is a major indicator of fecal contamination. Due to the prevalence in animal feces, *Salmonella enterica* and *Vibrio cholerae*, the initial pathogenic bacteria from water found in the middle of the nineteenth century, significantly increase death and disability rates worldwide [17]. It has been reported that, one of the main pools and channels for the propagation of antibiotic resistance is the aquatic environment. These microorganisms lead the engulfment of the antibiotic residues through the lateral gene transfer and become more resistant to those particular antibiotics (As Per Following Table 2).

**Table 2:** Studies on water-borne pathogen, their source and effect and antibiotic resistant

| Water borne pathogen | Antibiotic resistant | Source and effect | References |
|---|---|---|---|
| 1. *E. coli* | Cefotaxime, Ampicillin, Cefoperazone, Streptomycin, Tetracycline, Chloramphenicol | Commonly found in food and untreated water; cause diarrhea, stomac and urinary infection | [18] |
| 2. *Vibrio cholerae* | Polymyxin, Doxycycline, Bacitracin, Aminoglycosides, Meropenem, Rifampicin, Tetracycline, Erythromycin, Gentamycin | Found in places with inadequate water treatment, poor sanitation; can cause intestinal infection | [19] |
| 3. *Shigella* sp | Carbapenem, Norfloxacin | Developing from poor sanitation, contaminated food, shallow wells; can cause infection | [20, 21] |
| 4. *Campylobacter sp* | Erythromycin, Tetracycline, Ciprofloxacin | Found in food, feces and water; can cause infectious disease | [22, 23] |

**Role of nano-materials to treat antibiotics and water pathogen**

Microorganisms' resistance to antibiotics is a serious public health concern that calls for innovative methods of observing microbial behavior. One such method is the use of nano-materials. Nano-particles that have sizes smaller than 100 nm and are distinguished by their charge, shape, and size on surfaces [24]. With the help of nano-materials, the use of nanotechnology in environmental pollution remediation is growing steadily. Piezo-catalysis is a method developed using piezo-materials, which generate electron-hole pairs in response to stress, initiating redox processes [25]. The use of piezocatalytic nanomaterials for the removal of antibiotics and waterborne pathogens involves a combination of mechanical and catalytic processes, which involves- generation of electric charges, formation of reactive oxygen species,

degradation of contaminants and removal of by-products. Some piezo-catalytic nano-particles and their synthesis and effect on the respective antibiotics are discussed as per following Table 3.

**Table 3:** Different Piezo-catalytic nanomaterial and their effect on antibiotics

| Piezo-catalytic nano-particle (NP) | Synthesis | Effect | References |
|---|---|---|---|
| 1. Bi2WO6/$Ag_2O$ composite | Mixing of Bi2WO6 np with the Ag2O np in appropriate ratio (1:1 to 1:10); heat the mixture for better adhesion and interaction to create the np-composite; the characterize the composite using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM). | Through the use of piezo-catalytic degradation to break down ciprofloxacin. | [26] |
| 2. $BaTiO_3$ | Barium and Titanium precursor to ethanol solution; which form gel like solution; then drying and calcinated at 600°C-900°C to induce crystalline structure and then characterized by SEM, TEM. | Helps to degradation of Tetracycline | [27, 28] |
| 3. Fe@3DWS2 nano composite | WS2 nano-sheet preparation (W:S=1:15), then synthesized Fe@3dWS2 through ball milling method; characterized by SEM, TEM, XRD. | Degradation of Levofloxacin | [29] |
| 4. FTO/$BaTiO_3$/AgNPs | In order to create a gray powder known as $BaTiO_3$/AgNPs, hydrothermally produced $BaTiO_3$ was dispersed in $AgNO_3$ solution, stirred for ten minutes in the dark, and then exposed to light for thirty minutes. | Degradation of Ciprofloxacin | [30] |

## Conclusions and future perspective

This review study summarizes and reports on four common piezo-catalytic nano materials (Bi2WO6/Ag2O, BaTiO3, Fe@3D-WS2, and

FTO/$BaTiO_3$/AgNPs) for treating antibiotic-resistant infections. In usage scenarios, piezocatalytic nanomaterials effectively treat antibiotic-resistant water pathogens by breaking down organic pollutants and producing reactive oxygen species due to their unique combination of catalytic and piezoelectric properties [31]. Future research should enhance nanomaterials' piezocatalytic performance by optimizing composition, morphology, and surface properties. This selective targeting of waterborne pathogens maintains ecological balance. Scaling up synthesis and production for real-world water treatment is crucial. Future studies should evaluate environmental impact and long-term sustainability, while combining treatments for antibiotic-resistant waterborne pathogens. Piezocatalytic nanomaterials offer innovative water treatment solutions for antibiotic-resistant pathogens, requiring continued research, interdisciplinary collaborations, and robust regulatory frameworks to fully realize their potential. Therefore, Piezocatalytic-based treatment is a promising, sustainable, and environmentally friendly solution for addressing antibiotic-resistant pathogens in water sources, ensuring safe drinking water access.

**References**


1. Danner, M. C., Robertson, A., Behrends, V., & Reiss, J. (2019). Antibiotic pollution in surface fresh waters: Occurrence and effects. *Science of the total environment*, *664*, 793-804.
2. Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016). Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in microbiology*, *7*, 1728.
3. Serwecińska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. *Water*, *12*(12), 3313.
4. Kariuki, S., Kering, K., Wairimu, C., Onsare, R., & Mbae, C. (2022). Antimicrobial resistance rates and surveillance in sub-Saharan Africa: where are we now? *Infection and drug resistance*, 3589-3609.
5. Aguilar, G. R., Swetschinski, L. R., Weaver, N. D., Ikuta, K. S., Mestrovic, T., Gray, A. P., ... & Naghavi, M. (2023). The burden of antimicrobial resistance in the Americas in 2019: a cross-country systematic analysis. *The Lancet Regional Health–Americas*, *25*.
6. Tang, K. W. K., Millar, B. C., & Moore, J. E. (2023). Antimicrobial resistance (AMR). *British Journal of Biomedical Science*, *80*, 11387.
7. Mogasale, V. V., Saldanha, P., Pai, V., Rekha, P. D., & Mogasale, V. (2021). A descriptive analysis of antimicrobial resistance patterns of

WHO priority pathogens isolated in children from a tertiary care hospital in India. *Scientific reports*, *11*(1), 5116.

8. He, J., Dong, C., Chen, X., Cai, H., Chen, X., Jiang, X., ... & Badsha, M. A. (2023). Review of Piezocatalysis and Piezo-Assisted Photocatalysis in Environmental Engineering. *Crystals*, *13*(9), 1382.
9. Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. *Pathogens*, *10*(10), 1310.
10. Zainab, S. M., Junaid, M., Xu, N., & Malik, R. N. (2020). Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water research*, *187*, 116455.
11. Adegoke, A. A., Madu, C. E., Aiyegoro, O. A., Stenström, T. A., & Okoh, A. I. (2020). Antibiogram and beta-lactamase genes among cefotaxime resistant E. coli from wastewater treatment plant. *Antimicrobial Resistance & Infection Control*, *9*, 1-12.
12. Zhang, S. H., Lv, X., Han, B., Gu, X., Wang, P. F., Wang, C., & He, Z. (2015). Prevalence of antibiotic resistance genes in antibiotic-resistant Escherichia coli isolates in surface water of Taihu Lake Basin, China. *Environmental Science and Pollution Research*, *22*, 11412-11421.
13. Chang, D., Mao, Y., Qiu, W., Wu, Y., & Cai, B. (2023). The source and distribution of tetracycline antibiotics in China: A review. *Toxics*, *11*(3), 214.
14. Maghsodian, Z., Sanati, A. M., Mashifana, T., Sillanpää, M., Feng, S., Nhat, T., & Ramavandi, B. (2022). Occurrence and distribution of antibiotics in the water, sediment, and biota of freshwater and marine environments: a review. *Antibiotics*, *11*(11), 1461.
15. Dinos, G. P. (2017). The macrolide antibiotic renaissance. *British journal of pharmacology*, *174*(18), 2967-2983.
16. Alegbeleye, O. O., & Sant'Ana, A. S. (2020). Manure-borne pathogens as an important source of water contamination: An update on the dynamics of pathogen survival/transport as well as practical risk mitigation strategies. *International journal of hygiene and environmental health*, *227*, 113524.
17. Some, S., Mondal, R., Mitra, D., Jain, D., Verma, D., & Das, S. (2021).

Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. *Energy Nexus*, *1*, 100008.

18. Taviani, E., van den Berg, H., Nhassengo, F., Nguluve, E., Paulo, J., Pedro, O., & Ferrero, G. (2022). Occurrence of waterborne pathogens and antibiotic resistance in water supply systems in a small town in Mozambique. *BMC microbiology*, *22*(1), 243.
19. Das, B., Verma, J., Kumar, P., Ghosh, A., & Ramamurthy, T. (2020). Antibiotic resistance in Vibrio cholerae: understanding the ecology of resistance genes and mechanisms. *Vaccine*, *38*, A83-A92.
20. Ranjbar, R., & Farahani, A. (2019). Shigella: antibiotic-resistance mechanisms and new horizons for treatment. *Infection and drug resistance*, 3137-3167.
21. Gu, B., Qin, T. T., Fan, W. T., Bi, R. R., Chen, Y., Li, Y., & Ma, P. (2017). Novel mutations in gyrA and parC among Shigella sonnei strains from Jiangsu Province of China, 2002-2011. *International Journal of Infectious Diseases*, *59*, 44-49.
22. Portes, A. B., Panzenhagen, P., Pereira dos Santos, A. M., & Junior, C. A. C. (2023). Antibiotic resistance in Campylobacter: a systematic review of South American isolates. *Antibiotics*, *12*(3), 548.
23. Fischer, G. H., Hashmi, M. F., & Paterek, E. (2024). Campylobacter Infection. In *StatPearls [Internet]*. StatPearls Publishing.
24. Dolai, J., Mandal, K., & Jana, N. R. (2021). Nanoparticle size effects in biomedical applications. *ACS Applied Nano Materials*, *4*(7), 6471-6496.
25. Djellabi, R., Ordonez, M. F., Conte, F., Falletta, E., Bianchi, C. L., & Rossetti, I. (2022). A review of advances in multifunctional XTiO3 perovskite-type oxides as piezo-photocatalysts for environmental remediation and energy production. *Journal of Hazardous Materials*, *421*, 126792.
26. Liu, B., Sun, L. Y., Chen, Y. X., Su, S. X., Wang, X. F., & Wang, X. (2024). A novel Ag2O/Bi2WO6 composite with enhanced piezocatalytic performance for ciprofloxacin removal. *Inorganic Chemistry Communications*, *159*, 111832.
27. Guo, Q., Gao, T., Padervand, M., Du, D., Zhao, K., Zhang, Y., ... & Wang, C. (2023). Piezo-Photocatalytic Degradation of Tetracycline by 3D BaTiO3 Nanomaterials: The Effect of Crystal Structure and Catalyst Loadings. *Processes*, *11*(12), 3323.

28. Masekela, D., Hintsho-Mbita, N. C., Sam, S., Yusuf, T. L., & Mabuba, N. (2023). Application of BaTiO3-based catalysts for piezocatalytic, photocatalytic and piezo-photocatalytic degradation of organic pollutants and bacterial disinfection in wastewater: A comprehensive review. *Arabian Journal of Chemistry*, *16*(2), 104473.

29. Meng, F., Ma, W., Duan, C., Liu, X., Chen, Z., Wang, M., ... & Zhang, Z. (2019). High efficient degradation of levofloxacin by edge-selectively Fe@ 3D-WS2: Self-renewing behavior and Degradation mechanism study. *Applied Catalysis B: Environmental*, *252*, 187-197.

30. Masekela, D., Hintsho-Mbita, N. C., Ntsendwana, B., & Mabuba, N. (2022). Thin films (FTO/BaTiO3/AgNPs) for enhanced piezo-photocatalytic degradation of methylene blue and ciprofloxacin in wastewater. *ACS omega*, *7*(28), 24329-24343.

31. Jiang, Z., Tan, X., & Huang, Y. (2022). Piezoelectric effect enhanced photocatalysis in environmental remediation: State-of-the-art techniques and future scenarios. *Science of the Total Environment*, *806*, 150924.

# Chapter - 3

## Inflammatory Bowel Disease and the Gut Microbiome: A Systematic Review of Current Evidence and Future Directions


**Authors**

**Ranit Acharya**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Sagnik Sardar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Akash Saha**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Debjit De**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 3

## Inflammatory Bowel Disease and the Gut Microbiome: A Systematic Review of Current Evidence and Future Directions

**Ranit Acharya, Sagnik Sardar, Akash Saha and Debjit De**

**Abstract**

In the twenty first century, the epidemiological changes in inflammatory bowel disease (IBD) a chronic metabolic disease incidence across many countries. The exact cause of IBD is unknown but it is throughout to be caused by a combination of genetic and other factors. Along with these factors alteration of gut microbiota distribution also played major role in IBD that causes inflammation in the gastrointestinal tract. The microbial community's structure is impacted by the consumption of different nutrients, which also supply substrates for microbial metabolism. Dietary variances make differences in the gut flora and small chemicals produced by the microbiota can be taken by the host and impact numerous vital physiological processes. Empirical data has indicated that immune system dysfunction related to gut microbiota occurs in the setting of host genetic predisposition in IBD, such as Crohn's disease (CD) and ulcerative colitis (UC). Recent developments have demonstrated that in western nations, rising rates of fat and sugar consumption correspond with a rise in CD cases in the latter half of the 20th century. In this review we provide a thorough explanation of how nutrition impacts the composition and metabolome of the human intestinal gut microbiome, and how this may impact on health or in development of IBD. This review also highlights the possibility of modifying the gut microbiota to treat IBD by use of successful precision microbiome approach like prebiotics, probiotics, or fecal microbiota transplantation.

**Keywords:** Inflammatory Bowel Disease (IBD), Microbiome, Gut Microbiota, Crohn's Disease (CD), Ulcerative Colitis (UC).

### Introduction

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative disease (UC), are proposed to result from an inappropriate immune

response to the gut microbes in a genetically suspectable host. It is a chronic inflammatory disorder of the intestinal tract of an unknown cause. This incidence of IBD has increased in the western world since the mid twentieth century. At the turn of twenty-first century, it plateaued of up to 0.5 % of the general population, but now it continuing to rise in developing nations. Etiological study on IBD shows that several factors including host genetics are immune responses, the gut microbiota, and the importance of environmental stimuli in disease pathogenesis. Gut dysbiosis shows the relation with IBD. Due to lot of experiments on gut microbes and IBD we are able to open the role of microbiome in development of IBD. These findings improve our knowledge on the functional mechanism of the microbiome in the pathogenesis of IBD.

**What is IBD?**

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine. It including Crohn's disease and ulcerative colitis (UC). Crohn's disease affects the small intestine and large intestine, as well (Source: The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247) as mouth, oesophagus, stomach, anus, and ulcerative colitis primarily affect colony and the rectum.

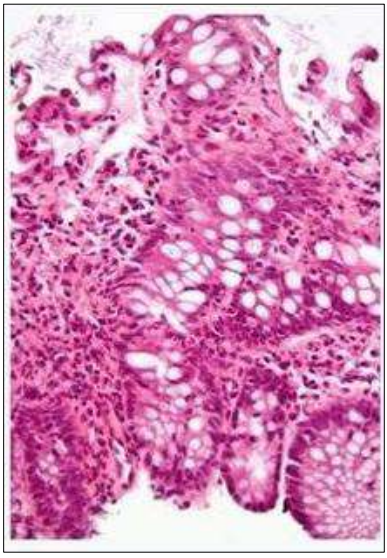

**Fig 1:** Inflammatory bowel disease (Google image)

**Signs and symptoms**

Crohn's disease and UC are very different diseases, but both have same symptoms like abdominal pain diarrhea, rectal bleeding, internal cramps in the region of pelvis and weight loss. Anemia is the most prevalent extra intestinal complication of IBD. Other disease including arthritis, (Source: The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247), pyoderma gangrenosum, non-thyroidal illness syndrome (NTIS) etc. Diagnosis is generally by assessment of inflammatory markers in stool following by colonoscopy with biopsy. Researches have been shows the prognosis.

**Causes of IBD**

**Bile acid**

Bile acid is important etiological agent in IBD pathogenesis. IBD patient have increase abundance of primary bile acid such as cholic acid and chenodeoxycholic acid and decrease abundance of secondary bile acid such as lithocholic asci and deoxycholic acid.

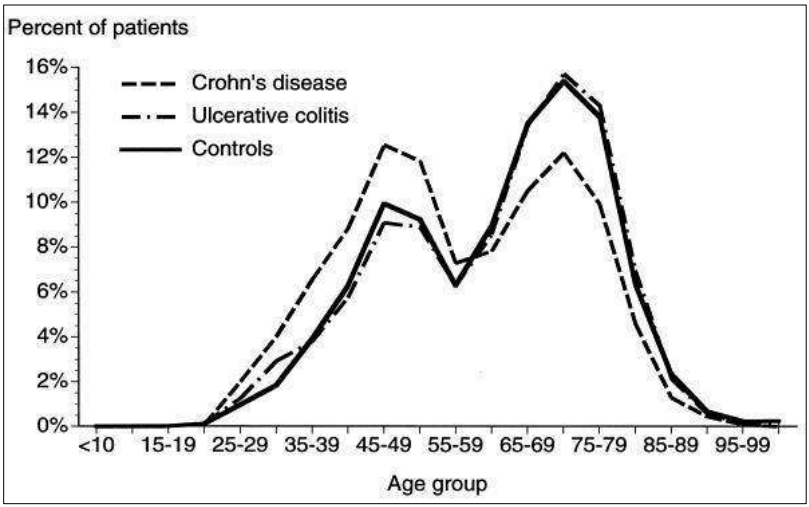


**Fig 2:** Age wise risk of IBD (Bäckhed *et al*., 2012)

**Oxidative stress and DNA damage**

Oxidative stress and DNA damage likely have a role in IBD. It increases the IBD colitis primarily affects the colon and the rectum.

**Microbiota**

Gut microbiota may contribute to IBD and it can control or balance the IBD in human body. Gut microbiome is microorganisms including bacteria, archaea, fungi and viruses that are live in the digestive tracts of animals. The gut is the main location of human microbiome. About 95 % of our microbiota is located in the GI tract.

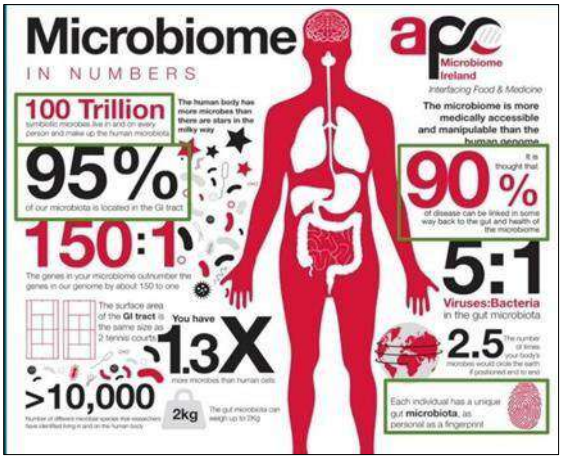


**Fig 3:** Body distribution of microbes (Bäckhed *et al*., 2012)

Composition of gut microbiota varies across the region. The colon contains (Source: The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247), highest microbial density. 99% of gut bacteria come in gut is about 10^13 – 10^14. It is established one to two years after birth. It has huge impact in our body like synthesis of vitamin that provide nutrients to gut epithelium, energy metabolism, glucose and lipid homeostasis, reduced short chain fatty acids linked to bowel inflammation. It also balances immunity. 70% of immune system depends on gut associated lymphoid tissue (GALT).

**Gut microbiota relation with IBD**

**Diet and gut microbiota**

Diet significantly impacts gut microbiota, with western diets linked to disease like IBD, diabetes and obesity. The protective mechanism of certain diet against IBD remain unclear amid urbanization risks. Long term and short-term diets influence gut microbiota composition and function. Diets promote higher microbiota (Source: Agus A., Denizot J., Thevenot J., Martinez-Medina

M., Messier S., Sauvanet P., *et al*. (2016). Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive, E. coli infection and intestinal inflammation. Sci. Rep. 6:19032. 10.1038/srep19032 [PMC free article] [PubMed] [Cross Ref] [Google Scholar]) diversity compared to western diets. Consuming a western diet, characterized by low Bacteroides to firmicutes ratio, is linked to increased susceptibility to adherest invasive E. coli infection and higher incidence of Crohn's disease. This correlation extends to consumption of animal products, bear, honey, animal fats and ghee. On the other hand, increase UC incidence is associated higher intake pineapples and coffee. Low fiber diet decreases microbial ecosystem. Purified prebiotic fibers diet variation do not alleviate mucus layer degradation.

**Bacterial microbiota**

Bacterial microbiota is the most well studied component of gut microbiota. In GI tract it contains upper level in in the colon of 10^11 – 10^12 cells/g. of luminal contents. Gut contains nearly 1000 bacteria species. This bacteria species plays important function in the host, including educating the immune response, secreting enzymes for digesting substrates. The *phyla firmicutes*, *Bacteroidetes*, *actinobacteria* and *verrucomicrobia* are predominant constituent of in healthy gut microbiota. The gut bacterial microbiota develops from a low diverse community at birth into a highly complex community with the introduction of diets by 9-12 months of age. Various factors including age, genetics, diet and drug intervene with gut microbiome. Gut microbiome was demonstrated to be an (Source: Agus A., Denizot J., Thevenot J., Martinez-Medina M., Messier S., Sauvanet P., *et al*. (2016). Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive, E. coli infection and intestinal inflammation. Sci. Rep. 6:19032. 10.1038/srep19032 [PMC free article] [PubMed] [Cross Ref] [Google Scholar]) essential factors in intestinal inflammation in IBD. It has been shown that in IBD there is a decrease in biodiversity, known as alpha diversity and in a community. The decreased diversity was partly linked to the temporal instability of the dominant taxa in IBD. There is a reduced diversity in inflamed vs non-inflamed region in CD patients.


**Fig 4:** Gut microflora

A multicentre study that they investigated that 1000 treatment native paediatric CD sample that change in bacteria including *veillonellaceae*, *clostridial* strongly correlated with disease status. Human studies have been shown that abundance of specific bacteria taxa was altered in IBD. *Enterobacteriaceae* bacteria are augmented both in patients with IBD and in mice. E. coli particularly adherent invasive E. coli strain, were isolated from ideal CD biopsy and were also found in UC patients. Meanwhile, mucosal samples showed more pronounced enrichment then faecal samples. It indicates that the inflammatory environment in IBD may favor the growth of this bacterial clade, *Enterobacteriaceae*. Anti-inflammatory (Source: Ahmad M. S., Krishnan S., Ramakrishna B. S., Mathan M., Pulimood A. B., Murthy S. N. (2000). Butyrate and glucose metabolism by colonocytes in experimental colitis in mice. Gut 46, 493–499. 10.1136/gut.46.4.493 [PMC free article] [PubMed] [CrossRef] [Google Scholar]) drug- mesalamine, could attenuator intestinal inflammation and decrease the abundance of Escherichia/shigella in IBD.

There are a specific group of gut bacteria like *Lactobacillus*, *facealibacterium* have been shown to be protective role against IBD. It is protective of the host from mucosal inflammation via several mechanism, including the stimulation of the anti-inflammatory cytokine. *Faccalibacterium prausnitzii* has been shows to have anti-inflammatory properties and was under irrepresented in IBD. Epidemiological data in humans suggested a

protective effect of *Heliobacter pylori* infection against (Source: The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247) the development of autoimmune disease, including IBD. Laboratory data demonstrated that *H-pylori* could induce immune tolerance and limit inflammatory responses. Some members of the gut microbiota have the ability to ferment dilatory fibre resulting in the production of short chain fatty acid (SCFAs) including acetate, propionate and butyrate. SCFAs has an effect on energy source for colonic epithelial cells. Lack of dietary fibre intake has been associated with the development of IBD. In addition, low fiber diet is associated with low concentration of SCFAs.

**Table:** List of microbial taxa and their changes in disease condition

| | **Decreased in IBD** | **Increased in IBD** |
|---|---|---|
| **Microbial composition** | *Bifidobacterium sp.*<br>Groups IV and XIVA *Clostridium*<br>*Faecalibacterium Prausnitzii*<br>*Roseburia species*<br>*Suterella species*<br>*Bacteroides*<br>*Saccharomyces cerevisiae* | *Probacteria*<br>*Escherichia coli*<br>*Fusobacterium species*<br>*Ruminococcus gnavusa*<br>*Pasteurellaceae* |

**Fungal microbiota (mycobiota)**

Fungi account for <0.1% of the total gut microbiota. It is underestimated due to our current challenge to annonate fungi as a result of an incomplete fungal genomic database. But target region sequencing of marker genes, such as internal transcribed spacer (ITS) and 18s rRNA, mapping to annotated databases has contributed to an improved understanding of gut microbiota. The composition of fungi in different body part is different, in GI tract, urogenital tract (Source: Albenberg L. G., Wu G. D. (2014). Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 146, 1564–1572. 10.1053/j.gastro.2014.01.058 [PMC free article] [PubMed] [CrossRef] [Google Scholar]) and oral cavity have the largest number of taxa consisting up to 160 species. *Candida albicans*, *C. parasitosis* and *C. blaberata* are common in human. In human long-term use of antibiotics can promote fungal overgrowth and infection that supports a role for gut mycobiota in the development of immune-mediated diseases. In healthy individuals, *saccharomyces, candida* and *cladosporium* were the most predominant genera but Ascomycota, C. albicans increase IBD. *Xylariales* were increased in inflammed mucosa of CD. Overall, these data suggest that an increased fungal load of candida species and altered bacteria

diversity may be associated with the pathogenic feature of CD. Therefore, emerging evidence favouring a role for gut mycobiota in IBD pathogenesis.

**Viral microbiota (virobiota)**

The virobiota comprises both eukaryotic viruses and prokaryotic bacteriophages and contain more diverse biological entities then the gut bacterial microbiota. Bacteriophages have a great role in IBD. Patients with CD exhibited a lower diversity but higher variability of the (Source: Albenberg L. G., Wu G. D. (2014). Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 146, 1564–1572. 10.1053/j.gastro.2014.01.058 [PMC free article] [PubMed] [CrossRef] [Google Scholar]) gut virome related to controles. Statistics shows that UK, Chicago and Boston richness of virome increase CD and UC patients. The expension in bacteriophages in IBD could arise from commensal microbes that enter lytic cycle or from new viruses introduction from surrounding environment. Composition of bacteriophage have the effect on gut bacterial microbiota that's why the bacteria who are decreasing the IBD are decreased that increase IBD.

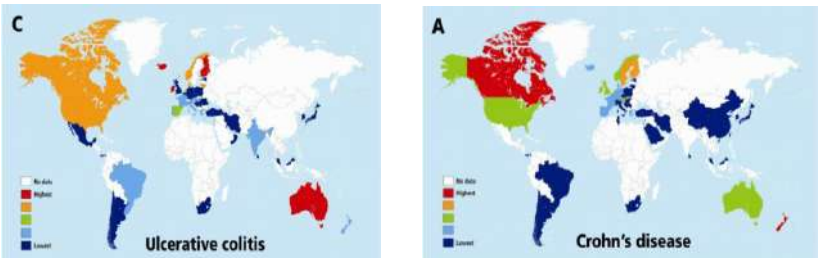


**Fig 5:** World distribution of CD and UC (Bäckhed *et al*., 2012)

**Helminths**

Helminths are worm-like parasites and a crucial component of gut microbial diversity. The hygiene hypothesis suggested that reduced exposure to such organism in early childhood may contribute to immune-mediated disease later in life, particularly in developed countries. The decline in helminth colonization in industrialized nations correlates with an increase in autoimmune disease like IBD. Studies indicate that helminths play an immunoregulatory role, protecting against IBD development. Helminths like *Trichuris muris* and *Heligmosoides polygyrus* demonstrate a protective effect by (Source: The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247) influencing inflammatory responses. Helminth

colonized individuals exhibit higher gut bacterial diversity suggesting a potential link between helminth presence and microbiome structure. The contrast in helminth prevalence and microbiome conjugation between rural and urban dwellers implies a protective role for helminths in rural population against IBD microbiota. This highlights, the indicate interplay between helminths, gut bacteria, and immune system in shaping health outcomes.

**Using gut microbiome to cure IBD**

In the treatment of inflammatory bowel disease (IBD) antibodies show modest effect in Crohn's disease (CD) but are generally ineffective in ulcerative colitis (UC). Combination of antibiotics may improve outcomes, but long-term use can lead to antibiotic resistance in gut microbes. Traditional probiotics have limited effectiveness (Source: Arnold I. C., Lee J. Y., Amieva M. R., Roers A., Flavell R. A., Sparwasser T., *et al*. (2011). Tolerance rather than immunity protects from Helicobacter pylori–induced gastric preneoplasia. Gastroenterology 140, 199–209. 10.1053/j.gastro.2010.06.047 [PMC free article] [PubMed] [CrossRef] [Google Scholar]) but *E. coli Nissle* show promise in reducing inflammation and sustaining remission in UC.

*F. prausnitzii* and other beneficial microbes may have protective effect by producing SCFAs and inhibiting immune responses. Blocking virulance products of pathogenic microbes or their activity may diminish dysbiotic bacteria in the gut. Microbial markers are crucial for predicting treatment responses and disease progression in conditions like CD and UC. Specific biomarkers such as apolipoprotein A1 and *F. prausnitzii* concentration, aid in predicting outcomes like steroid-free remission and post- operative recurrence. Additionally, microbial configuration correlate with therapy response and risk of relapse. Lifestyle factors, including antibiotic (Source: The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, published online 2018 Sep 25 doi: 10.3389/fmicb.2018.02247) exposure and urbanization increase the risk of IBD. Human migration studies and population-based studied highlight the link between early-life exposures, microbial colonization, and IBD risk, emphasizing the role of gut microbes in childhood IBD pathogenesis.

Faecal microbiota transplantation: Fecal microbiota transplantation (FMT), proved effective for recurrent clostridium defficile infection (CFI), has generated interest as a treatment of IBD. While successful in restoring microbial balance in CDI evidence for FMT in IBD remain equivocal. A systematic analysis of 18 (Source: Angelberger S., Reinisch W., Makristathis A., Lichtenberger C., Dejaco C., Papay P., *et al*. (2013). Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal

microbiota transplantation. Am. J. Gastroenterol. 108, 1620–1630. 10.1038/ajg.2013.257 [PubMed] [CrossRef] [Google Scholar]) studies including 122 patients with IBD found that around 36 - 45% patients achieved clinical remission during follow up. The clinical remission rate is 22% for UC and 61% for CD. More research can improve the power of treating IBD.

**Conclusion and future perspective**

Efforts thus far have successfully delineated the various elements comprising the human gut microbiota in both health and IBD, with a predominant focus on bacterial components. However, studies at the functional and strain-specific levels are still lacking. The less explored realms of gut microbiota, namely viruses and fungi, and their cross-kingdom interaction with bacteria, hold significant potential for influencing human health and IBD, yet remain in early stages of exploration. Studies involving advanced molecular microbiological techniques and animal models are essential. Understanding the role of different gut microbes in IBD pathogenesis and disease progression precise integration with host genetic polymorphisms and gene expression. While the prospect of microbe-based therapeutics is promising the effective application of probiotics, prebiotics, antibiotics and fecal microbiota transplantation (FMT) demands a personalized approach. Identifying specific subsets of patients who would derive the most benefit from such strategies is crucial for their successful implementation.

**References**


1. Agus A., Denizot J., Thevenot J., Martinez-Medina M., Massier S., Sauvanet P., *et al*. (2016). Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive, E. coli infection and intestinal inflammation. Sci. Rep. 6:19032. 10.1038/srep19032 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
2. Ahmad M. S., Krishnan S., Ramakrishna B. S., Mathan M., Pulimood A. B., Murthy S. N. (2000). Butyrate and glucose metabolism by colonocytes in experimental colitis in mice. Gut 46, 493–499. 10.1136/gut.46.4.493 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
3. Albenberg L. G., Wu G. D. (2014). Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology 146, 1564– 1572. 10.1053/j.gastro.2014.01.058 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
4. Angelberger S., Reinisch W., Makristathis A., Lichtenberger C., Dejaco

C., Papay P., *et al*. (2013). Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. Am. J. Gastroenterol. 108, 1620– 1630. 10.1038/ajg.2013.257 [PubMed] [CrossRef] [Google Scholar]

5. Arnold I. C., Lee J. Y., Amieva M. R., Roers A., Flavell R. A., Sparwasser T., *et al*. (2011). Tolerance rather than immunity protects from Helicobacter pylori–induced gastric preneoplasia. Gastroenterology 140, 199–209. 10.1053/j.gastro.2010.06.047 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

6. The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease, Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247

7. Bäckhed F., Fraser C. M., Ringel Y., Sanders M. E., Sartor R. B., Sherman P. M., *et al*.. (2012). Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe 12, 611–622. 10.1016/j.chom.2012.10.012

8. Backhed F., Roswall J., Peng Y., Feng Q., Jia H., Kovatcheva-Datchary P., *et al*.. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 17:852. 10.1016/j.chom.2015.05.012

9. Barr J., Auro R., Furlan M., Whiteson K., Talago N., Paul L., *et al*. (2013). Bacteriophage adhered to mucus provide a novel mucosal immune system. J. Immunol. 190:61.8.10.

10. Barr J. J., Auro R., Furlan M., Whiteson K. L., Erb M. L., Pogliano J., *et al*.. (2013). Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc. Natl. Acad. Sci. U.S.A. 110, 10771–10776. 10.1073/pnas.1305923110

11. Barreiro-de Acosta M., Alvarez Castro A., Souto R., Iglesias M., Lorenzo A., DominguezMunoz J. E. (2011). Emigration to western industrialized countries: a risk factor for developing inflammatory bowel disease. J. Crohns Colitis 5, 566–569. 10.1016/j.crohns,.2011.05.009

12. Benchimol E. I., Manuel D. G., Guttmann A., Nguyen G. C., Mojaverian N., Quach P., *et al*.. (2014). Changing age demographics of inflammatory bowel disease in ontario, canada: a population-based cohort study of epidemiology trends. Inflamm. Bowel Dis. 20, 1761–1769. 10.1097/MIB.0000000000000103

13. Bibiloni R., Fedorak R. N., Tannock G. W., Madsen K. L., Gionchetti P., Campieri M., *et al*.. (2005). VSL# 3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am. J. Gastroenterol. 100, 1539–1546. 10.1111/j.1572-0241.2005.41794.x

# Chapter - 4

## Microbial Approach for Lead Bioremediation in Paint Industry Waste

**Authors**

**Aakanksha Chourasia**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Pritha Pal**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 4

## Microbial Approach for Lead Bioremediation in Paint Industry Waste

**Aakanksha Chourasia and Pritha Pal**

**Abstract**

Plenty of research has been conducted in recent years on the elimination of lethal heavy metal ions from industrial effluent. The paint industry is one of several industries whose wastewater may contain heavy metal contamination. When heavy metals like lead (Pb) are released into industrial wastewater, they pose a serious risk to human health as well as the health of other living things. Aquatic plants and animals can absorb this heavy metal due to its high solubility in watery environments. The human body may absorb high doses of this heavy metal if it gets into the food chain. When a heavy metal intake exceeds the allowed limit, it can lead to major health problems, such as cancer. Therefore, before releasing metal-contaminated wastewater into the environment, it must be treated. To achieve technology-based treatment criteria, technologies for toxicity reduction are frequently used in industrial wastewater treatment methods including heavy metals. This study focused on the latest advancements and implementation of several treatment methods for eliminating heavy metals from industrial wastewater. When it comes to eliminating heavy metals from wastewater, the bioremediation approach is the most environmentally acceptable substitute for physiochemical procedures, in which microorganisms are essential. In this work, lead contamination of wastewater from the paint industry is addressed by the use of microorganisms, particularly bacterial species.

**Keywords:** Hazardous heavy metal, industrial wastewater, paint industry, cancer, bioremediation, bacterial species.

### Introduction

Paint is a synthetic liquid material that forms a thin, cohesive film that adheres to infrastructure, furniture, and utensils. The United Nations Environment Programme (UNEP), World Health Organization (WHO), and international scientific community are working to phase out lead-based paint

globally, recognizing the significant health risks associated with it (Connor *et al*. 2018). Paints are susceptible to microbial attack because they provide a surface for microbial development, regardless of whether they include harmful substances such as solvents and biocides. Biodeteriorators like bacteria, fungi, algae, and cyanobacteria can colonize and degrade paint coatings (Obidi *et al*. 2017). Colonization and biodeterioration rates are influenced by indigenous microbial diversity, environmental factors such as humidity, temperature, wind, light, oxygen, nitrogen, pH, and substrate composition (organic, inorganic, mineral, texture, pH, moisture) (Duan *et al*. 2018). Environmentalists argue that bacteria are the first biodeteriogens to colonize paint due to their low nutritional requirements and ability to support subsequent colonizers, such as fungi. The paint substrates are tainted with particulate matter such as dust, dirt, soot, and deceased cells, as well as volatile organic chemicals emitted by respiration and vapors, which can serve as nourishment. Microbial colonization causes deterioration and disintegration of organic and inorganic components in mural paintings (Rosado *et al*. 2015). The demand and widespread usage of chemicals such as dyes, solvents, and pigments in daily life have led to an expansion in their production through industrialization. According to the International Agency for Research on Cancer (IARC), painters and paint industry workers are exposed to a variety of potentially carcinogenic materials, including organic solvents (aliphatic, aromatic, and chlorinated), metals (lead, chromium, and cadmium), pigments, dyes, drying oils, biocide, and additives (Ezhilarasu *et al*. 2016). Lead, a material that has been utilized for thousands of years, is currently extensively employed in many products including paints, pigments, pipes, batteries, vinyl, cables, and radiation shielding. Lead is a perilous and enduring environmental poison that can damage many organs, human systems, and the environment. The worldwide illness burden from lead poisoning is estimated to be around 0.6%. Lead poisoning was first recognized over a century ago, affecting millions of youngsters. Lead-based paint has been outlawed in the US for decades (Amara *et al*. 2018).

Bioremediation is an eco-friendly method that uses the energy of naturally occurring prokaryotes and eukaryotes, such as bacteria, algae, fungus, plants, and enzymes, to restore the environment by eliminating or attenuating xenobiotics (Brar *et al*. 2017). Excessive exposure to xenobiotics can harm ecosystems, plants, animals, and human health. Conventional techniques of pollutant removal, such as disposal and land filling, are not environmentally friendly and can introduce contaminants into the food chain. Therefore, new and innovative approaches are necessary to maintain a healthy ecosystem (Adams *et al*. 2015). Bioremediation is an uncomplicated and economical

approach that necessitates minimal energy and equipment. It is widely accepted and has piqued the interest of environmentalists. Microbes, like biological systems, vary in nature and can absorb a variety of xenobiotics (Lawniczak *et al*. 2020). Microbes rely on xenobiotics for energy and have developed adaptation mechanisms to deal with substrate toxicity. Bioremediation is more effective than traditional approaches for treating pollutants in water, soil, and sediments. Enzymes produced by microbes can effectively remove contaminants and are extremely selective to their substrates (Phulpoto *et al*. 2021).

**Environmental pollution and health hazards from paint industry**

According to the World Health Organization (WHO), approximately 20-40% of workers in the industrial sector have a potential risk of developing cancer and neurological disorders. PCB-laden paint can lead to environmental contamination. Volatile organic compounds, hazardous vapours, and heavy metals such as lead, cadmium, chromium, and barium pose a threat to environmental health and contribute to ozone layer depletion (Amara *et al*. 2018). The process of industrialization has resulted in a surge in the manufacturing of chemicals, such as dyes, solvents, and pigments, in response to the growing demand and extensive utilization of these substances in everyday activities. In accordance to the International Agency for Research on Cancer (IARC), painters and paint industry workers are exposed to potentially carcinogenic compounds such as organic solvents (aliphatic, aromatic, and chlorinated), metals (lead, chromium, and cadmium), pigments, dyes, drying oils, biocide, and additives (Ezhilarasu *et al*. 2016). Despite the prohibition of lead use, numerous developed and emerging nations continue to impose limitations on its levels in paints, including those utilized for building interiors, toys, and anti-corrosive coatings. Lead-based paints cause health and environmental risks when found in consumer products such as toys, deteriorating paint chips, dust, and dirt (Connor *et al*. 2018). Paint pollution threatens both environmental health and biodiversity. Industrial effluents often contain hazardous dyes and intermediates that can cause cancer and mutations. Consequently, the presence of these substances diminishes the overall standard of living and causes damage to the ecosystem. Dyes pose challenges for biodegradation due to their synthetic composition and intricate aromatic chemical configurations. Dyes are categorized as persistent organic pollutants because of their exceptional stability in oxygen-deprived environments and when exposed to light. Conventional wastewater treatment technologies cannot fully remove dyes. To prevent toxicity, pollution, and carcinogenic consequences, dye-containing effluents should be processed before disposal or release (Phulpoto *et al*. 2021). After painting, solvents evaporate and enter the atmosphere, posing environmental and health risks (Amara *et al*. 2018).

Solvents in paint formulations can have short- and long-term environmental implications, as well as occupational health and neurobehavioral effects on workers (e.g., visual performance, memory impairment, behavioral symptoms) (Lin *et al*. 2019). Prolonged exposure to solvents has been associated with cognitive and emotional impairments, as well as immediate and long-lasting health effects such as asthma, liver and kidney harm, and cancer affecting several organs. Solvents such as benzene, toluene, xylenes (BTXs), and formaldehyde have been linked to cancer, making it crucial to address their emissions (Batterman *et al*. 2014). Benzene is a highly hazardous human carcinogen found in volatile organic chemicals commonly used in paints.The peoples in indoor environment without ventilation are at high risk of exposure with benzene fumes generated from gasoline, glues, solvents, paints and art supplies (Branch *et al*. 2016). Prolonged exposure to benzene can result in the development of leukemia, as well as symptoms such as drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, unconsciousness, damage to bone marrow, reduced formation of red blood cells, anemia, excessive bleeding, impaired immune system function, susceptibility to infections, mortality, and cancer affecting organs involved in blood formation. Toluene is an essential solvent used in the production of paints, coatings, adhesives, lubricants, and polymers. Inhaling toluene-containing paint fumes is a serious kind of inhalant abuse that can disrupt sleep, cause light-headedness, kidney failure, unconsciousness, and even death (Phulpoto *et al*. 2021). Ethylbenzene, a transparent liquid, can induce vertigo, irritation of the mouth and eyes, and impact the nervous system, liver, and kidneys. It is present in many products such as gasoline and paint. This study found that solvents such as hexane, heptane, and octane can also damage the central nervous system (Keegan *et al*. 2013). The worldwide illness burden from lead poisoning is estimated at 0.6%. Over a century ago, millions of children were afflicted by lead poisoning, prompting the recognition of the issue. For decades, the United States has prohibited the use of lead paint. Prolonged exposure to lead can have detrimental effects on the central nervous system (CNS), blood pressure regulation, vitamin D metabolism, gastrointestinal function, kidney function, and reproductive system. Lead can enter the body through various ways, including polluted dust, soil, water, and food. Paints with high concentrations contain mercury, chromium, and cadmium, followed by lead (Amara *et al*. 2018).

**Classification of paints used in paint industry**

Paint is a synthetic liquid substance that, upon application, solidifies into a cohesive and adhesive thin coating. This artificial substance ensures consistency in the appearance and quality of infrastructure, furniture, and

utensils used on a daily basis. Paint is a complex amalgamation of pigments, solvents, binders, and additives. Solvents facilitate the dispersion of paint upon application and drying, while preserving the integrity of the binder and pigment on the painted surface. Binder, a polymer that creates a uniform layer of variable thicknesses on the surface of the substrate, guarantees the adhesion of the paint film and protects the paint components (Phulpoto *et al*. 2021). Paints are categorized into four distinct types according to the binders they include (table 1).

**Table 1:** Classification of paints used in paint industry based on the binders (resins) (Phulpoto *et al*. 2021)

| Types of paints | Description |
|---|---|
| Acrylic paints or water-based paints | Acrylic encompasses both homopolymers and copolymers derived from acrylic acid and methacrylic acid esters. Water-based paints, also referred to as acrylic or emulsion paint, are commonly used. The inaugural acrylic paint emulsion for artists, known as Liquitex, was developed in 1956. Water-based polymers (WBPs) exhibit rapid evaporation and possess minimal toxicity when combined with water. WBPs are employed for aesthetic applications such as automotive painting, surface preservation, and residential construction and housing. |
| Polyvinyl acetate paints | Polyvinyl acetate (PVA) is extensively used in the manufacturing and conservation of paint. The low molecular weight of PVA results in accelerated drying. PVA paint, which is solventless, provides an attractive substitute for paint that is based on organic solvents. Polyvinyl acetate (PVA) emulsions are utilized for coating porous materials like wood, paper, linen, and building stone that have porous properties. PVA emulsions necessitate plasticization in order to adequately soften the polymer particles and create a paint covering. |
| Nitrocellulose paints | Nitrocellulose paints (NCPs) and pyroxylin refer to lacquers and paints that consist of cellulose nitrate. NCPs exhibit solubility in solvents such as esters, alcohols, ketones, and glycol ethers, but they are insoluble in linear (aliphatic) and aromatic mineral spirits. The primary characteristic of this paint is its capacity to readily dissolve in compatible solvents. After being brought into commercial use, NCPs were mostly employed for automobile coating and furniture lacquer. Moreover, their rapid drying and resilience rendered them highly sought-after in the paint sector. |
| Alkyd paints or oil-based paints or orthodox | Alkyd paints utilize a synthetic resin called alkyd resin as the binder. Polyesters that consist of unsaturated fatty acid chains, including oleic, linoleic, linolenic, and ricinoleic acid, serve as precursors for alkyd resin in oil-based paints (OBP). During the drying process, the volatile solvent rapidly vaporizes, and the binder undergoes a chemical reaction to solidify into a cohesive layer. Historically, alkyd paint formulas were composed of organic compounds and had an approximate solvent content of |

| | |
|---|---|
| | 50% VOC (volatile organic compounds). Alkyd paints are commonly diluted with white spirit at a concentration of 15-20% by weight. |

**Bioremediation as a sustainable approach:**

Bioremediation is a straightforward, economical, and environmentally beneficial technique that necessitates minimal energy and equipment. The idea has gained widespread acceptance and has generated significant interest among environmentalists (Lawniczak *et al*. 2020). Microbes, like biological systems, can ingest a variety of xenobiotics, making them versatile and adaptable to different ecosystems. Microbes use xenobiotics as a source of energy and have adaptation mechanisms to resist substrate toxicity. Bioremediation is more effective than traditional approaches for treating and removing pollutants in water, soil, and sediments (Brar *et al*. 2017). Enzymes produced by microbes can effectively remove contaminants and are extremely selective to their substrates (Lawniczak *et al*. 2020). The bioremediation process involves two approaches: Bioaugmentation combines artificial microorganisms with indigenous diversity to accelerate biodegradation for environmental cleanup, whereas bio-stimulation adjusts physical variables and nutrients to stimulate microbial growth and pollutant removal. Bioremediation is categorized into ex situ and in situ methods, depending on the location of the polluted site. Ex situ bioremediation takes place away from the pollution site, while in situ bioremediation occurs at the original location (Phulpoto *et al*. 2021).

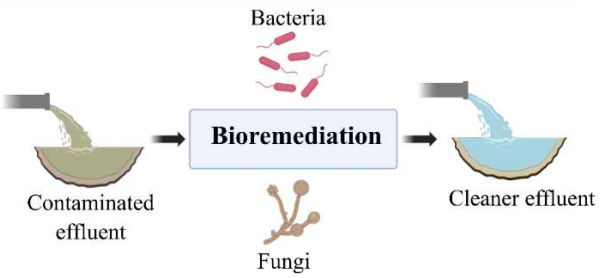


**Fig 1:** Pictorial representation of microbial bioremediation of lead contaminated paint industry wastewater

**Microbial bioremediation by bacteria**

Bacteria such as *Bacillus, Enterobacter, Pseudomonas, Proteus, Escherichia, Cyanobacteria, Sphingomonas, Thiobacillus, Mycobacterium, Clostridium, Alcaligenes, Micrococcus, Serratia, Aeromonas, Actinomycetes, Flavobacterium, Arthrobacter, Gallionella, Staphylococcus,* and others are

commonly isolated (Mukhaifi *et al*. 2019; Phulpoto *et al*. 2017). *Bacillus, Pseudomonas, Aeromonas, Halobacter,* and *Enterobacter* are known to detoxify and decolorize azo dyes more effectively than phenylamine, benzenediazonium chloride, or phenol (Phulpoto *et al*. 2021). *Pseudomonas* is extensively employed for industrial and environmental remediation owing to its capacity to thrive on a diverse range of organic compounds. The term "oil-eating bug" (OEB) is commonly used in the crude oil industry (Mitova *et al*. 2015).

**Table 2:** Microbial bioremediation of lead contaminated waste by bacteria (Phulpoto *et al*. 2021)

| Substrate | Bacteria |
|---|---|
| Water based paints | *Stenotrophomonas maltophilia* |
| Oil based paints | *B. subtilis* |
| Nitrocellulose paint | *Bacillus, Pseudomonas, Aeromonas, Halobacter, Flavobacterium, Escherichia, Enterobacter, P. Putidia, Bacillus subtilis.* |

## Microbial bioremediation using fungi

Fungal growth and metabolism produce several acids, including citric, gluconic, succinic, malic, oxalic, and itaconic acid. Acids react with paint components, either through metal ion chelation or cation solubilization (Unković *et al*. 2019). Fungi and actinomycetes produce mechanical harm via penetrating hyphae or mycelia. Paint waste deterioration is mostly caused by fungi from *Penicillium, Aspergillus, Fusarium, and Alternaria* species (Phulpoto *et al*. 2021).

Fungi reproduce through the dispersal of spores and have the potential to negatively impact the ability of paint to adhere to surfaces. Fungal species found on deteriorating paint film include *Apergillus, Trichoderma, Altemaria, Cladosporium, Aureobasidium, Acremonium, Scopulariopsis, Penicillium, Curvularia, Geotrichum, Mucor, Rhizopus, Stemphylium Drechslera,* slime-molds, *Fusarium, Gliomastk, Chaetomium,* hyphochytrids, chytrids, oomycetes, zygomycetes, and dikaryomycetes (Obidi *et al*. 2017).

**Table 3:** Microbial bioremediation of lead contaminated waste by fungi (Phulpoto *et al*. 2021)

| Substrate | Fungi |
|---|---|
| Water based paints | *Penicillium, Pleospora, Trichotecium, Fusarium sp., Rhizopus sp., Aspergillus sp., Mucour sp., Monas cus, Nigrospora.* |

| | |
|---|---|
| Oil-based paints | *Rhizopus, Aspergillus, Cladosporium, Trichoderma, Alternaria, Pestalotia, Penicillium, Fusarium.* |
| Nitrocellulose paint | *Aspergillus Ustus, Rhizopus arrhinus, Aspergillus niger, Pencillium citrinum.* |
| Emulsion paint | *Alternaria sp., Aspergillus sp., Penicillium sp., Cladosporium sp.* |

**Conclusion**

Paint's complicated composition poses environmental health risks, necessitating treatment before disposal. Furthermore, microbial-induced biodeterioration causes cultural and architectural loss. Bioremediation is a relatively new treatment option for environmentalists globally. Initially, microbial metabolic processes and degradation pathways were discovered to effectively clean up selected pollutants. Advancements in biotechnology have led to the development of genetically modified organisms (GMOs) with enhanced bioremediation capabilities. In addition, the use of immobilized cells and enzymes for environmental remediation has been investigated. The results of the consortia research revealed that bacterial consortia were the predominant choice for paint bioremediation, whereas there were limited studies that investigated the interaction between bacteria and fungi or the use of fungal consortia alone. The consortia research proved to be fruitless since the microbes exhibited antagonistic behaviour. Metagenomics analyzes the functional genes of bacteria in various physiological situations using DNA from the microbiome. While metagenomics can anticipate community functioning potential, it has limitations in understanding environmental clean-up efforts. Our hypothesis is that understanding the metaphenome is key to the future of bioremediation.

**Future prospects**

There is a need to find more effective microbes that can detoxify and break down paint waste, especially the harmful substances that cause damage to the environment, aquatic ecosystems, soil, and public health, but are now unidentified. It is imperative to search for more effective microorganisms for the purification and decomposition of lead prior to its ultimate discharge into the environment. Lead has detrimental effects on soil, aquatic ecosystems, and human health. In order to design successful bioremediation procedures, it is crucial to comprehend their genetic structure and biochemistry. Consequently, the long-term survival of natural ecosystems on land and in aquatic environments will be enhanced. In order to develop effective bioremediation solutions, it is imperative to have a comprehensive understanding of their genetic composition and biochemical processes. This enhances the likelihood

that natural ecosystems on land and in the ocean will endure for an extended period. Every industry that discharges lead in wastewater must adopt more economically efficient and environmentally sustainable waste treatment methods.

**Acknowledgement**

I like to extend my heartfelt appreciation to the esteemed authorities of Swami Vivekananda University, Dr. Pritha Pal, Head of the Department of Microbiology, and my Ph.D. mentor for her invaluable guidance and assistance in completing this review project.

**Conflict of interest**

The authors disclose no potential conflict of interest.

**References**

1. Adams, G. O., Fufeyin, P. T., Okoro, S. E., & Ehinomen, I. (2015). Bioremediation, biostimulation and bioaugmention: a review. International Journal of Environmental Bioremediation & Biodegradation, 3(1), 28-39. DOI:10.12691/ijebb-3-1-5
2. Amara, I., Miled, W., Slama, R. B., & Ladhari, N. (2018). Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. Environmental toxicology and pharmacology, 57, 115-130. https://doi. org/10.1016/j.etap.2017.12.001.
3. Batterman, S., Su, F. C., Li, S., Mukherjee, B., & Jia, C. (2014). Personal exposure to mixtures of volatile organic compounds: modeling and further analysis of the RIOPA data. Research Report (Health Effects Institute), (181), 3. PMID: 25145040; PMCID: PMC4577247.
4. Brar, A., Kumar, M., Vivekanand, V., & Pareek, N. (2017). Photoautotrophic microorganisms and bioremediation of industrial effluents: current status and future prospects. 3 Biotech, 7, 1-8. https ://doi. org/10.1007/s13205-017-0600-5
5. Branch H, Hamedan I (2016) The effects of oil paint vapor on thermal pain sensitivity in male rats
6. Duan, Y., Wu, F., Wang, W., Gu, J. D., Li, Y., Feng, H., ... & An, L. (2018). Differences of Microbial Community on the wall paintings preserved in situ and ex situ of the Tiantishan Grottoes, China. International Biodeterioration & Biodegradation, 132, 102-113. https ://doi. org/10.1016/j.ibiod.2018.02.013

7. Ezhilarasu, A. (2016). Textile industry Dye degrading by bacterial strain Bacillus sp. Int J Adv Res Biol Sci, 3(3), 211-226.

8. Keegan, T. J., Bunch, K. J., Vincent, T. J., King, J. C., O'Neill, K. A., Kendall, G. M., ... & Murphy, M. F. G. (2013). Case-control study of paternal occupation and social class with risk of childhood central nervous system tumours in Great Britain, 1962–2006. British journal of cancer, 108(9), 1907-1914. https://doi.org/10.1038/bjc.2013.171

9. Ławniczak, Ł., Woźniak-Karczewska, M., Loibner, A. P., Heipieper, H. J., & Chrzanowski, Ł. (2020). Microbial degradation of hydrocarbons—basic principles for bioremediation: a review. Molecules, 25(4), 856. https://doi.org/10.3390/molecules25040856

10. Lin, C. H., Lai, C. H., Peng, Y. P., Wu, P. C., Chuang, K. Y., Yen, T. Y., & Xiang, Y. K. (2019). Comparative health risk of inhaled exposure to organic solvents, toxic metals, and hexavalent chromium from the use of spray paints in Taiwan. Environmental Science and Pollution Research, 26, 33906-33916.

11. Mukhaifi, E. A., Al-Tamimi, W. H., & Burghal, A. A. (2019). Detection of Bacillus sp. from Wall Paint. Int J Curr Microbiol App Sci, 8, 2418-2422. https://doi.org/10.20546/ijcmas.2019.807.297

12. Mitova, M., Iliev, M., & Groudeva, V. (2015). Identification of Bacillus strains isolated from rock paintings in Magoura cave, Bulgaria. Journal of BioScience & Biotechnology.

13. O'Connor, D., Hou, D., Ye, J., Zhang, Y., Ok, Y. S., Song, Y., ... & Tian, L. (2018). Lead-based paint remains a major public health concern: A critical review of global production, trade, use, exposure, health risk, and implications. Environment international, 121, 85-101. https ://doi.org/10.1016/j.envint.2018.08.052

14. Obidi, O., & Okekunjo, F. (2017). Bacterial and fungal biodeterioration of discolored building paints in Lagos, Nigeria. World journal of microbiology and biotechnology, 33, 1-9. https://doi.org/10.1007/s11274-017-2362-y

15. Phulpoto, A. H., Qazi, M. A., Mangi, S., Ahmed, S., Phul, A. R., & Kanhar, N. A. (2017). Bioremediation of oil-based paint from aqueous media by novel indigenous Brevibacillus parabrevis strain NAP3 and its toxicity assessment. Polish Journal of Environmental Studies, 26(1). DOI: 10.15244/pjoes/62905

16. Phulpoto, A. H., Maitlo, M. A., & Kanhar, N. A. (2021). Culture-

dependent to culture-independent approaches for the bioremediation of paints: a review. International Journal of Environmental Science and Technology, 18, 241-262. https://doi.org/10.1007/s13762-020-02801-1

17. Rosado, T., Mirão, J., Candeias, A., & Caldeira, A. T. (2015). Characterizing microbial diversity and damage in mural paintings. Microscopy and Microanalysis, 21(1), 78-83. https://doi.org/10.1017/S1431927614013439

18. Unković, N., Ljaljević Grbić, M., Stupar, M., Vukojević, J., Subakov-Simić, G., Jelikić, A., & Stanojević, D. (2019). ATP bioluminescence method: Tool for rapid screening of organic and microbial contaminants on deteriorated mural paintings. Natural product research, 33(7), 1061-1069. https ://doi.org/10.1080/14786419.2015.1108975

# Chapter - 5

## A Brief Overview of DNA Damage and Apoptosis Caused by Provoked Oxidative Stress

**Authors**

**Akash Singha Roy**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Priyankar Pal**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 5

## A Brief Overview of DNA Damage and Apoptosis Caused by Provoked Oxidative Stress

**Akash Singha Roy and Priyankar Pal**

**Abstract**

An imbalance between a biological system's reactive oxygen species capacity for damage fix or detoxification is known as oxidative stress. Free radicals are generated due to oxidative stress results harms DNA, lipids, and proteins in cells. Base damage is primarily indirect and results from cellular signalling pathways being disrupted. A chemical change in DNA, such as a break in the DNA strand, a nucleobase missing from the DNA backbone, or a base that has been chemically altered (e.g., 8-OHdG), is referred to as DNA damage. While both are examples of DNA errors, DNA damage differs from mutation in that it can occur naturally or be triggered by external factors. DNA mutations are variations in paired sequences, whereas DNA damage is an unknown chemical process in DNA. Damage to DNA results in modifications to the genetic code and stops normal replication. A cell's response to damage is triggered by DNA, which results in the DNA damage response (DDR), a sophisticated signalling mechanism. DNA deterioration can result from an imbalance between the body's antioxidant and free radical reserves. The majority of organisms with many cells depend on apoptosis, or coordinated cell death, for their survival. Apoptosis can be triggered by intrinsic pathways or weak extrinsic signals, which activate caspases and degrade proteins. Both routes cause the activation of initiator and executioner caspases, which indiscriminately cleave proteins to kill cells. This review shed light on the connection between apoptosis, DNA damage, and oxidative stress.

**Keywords:** Free radicals, oxidative stress, DNA damage, apoptosis, extrinsic pathway, intrinsic pathway.

### Introduction

A global term in redox biology and medicine, oxidative stress, was first introduced in 1985. There has been a growing body of literature spanning a wide range of topics, as well as criticism and interest in it. It has drawn

attention and criticism, with a wealth of literature across many disciplines. The objective of this commentary is to discuss the present level of knowledge in practical areas, with a particular emphasis on translational applications in the context of health and illness. All life depends on oxygen for proper cellular operation, yet oxygen also damages biomolecules by producing Radicals that are reactive (ROS) and free radicals. ROS, a necessary for preserving cellular homeostasis, but excessive generation can lead to oxidative damage to the mitochondria and other cell constituents. Oxygen is essential for mitochondrial oxidative phosphorylation, which is controlled by mitochondrial DNA.

The cellular environment is constantly replenished with oxygen and other free radicals. The body defence system, including antioxidants like glutathione, superoxide dismutase, vitamins E, C, A, zinc, taurine, creatine and polyphenols from tea extract, co-evolves to combat or mitigate the consequences of ROS. Oxidative stress (OS) in the brain, a major producer of reactive oxygen species (ROS), can lead to neurodegeneration when ROS production surpasses antioxidant defences (Chiurchiù *et al*., 2016; El-Bachá *et al*., 1998; Singh *et al*., 2019; von Arnim *et al*., 2010).

Cells generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) during metabolism. Cells possess a variety of antioxidant systems to preserve these signalling pathways and stop oxidative damage. These mechanisms reduce the creation of ROS and RNS and detoxify cognizant metabolites. NADPH supports glutathione (GSH) and thioredoxin (TXN), which are used by cells to combat oxidative stress. Through genetic and metabolic reprogramming, to oxidative stress cells react. Stress is lessened when ROS are present because G6PD produces NADPH. Stress is lessened when ROS are present because G6PD is an enzyme that produces NADPH. Cells activate G6PD in response to low H2O2 levels, which reroutes glucose metabolism and converts NADP+ to NADPH. Thioredoxin reductase (TXNRD1/2) and GSR1, or glutathione reductase, are able to improve antioxidant systems due to this quick metabolic rerouting, which also lessens negative feedback regulation. By inhibiting glycolysis and enhancing glucose catabolism through the pentose phosphate pathway (PPP), cells exploit redox switches in kinase M2 (pyruvate) and dehydrogenase of glyceraldehyde 3-phosphate (GAPDH). As a result, 6-phosphate glucose spills over into the oxidative arm of the PPP, higher glycolysis intermediates accumulate, and G6PD produces more NADPH. The cysteine (Cys) residues' oxidation in mitochondrial electron transport chains is synchronised with ROS impacts on these activities.

ROS are able to trigger programmed cell death via triggering these three pathways- intrinsic mitochondrial, the extrinsic death receptor, and the endoplasmic reticulum stress. They result in cytochrome c release, disturbance caspases activation and membrane potential, and loss of permeability in the inner mitochondrial membrane.

ROS are harmful to cellular integrity, damaging DNA, lipids, and proteins. It is crucial for keep a balance in cells because oxidative stress, is characterised as surplus of reactive oxygen species in comparison to antioxidants, has been connected to a number of diseases. They are produced by exogenous sources like ionising radiation and environmental pollutants, as well as endogenous sources like the mitochondrial electron transport chain and cytosolic enzyme systems. Many disorders have been linked to upraised of reactive oxygen species (ROS), such as cancer, heart and neurological conditions, and ageing. Here, in this article we discussed about how "Oxidative Stress induces DNA damage & Apoptosis". Before going to topic, first let's discussed about "Oxidative Stress" and its concept and its role which helps us to understand detailing of this topic.

**Concept of oxidative stress**

A biological system's imbalance between oxidants and antioxidants, resulting from an excess of reactive oxygen species or aberrant antioxidant system operation, known as oxidative stress (OS). In form of definition, it says that, "Reactive oxygen species production and the biological system's ability to detoxify are out of balance, is known as oxidative stress".

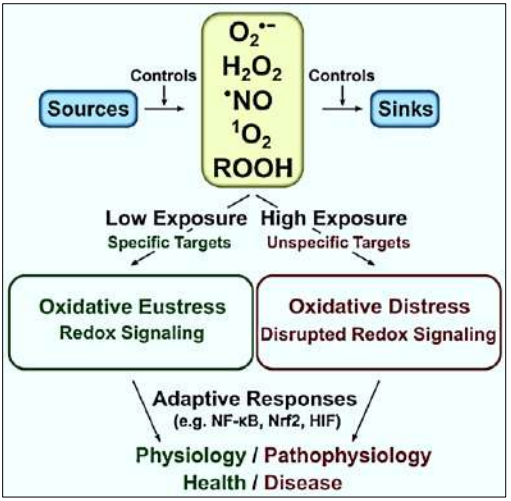


**Fig 1:** Oxidative stress and its relation with redox signalling

Since 1985, it has changed and now encompasses three types of abnormalities: physiological (called "oxidative eustress"), supraphysiological (called "oxidative distress"), and departures from a steady-state redox balance (called "reductive stress"). This idea is similar to redox homeostasis, also referred to as the "golden mean".

For redox management and redox signalling, oxidative stress refers to perturbations from the equilibrium, such as reductive stress, physiological deviations (oxidative eustress), and supraphysiological deviations (oxidative dis-stress) (Figure 1).

Redox signalling's oxidative equivalents target regulatory pathways, especially those that transcription factors influence. One of the main metabolites is hydrogen peroxide; other oxidants have different functions, such as radicals such as superoxide and nitric oxide, Hydrogen sulfide (H2S), peroxy-nitrite, and singlet molecular oxygen. While longer-term effects include altering enzyme patterns and stimulating gene transcription, short-term effects entail activating enzymes or ion channels.

The body defence system, including antioxidants like glutathione, superoxide dismutase, vitamins E, C, A, zinc, taurine, creatine and polyphenols from tea extract, co-evolves to combat or mitigate the impact of ROS. Oxidative stress (OS) in the brain, a major producer of reactive oxygen species (ROS), can lead to neurodegeneration when ROS production surpasses antioxidant defences (Chiurchiù *et al*., 2016; El-Bachá *et al*., 1998; Singh *et al*., 2019; von Arnim *et al*., 2010).

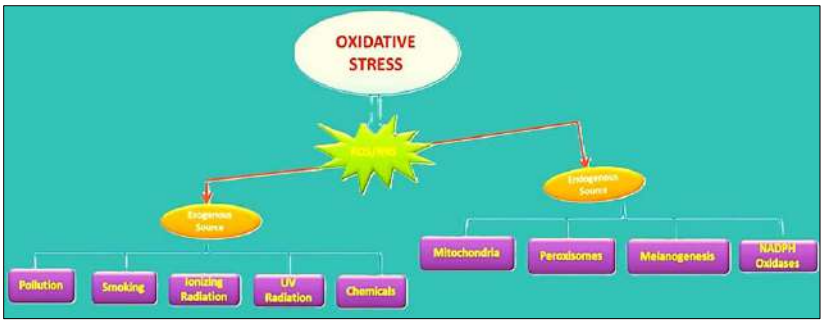


**Fig 2:** Illustration of both internal and external origins of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

**Biomarker and sensor of oxidative stress**

Biomolecules are significantly impacted by oxidative stress (OS), which is mostly caused by the brain's high oxygen consumption and weak antioxidant

defence mechanism. Due to their sensitivity to free radicals (ROS/RNS), these biomolecules which include lipids, proteins, DNA/RNA, and enzymes may serve as potential indicators of OS. Increased amounts of oxidative damage are seen in brains, and lipids and proteins especially guanine are more vulnerable to it. Proteins, enzymes, and receptors can become damaged due to lipid peroxidation, which is more likely to occur when lipids, especially those in the plasma membrane, are compromised (Chiurchiù *et al*., 2016). These biomolecules' deterioration may function as important OS indicators. Here are some oxidative stress sensors and biomarkers (Singh *et al*., 2019), like-

- **8-Hydroxydeoxyguanosine** - a biomarker for free radical-induced DNA damage. A portable biosensor can detect 8-OHdG.
- **Antioxidant enzymes** - These enzymes, including SOD, GPX, and CAT, are indicators of oxidative stress.
- **MDAP-1** - a fluorescent probe with low-concentration MDA detection capabilities *in vitro*.
- **Protein carbonyl -** an established oxidative stress indicator. Protein carbonyl levels are detected by 2,4-dinitrophenyl hydrazine (DNPH).
- **Lipo-peroxides (LP) -** act as Lipid indicators and oxidative stress.

**Mitochondria and oxidative stress**

Mitochondria are important organelles in cells that produce energy, thermogenesis, calcium homeostasis, metabolite formation, redox signalling, oxidative stress resistance, and programmed cell death (Singh *et al*., 2019). ATP is crucial for cell function and energy currency. Redox enzyme enrichment and mitochondrial dysfunction lead to reactive oxygen species (ROS). Proapoptotic protein release and mitochondrial dysfunction are largely due to reactive oxygen species or ROS, attack cardiolipin, a phospholipid in the mitochondrial membrane. Biological processes can be hampered, and a number of disorders can result from mitochondrial malfunction brought on by elevated ROS levels (Brown & Murphy, 2009; Long Hu *et al*., 2009).

**Function of oxidative stress**

Oxidative stress plays a multitude of roles. This section discusses the function of oxidative stress,

**Role regarding pro-oxidants in oxidative stress**

Fruits and vegetables, which are pro-oxidants, are important in oxidative stress. These antioxidants are vital for everyday meals because they are high in polyphenols. But neurodegenerative illnesses and long-term health issues

imply that pro-oxidants might possibly be important. Pro-oxidants fall into several subcategories, including medications, toxicants, infections, and food components. They can also be endogenous or exogenous. In addition, ion flux, climate, drug metabolites, anxiety, and environmental contamination all contain them. Consequently, in order to counteract oxidative stress, it is imperative that we include these components in our regular routines.

In the presence of transition metals, flavonoids, which are antioxidants, can also function as pro-oxidants. The flavonoid structure is important for these characteristics, and the antioxidant effects of -OH substitution are enhanced. Due to their absence of -OH substitution, flavone and flavanone are not copper-initiated pro-oxidants or antioxidants. The deoxyribose degradation assay was used by Chobot *et al*. to investigate the pro-oxidant and antioxidant characteristics of the flavonoid myricetin. Because they neutralise harmful free radical reactions and quench reactive metals, flavonoids and polyphenols have the ability to scavenge pro-oxidants in the diet and protect against oxidatively-induced illnesses. One important pro-oxidant that can function both as an antioxidant and ascorbic acid is a pro-oxidant that is dose-related. Auto-oxidation, on the other hand, may potentially have a harmful effect via changing gene expression. To fight oxidative stress and illnesses, it is essential to be aware of pro-oxidants.

**Heavy metal's function in oxidative stress**

Heavy metal's significant effects on various systems like respiratory, cardiovascular, reproductive, and central neurological, as well as key organs such the kidneys, lungs, liver, and brain, caused by oxidative stress. Oxidative stress is brought on by excessive concentrations of heavy metals, such as lead and mercury, and low amounts of necessary metals, such as zinc and selenium. This unbalanced redox condition in cells damages biomolecules and critical liver, kidney, and central nervous system are examples of organs.

While there is no need for mercury (Hg) in biological processes, its exitance and buildup can be detrimental to living things. Oxidative stress results in damage to membranes, biomolecule oxidation, and the invigorating regarding $H_2O_2$, lipid peroxidation, and protein oxidation. Mercury is a neurotoxin that can cause nervous system difficulties with extended exposure. It can also cause shyness, tremors, memory loss, hearing loss, and visual alterations.

One harmful metal that can harm human health and cause mitochondrial dysfunction is lead (Pb). It can cause oxidative stress. Lead exposure is impacted by several factors, encompassing age, dose, amount, as well as

health state. Owing to lead's strong affinity for metal cofactors and the –SH group, antioxidant enzyme activity is decreased, which increases oxidative stress and increases the risk of organ failure. In humans, arsenic (As) can cause cytotoxicity, genotoxicity, and cancer. It is also poisonous.

Reduced glutathione (GSH) is changed into oxidised glutathione (GSSG) and H2O2 is produced when it attaches to glutathione's –SH group. Fatty acid oxidation and gluconeogenesis are brought on by arsenic's inhibition of glucose absorption. Moreover, it disrupts the Kreb cycle, which results in malfunctioning mitochondria. Reactive oxygen species (ROS/RNS) and oxidative stress are produced by heavy metals, can lead to cell division, proliferation and death by damaging biomolecules and mitochondria.

**Apoptosis by oxidative stress**

Apoptosis is a crucial process in multicellular organisms, removing damaged cells to maintain normal development and homeostasis. However, excessive apoptosis can lead to diseases like rheumatoid arthritis, cancer, and AIDS. Apoptosis is triggered by factors like receptor-mediated signals, growth factor withdrawal, anti-tumour drugs, and DNA damage. Multiple signalling pathways may converge, predisposing cells to apoptosis, including mitochondria, radiation, cytotoxic chemicals, and drugs.

Cytotoxicity can result from excess oxidative stress through necrosis or programmed cell death, with alterations in redox status occurring before caspase activation. Anti-oxidants like N-acetylcysteine and Bcl-2 can block apoptosis. Under normal conditions, cells have antioxidant defence mechanisms, but when pro-oxidants overwhelm these, oxidative stress occurs. Apoptosis may serve as a fail-safe mechanism to prevent uncontrollable cell proliferation (Susin *et al*., 1998).

**Oxidative stress-related mechanism of cell death**

This section reviews cell death mechanisms via *in vitro* miniatures of oxidative stress, focusing on $H_2O_2$ and substances that cycle redox. Lipid metabolites are regarded as an external stressor and the physiological effects of cellular stress. Reactive oxygen species' function in programmed cell death is discussed. Cell death pathways are also discussed in response to radiation, photodynamic therapy, and cigarette smoke exposure (Singh *et al*., 2019).

**Hydrogen Peroxide induced apoptosis**

$H_2O_2$, a weak oxidant, is a crucial signalling molecule due to its long half-life and solubility in lipid and aqueous media. Recent research suggests that one possible paracrine modulator of apoptosis is $H_2O_2$, allowing it to diffuse

to cellular targets. Following therapy of different cell types with these model chemicals, both physiological and anatomical characteristics of programmed cell death found. Research has demonstrated that in cardiomyocytes, H2O2 triggers apoptotic cell death, whereas overproduction of Bcl-2 or Bcl-XL boosts the generation of $H_2O_2$ specifically for mitochondria. Catalase is one example of an antioxidant that often prevents oxidant-induced apoptosis (Ryter *et al*., 2007).

**Glutamate induced apoptosis**

Neurons can be harmed by the overproduction of endogenous chemicals such as glutamate, catecholamines, and epinephrine, which can emit reactive oxygen species. Heightened production of ROS, excitotoxicity, as well as damage to neuronal cells are the outcomes of this glutamatergic neuron overstimulation. Additionally, glutamate depletes intracellular GSH, which results in apoptosis-like cell death. Glutamate-induced neurotoxicity is prevented by N-acetylcysteine treatment.

Recent data, 4-HNE might be involved in protein modification in illnesses like alcohol-induced liver disease.

**Ultra-violet radiation (UVR) caused cell death**

The mechanisms of apoptosis following UVR unveiling are wavelength-specific, as demonstrated in murine lymphoma cells exposed to long-wave ultraviolet A1, Ultraviolet B, and Ultraviolet-C iso-toxic doses. Long-wave ultraviolet A(UVA)1 radiation induces both immediate and delayed apoptosis, although UVB or UVC luminousness only induces postponed death without first element. Programmed cell death after UVA radiation followed in various skin cell replicas, with extrinsic and intrinsic pathways reported.

**Smoking**

Heavy metals, aldehydes, aromatic hydrocarbons and phenolics are among the more than 4700 complex ingredients make up cigarette smoke (CS). It contains elevated level of ROS and free radicals, as well as additional oxidants. Asthma that causes chronic obstructive pulmonary disease (COPD) major source of demise globally, is primarily brought on by cigarette smoke. The exact pathophysiology of COPD remains unknown, and few effective treatment modalities exist (Church & Pryor, 1985). Exposure to cyber-threats may cause smokers who are sensitive to develop emphysema, lung tissue damage, and persistent airway irritation, blockage, and inflammation of the alveolar wall.

All cell types, including lung epithelial cells, undergo apoptosis when

exposed to cigarette smoke. When cigarette smoke extract quantities are low, it can induce apoptosis, and when it is higher, it can cause necrosis (Wickenden *et al*., 2003). Phosphorus-dependent or p53-independent pathways are involved in dose-dependent apoptosis, which is linked to elevated reactive oxygen species (ROS) (Carnevali *et al*., 2003; Raveendran *et al*., 2005; Wang *et al*., 2003).

Chronic stressors like nicotine might alter cell death pathways by lowering intracellular ATP levels and promoting neuronal apoptosis during development. This could lead to tissue loss caused by CS. Apoptosis can be induced *in vivo* by persistent exposure to CS; in rats, this results in a substantial increase in apoptotic cells inside the bronchial and bronchiolar epithelium (D'Agostini *et al*., 2001). Moreover, CS therapy promotes apoptosis via ap53-independent and ROS-mediated mechanisms in the stomach mucosa (Wang *et al*., 2000). It is challenging to normalise smoke concentrations for laboratory comparisons due to the potential for chronic CS exposure to cause mitochondrial dysfunction, decreased respiration, and increased oxidative stress (Anbarasi *et al*., 2005; Rangasamy *et al*., 2004).

**DNA damage mechanism**

**Concept**

DNA damage is a structural alteration that changes the coding characteristics of DNA and disrupts cell metabolism. One of the main causes of DNA damage is oxidative stress, which results in an imbalance between the quantity of reactive oxygen species (ROS) and an organism's capacity for detoxification (Migliore & Coppedè, 2009). ROS have the ability to change the structure of macromolecules. These include free radicals and oxidising agents (Cadet *et al*., 2011). Like hydroxyl radicals, superoxide and hydrogen peroxide can also cause ROS since they are not readily redox-compatible with DNA (Gonzalez-Hunt *et al*., 2018).

Comprehensive descriptions of these lesions and the mechanisms responsible for their formation can be found in Y Yu *et al*., J Cadet *et al*., M Dizdaroglu and P Jaruga and MD Evans *et al* (Gonzalez-Hunt *et al*., 2018). DNA strand breaks can arise from a range of mutilation to DNA caused by ROS, including base and sugar changes, sugar base cyclization, DNA-protein cross-links, and intra-and inter stand cross-links. Air pollution, ionising radiation, UV light, certain lifestyle choices (such as smoking and eating), and exposure to metals and pesticides are examples of exogenous factors that can harm DNA through oxidative stress. The significance of highlighting is that endogenous agents, including ROS originating from cell activities (oxidative

phosphorylation and the inflammatory reaction) and spontaneous or enzymatic conversions, can also damage DNA, especially when these mechanisms become dysfunctional.

**Definition**

DNA damage is a modification of the molecule's structure. It may consist of a base that has altered chemically, a missing nucleobase, or a break in a DNA strand.

**Types of DNA damage**

A cell's regular metabolic activities and external stressors can cause DNA damage, which occurs daily at the rate of 1000-1000000 molecular lesions. Even though, only makes up of approximately 6 billion, 0.000165% bases (3 billion base pairs) that comprise the human genome, lack of repaired, it can result in changes to significant genes (such as tumour suppressor genes), can impair a cell's functional ability and significantly raise threat like tumour generation and disease states like cancer.

DNA damage typically involves modifications to the bases, altering the double helix's structure and introducing bulky adducts that break the helical structure. DNA typically lacks tertiary structure, unlike proteins and RNA. However, in eukaryotes, DNA is twisted around histones, making both superstructures susceptible to genotoxicity effects.

DNA damage caused by oxidization, base alkene, base loss from hydrolysis, bulky adducts, DNA crosslinking, and breaks in DNA strands, including fractures of single and double stranded. It can also result from exposure to the environment or from biological functions.

**DNA damage by oxidative stress**

Leading source of cellular stress and damage, including oxidative DNA deterioration, is reactive oxygen species. Because they are initiated by enzymatic procedures or UV and ionising radiation, hydroxyl radicals (•OH) are reactive and electrophilic ROS. They can produce the oxidative stress indicator, 8-oxo-7,8-hydroxyguanine (8-oxoG) from guanine residues. Up to 100,000 8-oxo-dG lesions can occur in each cell, making guanine the most easily oxidised DNA base. Further oxidation and secondary oxidation products are permitted since 8-oxo-dG has a lower reduction potential than guanosine.

As was previously indicated, elevated tissue levels of 8-oxo-dG can function concerning biomarker with relation to oxidative stress. Moreover, elevated 8-oxo-dG levels are commonly linked to emergence of cancer and

other sickness conditions. In replicating process, DNA containing 8-oxo-dG, adenine is typically integrated on the opposite side of the lesion. During the repair phase that follows replication, thymine is inserted in place of the 8-oxo-dG. For this reason, G to T transversions commonly caused by 8-oxo-dG mutations.

Oxidative DNA damage and the resulting mutations cause age-related disorders such as cancer (Tubbs & Nussenzweig, 2017), dementia (Lodato *et al*., 2018), and other conditions that worsen with age (Church & Pryor, 1985; Ryter *et al*., 2007). Breaks in DNA strands and oxidative damage to DNA are caused by majority of cancer treatments; hence, oxidative DNA damage plays an impact on cancer survivors' long-term side effects.

**Conclusion**

Throughout many cell systems, oxidative stress is the mediator of apoptotic cell demise, and mitochondria are important in both the formation of reactive oxygen species or ROS and this process. When the antioxidant defences in our body are overpowered by the production of free radicals and reactive oxygen species (ROS), oxidative stress is the result. This imbalance possesses the capacity to harm tissue and contribute to the development of certain illness, including cancer and cardio-vascular disease.

Dietary and lifestyle choices could help to reduce oxidative stress, like-

- Consume a nutritious, well-balanced diet high in fruits and vegetables.
- Limit consumption of processed foods, especially those with high sugar and fat content
- Exercise daily
- Quit puffing
- Reduce anxiety
- Take dietary supplements containing vitamins including vitamin C and E.

**References**

1. Anbarasi, K., Vani, G., & Devi, C. S. S. (2005). Protective effect of bacoside A on cigarette smoking-induced brain mitochondrial dysfunction in rats. *Journal of Environmental Pathology, Toxicology and Oncology : Official Organ of the International Society for Environmental Toxicology and Cancer*, *24*(3), 225–234. https://doi.org/10.1615/JENVPATHTOXONCOL.V24.I3.80

2. Brown, H. A., & Murphy, R. C. (2009). Working towards an exegesis for lipids in biology. In *Nature Chemical Biology* (Vol. 5, Issue 9). https://doi.org/10.1038/nchembio0909-602

3. Cadet, J., Douki, T., & Ravanat, J. L. (2011). Measurement of oxidatively generated base damage in cellular DNA. In *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis* (Vol. 711, Issues 1–2). https://doi.org/10.1016/j.mrfmmm.2011.02.004

4. Carnevali, S., Petruzzelli, S., Longoni, B., Vanacore, R., Barale, R., Cipollini, M., Scatena, F., Paggiaro, P., Celi, A., & Giuntini, C. (2003). Cigarette smoke extract induces oxidative stress and apoptosis in human lung fibroblasts. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, *284*(6). https://doi.org/10.1152/AJPLUNG.00466.2001

5. Chiurchiù, V., Orlacchio, A., & Maccarrone, M. (2016). Is modulation of oxidative stress an answer? the state of the art of redox therapeutic actions in neurodegenerative diseases. In *Oxidative Medicine and Cellular Longevity* (Vol. 2016). https://doi.org/10.1155/2016/7909380

6. Church, D. F., & Pryor, W. A. (1985). Free-radical chemistry of cigarette smoke and its toxicological implications. *Environmental Health Perspectives*, *VOL. 64*. https://doi.org/10.1289/ehp.8564111

7. D'Agostini, F., Balansky, R. M., Izzotti, A., Lubet, R. A., Kelloff, G. J., & De Flora, S. (2001). Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats. *Carcinogenesis*, *22*(3), 375–380. https://doi.org/10.1093/CARCIN/22.3.375

8. El-Bachá, R. S., De-Lima-Filho, J. L., & Guedes, R. C. A. (1998). Dietary antioxidant deficiency facilitates cortical spreading depression induced by photoactivated riboflavin. *Nutritional Neuroscience*, *1*(3). https://doi.org/10.1080/1028415X.1998.11747230

9. Gonzalez-Hunt, C. P., Wadhwa, M., & Sanders, L. H. (2018). DNA damage by oxidative stress: Measurement strategies for two genomes. In *Current Opinion in Toxicology* (Vol. 7). https://doi.org/10.1016/j.cotox.2017.11.001

10. Lodato, M. A., Rodin, R. E., Bohrson, C. L., Coulter, M. E., Barton, A. R., Kwon, M., Sherman, M. A., Vitzthum, C. M., Luquette, L. J., Yandava, C. N., Yang, P., Chittenden, T. W., Hatem, N. E., Ryu, S. C., Woodworth, M. B., Park, P. J., & Walsh, C. A. (2018). Aging and neurodegeneration are associated with increased mutations in single

human neurons. *Science*, *359*(6375). https://doi.org/10.1126/science.aao4426

11. Long Hu, Jin Ying, Lin Mu, Sun Yu, Zhang Liang, & Clinch Carole. (2009). Fluoride toxicity in the male reproductive system. *Fluoride*, *42*(4), 260–276.

12. Migliore, L., & Coppedè, F. (2009). Environmental-induced oxidative stress in neurodegenerative disorders and aging. In *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* (Vol. 674, Issues 1–2). https://doi.org/10.1016/j.mrgentox.2008.09.013

13. Rangasamy, T., Cho, C. Y., Thimmulappa, R. K., Zhen, L., Srisuma, S. S., Kensler, T. W., Yamamoto, M., Petrache, I., Tuder, R. M., & Biswal, S. (2004). Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *Journal of Clinical Investigation*, *114*(9). https://doi.org/10.1172/JCI200421146

14. Raveendran, M., Wang, J., Senthil, D., Wang, J., Utama, B., Shen, Y., Dudley, D., Zhang, Y., & Wang, X. L. (2005). Endogenous nitric oxide activation protects against cigarette smoking induced apoptosis in endothelial cells. *FEBS Letters*, *579*(3). https://doi.org/10.1016/j.febslet.2004.12.052

15. Ryter, S. W., Hong, P. K., Hoetzel, A., Park, J. W., Nakahira, K., Wang, X., & Choi, A. M. K. (2007). Mechanisms of cell death in oxidative stress. In *Antioxidants and Redox Signaling* (Vol. 9, Issue 1). https://doi.org/10.1089/ars.2007.9.49

16. Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019). Oxidative stress: A key modulator in neurodegenerative diseases. In *Molecules* (Vol. 24, Issue 8). https://doi.org/10.3390/molecules24081583

17. Susin, S. A., Zamzami, N., & Kroemer, G. (1998). Mitochondria as regulators of apoptosis: Doubt no more. *Biochimica et Biophysica Acta - Bioenergetics*, *1366*(1–2). https://doi.org/10.1016/S0005-2728(98)00110-8

18. Tubbs, A., & Nussenzweig, A. (2017). Endogenous DNA Damage as a Source of Genomic Instability in Cancer. In *Cell* (Vol. 168, Issue 4). https://doi.org/10.1016/j.cell.2017.01.002

19. von Arnim, C. A. F., Gola, U., & Biesalski, H. K. (2010). More than the sum of its parts? Nutrition in Alzheimer's disease. In *Nutrition* (Vol. 26, Issues 7–8). https://doi.org/10.1016/j.nut.2009.11.009

20. Wang, H. Y., Ma, L., Li, Y., & Cho, C. H. (2000). Exposure to cigarette

smoke increases apoptosis in the rat gastric mucosa through a reactive oxygen species-mediated and p53-independent pathway. *Free Radical Biology and Medicine*, *28*(7). https://doi.org/10.1016/S0891-5849(00)00207-0

21. Wang, H. Y., Shin, V. Y., Leung, S. Y., Yuen, S. T., & Cho, C. H. (2003). Involvement of bcl-2 and Caspase-3 in Apoptosis Induced by Cigarette Smoke Extract in the Gastric Epithelial Cell. *Toxicologic Pathology*, *31*(2). https://doi.org/10.1080/01926230390183715

22. Wickenden, J. A., Clarke, M. C. H., Rossi, A. G., Rahman, I., Faux, S. P., Donaldson, K., & MacNee, W. (2003). Cigarette Smoke Prevents Apoptosis through Inhibition of Caspase Activation and Induces Necrosis. *American Journal of Respiratory Cell and Molecular Biology*, *29*(5). https://doi.org/10.1165/rcmb.2002-0235OC

# Chapter - 6

## Anticancer Peptides: Synthesis Methods, Modes of Action, and Future Potential Therapeutic uses for Cancer Treatment

**Authors**

**Pooja Kumari**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Semanti Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 6

## Anticancer Peptides: Synthesis Methods, Modes of Action, and Future Potential Therapeutic uses for Cancer Treatment

**Pooja Kumari and Semanti Ghosh**

**Abstract**

Cancer is the foremost cause of death worldwide. The worldwide encumbrance of cancer has increased due to the rising number of cancer diagnoses, inadequate treatment options, and the adverse effects of long-term medication. At present, serious side effects, toxicity, and drug resistance are brought on by immunotherapy, radiotherapy, surgical treatment, and chemotherapy. Anti-microbial peptides (AMPs) are widely known to have cancer-fighting properties. Anti-cancer peptides are found in several sources, including animals, insects, plants, and microbes. Two mechanisms are used to produce (ACPs) chemosynthesis and biosynthesis. ACPs, or anticancer peptides, have been known as a possible therapeutic strategy due to their fewer adverse effects great penetration, high degree of specificity, and simpler modification. Anticancer peptides target cancer cells specifically target cancer cells and act at various phases including initiation, promotion, and progression. ACPs, or Anticancer Peptides, exhibit anticancer activity through various mechanisms. ACPs can suppress the angiogenesis pathway, induce apoptosis (i.e., destruction of mitochondrial membranes), and engage and attract immune cells to destroy cancer cells. ACPs can stimulate vital proteins and encourage the formation of pores, which ultimately cause the cancer cells to lyse which enumerates the working mechanisms of ACP action. ACPs have a significant benefit over traditional chemotherapeutic drugs mainly because they do not seem to encourage the development of drug resistance. Some ACPs can boost cancer medication penetration and therapeutic benefits by delivering them directly to cells. An overview of various anti-cancer peptides, their underlying mechanisms of action, and, methods of synthesis, and uses, providing information on potentially useful therapeutic anticancer peptides for cancer treatment in the future.

**Keywords:** Anticancer peptides (ACPs), Antimicrobial peptides (AMPs),

cancer therapy, Mechanism of ACPs, Synthesis of ACPs, Application of ACPs.

**Introduction**

Cancer ranks among the world's most common causes of mortality. As per the World Health Organization (WHO), about 1 in 6 deaths in 2020 were related to cancer. Cancer is caused by genetic mutations in DNA, that cause uncontrolled proliferation of cells. Unchecked cell division is a hallmark of the cancer group of diseases. Cancer is not limited to humans; it may also affect animals and other living things. A normal cell dies by the process of apoptosis. When an abnormal cell (malignant cells) proliferates in an uncontrolled and haphazard manner, it leads to the development of lumps or malignant tumors. Metastasis is the process by which the cancer cells spread from one area of the body to another and proliferate. The main factor contributing to cancer-related deaths is widespread metastases. Breast cancer, lung cancer, prostate cancer, colon and rectum cancer, stomach cancer, liver cancer, and skin cancer (non-melanoma) are the most frequent cancers. Genetic factors and external agents can cause cancer. Three categories of external agents influence the changes in an individual's genetic factors. Physical cancer-causing agents include ultraviolet and ionizing radiation, chemical carcinogens like asbestos, tobacco smoke, alcohol, aflatoxin, and arsenic, and biological carcinogens like infections from viruses, bacteria, or parasites. According to the World Health Organization (WHO), unhealthy lifestyle habits such as alcohol consumption, tobacco use, inadequate fruit and vegetable intake, high body mass index, and lack of exercise may be responsible for up to one-third of all cancer deaths. Depending on the kind of cancer and its phases of progression, there are several treatment options available. Localized treatments like surgery or local radiation therapy are typically used. Systemic treatment like Chemotherapy, targeted therapy, immunotherapy, and other systemic medication therapies are used palliative care. Palliative care focuses on easing the discomfort and respiratory difficulties that come with cancer. Common methods of treatment include radiation therapy, chemotherapy, surgery, and stem cell, hormone therapy, immunotherapy (biological therapy), and targeted drug therapy. Most anticancer medications on the market target highly proliferating cells, sparing healthy cells that grow at a comparable pace. The emergence of Multi-Drug Resistant (MDR) cancer cells has significantly reduced therapeutic efficacy in the meantime. Resistant cells can transfer drug molecules outside of them (Riedl *et al*., 2011). Other ways by which cancer cells resist anticancer medicines include the repair of damaged DNA, resistance to oxidative stress

(ROS), and expression of enzymes that detoxify drugs in reaction. In summary, each of the current treatment approaches has pros, cons, and areas of use of its own. A single therapeutic approach may not always have the desired curative benefits, and combination therapy may, in some cases, provide a superior overall cure. Thus, there exists a pressing unfulfilled demand for evaluation and development of innovative, non-conventional therapeutic approaches, or anti-tumor medication.

Anti-cancer bioactive peptides (ACPs) are a novel approach to cancer treatment that has shown benefits over both medical as well as diagnostic uses. ACPs can increase the susceptibility of cancer cells to other therapeutic agents while also offering higher specificity, sensitivity, accuracy, and reduced toxicity.

Peptide ranges from 10 to 50 amino acid residues and are small, low-molecular-weight, cationic bioactive proteins that have a variety of biochemical functions in the body. In 1922, they were utilized for the first time in medicine to treat type 1 diabetes. 60 peptides have been licensed for use as medications after being utilized in over 600 clinical and preclinical trials (Lau & Dunn, 2018). They are used therapeutically in the treatment of cancer, drug delivery systems, modulating of the immune system, hormone control, modulation of inflammation, quorum sensing, and antibiotics. There are three types of sources for therapeutic peptides: natural, synthetic, and intentionally modified. They can be generated by chemical synthesis, recombinant genes, proteolysis, or living things. ACP screening makes use of computational techniques like deep learning and machine learning. There are different conformations of ACPs, including extended linear structures like that of Tritrpticin and Indolicidin. Some ACPs, such as LL-37, BMAP-27, BMAP-28, Cercopin A, etc. Their secondary structure includes α-helices or get folded into β-sheets (e.g., Defensins, Lactoferrin, etc.). Cancer cells contain a variety of characteristics, having a negative charge on their membranes, enhanced membrane mobility, and microvilli on their cell walls (Velayutham *et al.*, 2022). Through electrostatic interactions, ACPs can interact with these cells and cause necrosis, which selectively kills cancer cells. Antimicrobial peptides (AMPs) contribute significantly to innate immunity in many organisms. Usually, they are amphipathic, cationic compounds with a lot of hydrophobic residues. These features enable them to quickly engage with negatively charged microbial membranes, which are less likely to acquire AMP resistance, leading to microbial death. Since some AMPs have also shown anticancer action, they are also recognized as ACPs. Their interactions with the increased quantity of negatively charged molecules on the outer plasma membranes of cancer cells compared to normal cells, which includes

phosphatidylserine, glycoproteins, and glycolipids, are most likely the cause of their anticancer effect (Zhong *et al*., 2020). More than 20 ACPs have received FDA and EMA approval as of November 2019. Recently, ACPs such as Gallium Dotatoc Ga68, Kyprolis, SomaKit TOC, and Lutathera have been put on the market (Pan *et al*., 2020). Toxicity and poor targeting are drawbacks of many ACPs that significantly reduce their effectiveness. The research aims to increase the therapeutic capabilities and minimize the toxicity of ACPs by effective rebuilding or modification.

**Synthesis of ACPs**

Sources of anti-cancer peptides include animals, insects, plants, and microbes. Chemosynthesis and biosynthesis are two methods used for synthesizing anti-cancer peptides; chemosynthesis involves solid-phase peptide synthesis (SPPS) or solution-phase peptide synthesis (SuPPS). Enzymatic hydrolysis and recombinant DNA technology are two methods of biosynthesis. Improvements in SPPS automation and instrumentation have made chemosynthetic techniques more cost-effective (Stráner, n.d.). Biosynthetic techniques include reduced peptide toxicity, optimization, fusion protein partner selection, and expression system assessment of codons, protease selection that is suitable for enzymatic hydrolysis, as well as the optimization of the fermentation parameters and medium. Chemical synthesis is still expensive.

**Chemosynthesis**

**Solid-phase peptide synthesis**

The synthesis of longer and more complicated peptides is possible using solid-phase peptide synthesis (SPPS), a more advanced version of solution-phase peptide synthesis. Peptide synthesis phase. The synthesis process that SPPS prefers for short peptides with less than 50 amino acid residues. Its efficiency and simplicity of use make it an industrial decision for extensive manufacturing. Polystyrene resin is one type of solid support material that SPPS employs to bind and immobilize the produced peptide. Peptides undergo chemical modification at their side chains or N-terminus to add protective groups during production (Ma *et al*., 2022). Unfortunately, because of contamination and blockage of the reactive N-terminus, it is ineffective for synthesizing peptides longer than 70 amino acids and presents challenges for purification. Using solid support, the protected amino acid is loaded, coupled with a coupling reagent, deprotected, and then the peptide is purified and assessed. SPPS may be optimized to certain peptide sequences and it is utilized in the synthesis of anti-cancer peptides.

**Solution-phase peptide synthesis**

Solution-phase peptide synthesis (SuPPS) is a process that creates bioactive peptides by creating peptide bonds between amino acids. This process requires protected side-chain functional groups and protected substrates to prevent undesirable products from forming. SuPPS involves four phases: amino group protection, coupling of protected amino acids, deprotection of the amino group, and purification. The process can be customized to synthesize specific peptide sequences and is useful for producing large amounts of peptides. SuPPS is a multipurpose and effective way to make peptides that fight cancer.

**Biosynthesis**

**Enzymatic hydrolysis**

Enzymatic hydrolysis is an effective method to produce anti-cancer peptides since it has an excellent specificity, gentle response circumstances, and the absence of toxic chemicals. There are two sub-techniques: reversible hydrolysis reaction and enzymatic peptide synthesis. However, both approaches have drawbacks, including the inability to shift equilibrium and the restricted synthesis of shorter peptides. The following steps are involved in the synthesis of anti-cancer peptides: Selection of an appropriate protein source, extracting the protein using techniques like acid or alkali treatment, and enzymatically hydrolyzing it with proteases. With the use of methods like size-exclusion chromatography, ion-exchange chromatography, and ultrafiltration, the resultant mixture is separated and purified (Perez Espitia *et al*., 2012). Techniques like high-performance liquid chromatography (HPLC) and mass spectroscopy are used to characterize the separated peptides and assessed utilizing *in vitro* and *in vivo* tests for their anti-cancer efficacy. Enzymatic hydrolysis is a straightforward, highly effective technique that yields anti-cancer peptides. It can be enhanced further by combining it with chemical synthesis (da Silva, 2018).

**Recombinant DNA technology**

The recombinant DNA technique produces anti-cancer peptides by genetic engineering; foreign protein synthesis is carried out on bacterial hosts such as *E. coli*. This recombinant method provides an affordable, substitute for the large-scale synthesis of bioactive peptides. One effective way to produce anti-cancerous peptides with possible anti-tumor efficacy by using recombinant DNA technology. A peptide possessing possible anti-cancer properties is chosen, the gene is cloned using methods like polymerase chain reaction (PCR) and restriction enzyme digestion, an vector of expression is

created, the vector is transformed by inserting it into a host cell, the peptide is extracted and purified, and the final product is characterized and assessed. Recombinant DNA technology is a dynamic technique for producing peptides with conceivable anti-cancer action, particularly when it comes to producing anti-cancerous peptides (Li, 2011). This method may quickly generate vast amounts of peptides while enabling the optimization of peptide structure. The anti-cancer efficacy of the peptide is then investigated utilizing both *in vitro* and *in vivo* experiments.

**Reconstruction and modification**

Rebuilding the main chain or changing the side chains of ACPs might improve them (Xie *et al*., 2020). Peptide activity and selectivity are changed by main chain transformation because of changes in their conformations, hydrophilicities, and net charges. Higher-quality ACPs can be produced by substituting non-natural amino acids. Cancer cell penetration is facilitated by the incorporation of cholesterol in side chains. Polyethylene glycol (PEG) coupling lowers toxicity, boosts selectivity, and increases diameters. Phosphorylation occurs on the side chains of tyrosine, serine, and other amino acids (Sanyal *et al*., 2019); glycosidic linkages are used to connect sugars to certain amino acids.

**Computational methods for synthesizing ACPs**

AI is replacing labor-intensive, nonautomated, and high-priced traditional lab approaches in the prediction of high anti-cancer peptide sequences. Three AI methodologies are now in use: traditional machine learning, deep learning, and hybrid methods (Yi *et al*., 2019). This progress is mostly based on amino acid sequences (Ballester & Mitchell, 2010).

**Traditional machine learning**

The prediction of anticancer agents (ACPs) from amino acid sequences is done by supervised machine learning (SML) and unsupervised machine learning. In-depth data analysis is required for feature extraction to determine attributes such as the composition of amino acids, dipeptides, atoms, and physicochemical characteristics (Hajisharifi *et al*., 2014). Several machine learning techniques are employed, such as generalized neural networks (GNN), random forests (RF), k-nearest neighbor (KNN), and support vector machines (SVM). Jackknife cross-validation tests are used to evaluate prediction performance (Chou & Shen, 2007). The AntiCP, MLACP, and DRACP models each with unique advantages and disadvantages are all implemented using SVM models.

**Deep learning**

Deep learning (DL) methods are being utilized to find Anticancer Peptides (APPs) utilizing a variety of computational methodologies and machine learning algorithms (Agrawal *et al*., 2020). This includes Convolutional neural networks (CNN), recurrent neural networks (RNN), attention models, long short-term memory (LSTM), and CNN-RNN. While the DeepACP model employs CNN, CNN-RNN, and bidirectional RNN architectures, the ACP-DL predictor uses RNN to presage ACPs. Learnable and adaptive embedding is used by the ACP-red LAF model (Formulated in 2021) to enhance feature representation performance (He *et al*., 2021).

**Hybrid approach**

For data splitting, embedding, and feature extraction, hybrid learning combines deep learning with traditional machine learning. In ACP-DA, traditional machine learning enhances prediction performance by enriching data. ACP-GCN treats prediction as a graph classification and predicts ACPs using graph convolution networks (Rao *et al*., 2020). Based on this work, the xDeep-AcPEP model, created in 2021, forecast how ACPs would behave biologically against six tumor cells against six tumor cells. It shows that multi-tasking learning models surpass traditional single-tasking models regarding of prediction interpretation (Chen *et al*., 2021).

**Modes of exertion of ACPs**

Anti-cancer peptides (ACPs) perform at distinct phases, including induction, preferment, and consecution, and they particularly target cancer cells. ACPs provide several advantages over chemotherapeutic drugs, including increased specificity, deeper tumor penetration, fewer adverse effects, and ease of modification.

**The suppression of vasculogenesis**

Anti-angiogenic peptides (AAPs) are a class of ACPs that have the capability to be used in cancer therapies by hampering vasculogenesis because of less immunogenic, small, highly permeable, and selective. AAPs may particularly interconnect with growth factors, enzymes, and receptors involved in the development of blood vessels. Platelet-derived growth factor, vascular endothelial growth factor (VEGF), and angiopoietins are the primary targets of AAPs (Shin *et al*., 2022). AAPs prevent the construction of new veins by inhibiting the downstream signalling pathways of their targets.

**Disruption of the cell replication and cell propagation**

Targeting certain proteins necessary for the division and development of

cells, several ACPs halt the cell replication and prevent cell propagation to achieve their anticancer properties. By altering the activity of cyclin and cyclin-dependent kinase (CDK) or the cyclin/CDK complexes and their aims, the ACPs exert their effects. The majority of ACPs prevent cell division by stopping cells in the G1 phase, preventing the S phase conversion, and inducing programmed cell death. For example, Gonearrestide, suppresses the development of cancer cells by inducing G1 phase arrest via CDK4 inhibition (Ma *et al*., 2022), while rapeseed peptide triggers G0/G1 phase seize and suppresses cell propagation via controlling the P53 signaling pathway (Guo *et al*., 2022).

**Plasma membrane disruption and activation of programmed cell death**

The selectivity of ACPs in malignant cells stems from variations in cell membrane characteristics. Cancer cells contain net negative charge because of anionic substances, greater membrane fluidity, and larger cell surface areas. ACPs enter cells directly or via endocytic pathways, causing cytotoxicity by explicitly harms to the plasma membrane, necrosis, mitochondrial enlargement, apoptosis, or caspase activation. Some ACPs can activate both apoptotic pathways (Zhou *et al*., 2016).

**Reticence of relocation, aggression, and metastasis**

Relocation, aggression, and metastasis are interdependent steps that include extracellular matrix disintegration and epithelial-mesenchymal transition (EMT). ACPs suppress cancer cells' migration, invasion, and metastasis by regulating PI3K/AKT/mTOR and Wnt pathways. Peptides significantly inhibit cell migration, invasion, and metastasis (Guo *et al*., 2022).

**Instruction of white blood cells and immune response transformation**

Exempt suppression is a survival strategy used by cancer cells. Anticancer peptides (APCs) can alter immune cells like TAMs, Tregs, T cells, NK cells, and DCs. ACPs can facilitate tumor immunotherapy by transforming the stifling tumor microhabitat into an anticancer immune microhabitat (Zhang *et al*., 2023). ACPs can alter cytokine expression patterns, reprogramming or removing M2-like TAMs, enhancing $CD8^+$ T cell infiltration and activation, reducing immunosuppressive $CD4^+$, reducing $CD4^+$ Treg cells inhibiting the immune system, NK cell activation, and disrupting protein-ligand interactions (Podlesnykh *et al*., 2021). Peptide-based anti-cancer vaccines targeting tumor-specific antigens (TSAs), or tumor-associated antigens (TAAs) are being developed. Artificial peptides generated from these neoplasm epitopes are chosen for their capacity to trigger $CD8^+$ cytotoxic and $CD4^+$ effector T-cell responses. Peptides' biocompatibility and

potential uses in various diseases make them promising candidates for cancer immunotherapies.

## Conclusion

Although several beneficial options for cancer cure, such as immunotherapy, chemotherapy, radiation, and surgery, malignant tumors is the supreme cause of death. Chemo-therapeutics lack specificity and may cause cancer resistance, whereas early-stage surgery is ineffective and might cause metastasis. Promising results have been observed in the use of advanced cellular targeting (ACPs) as diagnostic and therapeutic instruments. ACPs have a unique therapeutic role because of their molecular targeting capabilities, which are derived from AMPs. Large-scale adoption is hampered, meanwhile, by their expensive cost, vulnerability to proteolytic cleavage, and distress about utilizing sequences that are close to those of human and natural AMPs. Despite these difficulties, ACPs have proven to be cytotoxically effective, guaranteeing their position in the curative anti-cancer toolbox. Innovative molecular representations, advanced computational techniques, and an awareness of the connections between symmetry and actions may all be useful tools in moving ACPs toward theranostic success.

## References


1. Agrawal, P., Bhagat, D., Mahalwal, M., Sharma, N., & Raghava, G. P. S. (2020, August 6). AntiCP 2.0: an updated model for predicting anticancer peptides. Briefings in Bioinformatics, 22(3). https://doi.org/10.1093/bib/bbaa153

2. Ballester, P. J., & Mitchell, J. B. O. (2010, March 17). A machine learning approach to predicting protein–ligand binding affinity with applications to molecular docking. Bioinformatics, 26(9), 1169–1175. https://doi.org/10.1093/bioinformatics/btq112

3. Chen, J., Cheong, H. H., & Siu, S. W. I. (2021, July 30). xDeep-AcPEP: Deep Learning Method for Anticancer Peptide Activity Prediction Based on Convolutional Neural Network and Multitask Learning. Journal of Chemical Information and Modeling, 61(8), 3789–3803. https://doi.org/10.1021/acs.jcim.1c00181

4. Chou, K. C., & Shen, H. B. (2007, November). Recent progress in protein subcellular location prediction. *Analytical Biochemistry*, *370*(1), 1–16. https://doi.org/10.1016/j.ab.2007.07.006

5. da Silva, R. R. (2018, April 16). Enzymatic Synthesis of Protein Hydrolysates from Animal Proteins: Exploring Microbial Peptidases. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.00735

6. Guo, R., Liu, J., Chai, J., Gao, Y., Abdel-Rahman, M. A., & Xu, X. (2022, June 28). Scorpion Peptide Smp24 Exhibits a Potent Antitumor Effect on Human Lung Cancer Cells by Damaging the Membrane and Cytoskeleton *in vivo* and *in vitro*. Toxins, 14(7), 438. https://doi.org/10.3390/toxins14070438

7. He, W., Wang, Y., Cui, L., Su, R., & Wei, L. (2021, July 29). Learning embedding features based on multisense-scaled attention architecture to improve the predictive performance of anticancer peptides. Bioinformatics, 37(24), 4684–4693. https://doi.org/10.1093/bioinformatics/btab560

8. Lau, J. L., & Dunn, M. K. (2018). Therapeutic peptides: Historical perspectives, current development trends, and future directions. *Bioorganic & medicinal chemistry*, *26*(10), 2700–2707. https://doi.org/10.1016/j.bmc.2017.06.052

9. Li, Y. (2011, December). Recombinant production of antimicrobial peptides in Escherichia coli: A review. Protein Expression and Purification, 80(2), 260–267. https://doi.org/10.1016/j.pep.2011.08.001

10. Ma, B., Fan, Y., Zhang, D., Wei, Y., Jian, Y., Liu, D., Wang, Z., Gao, Y., Ma, J., Chen, Y., Xu, S., & Li, L. (2022, August 15). De Novo Design of an Androgen Receptor DNA Binding Domain-Targeted peptide PROTAC for Prostate Cancer Therapy. Advanced Science, 9(28). https://doi.org/10.1002/advs.202201859

11. Ma, K., Wang, Z., Ju, X., Huang, J., & He, R. (2022, October 10). Rapeseed peptide inhibits HepG2 cell proliferation by regulating the mitochondrial and P53 signaling pathways. Journal of the Science of Food and Agriculture, 103(3), 1474–1483. https://doi.org/10.1002/jsfa.12243

12. Pan, X., Xu, J., & Jia, X. (2020, January 1). <p>Research Progress Evaluating the Function and Mechanism of Anti-Tumor Peptides</p> Cancer Management and Research. https://doi.org/10.2147/cmar.s232708

13. Perez Espitia, P. J., de Fátima Ferreira Soares, N., dos Reis Coimbra, J. S., de Andrade, N. J., Souza Cruz, R., & Alves Medeiros, E. A. (2012, February 29). Bioactive Peptides: Synthesis, Properties, and Applications in the Packaging and Preservation of Food. Comprehensive Reviews in Food Science and Food Safety, 11(2), 187–204. https://doi.org/10.1111/j.1541-4337.2011.00179.x

14. Podlesnykh, S. V., Abramova, K. E., Gordeeva, A., Khlebnikov, A. I., & Chapoval, A. I. (2021, January 6). Peptide Blocking CTLA-4 and B7-1 Interaction. Molecules, 26(2), 253. https://doi.org/10.3390/molecules26020253

15. Rao, B., Zhang, L., & Zhang, G. (2020). ACP-GCN: The Identification of Anticancer Peptides Based on Graph Convolution Networks. IEEE Access, 8, 176005–176011. https://doi.org/10.1109/access.2020.3023800

16. Riedl, S., Rinner, B., Asslaber, M., Schaider, H., Walzer, S., Novak, A., Lohner, K., & Zweytick, D. (2011, November). In search of a novel target — Phosphatidylserine exposed by non-apoptotic tumor cells and metastases of malignancies with poor treatment efficacy. Biochimica Et Biophysica Acta (BBA) - Biomembranes, 1808(11), 2638–2645. https://doi.org/10.1016/j.bbamem.2011.07.026

17. Sanyal, A., Dutta, S., Camara, A., Chandran, A., Koller, A., Watson, B. G., Sengupta, R., Ysselstein, D., Montenegro, P., Cannon, J., Rochet, J. C., & Mattoo, S. (2019, May). Alpha-Synuclein Is a Target of Fic-Mediated Adenylylation/AMPylation: Possible Implications for Parkinson's Disease. Journal of Molecular Biology, 431(12), 2266–2282. https://doi.org/10.1016/j.jmb.2019.04.026

18. Shin, M. K., Jang, B. Y., Bu, K. B., Lee, S. H., Han, D. H., Oh, J. W., & Sung, J. S. (2022, December 9). De Novo Design of AC-P19M, a Novel Anticancer Peptide with Apoptotic Effects on Lung Cancer Cells and Anti-Angiogenic Activity. International Journal of Molecular Sciences, 23(24), 15594. https://doi.org/10.3390/ijms232415594

19. Stráner, P. (n.d.). Bacterial expression and/or solid phase peptide synthesis of 20-40 amino acid long polypeptides and miniproteins, the case study of Class B GPCR ligands. http://www.eurekaselect.com. https://dx.doi.org/10.2174/1389203716666151102105215

20. Velayutham, M., Sarkar, P., Sudhakaran, G., Al-Ghanim, K. A., Maboob, S., Juliet, A., Guru, A., Saravanan, M., & Arockiaraj, J. (2022, October 28). Anti-Cancer and Anti-Inflammatory Activities of a Short Molecule, PS14 Derived from the Virulent Cellulose Binding Domain of *Aphanomyces invadans*, on Human Laryngeal Epithelial Cells and an In Vivo Zebrafish Embryo Model. Molecules. https://doi.org/10.3390/molecules27217333

21. Xie, M., Liu, D., & Yang, Y. (2020, July). Anti-cancer peptides: classification, mechanism of action, reconstruction, and modification. Open Biology, 10(7). https://doi.org/10.1098/rsob.200004

22. Yi, H. C., You, Z. H., Zhou, X., Cheng, L., Li, X., Jiang, T. H., & Chen, Z. H. (2019, September). ACP-DL: A Deep Learning Long Short-Term Memory Model to Predict Anticancer Peptides Using High-Efficiency Feature Representation. Molecular Therapy - Nucleic Acids, 17, 1–9. https://doi.org/10.1016/j.omtn.2019.04.025

23. Zhang, Y., Liu, C., Wu, C., & Song, L. (2023, March). Natural peptides for immunological regulation in cancer therapy: Mechanism, facts, and perspectives. Biomedicine & Pharmacotherapy, 159, 114257. https://doi.org/10.1016/j.biopha.2023.114257

24. Zhong, C., Zhang, L., Yu, L., Huang, J., Huang, S., & Yao, Y. (2020). A Review for Antimicrobial Peptides with Anticancer Properties: Re-purposing of Potential Anticancer Agents. BIO Integration, 1(4). https://doi.org/10.15212/bioi-2020-0013

25. Zhou, X. R., Zhang, Q., Tian, X. B., Cao, Y. M., Liu, Z. Q., Fan, R., Ding, X. F., Zhu, Z., Chen, L., & Luo, S. Z. (2016, August). From a pro-apoptotic peptide to a lytic peptide: One single residue mutation. Biochimica Et Biophysica Acta (BBA) - Biomembranes, 1858(8), 1914–1925. https://doi.org/10.1016/j.bbamem.2016.05.012

# Chapter - 7

## Gene Enrichment, Network Mapping, And Identification of Potential Core Genes Associated with Parkinson's Disease

**Authors**

**Deep Saha**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Prabahan Nath**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Semanti Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 7

## Gene Enrichment, Network Mapping, and Identification of Potential Core Genes Associated with Parkinson's Disease

**Deep Saha, Prabahan Nath and Semanti Ghosh**

**Abstract**

"Parkinson's disease" (PD) is a progressive neurological ailment. It proceeds continuously and impacts around 4% of individuals who are 80 years old or more. Early-stage detection is exceptionally difficult. Hence, the goal of this work is to explore and identify unique key genes connected to PD by employing the technique of computational bioinformatics analysis of gene expression. GSE207713which is a gene expression profile data was acquired from the "Gene Expression Omnibus" (GEO). The collected data comprised four samples obtained from healthy individuals and six samples obtained from patients with Parkinson's disease. The marking of "Differential Expression Genes" (DEGs) between PD patients and control was carried out using the GEO2R Tool. Subsequently, the "Database for Annotation, Visualization and Integrated Discovery" (DAVID) was used to perform "Gene Ontology" (GO) analysis further pathway enrichment analysis was done using "Kyoto Encyclopaedia of Genes and Genomes" (KEGG). After that, a "protein-protein interaction" (PPI) network was developed and ran down with "STRING" and "Cytoscape" tools. In the end, all total 10 hub genes were ascertained from the network. A total of 2251 genes turned out to be differentially expressed, among them 844 genes exhibiting upregulation and 1407 genes exhibiting downregulation. The PPI network identified 10 hub genes. CCNB1, BUB1, AURKB, BUB1B, PLK1, CDK1, CDC45, KIF11, CCNB2, & CDC20. The10 hub genes identified and their corresponding metabolic pathways exhibited significant enrichment and likely involve in the development of PD.

**Introduction**

PD is a chronic neurological ailment marked by the gradual decimation of "dopaminergic neurons" from the substantia nigra as well as formation of "Lewy bodies" (Moore *et al*., 2005). This leads symptoms like muscular stiffness, abnormal posture, dyskinesia and resting tremor (Hu *et al*., 2020). In

addition to these movement-related symptoms, individuals with PD often experience hypoolusive olfactory function, depression, cognitive impairment and other mental problems (Hu *et al*., 2020). The disease is progressive and has significant contribution on a person's physical wellbeing, impacting their ability to perform simple duties or engage in communal activities (Megari, 2013). PD is difficult to diagnose in its early stages, and current treatments focus on boosting dopamine levels in the brain to alleviate symptoms, but do not provide a cure (Gesellschaft, 2003). Thus, the detection of molecular biomarkers for PD plays a key role for expanding diagnostic quality, keeping track of disease progress, and designing superior therapeutic approaches (Cai *et al*., 2020). Choosing molecular biomarkers of Parkinson's disease is vital for enhancing our comprehension of the condition and ultimately developing a remedy. Globally, PD stands second following Alzheimer's disease amongst neurodegenerative disease. The primary clinical symptoms are tremors at repose, atypical body positioning, muscle rigidity, and involuntary movements, along with cognitive decline, despair, reduced sense of smell, and certain psychiatric issues. Based on epidemiological inquiry and analysis, the incidence of PD in world is rising due to the rapid, accelerated population aging, economic growth and severe environmental pollution (Gesellschaft, 2003). By 2030, the global population of individuals with PD is projected to reach 8.7 million, which is a more than twofold increase compared to the growth observed in 2005 (Wang *et al*., 2019). Contemporary research indicates that PD arises from a blend of hereditary and nongenetic elements. Researchers have confirmed the association of specific genes, VPS35 and LRRK2, with the progression of PD, but the exact mechanism remains uncertain. There is a deficiency in comprehending the pivotal genes to discern the advancement of PD (Li *et al*., 2021). Hence, it is crucial to ascertain novel genes that may be implicated in the occurrence and advancement of PD. These genes have the potential to be utilized as novel targets for the therapeutic intervention of PD. The main goal of this research is to identify new significant genes linked to the development and progression of PD. To discern the DEGs in astrocytes produced from induced pluripotent stem cells (iPSCs) of patients diagnosed with PD in comparison to those derived from healthy persons (Gesellschaft, 2003). In order to discern genes that are expressed differently between patients with PD The study analysed gene expression profile data retrieved from the GEO database to compare individuals with PD with healthy individuals (Li *et al*., 2021). GO assigned specific functions to differential expression genes, while KEGG identified enriched biological pathways (Meng *et al*., 2021). A PPI network was created to identify key genes linked to PD (Quan *et al*., 2021).

## Webtools and methods

### Data resources

The gene expression datasets were obtained from the *website https://www.ncbi.nlm.nih.gov/geo* for GEO database Astrocyte cell types were chosen as specimens for evaluation on the basis of their ability to more precisely reflect the actual variance in expression of genes in case of PD. Following careful assessment, the series GSE207713 (Quan *et al*., 2021) was selected which complies of 6 sample and 4 control individual data. The data were readily accessible on the GEO database (Mao *et al*., 2021), and our analysis did not entail any trials with human or animal subjects.

### Data analysis of DEGs

The GEO2R an online analytic tool was implemented to identify DEGs between samples from people suffering from PD and samples from healthy individuals (Clough & Barrett, 2016). The tool identified the P value and absolute log fold change (|log FC|) of each gene. Genes which met the specified criteria, with a significance level P-value$< 0.05$ and $|\log FC| \geq 1.0$, were classified as DEGs (Dai *et al*., 2018).

### GO analysis & KEGG pathway enrichment analysis

GO analysis is a customarily employed technique for pursuing in-depth investigations into functional enrichment. The organisation classifies gene functions into three groups: KEGG a widely used repository for data on biological processes, genomes, diseases, chemical compounds, and medications, categorizing them into biological process, cellular component & molecular function (Lei *et al*., 2023; Yu *et al*., 2013). The DEGs in the present study underwent pathway enrichment study using KEGG and GO annotation using DAVID tool (https://david.ncifcrf.gov) (Robson *et al*., 2023). For analysing DEGs with GO annotation (Lingamgunta *et al*., 2023) P value taken $< 0.05$ and gene count of minimum 30 (Chomitz *et al*., 2017). Similarly, analysing DEGs using the KEGG P value taken $< 0.05$ and gene count of minimum 20 (Quan *et al*., 2021; Yang *et al*., 2022).

### Building a PPI network

We utilised the "STRING APP" to analyse PPI data (Rao *et al*., 2014). In order to evaluate the potential PPI link for our unique goals, we mapped the DEGs which were identified for PD in the STRING database (Li *et al*., 2020). PPI network built selecting confidence score threshold of >0.7. Resulting network was visualised by the Cytoscape tools (Shannon *et al*., 2003; Tang *et al*., 2018).

**Detection of key genes**

The centrality metrics suggested above may recognize the genes in a complex PPI network that have the most impact (Elango *et al*., 2023). These genes are able to swiftly pass along and receive information, and are highly responsive to both local and global changes. frequently it can also serve as a means for recognizing key genes. The computation of centrality parameters was performed using Cytoscape plugins, specifically cytoHubba and Network Analyzer.

**Results and Discussion**

The GSE207713 series was chosen for the study. GSE207713 consists of six samples from people suffering from PD and four samples from control. A total of 2251 DEGs were discovered from GSE207713, with $P < 0.05$ & $|\log FC| > 1.0$. Among these 844 were upregulated and 1407 were downregulated (Li *et al*., 2021). The DEGs involved analogized samples obtained from patients diagnosed with PD to samples obtained from individuals without the disease. Figure 1 shows a volcanic map depicting all identified DEGs. The DEGs were arranged in ascending order according to their P values, with smaller P values indicating a greater difference. Table 1 displays information regarding the top 20 genes (Li *et al*., 2023).

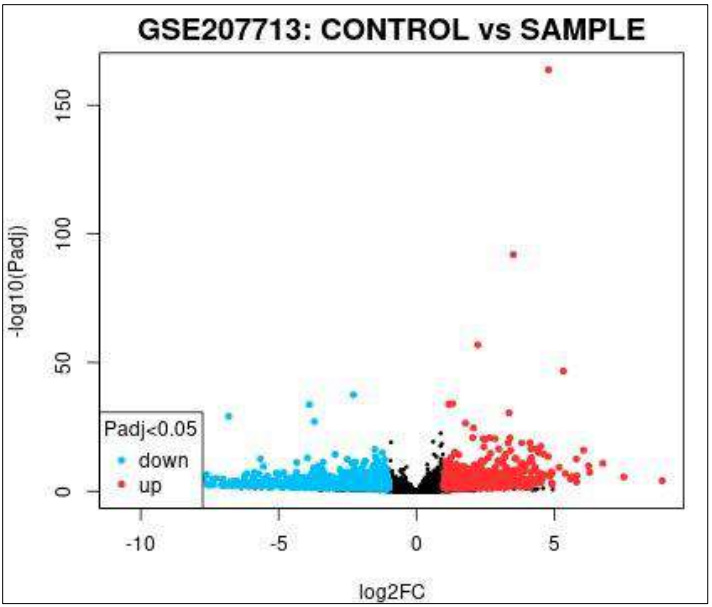


**Fig 1:** Maps displaying all differentially expressed genes related to volcanoes. red: Elevated genes, blue: Downregulated genes

**Table 1:** Top 20 DEGs list according to their P value

| Gene | P value | Log2FoldChange | Description |
|---|---|---|---|
| C5orf46 | 9.68E-169 | 4.7879774 | Up |
| SEMA3F | 1.29E-96 | 3.51902 | Up |
| ZNF699 | 2.47E-61 | 2.2291976 | Up |
| LINC01411 | 4.70E-51 | 5.3292002 | Up |
| COL5A3 | 9.65E-42 | -2.2831603 | Down |
| PPME1 | 3.12E-38 | 1.3088951 | Up |
| ACOX3 | 6.04E-38 | 1.1695804 | Up |
| FIBIN | 8.84E-38 | -3.8784736 | Down |
| ACTC1 | 1.52E-34 | 3.3605909 | Up |
| SPON1 | 4.19E-33 | -6.8103963 | Down |
| LSAMP | 4.86E-31 | -3.7030238 | Down |
| CEMIP2 | 2.27E-30 | 1.7817516 | Up |
| LARGE1 | 1.80E-28 | 2.0715876 | Up |
| SLC2A1 | 1.41E-24 | 2.0434625 | Up |
| STEAP3-AS1 | 1.47E-24 | 2.6496115 | Up |
| KCTD4 | 1.79E-24 | 3.3942519 | Up |
| PLCB4 | 3.92E-24 | 2.8505338 | Up |
| MYH11 | 4.01E-24 | 2.4210102 | Up |
| PMEPA1 | 7.13E-24 | 2.4918963 | Up |
| IGFL2 | 2.07E-22 | 4.1213987 | Up |

**Functional enrichment analyses of DEGs**

DAVID was utilised for conducting KEGG pathway enrichment and GO function studies for DEGs (Quan *et al*., 2021; Leng *et al*., 2024). In DAVID we selected species as *Homo Sapience.* The graphical representation of GO Function enrichment was plotted using the Power Bi (Figure 2), and the enrichment analysis of KEGG signalling pathway was plotted using the GeneCodis4 online tool (Figure 3). The enhanced GO theories are categorised into “Molecular Function” (MF) ontologies, “Cellular Component” (CC) and “Biological Process” (BP) (Hassani *et al*., 2021; Tang *et al*., 2019). From the GO analysis we can conclude that BP is the main category DEGs (Tang *et al*., 2019). The analysis of BP demonstrated that the DEGs were notably controlling factor in various BP s including cell differentiation, signal transduction, cell division, adhesion & proliferation, up regulation of gene

expression and cell cycle, intracellular signal transduction, nervous system development, and protein phosphorylation (Yu *et al*., 2020; Liu *et al*., 2021). Second in rank is MF. These classifications not only based on binding activity of proteins, identical proteins, ATPs, calcium, protein kinase, receptor binding, and actin binding, but also based on activity of transcription-factors, protein homodimerization, sequence-specific activity, protein serine/threonine/ tyrosine kinase activity etc. The co-localization analysis revealed that DEGs were prominently distributed in various cellular compartments, including the plasma membrane, cytoplasm, cytosol, exosome, extracellular space, extracellular region etc. (Wang *et al*., 2020). Furthermore, from the KEGG pathway analysis it can be concluded that the DEGs correspond to the pathways connected to cancer, "ECM-receptor interaction", "focal adhesion", "protein digestion", "Cell cycle" as well as "absorption", "PI3k-Akt signalling pathway", "Arrhythmogenic right ventricular cardiomyopathy", cell adhesion molecules, "small cell lung cancer", and "hypertrophic cardiomyopathy" (Huang *et al*., 2018; He *et al*., 2019).

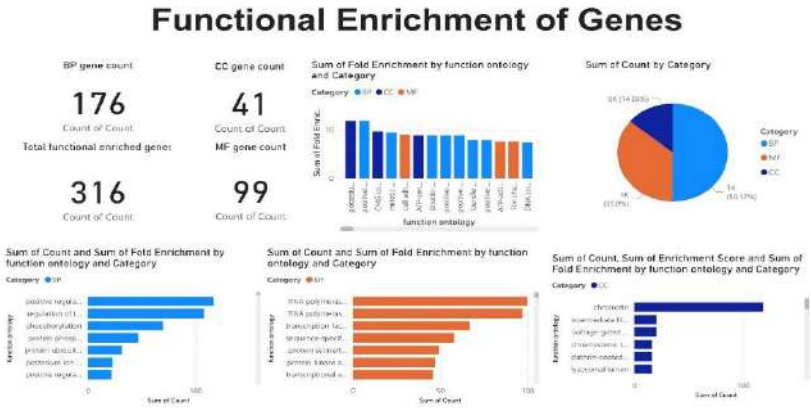


**Fig 2:** Perform gene ontology function enrichment study on differentially Expressed Genes (DEGs). Orange: MF, blue: CC, and sky blue: BP. x-axis values: gene counts

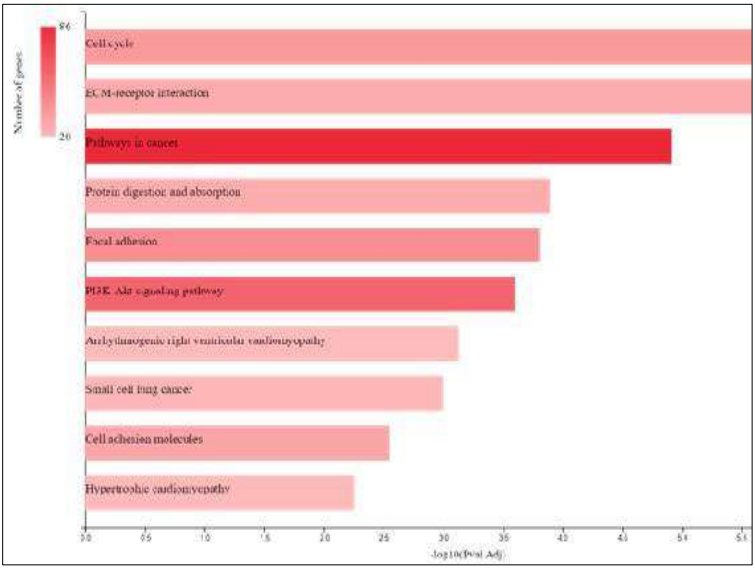


**Fig 3:** Enrichment analysis on the top 10 DEGs in the KEGG signalling pathway. The x-axis numbers: Padj Values and y-axis numbers: genes count

## Constructing a PPI network and identifying hub genes

The PPI of the DEGs were predicted and retrieved using the STRING APP in cystoscope (He *et al*., 2019; Zhang *et al*., 2010). The PPI network included 1812 nodes, as shown in Figure 4.

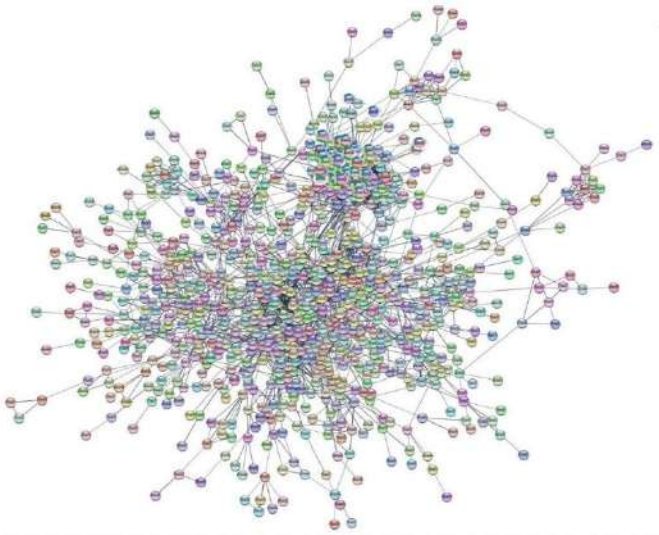

**Fig 4:** PPI Network of DEGs

The top ten connected hub genes, identified by their degree of connectedness in the PPI network, are listed in descending order in Table 2. CytoHubba, a plugin for Cytoscape, aids in identifying these genes by quantifying their degree in the PPI network and arranging them in order of connectivity. The findings indicated that CDK1 exhibited the highest gene connection degree of 123, followed by CCNB1 (degree= 101), BUB1 (degree= 98), CDC20 (degree= 97), PLK1 (degree= 94), BUB1B (degree= 93), CDC45 (degree= 90), KIF11 (degree= 89), CCNB2 (degree= 88), and AURKB (degree= 87) (Figure 5).

**Table 2:** List of top ten hub genes with descending order of degrees connectivity within PPI network

| Rank | Gene name | Degree |
|---|---|---|
| 1 | CDK1 | 123 |
| 2 | CCNB1 | 101 |
| 3 | BUB1 | 98 |
| 4 | CDC20 | 97 |
| 5 | PLK1 | 94 |
| 6 | BUB1B | 93 |
| 7 | CDC45 | 90 |
| 8 | KIF11 | 89 |
| 9 | CCNB2 | 88 |
| 10 | AURKB | 87 |

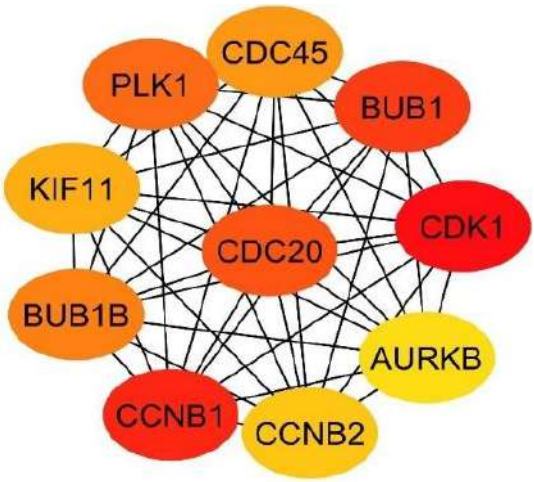


**Fig 5:** Top 10 hub genes with highest degree connection

Here, degree was utilised to quantify the significance of protein nodes in the PPI network. The nodes in the PPI network became more significant as the degree value increased (Yazdani *et al*., 2023).

## Conclusion

In summary, this study looks into unique fundamental genes associated with the onset of PD. Our study indicates that BP play a crucial part in the progression of PD. the identified 10 hub genes (CCNB1, BUB1, AURKB, BUB1B, PLK1, CDK1, CDC45, KIF11, CCNB2, & CDC20) that may contribute to the manifestation of PD (Gupta *et al*., 2023; Moore *et al*., 2021). Nevertheless, given the mentioned constraints of the study, additional research is required in the future. Therefore, we anticipate that our research will offer and foster curiosity and stimulate additional exploration of the discovered genes and pathways as potential drivers of PD.

## References


1. Cai, Y., Shen, H., Weng, H., Wang, Y., Cai, G., Chen, X., & Ye, Q. (2020, June 26). Overexpression of PGC-1α influences the mitochondrial unfolded protein response (mtUPR) induced by MPP+ in human SH-SY5Y neuroblastoma cells. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-67229-6

2. Chomitz, V. R., Brown, A., Lee, V., Must, A., & Chui, K. (2017, July 17). Healthy Living Behaviors among Chinese–American Preschool-Aged Children: Results of a Parent Survey. Journal of Immigrant and Minority Health. https://doi.org/10.1007/s10903-017-0619-z

3. Clough, E., & Barrett, T. (2016). The Gene Expression Omnibus Database. *Methods in molecular biology (Clifton, N.J.)*, *1418*, 93–110.

4. Dai, J., Lin, K., Huang, Y., Lu, Y., Chen, W. Q., Zhang, X. R., He, B. S., Pan, Y. Q., Wang, S. K., & Fan, W. X. (2018, October). Identification of critically carcinogenesis-related genes in basal cell carcinoma. OncoTargets and Therapy, Volume 11, 6957–6967. https://doi.org/10.2147/ott.s170504

5. Elango, R., Banaganapalli, B., Mujalli, A., AlRayes, N., Almaghrabi, S., Almansouri, M., Sahly, A., Jadkarim, G. A., Malik, M. Z., Kutbi, H. I., Shaik, N. A., & Alefishat, E. (2023, January). Potential Biomarkers for Parkinson Disease from Functional Enrichment and Bioinformatic Analysis of Global Gene Expression Patterns of Blood and Substantia Nigra Tissues. Bioinformatics and Biology Insights, 17, 117793222311662. https://doi.org/10.1177/11779322231166214

6. Gesellschaft e. V. Third Congress Deutsche Parkinson- Journal of Neural Transmission, 110(2), i–iv, (2003). https://doi.org/10.1007/s007020300000

7. Gupta, K., Lamba, T., & Garg, N. (2023). Predicting Parkinson's Disease Risk Through Protein and Peptide Level Analysis: An Evidence from EDA and Machine Learning Based Approach. https://doi.org/10.1109/icccis60361.2023.10425287

8. Hassani, B., Mollanoori, H., Pouresmaeili, F., Asgari, Y., & Ghafouri-Fard, S. (2021, September 29). Constructing mRNA, miRNA, circRNA and lncRNA Regulatory Network by Analysis of Microarray data in Breast Cancer. https://doi.org/10.21203/rs.3.rs-603108/v2

9. He, S., Sun, H., Huang, Y., Dong, S., Qiao, C., Zhang, S., Wang, C., Zheng, F., Yan, M., & Yang, G. (2019, June 25). Identification and Interaction Analysis of Significant Genes and MicroRNAs in Pterygium. BioMed Research International. https://doi.org/10.1155/2019/2767512

10. Hu, C., Ke, C. J., & Wu, C. (2020, August). Identification of biomarkers for early diagnosis of Parkinson's disease by multi-omics joint analysis. Saudi Journal of Biological Sciences, 27(8), 2082–2088.https://doi.org/10.1016/j.sjbs.2020.04.012

11. Huang, C., Huang, B. B., Niu, J. M., Yu, Y., Qin, X. Y., Yang, Y. L., Xiao, T. X., Chen, J., Ren, L. R., & Zhang, J. (2018). Global mRNA and Long Non-Coding RNA Expression in the Placenta and White Adipose Tissue of Mice Fed a High-Fat Diet During Pregnancy. Cellular Physiology and Biochemistry, 50(6), 2260–2271. https://doi.org/10.1159/000495086

12. Lei, C., Zhongyan, Z., Wenting, S., Jing, Z., Liyun, Q., Hongyi, H., Juntao, Y., & Qing, Y. (2023, May 22). Identification of necroptosis-related genes in Parkinson's disease by integrated bioinformatics analysis and experimental validation. Frontiers in Neuroscience, 17. https://doi.org/10.3389/fnins.2023.1097293

13. Leng, X., Xie, S., Tao, D., Wang, Z., Shi, J., Yi, M., Tan, X., Zhang, X., Liu, Y., & Yang, Y. (2024, January 8). Mouse Tspyl5 promotes spermatogonia proliferation through enhancing Pcna-mediated DNA replication. Reproduction, Fertility and Development, 36(3). https://doi.org/10.1071/rd23042

14. Li, J., Huang, G., Ren, C., Wang, N., Sui, S., Zhao, Z., & Li, M. (2021). Identification of differentially expressed genes-related prognostic risk

model for survival prediction in breast carcinoma patients. *Aging*, *13*(12), 16577–16599. https://doi.org/10.18632/aging.203178

15. Li, J., Li, R., Wu, X., Hoo, R. L. C., Lee, S. M. Y., Cheung, T. M. Y., Ho, B. S. Y., & Leung, G. P. H. (2021, February 3). *Amauroderma rugosum* Protects PC12 Cells against 6-OHDA-Induced Neurotoxicity through Antioxidant and Antiapoptotic Effects. Oxidative Medicine and Cellular Longevity, 2021, 1–15. https://doi.org/10.1155/2021/6683270

16. Li, J., Sun, Z., Lv, Z., Jiang, H., Liu, A., Wang, M., Tan, G., Guo, H., Sun, H., Wu, R., Xu, X., Yan, W., Jiang, Q., Ikegawa, S., & Shi, D. (2021, October 20). Microtubule Stabilization Enhances the Chondrogenesis of Synovial Mesenchymal Stem Cells. Frontiers in Cell and Developmental Biology, 9. https://doi.org/10.3389/fcell.2021.748804

17. Li, L., Wang, Z., Xu, K., Bai, X., Wu, W., Zhang, Z., & Chen, X. (2023, April 21). AUF1 modulates apoptosis via regulating transcription and alternative splicing of immune response genes in auditory hair cells. https://doi.org/10.21203/rs.3.rs-2807407/v1

18. Li, Q., Liang, J., & Chen, B. (2020, January 1). Identification of CDCA8, DSN1 and BIRC5 in Regulating Cell Cycle and Apoptosis in Osteosarcoma Using Bioinformatics and Cell Biology. Technology in Cancer Research & Treatment. https://doi.org/10.1177/1533033820965605

19. Lingamgunta, L. K., Aloor, B. P., Dasari, S., Ramakrishnan, R., Botlagunta, M., Madikonda, A. K., Gopal, S., & Sade, A. (2023). Identification of prognostic hub genes and therapeutic targets for selenium deficiency in chicks model through transcriptome profiling. *Scientific reports*, *13*(1), 8695. https://doi.org/10.1038/s41598-023-34955-6

20. Liu, T., Li, C. X., Zhong, J., Shu, D., Luo, D., Li, Z. M., Zhou, J. Y., Yang, J., Tan, H., & Ma, X. R. (2021, March 4). Exogenous 1′,4′-trans-Diol-ABA Induces Stress Tolerance by Affecting the Level of Gene Expression in Tobacco (*Nicotiana tabacum* L.). International Journal of Molecular Sciences, 22(5), 2555. https://doi.org/10.3390/ijms22052555

21. Mao, Q. Y., Xie, S., Wu, L. L., Xiang, R. L., & Cai, Z. G. (2021). MicroRNA-mRNA expression profiles and functional network after injection of botulinum toxin type A into submandibular glands. Toxicon: official journal of the International Society on Toxinology, 199, 31–40. https://doi.org/10.1016/j.toxicon.2021.05.011.

22. Megari K. (2013). Quality of Life in Chronic Disease Patients. *Health psychology research*, *1*(3), e27. https://doi.org/10.4081/hpr.2013.e27

23. Meng, F., Du, N., Xu, D., Kuai, L., Liu, L., & Xiu, M. (2021, July 21). Bioinformatics Analysis of the Molecular Mechanism and Potential Treatment Target of Ankylosing Spondylitis. Computational and Mathematical Methods in Medicine, 2021, 1–11. https://doi.org/10.1155/2021/7471291

24. Moore, A., Bandres-Ciga, S., Blauwendraat, C., & Diez-Fairen, M. (2021, March 12). A genetic and transcriptomic assessment of theKTN1gene in Parkinson's disease risk. https://doi.org/10.1101/2021.03.08.21252688

25. Moore, D. J., West, A. B., Dawson, V. L., & Dawson, T. M. (2005, July 21). MOLECULAR PATHOPHYSIOLOGY OF PARKINSON'S DISEASE. Annual Review of Neuroscience, 28(1), 57–87.

26. Quan, W., Li, J., Jin, X., Liu, L., Zhang, Q., Qin, Y., Pei, X., & Chen, J. (2021, September 17). Identification of Potential Core Genes in Parkinson's Disease Using Bioinformatics Analysis. Parkinson's Disease, 2021, 1–10. https://doi.org/10.1155/2021/1690341

27. Rao, V. S., Srinivas, K., Sujini, G. N., & Kumar, G. N. (2014). Protein-protein interaction detection: methods and analysis. *International journal of proteomics*, *2014*, 147648. https://doi.org/10.1155/2014/147648

28. Robson, J. K., Tidy, A. C., Thomas, S. G., & Wilson, Z. A. (2023, December 8). Environmental regulation of male fertility is mediated through Arabidopsis transcription factors bHLH89, 91, and 10. Journal of Experimental Botany. https://doi.org/10.1093/jxb/erad480

29. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research*, *13*(11), 2498–2504. https://doi.org/10.1101/gr.1239303

30. Tang, D., Zhao, X., Zhang, L., Wang, Z., & Wang, C. (2018, December 3). Identification of hub genes to regulate breast cancer metastasis to brain by bioinformatics analyses. Journal of Cellular Biochemistry, 120(6), 9522–9531.

31. Tang, F., Lu, Z., Wang, J., Li, Z., Wu, W., Duan, H., & He, Z. (2019, December). Competitive endogenous RNA (ceRNA) regulation network of lncRNAs, miRNAs, and mRNAs in Wilms tumour. BMC Medical Genomics, 12(1). https://doi.org/10.1186/s12920-019-0644-y

32. Wang, L., Li, S., Wang, Y., Tang, Z., Liu, C., Jiao, W., & Liu, J. (2020). Identification of differentially expressed protein-coding genes in lung adenocarcinomas. *Experimental and therapeutic medicine*, *19*(2), 1103–1111. https://doi.org/10.3892/etm.2019.8300

33. Wang, Q., Liu, J., Guo, Y., Dong, G., Zou, W., & Chen, Z. (2019). Association between BDNF G196A (Val66Met) polymorphism and cognitive impairment in patients with Parkinson's disease: a meta-analysis. Brazilian Journal of Medical and Biological Research, 52(8). https://doi.org/10.1590/1414-431x20198443

34. Yang, Y., Wang, Y., Wang, C., Xu, X., Liu, C., & Huang, X. (2022, November 8). Identification of hub genes of Parkinson's disease through bioinformatics analysis. *Frontiers in Neuroscience*, *16*.

35. Yazdani, F., Mottaghi-Dastjerdi, N., Ghorbani, A., Soltany-Rezaee-Rad, M., Montazeri, H., Khoshdel, F., & Guzzi, P. H. (2023, October 17). Identification of Key Genes and Pathways Governing T-DM1 Resistance in OE-19 Esophageal Cancer Cells through Bioinformatics Analysis. https://doi.org/10.21203/rs.3.rs-3423572/v1

36. Yu, H., Zheng, X., & Zhang, Z. (2013, June). Mechanism of Roux-en-Y Gastric Bypass Treatment for Type 2 Diabetes in Rats. Journal of Gastrointestinal Surgery, 17(6), 1073–1083. https://doi.org/10.1007/s11605-013-2188-3

37. Yu, M., Xu, W., Jie, Y., Pang, J., Huang, S., Cao, J., Gong, J., Li, X., & Chong, Y. (2020, December 30). Identification and Validation of Three Core Genes in p53 Signaling Pathway in Hepatitis B Virus-Related Hepatocellular Carcinoma. https://doi.org/10.21203/rs.3.rs-136505/v1

38. Zhang, M., & Lu, L. J. (2010). Investigating the validity of current network analysis on static conglomerate networks by protein network stratification. *BMC bioinformatics*, *11*, 466.

# Chapter - 8

## A Review on Bioinspired ZnO Nanoparticles for Environmental Remediation


**Authors**

**Dipti Das**
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India
Department of Botany, Kalna College, Purba Bardhaman, West Bengal, India

**Keya Mandal**
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India
Department of Environmental Science, Kalna College, Purba Bardhaman, West Bengal, India

**Supriya Kumar Bose**
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

**Arpita Chakraborty**
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

**Gopal Mistri**
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India
Department of Zoology, Kalna College, Purba Bardhaman, West Bengal, India

**Sima Orang**
The Chief Medical Office of Health, Purba Medinipur, West Bengal, India

**Sabyasachi Ghosh**
Department of Biotechnology, School of Life Science, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 8

## A Review on Bioinspired ZnO Nanoparticles for Environmental Remediation

**Dipti Das, Keya Mandal, Supriya Kumar Bose, Arpita Chakraborty, Gopal Mistri, Sima Orang and Sabyasachi Ghosh**

**Abstract**

The use of dyes has increased dramatically in some industries these days, including leather, textiles, food, medicine, paper, cosmetics, etc. The issues have arisen due to the disposal of the dyes in the environment, mostly into the water bodies along with the industrial wastes, which are detrimental for humans, animals and aquatic life. One of the big successes for resolving these problems is the application of nanoparticles (NPs) to environmental pollution. The synthesis of NPs using green methods is regarded as an economical, quick, easy, eco-friendly, biocompatible and safe. Here in, we studied the procedures of biosynthesizing of Zinc Oxide NPs from extract of various plant. Here, the role of this bio-inspired ZnO NPs is also noticed for eradication of several dyes. ZnO NPs are of great interest as they are easy and affordable to make, safe and have potential applications in respect of cleaning up the environment.

**Keywords:** ZnO nanoparticles, plant extract, biosynthesis, dye removal.

### Introduction

One of the most basic elements in the universe is water. To exist, all living things need clean water (Tanwar *et al*., 2021). A significant amount of pollution from man-made sources and agricultural and industrial effluents has affected natural water sources in recent years. Chemical processes and/or dye-related products such as textiles, paints, cosmetics, pulp and paper manufacture, dyeing of fabrics, printing, food, treating leather, photography, and pharmaceutical sectors, use a lot of clean water. Dyes damage water sources because they are colorants, persistent, stubborn and potentially harmful and carcinogenic substances. These pollutants will have detrimental effects on the health of humans, microorganisms, animals, and plants (Zafar *et al*., 2019). Numerous physical, biological and chemical techniques should be developed to remove toxic contaminants including dyes from

wastewater. In this regard, the effective application of NPs to eliminate dyes and cleanup of other environmental pollutants has received a lot of attention recently. However, the traditional approaches of synthesizing NPs are expensive, hazardous, and not good for the environment. To address these issues and ensure sustainability, scientists and researchers have taken a green strategy to manufacture environment-friendly NPs by avoiding the usage of hazardous materials or byproducts in the process. A large number of NPs namely silver (Ag), copper (Cu), platinum (Pt), gold (Au), titanium dioxide ($TiO_2$), zinc oxide (ZnO), and so on are produced by using of plant extracts (Jadoun *et al*., 2024). ZnO-NPs have drawn the most interest of all because of their more excitation energy, ratio of large surface area-to-volume, outstanding photosensitivity, non-toxicity, affordable price, distinctive morphological, optical and chemical properties and wide range of applications (Arif *et al*., 2020; Batra *et al*., 2022; Jadoun *et al*., 2024). With a 3.2 eV band gap and a high surface area, these NPs can generate both $O_2$ −* (superoxide) and OH* (hydroxide) radicals to originate electrons (e−) and holes (h+). This leads to the reduction - oxidation reactions in the valence band (VB), and conduction band (CB) thereby enabling photocatalysis to degrade dyes. The current research about the green based synthesis of ZnO-NPs which uses the parts of plant, like leaves, flowers, stems, roots, seeds, and fruits, and its implications to clean up the environment, including photocatalytic dye degradation, has been compiled here (Jadoun *et al*., 2024).

**Biosynthesis of ZnO NPs using plant extracts**

Green approach is becoming more and more integrated into bio-nanotechnology with the goal of developing environmentally friendly, simple, low-cost, and energy-efficient processes. This method yields better and more consistent results, and the precursors are safe, non-toxic, and environmentally friendly (Batra *et al*., 2022). Many researchers have investigated the green fabrication of ZnO-NPs as a safe, non-toxic, and environmentally friendly substitute that uses extracts from different plant parts releasing biomolecules and phytochemicals like phenolic acids, alcohols, flavonoids, and terpenoids, and chelating NPs to reduce metal ions and serve as a factor of capping or stabilization and reduction (Hoon Seo *et al*., 2019; Tanwar *et al*., 2021; Batra *et al*., 2022). Owing to their uniformity and size, plant-based NMs are recommended. Plant-assisted manufacturing can be carried out in mild experimental settings and with minimal reaction time due to the abundance of plant sources. On the other hand, when plant-inspired synthesis is employed for large-scale production, further caution is required since the size of the product may produce anisotropic features (Batra *et al*., 2022).

Transferring phytochemicals from a green source into a solution

essentially involves the extraction process. To get rid of any dirt and undesired contamination, the various plant parts are thoroughly cleaned with tap water. The parts of plants are then dried up, minced into smaller pieces, and ground into a dust. To make a liquid extract, these powders are boiled in an appropriate solvent (milli-Q water). Zn salt solution, the precursor for ZnO NPs, is mixed with the obtained plant extract at the optimal pH and temperature to start the synthesis process (Figure 1) (Bhattacharjee *et al*., 2022).

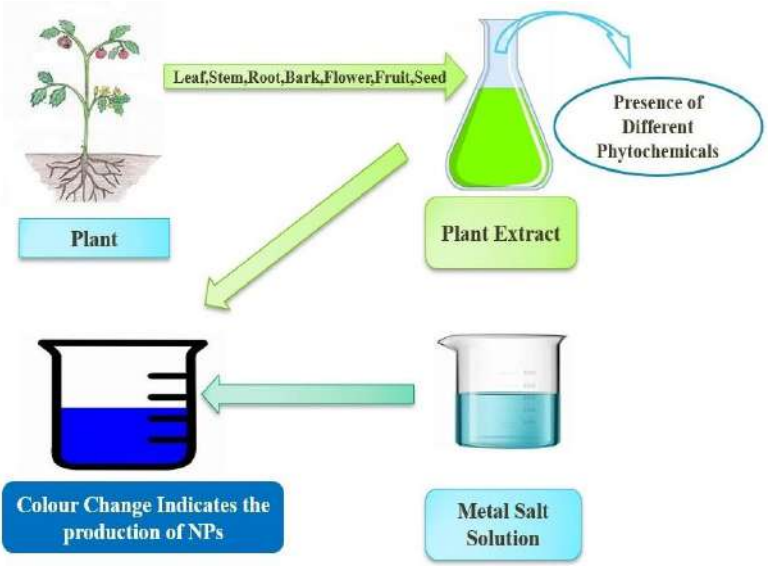


**Fig 1:** Plant-based synthesis of nanoparticles

With the aim of regulating the perfect reaction conditions to synthesized of ZnO NPs, parameter optimization is crucial for boosting high quality production and determine the highest activity for ZnO (Figure 2).

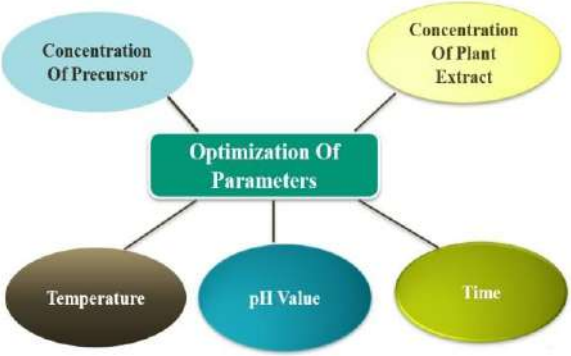


**Fig 2:** Optimization of parameters during plant-assisted synthesis of NPs

**Role of various plant extracts in bio-inspired NPs**

The use of plant extracts is influenced by existence of distinctive bioactive compounds namely phenolic acids, amino acids, flavonoids, alcohols, saponins, polyphenols, alkaloids, methylxanthines, terpenoids tannins and polysaccharides, which act as capping or stabilizing and reducing factors of metal oxide ions in their precursors during plant-mediated NP formation. Leaves, roots, stems, rhizomes, bark, flower, fruits, peels etc. can be utilized in the plant-assisted production of NPs. ZnO NPs were formed as a consequence of the reduction of the zinc salt, which was effectively aided by the active ingredients in *Carica papaya* leaf extract, including flavonoids, phenols, alkaloids, tannins, and glycosides (Gaur *et al*., 2023). The size, shape, and surface features of the resultant nanostructures may be uniquely influenced by each plant extract. While disassembly and post synthetic modification of surface NPs are facilitated by capping agents, redox mediators play a crucial role in the reduction of metal ions (Batra *et al*., 2022). In this case, selection of right solvent and adjusting the boiling temperature are crucial to extract phytochemicals without causing any structural damage. Some researchers emphasize the importance of distinct functional groups found in plant – derived components that support the production of NPs. Researcher workers put on their utmost interest on C-H, O-H, C = C groups, commonly found in secondary by-products like alkaloids, terpenoids and flavonoids. For example, the *Tectona grandis* leaf extract was used by Senthilkumar *et al*., (2017) to propose the ZnO NPs evolution method. Herein, zinc nitrate decomposes into ZnO NPs due to the presence of -OH groups in phenol and flavonoids. Primarily, to initiate the production of NPs, flavonoids and phenols in the leaf extract solution attach to the surface in a zinc salt (Bhattacharjee *et al*., 2022).

**Table 1:** Usage of plant- based ZnO-NPs for photocatalytic degradation of diverse dyes

| Plant | Plant parts used | Zn precursor | Shape | Size (nm) | Irradiation | Dye | Degradation rate (%)/time | Ref. |
|---|---|---|---|---|---|---|---|---|
| *Ruellia tuberosa* | Root extract | Zinc Sulfate | Rod-shaped | 40-50 | UV & Sunlight | MB & MG | 94%/150 & 92%/150 | Vasantharaj *et al*., 2021 |
| *Vitex negundo* | Leaf extract | Zinc Nitrate Hexahydrate | Spherical | 5-35 | Sunlight | MB | 98.50%/60 | enkatesan *et al*., 2024 |
| *Calotropis procera* | Leaf extract | Zinc Nitrate | Spherical | 15–25 | UV | MO | 81/100 | Gawade *et al*., 2017 |
| *Canna indica* | Flower extract | Zinc Nitrate | Spherical | 27.82 | Sunlight | MB | 94.23% / 150 | Nguyen *et al*., 2021 |
| *Acalypha* | Leaf | Zinc Nitrate | Spherical | 16 | Sunlight | MB | 96%/90 | Kamarajan |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *indica* | extract | | | | | | | *et al*., 2022 |
| *Passiflora foetida* | Fruit peels | Zinc Nitrate | Hexagonal | 58 | Sunlight | RhB | 91.06%/70 | Khan *et al*., 2021 |
| *Acacia caesia* | Bark extract | Zinc Nitrate Hexahydrate | Hexagonal | 32.32 | UV | MB | 92%/40 | Ashwini *et al*., 2021 |

(MO - methyl orange, MG - malachite green, MB - methylene blue, RhB - Rhodamine B)

**Procedure of photocatalytic dye removal using bioinspired ZnO NPs**

The photocatalytic dye degradation by ZnO NPs produced from plant extracts are provided in Table 1. When a photocatalyst is exposed to a light source with sufficient energy, electrons migrate from VB to CB and holes are produced in VB. Dyes can be broken down by redox processes using light-driven $e^-$ and $h^+$, which are produced when excitement of electrons occur from the VB to CB illumination of light (Tanwar *et al*., 2021). The mechanism has four stages: (a) absorption of photon to make $e^-$ **-** $h^+$ pairs; (b) separating the carrier that is charged; (c) movement of $h^+$ and $e^-$ to the surface area; and (d) using charges of surface area for redox processes. Equations for dye removal are as follows (Batra *et al*., 2022):

a) $ZnO + light \rightarrow h^+ + e^-$

b) $h^+ + H_2O \rightarrow OH^- + H^+$

c) $h^+ + OH^- \rightarrow OH^*$

d) $e^- + O_2 \rightarrow O^{2-}*$

e) $O^{2-}* + H^+ \rightarrow H_2O_2$

f) $H_2O_2 + O^{-2}* + e^- \rightarrow OH *$

g) $H_2O_2 + light \rightarrow OH^*$

h) $OH^* + Dye \rightarrow$ Degraded by-products ($CO_2 + H_2O$)

The steps involved in photodegrading dye molecules in the presence of light using ZnO-NPs photocatalyst are presented in Figure 3.

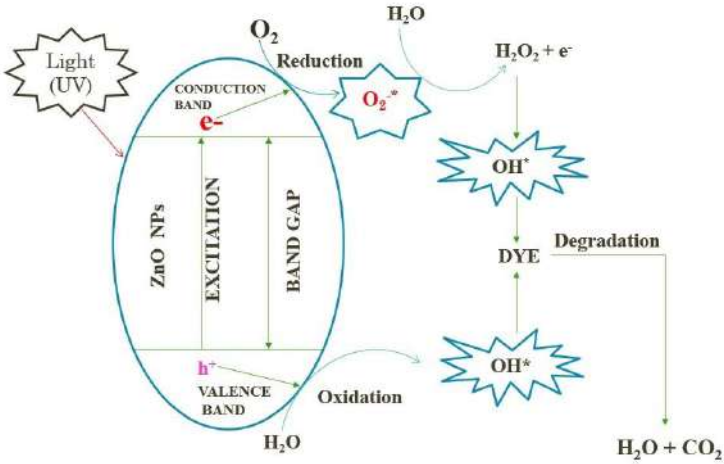


**Fig 3:** Schematic representation of photocatalytic dye degradation

## Conclusion

The plant-based NPs have a wide range of applications and therefore developed into an important field of study. The study represents a simple green synthesis method for producing ZnO NPs using different plant extracts. Plant extract solution contains numerous phytochemicals, acting as a stabilizing and reducing factor for the synthesis ZnO-NPs. Multiple dyes are one of the major resources of environmental pollution including soil and water pollution. The dyes have eliminated using different methods, but all methods have some limitations. In this review, the usage of plant extract-inspired ZnO NPs has been emphasized on the harmful dye degradation through photocatalysis, as the plant derived ZnO-NPs are an inexpensive, safe, more eco-friendly and efficient choice for environmental remediation. In future, it becomes more necessary to focus on the improvement of this more promising approach for a safe planet.

## References

1. Arif, R., Jadoun, S., & Verma, A. (2020). Synthesis of nanomaterials and their applications in textile industry. Frontiers of textile materials: polymers, nanomaterials, enzymes, and advanced modification techniques, 117-133.

2. Ashwini, J., Aswathy, T. R., Rahul, A. B., Thara, G. M., & Nair, A. S. (2021). Synthesis and characterization of zinc oxide nanoparticles using *Acacia caesia* bark extract and its photocatalytic and antimicrobial activities. *Catalysts*, *11*(12), 1507.

3. Bhattacharjee, N., Som, I., Saha, R., & Mondal, S. (2024). A critical review on novel eco-friendly green approach to synthesize zinc oxide nanoparticles for photocatalytic degradation of water pollutants. *International Journal of Environmental Analytical Chemistry*, *104*(3), 489-516.

4. Batra, V., Kaur, I., Pathania, D., & Chaudhary, V. (2022). Efficient dye degradation strategies using green synthesized ZnO-based nanoplatforms: A review. Applied Surface Science Advances, 11, 100314.

5. Gaur, J., Kumar, S., Pal, M., Kaur, H., Batoo, K. M., & Momoh, J. O. (2023). Current trends: Zinc oxide nanoparticles preparation via chemical and green method for the photocatalytic degradation of various organic dyes. *Hybrid Advances*, 100128.

6. Gawade, V. V., Gavade, N. L., Shinde, H. M., Babar, S. B., Kadam, A. N., & Garadkar, K. M. (2017). Green synthesis of ZnO nanoparticles by using *Calotropis procera* leaves for the photodegradation of methyl orange. *Journal of Materials Science: Materials in Electronics*, *28*, 14033-14039.

7. Hoon Seo, K., Markus, J., Soshnikova, V., Oh, K. H., Anandapadmanaban, G., Elizabeth Jimenez Perez, Z., ... & Yang, D. C. (2019). Facile and green synthesis of zinc oxide particles by Stevia Rebaudiana and its *in vitro* photocatalytic activity. Inorganic and Nano-Metal Chemistry, 49(1), 1-6.

8. Jadoun, S., Yáñez, J., Aepuru, R., Sathish, M., Jangid, N. K., & Chinnam, S. (2024). Recent advancements in sustainable synthesis of zinc oxide nanoparticles using various plant extracts for environmental remediation. Environmental Science and Pollution Research, 1-25.

9. Kamarajan, G., Anburaj, D. B., Porkalai, V., Muthuvel, A., & Nedunchezhian, G. (2022). Green synthesis of ZnO nanoparticles using *Acalypha indica* leaf extract and their photocatalyst degradation and antibacterial activity. *Journal of the Indian Chemical Society*, *99*(10), 100695.

10. Khan, M., Ware, P., & Shimpi, N. (2021). Synthesis of ZnO nanoparticles using peels of *Passiflora foetida* and study of its activity as an efficient catalyst for the degradation of hazardous organic dye. *SN Applied Sciences*, *3*, 1-17.

11. Nguyen, D. T. C., Le, H. T., Nguyen, T. T., Nguyen, T. T. T., Bach, L.

G., Nguyen, T. D., & Van Tran, T. (2021). Multifunctional ZnO nanoparticles bio-fabricated from *Canna indica* L. flowers for seed germination, adsorption, and photocatalytic degradation of organic dyes. *Journal of Hazardous Materials*, *420*, 126586.

12. Senthilkumar, N., Nandhakumar, E., Priya, P., Soni, D., Vimalan, M., & Potheher, I. V. (2017). Synthesis of ZnO nanoparticles using leaf extract of *Tectona grandis* (L.) and their anti-bacterial, anti-arthritic, anti-oxidant and *in vitro* cytotoxicity activities. *New Journal of Chemistry*, *41*(18), 10347-10356.
13. Tanwar, N., Dhiman, V., Kumar, S., & Kondal, N. (2022). Plant extract mediated ZnO-NPs as photocatalyst for dye degradation: an overview. Materials Today: Proceedings, 48, 1401-1406.
14. Vasantharaj, S., Sathiyavimal, S., Senthilkumar, P., Kalpana, V. N., Rajalakshmi, G., Alsehli, M., ... & Pugazhendhi, A. (2021). Enhanced photocatalytic degradation of water pollutants using bio-green synthesis of zinc oxide nanoparticles (ZnO NPs). *Journal of Environmental Chemical Engineering*, *9*(4), 105772.
15. Venkatesan, S., Suresh, S., Arumugam, J., Ramu, P., Pugazhenthiran, N., Jothilakshmi, R., & Prabu, K. M. (2024). Sunlight assisted degradation of methylene blue dye by zinc oxide nanoparticles green synthesized using Vitex negundo plant leaf extract. *Results in Chemistry*, *7*, 101315.
16. Zafar, M. N., Dar, Q., Nawaz, F., Zafar, M. N., Iqbal, M., & Nazar, M. F. (2019). Effective adsorptive removal of azo dyes over spherical ZnO nanoparticles. Journal of Materials Research and Technology, 8(1), 713-725.

# Chapter - 9

## Surveying Indoor Air Pollution Status in an South Asian Developing City: A Study Report


**Authors**

**Anwesa Dutta**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Prerona Dutta**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Srishti Choudhury**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Saikat Manna**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Suraj Jyote**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Priti Nandi**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Sarmistha Saha**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Sabarni Sarkar**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

**Sandip Sankar Ghosh**
The Climate Thinker, Kolkata, West Bengal, India

**Srijan Haldar**
Department of Biotechnology, Swami Vivekananda University, Kolkata, West Bengal, India

# Chapter - 9

## Surveying Indoor Air Pollution Status in an South Asian Developing City: A Study Report

**Anwesa Dutta, Prerona Dutta, Srishti Choudhury, Saikat Manna, Suraj Jyote, Priti Nandi, Sarmistha Saha, Sabarni Sarkar, Sandip Sankar Ghosh and Srijan Haldar**

**Abstract**

The study report provides a comprehensive analysis of the indoor air quality in a developing urban setting. Focusing on a South Asian developing city, the study employs survey methods to assess the levels of indoor air pollution, identifying key sources and potential health implications. By utilizing low cost air pollution monitoring equipment and conducting household surveys, the report sheds light on the prevailing indoor air quality, contributing factors, and recommendations for mitigation. This research is instrumental in understanding the local context and lays the groundwork for informed policies and interventions aimed at improving indoor air quality in rapidly developing urban environments.

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

Indoor air pollution is a critical environmental and public health issue characterized by the deterioration of indoor air quality due to the presence of harmful chemicals and pollutants. Unlike outdoor pollution, indoor pollution can be particularly severe because enclosed spaces allow pollutants to accumulate to high levels. The sources of indoor air pollution vary but commonly include combustion processes, building materials, and biological agents. In many developing countries, indoor air pollution poses a significant health risk, often surpassing the impacts of outdoor air pollution. Statistics underscore the severity of indoor air pollution's impact. The reliance on solid fuels for cooking and heating exacerbates the issue, with biomass fuels such as firewood and cow dung being widely used. These fuels, when burned incompletely, release a cocktail of pollutants including particulate matter, carbon monoxide, and polyromantic hydrocarbons, among others. Such pollutants not only degrade air quality but also pose substantial health risks to inhabitants, particularly women and children who spend significant time

indoors. The health consequences of indoor air pollution are dire, contributing to millions of premature deaths globally each year. Respiratory ailments like pneumonia and chronic obstructive pulmonary disease (COPD) are prevalent among populations exposed to indoor pollutants. Furthermore, there is evidence linking indoor air pollution to adverse perinatal outcomes, cardiovascular diseases, and even certain cancers. Formaldehyde, a common indoor pollutant, is known to cause respiratory issues and is classified as a carcinogen. Indoor air pollution is a pressing concern due to widespread solid fuel usage, particularly in rural areas. The combustion of biomass fuels for cooking releases high levels of harmful substances, leading to a range of health problems. Studies have documented elevated rates of respiratory infections, low birth weight, and even tuberculosis linked to exposure to indoor pollutants. Vulnerable populations, such as women and children, bear the brunt of these health burdens.

Addressing indoor air pollution requires a multifaceted approach encompassing awareness campaigns, policy interventions, and technological innovations. Urgent action is needed to mitigate the health risks associated with indoor pollution, particularly in regions heavily reliant on solid fuels. In the current study we have reported the indoor pollution status of 50 individual homes of a south Asian developing 2 tire city Barrackpore, West Bengal, India. Our work revels that irrespective of socioeconomic status, Education status people of this homes live in a much poor indoor air condition (with respect to who guidelines) which may cause them multiple health issues in near future.

**Methodology**

Data collection and Survey area selection: For sampling process we have chosen few small areas of Barrackpore municipality. Although the town was old but recently in last 15-20 years aggressive developmental activities has been continues in the city especially towards to outer edge of city.

Survey process: The door-to-door indoor air pollution survey protocol is designed to systematically assess and analyze indoor air quality in a comprehensive manner. A small scale study has been carries out to understand a basic idea about the IAQ. 50 random house hold have been chosen in Barrackpore (North 24 Pargana, West Bengal.) municipal area including all types of socioeconomic status. The survey begins with informed consent from participants, followed by a structured questionnaire to gather information on household characteristics, cooking practices, and potential pollutant sources. Trained surveyors utilize portable air quality monitoring equipment to measure key pollutants, including Particulate Matter (PM2.5). Monitoring of

PM2.5 was conducted using the PMS5003 sensor. To ensure accuracy, it was pre-calibrated the sensors using a reference-grade monitor. Observations regarding ventilation, fuel types, and household activities are recorded.

Data analysis: A combination of quantitative and qualitative data collection approaches to gather comprehensive insights. For quantitative analysis, real-time monitoring devices were strategically placed in various indoor spaces to measure key pollutants such as Particulate Matter (PM2.5). Data collection spanned different times of the day to capture variations in pollutant levels. Statistical analysis, including descriptive statistics and correlation analyses, was conducted to identify patterns and relationships between pollutant levels and various contributing factors.

**Results:**

Cooking fuel and IAP: Cooking fuel is a critical factor influencing indoor air pollution due to its combustion characteristics. The type of fuel used, such as solid biomass (wood, coal) or cleaner alternatives like Liquefied Petroleum Gas (LPG), significantly impacts indoor air quality. As per data collected in the survey it was found that 26.87% population still don't use clean fuel LPG as cooking fuel at their home. Moreover, 30.35 % of Population Who is not utilising LPG as their primary fuel use wood and 69.65% uses coal for cooking. Traditional biomass fuels release high levels of particulate matter, carbon monoxide, and other pollutants during combustion, contributing to indoor air pollution. In contrast, cleaner fuels like LPG produce fewer harmful emissions choosing the right cooking fuel is crucial for ensuring better indoor air quality, reducing respiratory issues, and promoting overall well-being in households (Figure 1A).

Contribution of cooking area ventilation status for IAP: Ventilation in the kitchen plays a crucial role in mitigating indoor air pollution. Inadequate ventilation can lead to the accumulation of pollutants released during cooking, such as particulate matter, carbon monoxide, and volatile organic compounds. Proper ventilation, through the use of exhaust fans or range hoods, helps to expel these pollutants, ensuring better air circulation and quality. Although 61 % of population have a well-ventilated kitchen space it was noticed that 39% don't have proper air circulation system. Efficient ventilation reduces the concentration of harmful substances in the indoor environment, minimizing health risks associated with prolonged exposure. Adequate airflow not only enhances the comfort of the kitchen but also contributes significantly to creating a healthier living space by addressing indoor air pollution concerns (Figure 1B).

Incorporation of air pollutants due to indoor life style habit: Indoor air Pollution results due to worship sticks, mosquito coils, and room fresheners of the use of common household items such as worship sticks, mosquito coils, and room fresheners can contribute to indoor air pollution. Burning worship sticks releases particulate matter (PM) and volatile organic compounds (VOCs), potentially affecting respiratory health. Mosquito coils, while effective in repelling insects, emit PM and may contain harmful compounds. Room fresheners, often containing VOCs, can compromise indoor air quality and trigger respiratory irritation. Among LPG users only Insense stick usage is 83 % and followed by mosquito coil and room freshener usage 51.35% and 5.41% respectively (Figure 1C). However in case of Non–LPG users the usage of Insense stick, mosquito coil, population is 100%, 88.93%, respectively (Figure 1D). None from Non –LPG users use room freshener as a source or fragments at their home. As per study data it was evident the worship sticks, mosquito coils used irrespective of socio economic status. However usage tendency of mosquito coil is bit higher in non-LPG users. As most of the non-LPG users belong to lower earning group their home location is more infested with mosquito due to lack of civic senses. The same economic status actually restricts them regarding room freshener use which helps them to keep away from this indoor pollution.

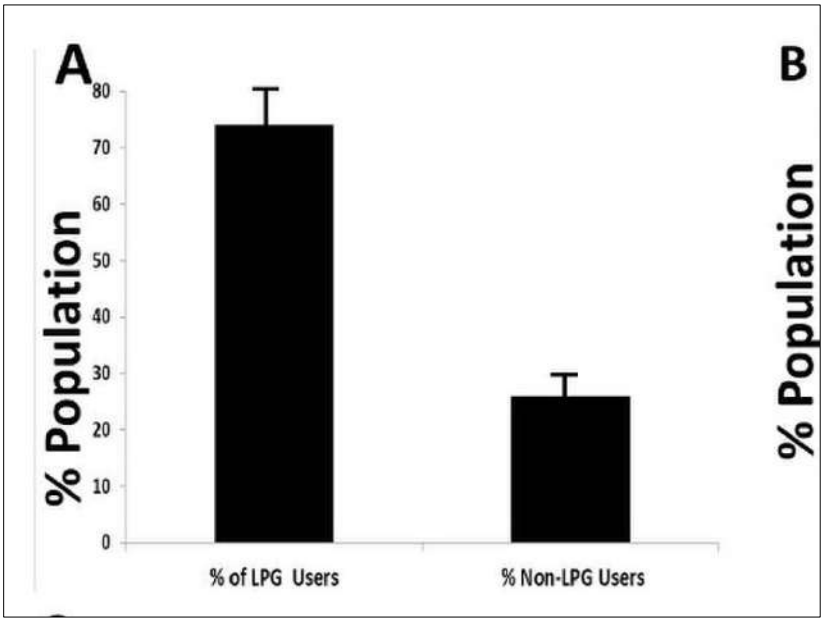


**Fig 1:** Status of different Air pollution source for indoor air pollution. A % of LPG user (Clean cooking fuel) vs. % of Non-LPG (polluted cooking fuel) user, B. Ventilation

status at kitchen area, C. Lifestyle induced indoor air pollution source and their usage of LPG user, D. Lifestyle induced indoor air pollution source and their usage of non-LPG user.

## Conclusion

The indoor air pollution study underscores the multifaceted nature of this issue and its potential implications for human health. Identifying specific sources and understanding the role of ventilation provides actionable insights for mitigating indoor air pollution. Implementing strategies to promote cleaner cooking practices and enhance ventilation systems can significantly improve indoor air quality, reducing associated health risks. The study emphasizes the need for targeted interventions, awareness campaigns, and policy measures to address indoor air pollution comprehensively and enhance the overall well-being of indoor occupants.

Conflict of interest: Authors declare no conflict of interest.

Funding: The work is funded by Swami Vivekananda University, Kolkata, India and The Climate Thinker, Kolkata, India

# Chapter - 10

## Precision Therapy: CRISPR-Cas9 Gene Editing in Sickle Cell Disease


**Authors**

**Santanu Biswas**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Semanti Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Bidisha Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Subhasis Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Suranjana Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 10

## Precision Therapy: CRISPR-Cas9 Gene Editing in Sickle Cell Disease

**Santanu Biswas, Semanti Ghosh, Bidisha Ghosh, Subhasis Sarkar and Suranjana Sarkar**

### Abstract

Sickle cell disease (SCD) presents significant health challenges globally, necessitating the exploration of more effective treatments. CRISPR-Cas9 gene editing technology offers a promising avenue for targeted therapy. This abstract provides an overview of current research on CRISPR-Cas9-based approaches for curing SCD. By precisely targeting the genetic mutation responsible for abnormal hemoglobin production, CRISPR-Cas9 holds the potential to rectify the underlying defect. Preclinical studies utilizing animal models have shown promising results, demonstrating successful correction of the sickle cell mutation and subsequent restoration of hemoglobin function, leading to symptom alleviation. Early results from clinical trials also suggest the safety and feasibility of CRISPR-Cas9-based therapies in human subjects. Nevertheless, challenges such as off-target effects and delivery methods warrant further investigation. Nonetheless, CRISPR-Cas9 gene editing emerges as a transformative approach for SCD treatment, offering hope for a definitive cure and improved quality of life for affected individuals. Continued research and development in this field are essential for maximizing the potential of CRISPR-Cas9 in addressing SCD.

**Keywords:** CRISPR-Cas9, gene editing, hemoglobin, sickle cell disease.

### Introduction

Precision therapy has revolutionized the field of medicine by providing targeted and personalized treatments for a range of genetic disorders. One of the most significant advancements in this area is CRISPR-Cas9 gene editing, a technology that allows for the precise correction of genetic mutations. A particularly promising use of CRISPR-Cas9 is in the treatment of sickle cell disease (SCD), a genetic blood disorder characterized by the production of anomalous hemoglobin. This condition is caused by a single point mutation in the beta-globin gene, which leads to the formation of rigid, sickle-shaped red

blood cells that can obstruct blood flow and result in serious health complications. Traditional treatments for SCD have mainly focused on symptom management and bone marrow transplants, which are not universally available or effective. The introduction of CRISPR-Cas9 presents a new approach by allowing precise correction of the defective gene, potentially offering a permanent cure for those affected by this debilitating disease. This introduction highlights the significance of CRISPR-Cas9 in the treatment of SCD, emphasizing its potential to revolutionize patient outcomes through precise genetic alterations (Elendu *et al*., 2023).

**Sickle cell disease progression**

Sickle cell anemia is a genetic abnormally resulting from a mutation in the HBB gene, encoding the beta-globin subunit of the hemoglobin molecule. This mutation produces an irregular structured hemoglobin which is responsible for the disorder, called hemoglobin S (HbS). The pathophysiology of sickle cell anemia is complex, involving several key mechanisms that lead to the clinical manifestations of the disease, explained below (Kavanagh *et al*., 2022; Inusa *et al*., 2019).

**Genetic Mutation and Hemoglobin S (HbS) formation**

Mutation leading to sickle cell anemia is the substitution of single nucleotide (adenine to thymine) in the 6$^{th}$ codon of the beta-globin gene, causing to the substitution of Glutamic acid to Valine. This seemingly small change significantly alters the properties of the hemoglobin molecule. Under normal oxygenated conditions, HbS functions similarly to normal hemoglobin (HbA). However, when oxygen levels drop, HbS molecules tend to polymerize, forming long, rigid chains. This polymerization process distorts the shape of red blood cells, transforming them from flexible, biconcave discs into rigid, sickle-shaped cells (Kirkham *et al*., 2023).

**Polymerization and red blood cell deformation**

The polymerization of HbS is the root cause in the progression of sickle cell anemia. The sickling of red blood cells occurs when these HbS polymers form, causing the cells to become rigid and lose their normal elasticity. Unlike normal red blood cells, which can easily navigate through the microvasculature, these stiff, sickle-shaped cells can become trapped in small blood vessels.

**Vascular occlusion and ischemia**

Blood vessels obstruction by the irregularly shaped RBCs leads to wide range of complications. Vascular occlusion reduces blood flow, causing

ischemia and tissue hypoxia. The reduced blood flow can lead to intense pain episodes known as sickle cell crises or vaso-occlusive crises, which are distinctive features of sickle cell anemia. These painful events can affect multiple parts of the body, including bones, lungs, spleen, liver, and brain.

**Hemolysis and Anemia**

Chronic hemolytic anemia is the prime indicative signature associated with Sickle cell anemia. The RBCs with a rigid and unusual shape have a significantly reduced lifespan, often living only 10-20 days compared to the normal 120 days for healthy red blood cells. This rapid turnover leads to a constant state of anemia as the bone marrow cannot compensate by producing new red blood cells quickly enough. The breakdown of these sickled cells releases free hemoglobin into the bloodstream, which can bind to and deplete nitric oxide, a molecule that helps maintain the dilation of blood vessels. This depletion further contributes to vascular complications by promoting vasoconstriction and endothelial dysfunction.

**Inflammation and immune response**

Persistent hemolysis and repetitive vaso-occlusive crises instigate inflammatory responses within the system. This stimulation triggers the endothelial cells lining the blood vessels to generate adhesion molecules, facilitating the binding of sickled cells to the vessel walls. Consequently, this exacerbates the process of vessel occlusion, leading to further hindrance in blood circulation. The persistent inflammatory state can lead to organ damage over time.

**Organ damage and complications**

The recurrent episodes of ischemia and the constant hemolysis cause cumulative damage to various organs. The spleen is often one of the first organs affected, leading to functional asplenia and increased susceptibility to infections. The kidneys, liver, lungs, heart, and brain are also at risk of damage due to the ongoing ischemia and oxidative stress. Pulmonary complications, such as acute chest syndrome, are particularly severe and can be life-threatening (Hardouin *et al*., 2023).

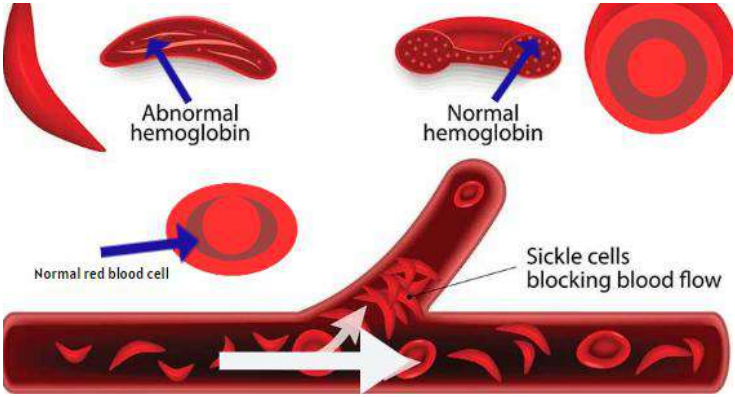


**Fig 1:** Sickle cell anemia progression

**Traditional SCD treatment**

Sickle cell disease (SCD) management requires a comprehensive treatment approach. Traditional therapies are centered around alleviating symptoms, preventing complications, and improving patients' quality of life. Key elements of traditional SCD treatment include (Tisdale *et al*., 2020; Abdel-Hadi *et al*., 2023):

**Pain management**

Painful episodes, known as sickle cell crises or vaso-occlusive crises, are a hallmark of SCD. These occur when sickled red blood cells obstruct blood flow, leading to ischemia and severe pain. Strategies for pain management include:

**Analgesics:** Nonsteroidal anti-inflammatory drugs (NSAIDs) are used for mild to moderate pain, while opioids such as morphine or hydromorphone may be necessary for severe pain.

**Hydration:** Maintaining adequate hydration helps reduce the frequency of pain crises by decreasing blood viscosity.

**Heat therapy:** Applying heat to painful areas can provide relief.

**Infection prevention**

Individuals with SCD, particularly children, are at higher risk for infections due to functional asplenia (impaired spleen function). Preventive measures include:

**Vaccinations:** Patients should receive all standard childhood immunizations, as well as additional vaccines such as pneumococcal, meningococcal, and influenza vaccines.

**Antibiotics:** Prophylactic penicillin is often prescribed for young children until at least five years of age to prevent pneumococcal infections.

**Blood transfusions**

Regular blood transfusions aid to decrease the threat of stroke and other complications in patients with severe SCD. Transfusion increases the count of normal RBCs, thus reducing the proportion of sickled cells. However, chronic transfusion therapy can lead to iron overload in the body, requiring chelation therapy to expel additional iron.

**Hydroxyurea therapy**

Hydroxyurea is a disease-modifying drug that has become a cornerstone in SCD management. It functions by:

- **Increasing Fetal Hemoglobin (HbF):** Hydroxyurea stimulates the production of fetal hemoglobin, reducing the polymerization of sickle hemoglobin (HbS) and decreasing the frequency of pain crises.
- **Reducing Complications:** Clinical studies have shown that hydroxyurea can reduce the incidence of acute chest syndrome, transfusions, and hospitalizations.

**Managing acute chest syndrome**

Acute chest syndrome presents as a critical complication marked by chest discomfort, elevated body temperature, and pulmonary infiltrates. Management includes:

**Antibiotics:** Broad-spectrum antibiotics are used to treat potential infections.

**Oxygen therapy:** Supplemental oxygen is administered to maintain adequate oxygenation.

**Bronchodilators:** These can be used to open airways and improve breathing.

**Blood transfusions:** Exchange transfusions may be necessary in severe cases to reduce the concentration of sickled cells.

**Iron chelation therapy**

For patients undergoing regular blood transfusions, iron overload is a significant risk. Iron chelation therapy involves using medications such as deferoxamine, deferasirox, or deferiprone to bind excess iron and facilitate its excretion.

**Bone marrow transplantation**

This treatment option removes the patient's defective bone marrow and replaces it with a compatible and healthy one from a donor. However it is only suitable for a limited number of patients due to the risks involved and the need for a closely matched donor.

**Precision therapy**

Precision therapy for sickle cell anemia marks a significant departure in the management of this genetic condition. Utilizing advancements in gene editing technologies like CRISPR/Cas9, precision therapy seeks to rectify the underlying genetic mutation responsible for the disorder. Sickle cell anemia arises from HBB gene alteration in specific location, culminating in the production of oddly shaped hemoglobin (HbS) carrying RBCs. This anomalous hemoglobin prompts red blood cells to adopt a sickle shape, precipitating vaso-occlusive crises, anemia, and organ impairment. Precision therapy entails targeted modifications to the patient's hematopoietic stem cells (HSCs), the precursors to all blood cells. Employing CRISPR/Cas9, scientists can accurately edit the HBB gene, rectifying the mutation and reinstating normal hemoglobin synthesis. This therapeutic approach holds promise as a potential curative treatment for SCD, addressing the root cause of the ailment at the genetic level. The procedure commences with the isolation of the patient's HSCs, which are then subjected to precise modifications using CRISPR/Cas9 to rectify the alteration in the HBB gene. Subsequently, these modified cells are reintroduced into the patient's body, where they can generate healthy red blood cells with normal hemoglobin. This method offers the prospect of enduring relief from sickle cell anemia symptoms and a markedly enhanced quality of life for affected individuals (El *et al*., 2022).

**CRISPR treatment**

The incorporation of CRISPR-Cas9 gene editing technology represents a significant advancement in the treatment of sickle cell disease (SCD), a hereditary condition characterized by the presence of abnormal hemoglobin (HbS). This innovative approach aims to correct the genetic mutation underlying SCD, offering a promising path towards a lasting remedy through precise genomic modifications. CRISPR-Cas9 functions as a highly accurate gene-editing system, comprising the Cas9 endonuclease and a guide RNA (gRNA). The gRNA is precisely designed to target a specific DNA sequence, guiding the Cas9 enzyme to induce a precise double-strand break at the intended site. Following the break, the cell's intrinsic DNA repair mechanisms can be utilized to introduce the desired genetic correction or alteration (Frangoul *et al*., 2021).

**Targeting the genetic basis of SCD**

The CRISPR-Cas9 treatment is utilized in two primary ways to address this genetic defect:

**Direct gene correction:** This approach involves the HBB gene correction in hematopoietic stem cells (HSCs) to retract the sickle cell mutation. By precisely altering the mutated nucleotide, normal HbA production can be restored, thereby reducing the prevalence of sickled erythrocytes.

**Induction of Fetal Hemoglobin (HbF):** An alternative strategy focuses on reactivating the expression of HbF, which is not prone to sickling. This can be achieved by disrupting the regulatory elements that repress HbF production postnatally. Elevated HbF can restructure the symptoms of SCD by compensating for the defective HbS.

**Mechanism of CRISPR/CAS9 treatment**

**Cell culture**

Human hematopoietic stem and progenitor cells (HSPCs) expressing the CD34 marker are sourced from mobilized peripheral blood, bone marrow, or umbilical cord blood. Predominantly, peripheral blood-derived CD34+ HSPCs have been utilized in genome editing studies. Before gene editing commences, CD34+ cells are cultured in pre-stimulation media supplemented with cytokines to enhance their efficiency. Subsequently, the corrected CD34+ cells are cultured in a medium conducive to erythroid differentiation for further analysis.

**Gene editing and reagent delivery**

The application of CRISPR/Cas9 derived from Streptococcus pyogenes (Spy Cas9) has been extensively employed in scientific investigations. This particular system relies on a protospacer adjacent motif (PAM) sequence of 5′-NGG-3′ for target recognition. Over time, significant advancements have been achieved in enhancing the safety and efficacy of gene editing in hematopoietic stem and progenitor cells (HSPCs) using CRISPR/Cas9 technology. Initial experiments utilizing plasmid DNA-based systems resulted in adverse outcomes such as toxicity and suboptimal editing efficiency. However, the introduction of pre-complexed ribonucleoproteins (RNPs) consisting of gRNA and Cas9 has reduced toxicity and improved editing specificity. Electroporation via nucleofection is a common method for delivering RNPs into HSPCs due to its efficiency in delivering the components into the cell nucleus.

Chemical alterations to guide RNAs (gRNAs) have played a crucial role in enhancing genome editing efficiency while simultaneously addressing concerns related to toxicity. Additionally, the creation of high-fidelity Cas9 variants has been pivotal in reducing off-target effects without compromising on-target activity. Concurrently, endeavors have been made to optimize methodologies aimed at augmenting the ratio of homology-directed repair (HDR) to non-homologous end joining (NHEJ), thus facilitating more efficient correction of disease-associated mutations. Commercially available high-fidelity Cas9 protein and chemically modified synthetic gRNAs have facilitated the optimization of gene editing parameters for CD34+ cells. In therapeutic applications, gene editing can correct disease-causing mutations by introducing CRISPR gRNA/Cas9 RNP complexes along with a DNA donor template into HSPCs isolated from patients with sickle cell disease (SCD). This correction is achieved through HDR, offering a potential treatment strategy for SCD (Park *et al*., 2021). Mechanism of the treatment has been explained in fig 2 below.

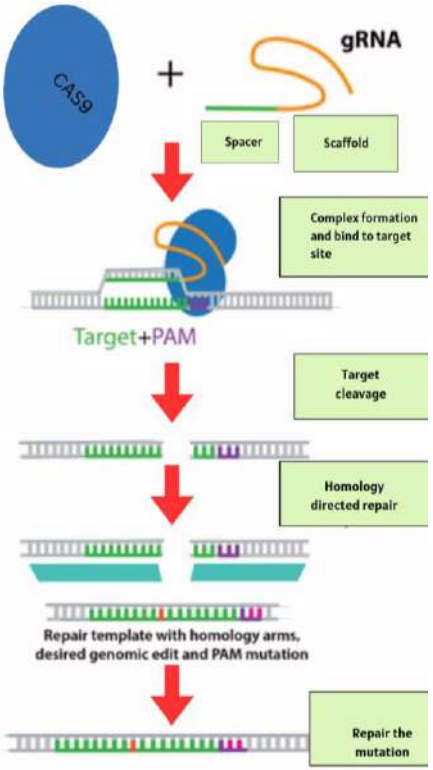


**Fig 2:** Mechanism of CRISPR/cas9 SCD Treatment

**Designing a flowchart for CRISPR-Cas9 Treatment**

1. Hematopoietic Stem Cell (HSC) Extraction: Procurement of HSCs from sources like peripheral blood, bone marrow, or cord blood.
2. Preparation of CRISPR Components:
    - Assembly of essential CRISPR-Cas9 elements:
    - Cas9 enzyme
    - Guide RNA (gRNA) for targeting the mutated gene linked to sickle cell disease
    - Donor DNA template to facilitate homology-directed repair (HDR)
3. HSC Culturing and Stimulation:
    - HSCs are cultured in a controlled laboratory environment.
    - Pre-stimulation with cytokines may be performed to augment gene editing efficacy.
4. CRISPR Component Delivery: Introduction of CRISPR components into cultured HSCs via techniques like electroporation or viral vectors.
5. Gene Editing Phase:
    - Binding of Cas9 enzyme to the target DNA sequence under the guidance of gRNA.
    - Initiating a double-strand break (DSB) precisely at the predetermined location within the mutated gene.
6. Homology-Directed Repair (HDR):
    - Utilization of the donor DNA template to promote HDR, enabling precise correction of the mutant gene.
    - Integrating the DNA from donor into the genome at the DSB site, substituting the mutant sequence with the corrected one.
7. Cell Expansion and Selection:
    - Expansion of edited HSCs in culture to amplify cell numbers.
    - Identification and isolation of cells harboring the corrected gene using genetic markers or other selection methods.
8. Quality Control and Characterization:

- Evaluation of edited HSCs for parameters such as:
- Efficiency of gene correction
- Presence of off-target effects
- Viability and purity

9. Transplantation of Edited HSCs:
    - Infusion of corrected HSCs back into the patient's body.
    - Migration of HSCs to the bone marrow and differentiation into mature blood cells, including red blood cells with normal hemoglobin.

**Future perspectives**

The future of sickle cell disease (SCD) treatment holds considerable promise, driven by ongoing advancements in genome editing technology. A key focus lies in enhancing the efficiency and precision of genome editing methods, particularly in hematopoietic stem and progenitor cells (HSPCs), which are pivotal targets for therapeutic intervention. Researchers are exploring innovative delivery approaches, such as nanoparticle-based systems, to optimize the transport of CRISPR/Cas9 components into target cells, thereby improving editing efficacy. Furthermore, future strategies may extend beyond merely correcting the underlying genetic mutation to addressing disease modifiers or pathways implicated in SCD pathophysiology, potentially offering avenues for alleviating disease severity and complications. Advancements in *in vivo* gene editing techniques hold promise for directly correcting mutations within the body, potentially transforming treatment paradigms. Combinatorial therapies, which integrate gene editing with other modalities like gene therapy or pharmacological agents, may emerge as synergistic approaches for tackling various aspects of SCD. Moreover, the concept of personalized medicine is expected to play a pivotal role, tailoring treatment strategies based on individual patient characteristics and genetic profiles. As these innovations progress, it will be crucial to translate promising preclinical findings into clinical applications through rigorous evaluation in clinical trials to ensure safety, efficacy, and long-term outcomes. Additionally, ensuring equitable access to emerging gene editing therapies globally will be essential, necessitating the resolution of logistical challenges and the establishment of regulatory frameworks to facilitate widespread adoption and accessibility.

## Conclusion

**S**ickle cell disease (SCD) presents a multifaceted genetic anomaly accompanied by notable clinical complexities. However, recent advancements in gene editing technologies show promise in revolutionizing treatment outcomes. The emergence of CRISPR/Cas9 genetic correction has fundamentally transformed the landscape of SCD treatment, enabling precise modifications to the genetic anomalies underlying the condition. Through targeted interventions on hematopoietic stem and progenitor cells (HSPCs), researchers have successfully confirmed the feasibility of rectifying the disease-causing mutation, thereby restoring normal hemoglobin functionality in preclinical studies. Nonetheless, despite these encouraging findings, numerous hurdles need to be addressed before gene editing therapies can be widely adopted in clinical trials. Challenges include refining delivery techniques, ensuring the safety and efficacy of treatments, addressing potential off-target effects, and navigating regulatory requirements. Moreover, the development of personalized treatment strategies tailored to individual patient characteristics holds immense potential for enhancing therapeutic outcomes. Despite these obstacles, the future outlook for SCD treatment appears promising, with ongoing research endeavors poised to translate innovative gene editing technologies into transformative therapies that offer renewed hope for individuals afflicted with SCD. Achieving this vision will necessitate collaborative efforts among scientists, healthcare professionals, policymakers, and patient advocates to ensure equitable access to these groundbreaking treatments for all affected individuals.

## References


1. Elendu, C., Amaechi, D. C., Alakwe-Ojimba, C. E., Elendu, T. C., Elendu, R. C., Ayabazu, C. P., Aina, T. O., Aborisade, O., & Adenikinju, J. S. (2023). Understanding Sickle cell disease: Causes, symptoms, and treatment options. *Medicine*, *102*(38), e35237.
2. Inusa, B. P. D., Hsu, L. L., Kohli, N., Patel, A., Ominu-Evbota, K., Anie, K. A., & Atoyebi, W. (2019). Sickle Cell Disease-Genetics, Pathophysiology, Clinical Presentation and Treatment. *International journal of neonatal screening*, *5*(2), 20.
3. Kavanagh, P. L., Fasipe, T. A., & Wun, T. (2022). Sickle Cell Disease: A Review. *JAMA*, *328*(1), 57–68.
4. Kirkham, J. K., Estepp, J. H., Weiss, M. J., & Rashkin, S. R. (2023). Genetic Variation and Sickle Cell Disease Severity: A Systematic Review and Meta-Analysis. *JAMA network open*, *6*(10), e2337484.

5. Hardouin, G., Magrin, E., Corsia, A., Cavazzana, M., Miccio, A., & Semeraro, M. (2023). Sickle Cell Disease: From Genetics to Curative Approaches. *Annual review of genomics and human genetics*, *24*, 255–275.

6. Tisdale, J. F., Thein, S. L., & Eaton, W. A. (2020). Treating sickle cell anemia. *Science (New York, N.Y.)*, *367*(6483), 1198–1199.

7. Abdel-Hadi, L., Ventura Carmenate, Y., Castillo-Aleman, Y. M., Sheikh, S., Zakaria, A., & Phillips, J. (2023). Treatment of sickle cell disease - options and perspective. *American journal of blood research*, *13*(2), 61–70.

8. El Hoss, S., El Nemer, W., & Rees, D. C. (2022). Precision Medicine and Sickle Cell Disease. *HemaSphere*, *6*(9), e762.

9. Frangoul, H., Altshuler, D., Cappellini, M. D., Chen, Y. S., Domm, J., Eustace, B. K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., Ho, T. W., Kattamis, A., Kernytsky, A., Lekstrom-Himes, J., Li, A. M., Locatelli, F., Mapara, M. Y., de Montalembert, M., Rondelli, D., Sharma, A., … Corbacioglu, S. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. *The New England journal of medicine*, *384*(3), 252–260.

10. Park, S. H., & Bao, G. (2021). CRISPR/Cas9 gene editing for curing sickle cell disease. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*, *60*(1), 103060.

# Chapter - 11

## Unveiling the Therapeutic Potential of *Mycobacterium vaccae* for Mental Health


**Authors**

**Bidisha Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Ankit Pal**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Semanti Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Subhasis Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Suranjana Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 11

## Unveiling the Therapeutic Potential of *Mycobacterium vaccae* for Mental Health

**Bidisha Ghosh, Ankit Pal, Semanti Ghosh, Subhasis Sarkar and Suranjana Sarkar**

**Abstract**

Recent studies increasingly emphasize its ability to positively impact mood, anxiety, and depression symptoms. Mechanistically, *M. vaccae* triggers serotonin release, a pivotal neurotransmitter essential for mood regulation and emotional well-being. Furthermore, its immunomodulatory properties exhibit anti-inflammatory effects, potentially mitigating stress-related disorders. Animal studies further highlight its capacity to enhance cognitive function and bolster stress resilience. Encouragingly, ongoing human trials indicate promising outcomes in leveraging *M. vaccae* as a therapeutic intervention for mental health conditions. While further exploration is warranted to elucidate precise mechanisms and optimal dosing protocols, existing evidence underscores *M. vaccae's* potential as an adjunctive therapy for mental health enhancement. This review underscores the growing interest in *M. vaccae* and its promising implications for mental health interventions, emphasizing the need for continued research to fully harness its therapeutic benefits.

**Keywords:** Immunomodulation, *Mycobacterium vaccae*, mental health, therapeutic potential.

### Introduction

In recent times, mental health has become a pressing concern worldwide. Mental health encompasses an individual's emotional, psychological, and overall well-being, playing a crucial role in maintaining a high quality of life, fostering healthy relationships, and contributing positively to society. However, the prevalence of mental health issues poses a significant burden on modern society, impacting both personal and professional spheres. Disorders related to mental health, like depression, anxiety, schizophrenia, among others, negatively influence individuals across a wide spectrum and can lead to severe consequences if left untreated. According to a study conducted in 2017 by Sasi and Ref, 2023, approximately 197.3 million people in India,

constituting 14.6% of the population, were affected by mental disorders. Additionally, research conducted in 2022 revealed a suicide rate of 11.6 per 100,000 individuals in India. Globally, mental illness accounts for approximately 14.3% of all deaths, amounting to 8 million fatalities annually. These statistics underscore the urgent need for increased awareness, support, and resources to address mental health issues and alleviate their societal impact.

**Mental disorders**

In recent times, mental disorders have become increasingly prevalent across all age groups, with the onset of such conditions occurring at an average age of 14.5 years (Solmi *et al*., 2022). Psychiatric disorders associated with stress often exhibit modified metabolic function and persistent low-grade inflammation (Loupy *et al*., 2021). Stress-related psychiatric disorders, including major depressive disorder and anxiety disorders, are commonly linked to psychosocial stress as a significant risk factor (Fan *et al*., 2015). Furthermore, anxiety disorders, affective disorders, and conditions related to trauma and stress, such as posttraumatic stress disorder (PTSD), are common within stress-related mental health conditions. In addition to the aforementioned disorders, several others warrant consideration:

**Bipolar disorder:** Abnormal mood fluctuations, energy, and movement rates are characteristics of this disorder. Individuals often struggle to focus on tasks and are at an increased risk of suicide.

**Eating disorders:** Conditions like anorexia nervosa and bulimia nervosa involve abnormal eating patterns, preoccupation with food, and profound concerns regarding body image and weight. Typically emerging during adolescence, these disorders lead to various health complications and may precipitate suicidal ideation.

**Disruptive behavior and dissocial disorders:** Often termed conduct disorder, this condition typically manifests in childhood and is characterized by persistent behavioral issues such as defiance, violation of others' rights, and disregard for rules and regulations.

**Neurodevelopmental disorders:** This category encompasses conditions that affect an individual's ability to acquire and perform specific intellectual, motor, language, or social tasks like attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and intellectual developmental disorders.

The "Old Friends" hypothesis, proposed by Graham Rook in 2003, posits

that urbanization diminishes exposure to immunoregulatory microorganisms, thereby disrupting the balance between regulatory and effector T cells. This hypothesis suggests that reduced microbial exposure in modern city environments may elevate stress levels and promote maladaptive behavioral traits by impeding immunoregulation (Hassell *et al*., 2023; Holbrook *et al*., 2023; Loupy *et al*., 2021).

**Current treatment methods**

Various approaches are utilized to manage different mental disorders. Psychotherapy involves engaging in therapeutic discussions with a trained therapist to comprehend and address symptoms effectively. Psychiatrists may prescribe psychiatric medications such as mood stabilizers, anxiolytics, antidepressants, and antipsychotics to alleviate symptoms associated with various mental disorders. Incorporating lifestyle modifications, such as engaging in regular physical activity, maintaining a well-balanced diet, ensuring adequate rest, refraining from substance abuse, and practicing stress-relief techniques like mindfulness or meditation, play an integral role in promoting mental wellness. Social involvement through peer support groups or maintaining connections with friends and family provides valuable coping mechanisms and emotional support. Hospitalization may be necessary in severe cases to ensure appropriate care and stabilization. When conventional treatments fall short, alternative therapies such as electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS), and vagus nerve stimulation (VNS) are explored. Tailored to the unique requirements of each individual, these approaches form part of holistic treatment regimens aimed at tackling the distinct complexities associated with mental health conditions.

**Challenges with traditional treatments**

Despite improvements in mental health care, there are still a number of obstacles to overcome:

Access to mental health care presents numerous challenges for individuals, encompassing obstacles such as high costs, prolonged wait times for appointments, and a scarcity of mental health specialists, particularly in rural regions. Furthermore, the stigma surrounding mental illness may deter individuals from seeking help or adhering to treatment plans, resulting in untreated or poorly managed disorders. Additionally, not all patients respond uniformly to treatment, necessitating a trial-and-error approach to identify the most effective counseling, medication, or other interventions. The presence of comorbidity, where individuals with mental health issues also contend with physical health conditions, emphasizes the necessity of integrated treatment

strategies. Nevertheless, limitations in resources frequently impede access to appropriate assistance and treatment options for those in need. Despite advancements in teletherapy and digital mental health technologies, concerns persist regarding data security, privacy, and disparities in technology access.

**Alternative treatment using *Mycobacterium vaccae***

*Mycobacterium vaccae* is a naturally occurring non-pathogenic bacterium found in soil, belonging to the Mycobacteriaceae family. Discovered by Dr. John L. Stanford and Roger C. Paul in the Kyoga Nile valley near Lake Kyoga in Uganda, this bacterium has garnered interest in various fields of research, including immunotherapy for conditions such as allergic asthma, depression, dermatitis, eczema, psoriasis, leprosy, and cancer (Graham and Stanford, 1988). Interestingly, *Mycobacterium vaccae* shares its genus with Mycobacterium tuberculosis, the causative agent of tuberculosis. Researchers have observed that certain strains of *Mycobacterium vaccae* possess an outer layer that inhibits the synthesis of Th-1 cytokines, key components in certain types of T-helper cell immune responses (Rodríguez-Güell *et al*., 2006).

A particular strain of Mycobacterium vaccae, identified as NCTC 11659, displays saprophytic environmental traits alongside properties that regulate the immune system and alleviate inflammation. Researchers have found the potential of *Mycobacterium vaccae* NCTC 11659 as an immunoregulatory therapy where inflammation is a prominent factor. Studies have indicated encouraging outcomes, demonstrating the strain's ability to decrease inflammation and bolster resilience to stress (Holbrook *et al*., 2023).

**Mechanism of treatment**

When conventional treatments fall short, alternative therapies such as electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS), and vagus nerve stimulation (VNS) are explored. Tailored to the unique requirements of each individual, these approaches form part of holistic treatment regimens aimed at tackling the distinct complexities associated with mental health conditions (Zuany-Amorim, *et al*., 2002; Hunt, *et al*., 2005).

Experimental studies suggest that M. vaccae NCTC 11659 may induce an anti-inflammatory or immunoregulatory response under hyper-inflammatory conditions induced by factors such as allergic airway inflammation, severe trauma, stress, or disorders like post-traumatic stress disorder (PTSD) (R. M. Voigt, *et al*., 2022).

Pre-exposure to heat-killed *M. vaccae* NCTC 11659 attenuated inflammation triggered by a lipopolysaccharide (LPS) challenge. This

pretreatment reduced proinflammatory cytokine mRNA levels and increased anti-inflammatory to proinflammatory cytokine mRNA ratios (A. Viola, *et al*., 2019). Human monocyte-derived macrophages, when exposed to *M. vaccae* NCTC 11659 before encountering high levels of LPS, showed reduced expression of IL12A, IL12B, and IL23A, while exhibiting increased TGFB1 mRNA expression. This shift from a proinflammatory to an anti-inflammatory phenotype was particularly evident at higher LPS concentrations (Holbrook, E. M., *et al*., 2023). These findings suggest that *M. vaccae* NCTC 11659-stimulated macrophages may alter tissue T-cell responses locally, leading to immunoregulation at inflammation spot. Consequently, *M. vaccae* NCTC 11659 could potentially serve as an intervention to mitigate stress-induced inflammation and neuroinflammation, associated with the development and progression of inflammatory diseases and stress-related psychiatric disorders (Holbrook, E. M., *et al*., 2023).

**Current status**

Presently, investigations into the therapeutic applications of *Mycobacterium vaccae* for mental health are actively progressing and showing encouraging outcomes. Research endeavors have delved into the impacts of *Mycobacterium vaccae* on diverse facets of mental well-being, encompassing stress resilience, mood modulation, and neuroinflammatory processes. Evidence suggests that exposure to *Mycobacterium vaccae* may lead to reductions in stress-induced inflammation and enhancements in stress resilience. Through its ability to modulate the immune system and stimulate anti-inflammatory responses, *Mycobacterium vaccae* holds potential for mitigating symptoms associated with stress-related psychiatric disorders like depression, anxiety, and post-traumatic stress disorder (PTSD).

Moreover, preclinical investigations have indicated that *Mycobacterium vaccae* could exert antidepressant-like effects by influencing neurotransmitter pathways implicated in mood regulation. These findings have spurred interest in exploring *Mycobacterium vaccae* as a novel therapeutic avenue for mental health conditions. Ongoing clinical trials assessing the utility of *Mycobacterium vaccae* as a treatment for mental health disorders will provide valuable insights into its potential benefits and limitations. In summary, while the current status of research on *Mycobacterium vaccae* for mental health is promising, additional evidence is necessary before its widespread adoption as a treatment modality.

**Future prospect**

The future of mental health treatment holds significant promise with the

emergence of innovative technologies across various domains. These advancements encompass a spectrum of approaches, including digital therapeutics, artificial intelligence (AI) and machine learning, virtual reality (VR) therapy, neurofeedback and brain stimulation, genomics and personalized medicine, telepsychiatry and teletherapy, biometric monitoring, and precision psychiatry. Digital therapeutics, such as smartphone applications and wearable devices, deliver personalized interventions and real-time support for patients. AI and machine learning analyze data to tailor treatment plans and forecast outcomes. VR therapy provides immersive environments for exposure therapy and skills training. Non-invasive techniques like neurofeedback and transcranial magnetic stimulation target brain activity to alleviate symptoms. Genomics facilitates personalized treatments based on genetic profiles, while telepsychiatry extends access to care through remote consultations. Wearable devices monitor physiological markers to provide insights into mental health status, and precision psychiatry customizes interventions to individual characteristics for optimal outcomes. These innovations are poised to revolutionize mental healthcare delivery, offering personalized, accessible, and effective treatments for individuals with mental health disorders.

**Conclusion**

In summary, the rapid progression of technology presents promising opportunities for transforming mental health treatment. These advancements span a wide array of innovations, including digital therapeutics, AI-driven interventions, personalized genomics, and telepsychiatry, among others. These breakthroughs offer potential solutions to longstanding challenges in mental healthcare delivery, such as access, effectiveness, and personalization. By harnessing these cutting-edge technologies, we have the potential to enhance care delivery, improve treatment outcomes, and empower individuals to manage their mental well-being more effectively. However, it is essential to ensure that these innovations are developed ethically, rigorously tested, and made accessible to all who need them. Moving forward, collaborative efforts among researchers, clinicians, policymakers, and technology developers will be crucial in fully realizing the benefits of these advancements and building a more equitable and inclusive mental healthcare system for the future. Preliminary mouse studies indicate that this bacterium may have antidepressant-like effects by promoting the synthesis of serotonin, a neurotransmitter involved in mood regulation. Furthermore, in tests on animals, exposure to *Mycobacterium vaccae* has been associated with decreased anxiety and enhanced cognitive performance. It is essential to

remember that, even though these results are encouraging, additional research work is still required to fully understand the mechanisms and potential therapeutic role of *Mycobacterium vaccae* in the treatment of mental disorders.

**References**


1. Fan, L. B., Blumenthal, J. A., Watkins, L. L., and Sherwood, A. (2015). Work and home stress: associations with anxiety and depression symptoms. Occup. Med. 65, 110–116.
2. Hassell, J. E., Baratta, M. V., Fallon, I. P., Siebler, P. H., Karns, B. L., Nguyen, K. T., Gates, C. A., Fonken, L. K., Frank, M. G., Maier, S. F., & Lowry, C. A. (2023). Immunization with a heat-killed preparation of *Mycobacterium vaccae* NCTC 11659 enhances auditory-cued fear extinction in a stress-dependent manner. *Brain, behavior, and immunity*, *107*, 1–15.
3. Holbrook, E. M., Zambrano, C. A., Wright, C. T. O., Dubé, E. M., Stewart, J. R., Sanders, W. J., Frank, M. G., MacDonald, A. S., Reber, S. O., & Lowry, C. A. (2023). *Mycobacterium vaccae* NCTC 11659, a Soil-Derived Bacterium with Stress Resilience Properties, Modulates the Proinflammatory Effects of LPS in Macrophages. *International journal of molecular sciences*, *24*(6), 5176.
4. Hunt, J.R.; Martinelli, R.; Adams, V.C.; Rook, G.A.; Brunet, L.R. Intragastric administration of *Mycobacterium vaccae* inhibits severe pulmonary allergic inflammation in a mouse model. Clin. Exp. Allergy 2005, 35, 685–690.
5. Loupy, K. M., Cler, K. E., Marquart, B. M., Yifru, T. W., D'Angelo, H. M., Arnold, M. R., Elsayed, A. I., Gebert, M. J., Fierer, N., Fonken, L. K., Frank, M. G., Zambrano, C. A., Maier, S. F., & Lowry, C. A. (2021). Comparing the effects of two different strains of mycobacteria, *Mycobacterium vaccae* NCTC 11659 and M. vaccae ATCC 15483, on stress-resilient behaviors and lipid-immune signaling in rats. Brain, behavior, and immunity, 91, 212–229.
6. Rodríguez-Güell, Elisabeth; Agustí, Gemma; Corominas, Mercè; Cardona, Pere-Joan; Casals, Isidre; Parella, Teodor; Sempere, Marco-Antonio; Luquin, Marina; Julián, Esther (2 May 2006). "The production of a new extracellular putative long-chain saturated polyester by smooth variants of *Mycobacterium vaccae* interferes with Th1-cytokine production". *Antonie van Leeuwenhoek*. 90 (1): 93–108.

7. Rook, Graham A. W. & Stanford, John L., "Immuno-therapeutic composition of killed cells from mycobacterium vaccae", issued February 9, 1988.

8. Sasi, N. and Rej, S. (2023). World mental health day 2023: increasing awareness of mental health in India & exciting opportunities for the future. Indian Journal of Medical Research, 158(4), 334.

9. Solmi, M., Radua, J., Olivola, M. *et al*. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. Mol Psychiatry 27, 281–295 (2022).

10. Viola, A.; Munari, F.; Sánchez-Rodríguez, R.; Scolaro, T.; Castegna, A. The metabolic signature of macrophage responses. Front. Immunol. 2019, 10, 1462.

11. Voigt, R.M.; Zalta, A.K.; Raeisi, S.; Zhang, L.; Brown, J.M.; Forsyth, C.B.; Boley, R.A.; Held, P.; Pollack, M.H.; Keshavarzian, A. Abnormal intestinal milieu in post-traumatic stress disorder is not impacted by treatment that improves symptoms. Am. J. Physiol. Gastrointest. Liver Physiol. 2022, 323, G61–G70

12. Zuany-Amorim, C.; Sawicka, E.; Manlius, C.; Le Moine, A.; Brunet, L.R.; Kemeny, D.M.; Bowen, G.; Rook, G.; Walker, C. Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells. Nat. Med. 2002, 8, 625–629.

# Chapter - 12

## Biofertilizer and their Importance in Sustainable Agriculture

**Authors**

**Sayani Das**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Bidisha Ghosh**
Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal

**Suranjana Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

**Subhasis Sarkar**
Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, West Bengal, India

# Chapter - 12

## Biofertilizer and their Importance in Sustainable Agriculture

**Sayani Das, Bidisha Ghosh, Suranjana Sarkar and Subhasis Sarkar**

**Abstract**

Biofertilizers are substances that are enriched with bacteria that aid in the growth of trees and plants by providing them with more vital nutrients. It is made up of living things. Microorganisms found in biofertilizer help the host plants receive an appropriate amount of nutrients, support healthy development, and maintain physiological balance. To prepare biofertilizers, living microorganisms are employed. Only those microbes that specifically aid in plant growth and reproduction are employed. Different kinds of microorganisms are employed in the production of biofertilizers. In addition to increasing soil fertility, biofertilizers have the ability to maximize resource efficiency and boost crop productivity in a sustainable manner. They boost soil fertility and sustainability over time by fixing atmospheric N2, mobilizing fixed macro and micronutrients, or converting insoluble P in the soil into forms that plants can use. These days, liquid biofertilizers are demonstrating excellent results in a wide range of crops, surpassing the traditional limitations associated with biofertilizer application. Certain types of microorganisms are used in conjunction with biofertilizers to improve the micronutrient's effectiveness of usage and to aid in the decomposition of organic residue. After all, we require an affordable and adaptable solution that may lessen the degradation of soil caused by chemical application, or what is known as an environmentally friendly solution.

**Keywords:** Biofertilizers, micro-organisms, soil fertility, plant growth, sustainable agriculture.

### Introduction

Since the middle of the 20th century, chemical fertilizers the primary product for supplementing soil nutrients with nutrients have been extensively utilized in agricultural production [Cleland, 2013; Godfray *et al*., 2010]. Food production can be swiftly and effectively increased by chemical fertilizers

[Brown, 1981; Velimirovic *et al*., 2021]. However, the rate of reaction is steadily declining when these fertilizers are added at an increasing pace [Chen *et al*., 2021; Tilman *et al*., 2002]. Furthermore, chemical fertilization leads to a host of other environmental and health issues, including radioactive and heavy metal-contaminated food and soil [Cheraghi *et al*., 2013; Latifi and Jalali, 2018; Savci, 2012a]; air pollution from NO, $N_2O$, $NO_2$, and other gasses; and nitrate-induced groundwater pollution [Goss *et al*., 1998; Rivers *et al*., 2012; *et al*., 2012; Zhao *et al*., 2019], as well as modifications to the pH and composition of the soil [Savci, 2012b; Xie *et al*., 2019].Sustainable agriculture is supported by environmentally friendly biofertilizers, which are a good substitute for chemical fertilizers [Olanrewaju *et al*., 2017]. A substance that contains living microorganisms that, when applied to soil, plant surfaces, or seeds, colonizes the rhizosphere or the interior of the plant and increases the supply or availability of primary nutrients to the host plant is known as biofertilizer [Vessey, 2003]. Plant-growth-promoting microbes (PGPM), plant-growth-promoting bacteria (PGPB), or plant-growth promoting rhizobacteria (PGPR) are the common names for these microorganisms. "Nitragin," a preparation comprising nitrogen-fixing rhizobium strains, marked the beginning of the commercial history of biofertilizers in 1895 [Soumare *et al*., 2020]. Phosphorus-dissolving bacteria first appeared in the 1950s. To serve as biofertilizers, transforming soil phosphorus into a form that plants can absorb [Wang *et al*., 2019]. According to reports up to this point, a variety of bacteria, fungi, and algae have the ability to promote plant growth through a variety of processes [Azizoglu, 2019; Barin *et al*., 2022; Gezgin *et al*., 2020; Ismail *et al*., 2021; Naeem *et al*., 2021; Sun *et al*., 2020]

Crop yields can be efficiently increased and chemical fertilizer use can be decreased in agriculture by applying biofertilizers. According to studies, biofertilizers can replace roughly 25%–30% of chemical fertilizers and boost crop yields by 10%–40% when used in conjunction with them [Pal *et al*., 2015]. According to research conducted by the Brazilian Agricultural Research Corporation, using a study by the Brazilian Agricultural Research Corporation showed that the use of biofertilizers for biological nitrogen fixation in soybean cultivation can completely replace chemical nitrogen fertilizers without reducing yields.According to a Brazilian Agricultural Research Corporation study, chemical nitrogen fertilizers can be totally replaced with biofertilizers for biological nitrogen fixation in soybean farming without lowering yields.

Chemical fertilizers, the principal product for adding nutrients to soil,

have been widely used in agricultural production since the mid-1900s [Cleland, 2013; Godfray *et al*., 2010]. Chemical fertilizers have the ability to quickly and efficiently improve food production [Brown, 1981; Velimirovic *et al*., 2021]. However, when these fertilizers are added at a faster rate, the rate of reaction is gradually decreasing [Chen *et al*., 2021; Tilman *et al*., 2002]. Chemical fertilization also causes radioactive and heavy metal-contaminated food and soil, among a host of other environmental and health problems [Cheraghi *et al*., 018; Savci, 2012]; nitrate-induced groundwater pollution [Goss *et al*., 1998; Rivers *et al*., 2012;] and air pollution from NO, $N_2O$, $NO_2$, and other gases.

**Potential Significance of beneficial microbes in sustainable agriculture**

**Some of the important bio fertilizer are mentioned below**

**Rhizobium**

It is the symbiotic bacteria that, through root nodules, fix atmospheric $N_2$ gas in plants and form beneficial relationships with their host plants. The bacteria receive newly produced compounds and necessary minerals from the roots of the plant. Rhizobium is said to be able to repair 50–300 kg N/ha. When paired with certain other legume species, a strain of Rhizobia that nodulates and fixes a lot of nitrogen in conjunction with one legume species may also do the same. Testing is necessary to confirm that. Leguminous plants that exhibit a comparable response to specific strains of Rhizobia are classified as belonging to the "effectiveness" group. (Wani and Lee, 2002).

**Azotobacter**

It is a free-living, non-symbiotic nitrogen-fixing bacteria that also produces chemicals that are purportedly beneficial to plant growth and antibodies that inhibit a variety of root diseases. is a member of the aerobic, heterotrophic Azotobacteriaceae family. Alkaline or neutral soils include azotobacter, with A. chroococcum being the most often found species in arable soils. Additional species that have been reported include *A. macrocytogenes*, *A. beijerinckii*, *A. insignis*, and *A. vinelandii*. The absence of organic matter and the existence of hostile microorganisms in the soil cause the amount of Azotobacter to seldom exceed 104 to 105 g-1 of soil. The bacterium generates antifungal antibiotics that stop a number of dangerous fungus from growing in the root area, preventing the death of seedlings.

**Plant growth promoting rhizobacteria (PGPR)**

A diverse group of soil bacteria that stimulate the growth of their host plant when they are grown together. Fixing N2, boosting nutrient availability

in the rhizosphere, favorably affecting root growth and shape, and encouraging more advantageous plant-microbe symbioses are examples of PGPR modes. According to several researches, PGPR frequently has several different ways of acting. When growing the aromatic grass palmarosa (*Cymbopogon martinii*) with an insoluble inorganic phosphate, [Ratti *et al*. 2001] discovered that a combination of the arbuscular mycorrhizal fungi *Glomus aggregatum*, the PGPR *Bacillus polymyxa*, and *Azospirillum brasilense* maximized biomass and P content.

**Cyanobacteria**

A class of aquatic creatures with one to many cells is known as cyanobacteria, and they are both free-living and symbiotic (blue green algae). They only grow rice and are located in damp, marshy environments. They can be red, purple, or brown in color and cannot withstand acidic environments. An essential supply for the marine ecosystem is provided by the activity of nitrogen-fixing organisms (Gonzalez *et al*. 2005). Cyanobacteria have the capacity to fix nitrogen and can withstand harsh environments. In addition, they enrich the soil with organic matter, release nutrients and auxin that stimulate development, release insoluble phosphate, and enhance the chemical and physical characteristics of the soil.

**Zinc solubilizers**

Phosphate solubilizing bacteria such as *B. magaterium, P. striata*, and phosphate mobilizing Mycorrhiza, as well as nitrogen fixers such as Rhizobium, Azospirillum, Azotobacter, and BGA, are generally acknowledged as bio-fertilizers [Subba Roa, 2001]. Though these only provide major nutrients, soil contains a variety of microorganisms that can transform micronutrients, such as zinc, iron, copper, and so on. These microorganisms can be used as biofertilizers to supply micronutrients like zinc, which is particularly important and is found in the earth's crust in amounts as low as 0.008%, but more than 50% of Indian soils show deficiencies in zinc, with content falling below the critical level of 1.5 ppm of available zinc [Katyal and Rattan, 1993]. Plants are able to overcome their limitations in taking zinc from the soil.

**Significance use of soil microbes in sustainable crop production**

Potential use of soil microbes in sustainable crop production. The beneficial soil micro-organisms sustain crop production either as biofertilizers [Singh *et al*. 1977] or symbiont [Goss *et al*.1998]. They perform nutrient solubilisation which facilitate nutrient availability and thereby uptake [Rivers *et al*. 2012]. It improves the plant growth by advancing the root architecture.

Their activity provides several useful traits to plants such as increased root hairs, nodules and nitrate reductase activity and [Cheraghi *et al*., 2013]. Efficient strains of Azotobacter, Azospirillum, Phosphobacter and Rhizobacter can provide significant amount of available nitrogen through nitrogen cycling [Zhoa *et al*., 2019]. The biofertilizers produced plant hormones, which include indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). Biofertilizers improve photosynthesis performance to confer plant tolerance to stress and increase resistance to pathogens [Goss *et al*. 1998] thereby resulting in crop improvement.

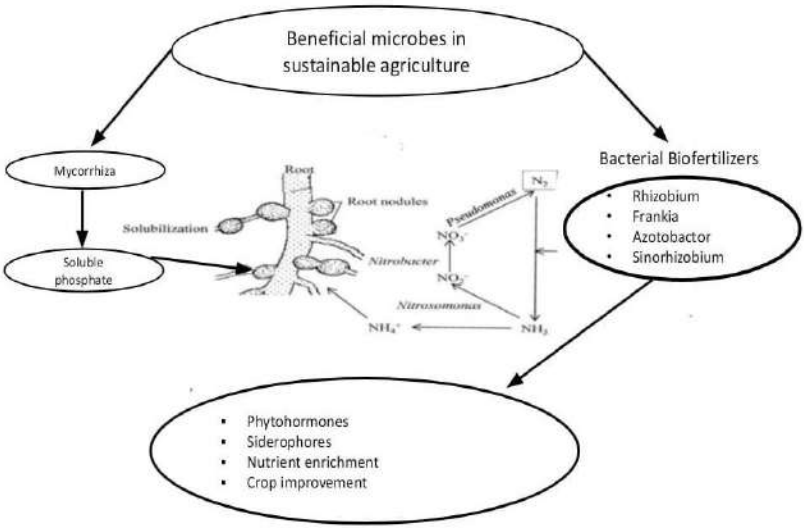


**Fig 1:** From: Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity

Possible application of soil microbes to environmentally friendly crop production. Crop production is supported by the helpful soil microorganisms acting as symbionts [Rana *et al*. 2013] or biofertilizers [Moghimi *et al*, 1978]. They solubilize nutrients, facilitating their availability and subsequent uptake [Wani & Lee *et al*. 2002]. By expanding the root architecture, it enhances plant growth [Ghosh *et al*. 2004]. Increased root hairs, nodules, and nitrate reductase activity are just a few beneficial characteristics that plants might benefit from their activity [Rana *et al*. 2013]. Through nitrogen cycling, effective strains of Azotobacter, Azospirillum, Phosphobacter, and Rhizobacter can contribute a sizable quantity of available nitrogen [Nyekha *et al*. 2015].

**Future perspectives of biofertilizers**

The most crucial and particular study requirements ought to emphasize the following:

Choosing multifunctional biofertilizers that are competitive and efficient for a range of crops. A quality assurance system for the inoculant production process and its use in their application and explore the benefits of plant micro organisms symbiosis. Research on the microbiological survival of biofertilizers in stressed soil environments 4. Agronomic, soil, and financial assessment of biofertilizers for various farming systems. 5. Bringing scientific expertise for the best formulation and industrial biofertilizer production to bear. 6. The "Bio-fertilizer Act" was established, and stringent guidelines for quality assurance in markets and applications were implemented. Use of biofertilizer is restricted (i) Constraints at the production level: absence of acceptable carriers, absence of effective strains, and mutation during fermentation. (ii) Market-level restrictions: insufficient and unskilled labor, lack of quality control, inconsistent and seasonal demand, and ignorance of farmers. (iii) Resource limitation: insufficient resources can be generated to produce biofertilizer. Limitations at the field level: Soil Climatic factors and native microbial population.

## Conclusion

Microorganism-plant interaction is essential. In the upcoming years, biofertilizer will be crucial to enhancing crop availability and nutrient supplies. They are inexpensive, non-bulky, environmentally acceptable agricultural inputs. A biofertilizer is an organic product made from the soil in the root zone (rhizosphere) or from the roots of plants that contains a concentrated version of a particular microorganism. *Acetobacter, Azospirillum*, and *Azotobacter* are the biofertilizers that are crucial for nitrogen. *Bacillus sp*., *Aspergillius sp*., Phosphate solubilization and additional nutrient-rich soil minerals. The goal will be aided by the present trend of sustainable agricultural systems with minimal chemical inputs. As a result, the use of inorganic chemical fertilizers was drastically cut, down to 30–50%. This aids in the realization environmental friendly and sustainable agriculture.

## References


1. Ghosh N. Promoting Bio-Fertilizers in Indian Agriculture, Research Project conducted at the Institute of Economic Growth for Ministry of Agriculture, 2004.
2. Ghosh N. Promoting Bio-Fertilizers in Indian Agriculture, Research Project conducted at the Institute of Economic Growth for Ministry of Agriculture, 2004.
3. Gonzalez LJB, Rodelas C, Pozo V, Salmeron MV, Mart Nez, Salmeron V. Liberation of amino acids by heterotrophic nitrogen fixing bacteria. Amino Acids. 2005; 28:363-367.

4. Gonzalez LJB, Rodelas C, Pozo V, Salmeron MV, Mart Nez, Salmeron V. Liberation of amino acids by heterotrophic nitrogen fixing bacteria. Amino Acids. 2005; 28:363-367. 6. Katyal JC, Rattan RK. Distribution of zinc in Indian soils. Fertilizer News. 1993; 38(6):15-26.
5. Katyal JC, Rattan RK. Distribution of zinc in Indian soils. Fertilizer News. 1993; 38(6):15-26.
6. Moghimi A, Tate ME, Oades JM. Characterization of rhizospheric products especially 2- Ketogluconic acid. Soil Biology and Biochemistry. 1978; 10:283-287.
7. Moghimi A, Tate ME, Oades JM. Characterization of rhizospheric products especially 2- Ketogluconic acid. Soil Biology and Biochemistry. 1978; 10:283-287.
8. Nyekha Nusakho, Sharma YK, Sharma SK, Gupta RC. Influence of phosphorus and PSB on performance of green gram and soil properties. Annals of Plant and Soil Research. 2015; 15(3):323-325.
9. Nyekha Nusakho, Sharma YK, Sharma SK, Gupta RC. Influence of phosphorus and PSB on performance of green gram and soil properties. Annals of Plant and Soil Research. 2015; 15(3):323-325
10. Ratti N, Kumar S, Verma HN, Gautams SP. Improvement in bioavailability of tricalcium phosphate to Cymbopogon martini var. motia by rhizobacteria, AMF and azospirillum inoculation. Microbiology Research. 2001; 156:145-149.
11. Ratti N, Kumar S, Verma HN, Gautams SP. Improvement in bioavailability of tricalcium phosphate to Cymbopogon martini var. motia by rhizobacteria, AMF and azospirillum inoculation. Microbiology Research. 2001; 156:145-149.
12. Singh PK. Multiplication and utilization of fern Asolla containing nitrogen-fixing algal symbiont as green manure in rice cultivation. Riso. 1977; 46:642-644.
13. Subba Roa, NS. An appraisal of biofertilizers in India. The biotechnology of biofertilizers, (ed.) S. Kannaiyan, Narosa Pub. House, New Delhi (in press), 2001.
14. Wani SP, Lee KK. Population dynamics of nitrogen fixing bacteria associated with pearl millet (*P. americanum* L.). In biotechnology of nitrogen fixation in the tropics. University of Pertanian, Malaysia, 2002,