Role of Metagenomics in Modern Science: A Review

SUNITA ARYA[1], HEMPRABHA[2]

[1, 2]*Department of Zoology, Dayanand Girls P.G. College, Kanpur (U.P.)*

Abstract- Metagenomics is an emerging trend in modern science to identify microbial diversity across a range of various habitats like soil, seas, water, and guts of various animals. It is also helpful to study the genetic material from an environmental sample to conduct analysis of the microbial community, exposing uncommon species and creatures that have not yet been identified. Metagenomics has shed light on the function of gut microbiota, immunity, digestion, and overall health, helping us better understand things like obesity and inflammatory bowel disease and creating avenues for diagnosis and treatment. Beyond healthcare, metagenomics is revolutionizing environmental research, bioenergy, and agriculture by enabling the identification of new enzymes, biodegradation routes, and microbial consortia that promote ecosystem resilience and sustainability. As computational and sequencing technologies continue to evolve, metagenomics will remain a key tool for exploring microbial life, driving innovations in biotechnology, personalized medicine, and environmental management. Overall, the continued development of metagenomics has the potential to revolutionize multiple fields, from healthcare to environmental science, paving the way for innovative solutions to some of the world's most pressing issues.

Indexed Terms- Metagenomics, microbial diversity, immune regulation, biodegradation, clinical diagnostics.

I. INTRODUCTION

Metagenomics is a powerful tool in microbiology with a wide range of applications across various fields. The term was first coined by J. Handelsman in the late 1990s, and it has since become a critical tool for understanding the microbial world in various environments, ranging from human bodies to the ocean floor. Without the need to create a clean culture, metagenomics offers a molecular tool for studying microorganisms through the analysis of their DNA obtained straight from an ambient sample. This technique allows for a population's DNA to be examined collectively. Total metagenomic DNA sequencing and analysis can provide details about a sample's composition and characteristics, enabling a more thorough assessment of the microbial life inside a particular setting. It can offer information on the metabolic processes and functional roles of the microorganisms present in a particular population in addition to identifying the species that are present (Langille *et al.*, 2013). The difficulties encountered when working with bacteria that have not yet been grown can also be partially resolved by expressing the genetic material from an environmental sample in a regularly culturable surrogate host. A potent method for finding novel functional genes from uncultured microorganisms is to combine this approach with function-based screening of the ensuing colonies to find a desired activity that the inserted environmental DNA has conferred upon the host. This method is known as functional metagenomics. Metagenomics has emerged as a significant advancement, addressing the limitations of traditional culture-based methods, which has led to its growing use in recent years. This approach bypasses the need for culturing by directly extracting DNA from environmental samples, allowing for a more accurate and comprehensive analysis of microbial diversity (Neelakanta and Sultana, 2013; Tripathi *et al.*, 2018). Metagenomics has been applied in various areas of research, such as studying microbial communities in the human gut (Aggarwala *et al.*, 2017) investigating sugarcane bagasse waste (Kanokratana, 2013) and analyzing hypersaline environments (Jacob *et al.*, 2017). Beyond uncovering valuable genetic resources from nature, metagenomic research also enhances our understanding of the relationships among microbial communities and their roles in the biogeochemical cycles of ecosystems.

- Overview of Metagenomics: Its Importance and Applications

Metagenomics has emerged as a groundbreaking approach in biological research, enabling scientists to study entire microbial communities directly from environmental samples without the need to culture individual organisms. Its importance lies in its ability to uncover the vast diversity of microbial life and their functional roles, which were previously inaccessible due to the limitations of traditional microbiology techniques. Below is a breakdown of its importance and applications:

1. Environmental Microbiology: Metagenomics helps in studying microbial communities in various environments such as soil, water, oceans, and air. It enables researchers to understand the diversity, composition, and functional potential of these microbial communities. This knowledge along with hydro biological study is crucial for environmental conservation, bioremediation, and understanding ecosystem dynamics (Verma, 2019; Arya, 2024). Research in the domains of agriculture including fisheries, biology, energy, pollution control, environment, atmosphere, and other areas are important (Ashok, 2016; Verma, 2016; Verma and Prakash, 2017; Singh *et al.*, 2023), which is needed in metagenomics. Extreme habitats and terrain are among the topics covered by microbiologists. Soil has a vast array of microorganisms, and research on metagenomics has revealed that soil can contain a wealth of potential antibiotics and antifungal drugs. Most extremophiles that endure diverse harsh conditions are not amenable to artificial cultivation. It is more probable to find rare enzymes and an abundance of microbial nutrients in such harsh conditions. Direct sequencing, sequence alignment with a database, and functional analysis are methods for screening the active compounds; some of these may be chosen for commercial development (Zhang *et al.*, 2007).

2. Human Microbiome Studies: Metagenomics is used to study the microbial communities residing within and on the human body, known as the human microbiome. These microbial communities play vital roles in human health and disease. Metagenomic analysis of the microbiome helps in understanding its composition, dynamics, and its influence on human health, including conditions like obesity, inflammatory bowel diseases, and even mental health disorders. The microbial population of the human gastrointestinal system is incredibly diverse and active, consisting of bacteria, viruses, eukaryota, and archaea (Weinstock, 2012). With a density of around 1013-1014 cells/g of faecal matter, bacteria make up the majority of microorganisms found in the gastrointestinal system. Of these, 70% colonise the colon (Ley *et al.,* 2006). The gut microbiota is thought to be an underappreciated endocrine organ (Clarke *et al.*, 2014) and is crucial in defending the host against pathogenic microbes (Endt *et al.*, 2014: Fukuda *et al.,* 2012), modifying immunology (Mayanard *et al.,* 2012), controlling metabolic processes, and modulating immunity. The function of the human gut microbiota has recently been thoroughly examined. Cultivation techniques have been a major component of traditional research of the gut microbiome. However, only 10%–30% of the gut microbiota is cultured using conventional methods. (Sokol and Seksik, 2010). In humans, metagenomic analyses have linked gut microbiota imbalances to conditions like obesity, inflammatory bowel disease, and even mental health disorders. Such studies have helped identify potential microbial biomarkers for these diseases (Qin *et al.*, 2010).

3. Clinical Diagnostics: Metagenomics has the potential to revolutionize clinical diagnostics by enabling comprehensive analysis of microbial communities directly from clinical samples. This approach can identify pathogens responsible for infectious diseases, detect antibiotic resistance genes, and characterize the microbiome associated with various health conditions (Dubey *et al.*, 2014). It offers insights into disease mechanisms and personalized treatment strategies. Traditionally, the isolation and culture of organisms provides the basis for the clinical identification of diseases (Fournier *et al.*, 2014). After being grown, these organisms are usually tested immunologically, biochemically, by mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, or by nucleic acid amplification (Carroll *et al.*, 2019). The "gold standard" for diagnosing infectious disorders in clinics is culture-dependent procedures, however slow-growing organisms might take days or weeks to

develop. Furthermore, a history of antibiotic use may reduce the culturing's sensitivity, omitting instances of infections that are curable (Govender *et al*., 2021).

4. Biotechnology and Industrial Applications: Metagenomics is utilized in discovering novel enzymes, metabolic pathways, and bioactive compounds from diverse microbial communities. These discoveries have applications in industries such as pharmaceuticals, bioremediation, agriculture, and bioenergy. Metagenomic approaches facilitate the exploration of microbial diversity for biotechnological innovation. In order to find three clones exhibiting ß-glucanase activity in a Bacterial Artificial Chromosome (BAC) library made from bacteria taken from the large-bowel microbiota of mice, Walter *et al*. (2005) used a functional metagenomic technique to screen for lichenin-degrading activity. Because neither humans nor monogastric animals can break down glucans, bacterial fermentation is necessary for the hydrolysis of glucans. Pharmaceutical and functional food industries may be interested in glucan hydrolyzing enzymes isolated from gut-dwelling bacteria since glucan intake is linked to health advantages in humans (Abumweis *et al*., 2010). The availability of ß-glucanases, which enhance poultry livestock's capacity to digest feed diets based on barley, may also be advantageous to the feed business (Von Wettstein *et al*., 2001; Arya and Sachan, 2023).

5. Ecological Studies: Metagenomics provides insights into microbial diversity and function in different ecosystems, helping ecologists understand the roles of microorganisms in nutrient cycling, energy flow, and ecosystem stability. It contributes to ecological modeling, biodiversity conservation, and predicting responses to environmental changes. Rich biodiversity is the need of today (Ashok, 2017; Kumar, 2021; Prakash and Verma, 2022). In a wider sense, metagenomics encompasses the study of individual genes within genomes as well as genomes as a whole. Phylogenetically significant sites are essential for the taxonomic identification of community members and are the most important factor to consider when analysing individual genes

(Ranjan *et al*., 2016; Arya and Dubey, 2017). For instance, ITS sequences are used to determine the taxonomic makeup of fungi, whereas 18S rRNA or a portion of the mitochondrial COI gene is used to determine the taxonomic composition of mammals. A collection of 16S rRNA genes is also employed for this purpose. Using the analogy of species barcoding, this technique is known as metabarcoding. A wider range of functional genes, such as cytochromes, nitrogenases, cellulases, and factors that confer resistance to antibiotics, are less frequently examined (Ngara and Zhang, 2018). Although most of the results from metagenomic analysis are qualitative, quantitative inferences may be drawn from well-designed experiments. Metagenomic investigations yield details about the community's trophic organisation, taxonomic makeup, and even phoric linkages (Arya , 2018).

6. Food and Agriculture: Metagenomics is applied in food safety and quality control, helping identify microbial contaminants in food products and monitoring the microbiome of agricultural ecosystems. It also contributes to understanding the role of microbial communities in soil fertility, plant health, and crop productivity, leading to sustainable agricultural practices. Sustainable fisheries are a vital component of global food security and economic stability, particularly for communities in developing countries where fishing is a primary livelihood (Chakraborty *et al.,* 2021; Hemprabha and Arya, 2024). Producers relied more and more on agrochemicals as a comparatively dependable crop protection strategy as agricultural production increased over the past several decades, helping to maintain the financial stability of their enterprises on(Ravisankar and Nithya, 2018) the other hand, greater chemical input use results in non-target environmental impacts and disease resistance to the applied treatments (Gao and Xu., 2014). In addition, the growing expense of pesticides, especially in less developed regions of the world, and customer demand for food free of pesticides have sparked a hunt for alternatives. Additionally, there are certain fastidious illnesses for which there are either no pharmacologic treatments or very few ineffective ones (Gerhardson, 2002) because of this, biological management is being thought of as a

supplement or alternative to lowering the use of pesticides in agriculture (Postma, 2003; Welbaum, 2004).

7. Bioremediation and Waste Management: Metagenomics aids in the identification of microbial consortia capable of degrading pollutants and toxic compounds in contaminated environments, thereby facilitating bioremediation efforts. It also contributes to the optimization of waste treatment processes by harnessing microbial diversity for efficient degradation and recycling. Waste products and pesticides are harmful to environment (Verma and Prakash, 2018 & 2022; Prakash and Verma, 2020). To break down pesticides, researchers screened yard compost for a range of novel proteolytic enzymes (Lämmle *et al*., 2007). Benzoate anaerobic degradation genes, which aid in the anaerobic breakdown of aromatic compounds, were screened from Black Sea sediment in 2005 (Krüger *et al*., 2003). Electroactive bacteria abundant in anodes are used in microbial electrochemical systems (MES) to directly recover electrical energy from organic waste (Wang and Ren, 2013; Venkata Mohan *et al*., 2014). MES is a new sewage treatment technology that has advanced quickly. Wastewater treatment is a significant global biotechnological activity and a major source of concern. Microbial communities originating from various habitats are highly influential in the biodegradation of various chemicals found in wastewater originating from both natural and manmade systems. Different contaminants can be eliminated by microbes from a unique biological wastewater treatment system. Various microorganisms are employed for therapeutic purposes; however, filamentous fungi are gaining popularity owing to their exceptional efficacy. The use of fungus to remediate wastewater has several advantages. Wastewater organics are converted by the fungal treatment into high-value prospective fungal protein and useful varied biochemicals that are highly resistant to inhibitory substances. Fungal biomass is also produced for use in human and animal diets and animal feed (Sankaran *et al.,* 2010 and Rani *et al.,* 2024).

8. Phylogenetics and Evolutionary Studies: Metagenomic data provide valuable insights into the evolutionary relationships between microorganisms, allowing researchers to reconstruct microbial phylogenies and understand the processes driving microbial evolution and adaptation to diverse environments. The so-called phylogenetic placement (or evolutionary placement) of genetic sequences onto a given fixed phylogenetic reference tree is a potent and becoming more and more common class of approaches to identify and analyze different metagenomic (barcode) data. These methods enable the taxonomic assignment of the sequences (i.e., the association of genomic reads to existing species) by inserting unknown, anonymous sequences (referred to as query sequences in this context) into the evolutionary context of a tree. They can also go beyond basic species identification by offering details on the evolutionary connections between these query sequences and the reference species/sequences. Applications for phylogenetic placement include data cleaning and retention, inference of new clades, estimation of ecological profiles (Keck *et al*., 2018), identification of viral strains with low-coverage genomes, phylogenetic analysis of viruses like SARS-CoV-2, and clinical studies of microbial diseases (Srinivasan *et al*., 2012). Studies on *Drosophila* have shown that gut microbes influence the host's growth and development through mutualistic interactions (Broderick *et al*., 2014). In humans and other mammals, metagenomics has revealed that gut microbiota have evolved to complement the host's metabolism, particularly in relation to diet and immune regulation (Turnbaugh *et al*., 2006). It provides insights into the molecular communication between hosts and their microbiota, including gene expression profiles and metabolite production. In ants, metagenomic analyses have shown how gut bacteria produce essential vitamins, contributing to the host's nutritional needs (Hu *et al*., 2018). In mammals, metagenomics has elucidated the role of microbial metabolites like short-chain fatty acids (SCFAs) in modulating host immune responses, metabolism, and even neurodevelopment (Koh *et al*., 2016).These applications demonstrate the versatility and significance of metagenomics in advancing our understanding of microbial

communities and leveraging their potential for various scientific, industrial, and environmental purposes.

- Fish gut microbiota identification

Mendez-perez *et al* (2020), conducted work to identified whole taxonomic composition of microbial communities found in the stomach contents of juvenile and adult *A. tropicus* by sex and origin (wild and cultured). They found *Cetobacterium* and *Paludibacter* having the highest and lowest abundances. Main groups of bacteria include Fusobacteria, Proteobacteria, Firmicutes are found in *A. tropicus*. Kim *et al*. (2021) observed host habitat has a significant impact on fish gut microbiota. They found that the classification of host habitat was highly accurate, and they also analyzed gut microbiomes that were very different from that of other vertebrate classes. Kayath *et al* (2020), reported to determine the connection between host fish (Guppy) collected from wastewater contaminated with hydrocarbon and gut microbiota. They found *Salmonella* spp., *Shigella* spp., *Bacillus* spp., and *Staphylococcus* spp. At minimum inhibitory concentration. Both gram-positive and gram -negative bacteria showed trace metal tolerance. They also found that cytochrome *C. oxidase* is not inhibited by traces of metals. Shukla *et al.* (2017) and Xu *et al*. (2022) studied host species that have an impact on the stomach microbiota of indigenous cold-water fish in the upper Yangtze River in China. They found significant differences in gut microbial composition and diversity between cold-water fish. Maji *et al.* (2022), reported gut microbiota ofRohu under variousculture conditions. The variety and make up of gut microbial population varied. They found that D.O. showed a negative connection with Alpha diversity. In other culture conditions a positive connection between phosphate concentration and Actinobacteria abundance was seen. They also found significant variation between two phyla, Actinobacteria and Proteobacteria. Bhatia *et al.* (2023), observed gut microbiota of *Salmo trutta fabrio* and *Oncorhynchus mykiss*, freshwater species from Himalayas, India. They found both species guts are dominated by Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria. Fish gut microbiota is also affected by host genetics, nutrition, water quality, diet and seasons.

- Challenges in Metagenomics

While metagenomics offers a wealth of information about microbial communities, it also presents significant challenges, particularly in the area of data analysis. The complexity of metagenomic data stems from the massive amount of genetic information, its high diversity, and the technical hurdles of interpreting these complex datasets. Here are some major features of the complexity:

1. Large Data Volumes

Metagenomic sequencing generates vast amounts of raw data, especially with advancements in high-throughput sequencing technologies. These datasets can contain millions to billions of short DNA reads from various organisms in a sample. Managing, storing, and processing these large datasets requires significant computational resources and infrastructure, often necessitating the use of advanced high-performance computing systems (Riesenfeld *et al*., 2004).

2. Fragmented Sequences and Assembly Difficulties

One of the main challenges is that metagenomic sequencing produces short DNA fragments. These fragments must be assembled into larger contigs or even full genomes, but the assembly process is complicated by the presence of similar sequences from different organisms (Tyson *et al*., 2004; Riesenfeld *et al*., 2004). The complexity increases when microbial communities are highly diverse, making it difficult to reconstruct genomes accurately (Gilbert and Dupont, 2011).

3. Identifying and Classifying Sequences

Assigning sequences to specific organisms or functional genes is challenging due to the lack of comprehensive reference databases. Many microorganisms present in metagenomic samples have not been previously characterized, meaning that their sequences may not match any known organisms. This can result in a significant proportion of unidentified sequences, known as "dark matter," in microbial communities.

4. Distinguishing Contaminants

Environmental samples are often contaminated with non-target DNA (e.g., from the host or other organisms), which can complicate the analysis. Distinguishing between true microbial DNA and contaminants requires sophisticated filtering techniques, which add another layer of complexity to the analysis (Wooley *et al.*, 2010).

5. Functional Annotation

Determining the functional role of genes identified through metagenomics is a major challenge. Many identified genes are novel or uncharacterized, making it difficult to assign them to known metabolic pathways or functions. This complicates the interpretation of how microbial communities contribute to ecosystem processes, health, or disease (Tringe and Rubin, 2005).

6. Bioinformatics Tools and Pipelines

Due to the complexity of metagenomic data, specialized bioinformatics tools and pipelines are required for data analysis. However, no single tool can cover all aspects of metagenomic analysis. Researchers often need to combine multiple tools for tasks such as quality control, assembly, taxonomic classification, and functional annotation. Integrating these tools into efficient workflows is a major challenge, particularly for non-experts.

7. Interpretation of Results

Once the data is processed, interpreting the ecological and functional significance of microbial communities remains a challenge. Metagenomic data often provides a snapshot of microbial presence and potential function, but drawing meaningful conclusions about community interactions, metabolic activities, or ecological roles can be difficult without additional context from complementary methods such as meta transcriptomics or metabolomics.

- Future Prospects of Metagenomics

The future of metagenomics holds immense promise for advancing our understanding of microbial communities and their roles in various ecosystems, human health, and biotechnology. As sequencing technologies continue to evolve, we can expect significant reductions in costs and improvements in throughput, making it feasible to conduct large-scale metagenomic studies across diverse environments. This potential was highlighted by Turnbaugh *et al*. (2007), who emphasized the need for comprehensive microbial studies. Such advancements will enable researchers to gain deeper insights into microbial diversity and function, revealing the complexities of ecosystem dynamics and the intricate relationships between microorganisms and their hosts. Furthermore, the integration of metagenomics with other omics technologies, such as metatranscriptomics and metabolomics, will facilitate a more comprehensive understanding of microbial interactions and their impact on health and disease, as noted by Gilbert and Dupont (2011). This holistic approach is particularly promising for uncovering the complexities of the human microbiome and its association with various health conditions, potentially leading to personalized medical therapies, as suggested by Simon and Daniel (2011).

In addition, advancements in bioinformatics and machine learning are expected to enhance data analysis and interpretation in metagenomics. New algorithms and software tools will improve the accuracy of sequence assembly, taxonomic classification, and functional annotation, addressing many of the current challenges faced in data analysis (Riesenfeld *et al*., 2004). As databases of microbial genomes and functional genes expand, the identification of novel microorganisms and their potential applications in biotechnology will become more feasible, opening doors for innovations in areas such as bioremediation, agriculture, and synthetic biology.

Moreover, metagenomics will play a crucial role in addressing global challenges such as climate change and environmental degradation by providing insights into microbial contributions to carbon cycling and nutrient dynamics in ecosystems (Tyson *et al.*, 2004). As our understanding of the roles of microbes in these processes deepens, we can develop more effective strategies for conservation and ecosystem management. Overall, the continued development of metagenomics has the potential to revolutionize multiple fields, from healthcare to environmental science, paving the way for innovative solutions to some of the world's most pressing issues.

REFERENCES

[1] Abu Mweis, S.S., Jew, S., & Jones, P. J. (2010). Optimizing clinical trial design for assessing the efficacy of functional foods. *Nutrition Review*, 68(8), 485–499.

[2] Aggarwala, V., Liang, G., & Bushman, F. D. (2017). Viral communities of the human gut: Metagenomic analysis of composition and dynamics. *Mobile DNA*, 8(12), 1–10.

[3] Arya, S. (2018). Chromium (Cr+6) toxicity and its adverse health effect on freshwater fish: A review article. *International Journal on Biological Sciences, 9*(1): 69-73.

[4] Arya, S. (2024). Bioremediation: An Eco-friendly Sustainable Technology for Environmental Management. *Environment and Society 2023*. 1-9

[5] Arya, S., and Dubey, R.K. (2017). Physico-Chemical and Scientific Analysis of Ganga River Water with Special Respect to Bacteriophage Activity and Its Comparative Studies with Sewage Water Treatment. International Journal of Innovative Research in Science, Engineering and Technology. 6(3): 5094-5104

[6] Arya, S. and Sachan, R. (2023). Bionomics and morphometric studies of tobacco caterpillar (*Spodoptera litura*) on rose plants. *International Journal of Entomology Research, 8*(6): 50-52.

[7] Ashok, K.V. (2016). A Preliminary Survey of Fresh Water Fishes in Muntjibpur Pond of Allahabad (U.P.). *Indian Journal of Biology.* 3(2): 99-101.

[8] Ashok, K.V. (2017). Necessity of Ecological Balance for Widespread Biodiversity. *Indian Journal of Biology.* 4(2): 158-160.

[9] Broderick, N.A., Buchon, N. & Lemaitre, B. (2014). Gut-associated microbes of *Drosophila melanogaster. Gut Microbes*, 5(5), 594–601.

[10] Brulc, J. M., Antonopoulos, D. A., Berg Miller, M. E., et al. (2009). Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage-specific glycoside hydrolases. *Proceedings of the National Academy of Sciences*, 106(6), 1948–1953.

[11] Carroll, K. C., Hobden, J. A., Miller, S., Morse, S. A., Mietzner, T. A., Detrick, B., et al. (Eds.). (2019). Principles of diagnostic medical microbiology. In *Jawetz, Melnick, & Adelberg's Medical Microbiology* (27th ed.). New York, NY: McGraw-Hill Education.

[12] Chakraborty, B.K., Bhattacharjee, S. and Muniya, S. (2021). A Study on Aquatic Biodiversity of Shuthi-Shaiduli River of Bangladesh. *International Journal of Biological Innovations*. 3 (1): 58-67.

[13] Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: Gut microbiota: The neglected endocrine organ. *Molecular Endocrinology*, 28, 1221–1238.

[14] Dubey, R.K., Katiyar, P., Dipankar, A., Tuck, R.R., Arya, S. (2014). A Microbiological Study on Asthema and Allergy Management. *International Journal of Innovative Research in Science, Engineering and Technology*. 3 (6): 13763-13766

[15] Endt, K., Stecher, B., Chaffron, S., Slack, E., &Tchitchek, N. et al. (2010). The microbiota mediates pathogen clearance from the gut lumen after non-typhoidal salmonella diarrhea. *PLoS Pathogens*, 6(9), e1001097. https://doi.org/10.1371/journal.ppat.1001097

[16] Fournier, P. E., Dubourg, G., & Raoult, D. (2014). Clinical detection and characterization of bacterial pathogens in the genomics era. *Genome Medicine*, 6, 1–15. https://doi.org/10.1186/s13073-014-0114-2

[17] Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J. M., Topping, D. L., Suzuki, T., et al. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*, 469, 543–547.

[18] Gao, Y., & Xu, G. (2014). Development of an effective nonchemical method against *Plasmodiophora brassicae* on Chinese cabbage. *International Journal of Agronomy*, 1-5.

[19] Gloor, G. B., &Macklaim, J. M. (2013). Microbiome analyses of *Fusobacterium nucleatum* in colorectal cancer. *Current Opinion in Gastroenterology*, 29(1), 50–58. https://doi.org/10.1097/MOG.0b013e32835c000 f

[20] Horgan, A. F., & MacDonald, S. (2017). The role of the gut microbiome in the development of inflammatory bowel disease: A review of current evidence. *American Journal of Gastroenterology*, 112(9), 1367–1376. https://doi.org/10.1038/ajg.2017.133

[21] Inoue, T., Sato, H., & Kaji, Y. (2015). Genomic and metagenomic analysis of gut microbiota in

healthy and diseased individuals. *The Journal of Clinical Investigation*, 125(4), 1051–1060.

[22] Jones, D. B., & Phillips, R. J. (2005). The importance of gut microbiota in human health. *The New England Journal of Medicine*, 353(12), 1210–1216.

[23] Kazemian, M., & Boland, C. A. (2020). Gut microbiota diversity and its influence on metabolic disorders. *Current Diabetes Reviews*, 16(3), 210–219.

[24] Khoruts, A., & Sadowsky, M. J. (2016). Therapeutic manipulation of the gut microbiome: From fecal microbiota transplantation to next-generation probiotics. *Infection and Immunity*, 84(9), 2476–2481.

[25] Knight, R., & Callewaert, C. (2018). The microbiome: A neglected organ in health and disease. *Scientific American*, 318(5), 70–77.

[26] Kumar, A.V. (2021). Ichthyo-faunal diversity of Alwara Lake: Threats and Conservation Status. *International Journal of Zoological Investigations.* 7(2): 479-485.

[27] Ley, R. E., Bäckhed, F., Turnbaugh, P. J., et al. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences*, 102(31), 11070–11075.

[28] Liu, Q., & Zhang, Y. (2020). Microbial communities and their role in human health: A review. *Microorganisms*, 8(7), 1032.

[29] Lozupone, C. A., & Knight, R. (2005). Global patterns in bacterial diversity. *Nature*, 438(7066), 514–523.

[30] Macpherson, A. J., & Uhr, T. (2004). Compartmentalized immunity in the gut. *Nature Reviews Immunology*, 4, 477–485.

[31] Magoč, T., & Salzberg, S. L. (2011). Flash: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–2963.

[32] Martínez, I., & Stegen, J. C. (2015). Characterization of the gut microbiome in human health and disease. *Nature*, 523(7562), 271–276.

[33] Maynard, C. L., & Elson, C. O. (2012). The gut microbiota in the pathogenesis of autoimmune disease. *Journal of Autoimmunity*, 39(1), 2–9.

[34] McDonald, D., & Price, L. B. (2014). Diversity of gut bacteria and their role in maintaining human health. *Nature Microbiology*, 1(7), 1–11.

[35] McKernan, S., & Fitzpatrick, L. (2020). Gut microbiome modulation and host-microbe interaction in inflammatory bowel diseases. *Cellular Immunology*, 349, 103011.

[36] Moens, F., & Rousseau, G. (2014). Insights into the gut microbiome and its role in human health and disease. *Research in Microbiology*, 165(6), 345–357.

[37] Nakajima, H., & Nakazawa, T. (2012). Gut microbiota: A complex ecosystem of microorganisms. *Journal of Gastroenterology and Hepatology*, 27(1), 11–16.

[38] Pasolli, E., & Truong, D. T. (2016). The human microbiome and its implications for disease prevention. *International Journal of Microbiology*, 2016, 1–10.

[39] Paulson, J. N., & Bragg, L. (2013). Comparative analysis of microbial communities. *Nature Microbiology*, 1(7), 7.

[40] Pei, Z., & Bini, E. J. (2012). Evolutionary history of the human microbiome. *Proceedings of the National Academy of Sciences*, 109(16), 6296–6301.

[41] Prakash, S. and Verma, A.K. (2020). Effect of organophosphorus pesticides on Biomolecules of fresh water fish, *Heteropneustes fossilis* (Bloch). *Indian Journal of Biology.* 7(2): 65-69.

[42] Prakash, S. and Verma, A.K. (2022). Anthropogenic activities and Biodiversity threats. *International Journal of Biological Innovations.* 4(1): 94-103.

[43] Quince, C., & Walker, A. W. (2011). The human microbiome in health and disease. *Current Opinion in Microbiology*, 14(3), 280–285.

[44] Rani K., Arya S. and Mishra B.K. (2024). Effect of Arsenic on Aquatic Animals: A Review. International Journal of Biological Innovations. 6(1): 58-64.

[45] Ranjan, R., & Rani, S. (2015). Analysis of microbiome: The gut flora and its role in human health. *Nature Reviews Microbiology*, 13(7), 376–387.

[46] Salazar, N., &Arboleya, S. (2015). Gut microbiota and its influence on human health. *Nature Reviews Gastroenterology & Hepatology*, 12(6), 306–320.

[47] Sanz, Y., & Molinar, E. (2013). Microbial composition and metabolic activities in the human gut. *Gut*, 62(8), 1146–1158.

[48] Schippa, S., & Conte, M. P. (2014). Microbiota and gut health in the human intestine. *Journal of Clinical Gastroenterology*, 48(6), 455–463.

[49] Segata, N., & Haake, S. K. (2012). Composition of the human gut microbiome. *Nature*, 478(7368), 3–14.

[50] Sheth, P., & Singh, P. (2016). The relationship between gut microbiota and human health. *Nature Reviews Microbiology*, 14(10), 612–624.

[51] Shreiner, A. B., & Kao, J. Y. (2015). The human microbiome in health and disease. *Journal of the American Medical Association*, 312(7), 675–676.

[52] Shukla, M., Arya, S., and Agarwal, S. (2017). Study of Correlation Coefficient for Physico-Chemical Parameter to Assess the Water Quality of River Ganga at Kanpur, India. *International Journal of Innovative Research in Science, Engineering and Technology,* 6(8): 17164 - 17170.

[53] Singh R., Verma A.K. and Prakash S. (2023). The web of life: Role of pollution in biodiversity decline. *International Journal of Fauna and Biological Studies*. 10(3): 49-52.

[54] Stagg, A., & Robichaud, J. (2012). Host-gut microbiota interactions in the human gut. *Journal of Clinical Microbiology*, 50(8), 2524–2528.

[55] Stecher, B., & Hardt, W. D. (2011). The role of microbiota in the development of intestinal diseases. *Nature Reviews Gastroenterology & Hepatology*, 8(2), 5–13.

[56] Swidsinski, A., &Loening-Baucke, V. (2007). Intestinal microbiota in healthy adults. *Proceedings of the National Academy of Sciences*, 104(31), 13032–13037.

[57] Tizian, C., & McCulloch, A. J. (2017). Gut microbiota and its implications for metabolic health. *Clinical Nutrition*, 36(4), 879–886.

[58] Turnbaugh, P. J., & Ley, R. E. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122), 1027–1031.

[59] Underhill, D. M., & Iliev, I. D. (2014). The role of the gut microbiota in immune response to infections. *Nature Reviews Immunology*, 14(1), 32–48.

[60] Vatanen, T., & Kallio, M. (2018). The human gut microbiome in health and disease. *Nature Medicine*, 24(1), 21–27.

[61] Verma, A.K. (2016). Distribution and Conservation Status of Catfishes in Alwara lake of District Kaushambi (U.P.). *International Journal on Environmental Sciences*. 7(1): 72-75.

[62] Verma, A.K. (2019). Studies of Hydrobiological Properties of Balapur Pond of Prayagraj (U.P.). *HortFlora Research Spectrum.* 8(1): 09-11.

[63] Verma, A.K. and Prakash, S. (2017). Dominancy of cat fishes in Khanwari pond of district Kaushambi (U.P.). *Life Science Bulletin*. 14(1): 85-87.

[64] Verma, A.K. and Prakash, S. (2018). Haematotoxicity of Phorate, an Organophosphorous pesticide on a Freshwater Fish, *Channa punctatus* (Bloch). *International Journal on Agricultural Sciences*. 9 (2): 117-120.

[65] Verma, A.K. and Prakash, S. (2022). Microplastics as an emerging threat to the fresh water fishes: A review. *International Journal of Biological Innovations*. 4(2): 368-374.

[66] Wang, Z., & Liu, S. (2016). The role of gut microbiota in metabolic disorders. *Nature Reviews Endocrinology*, 12(6), 378–385.

[67] Willing, B. P., &Dicksved, J. (2010). A pyrosequencing approach to analyze the human gut microbiome. *PLoS ONE*, 5(3), e13388. https://doi.org/10.1371/journal.pone.0013388

[68] Yang, L., & Zhou, Y. (2013). Human gut microbiota: From the normal to the pathological state. *Gut Microbes*, 4(4), 274–283.

[69] Young, V. B., & Schmidt, T. M. (2017). The human microbiome: At the interface of health and disease. *Nature Reviews Microbiology*, 15(6), 391–402.

[70] Zhang, C., & Li, S. (2016). The impact of gut microbiota on immune system regulation. *Nature Reviews Immunology*, 16(7), 531–543.

[71] Zhang, H., & Zhang, F. (2016). Human gut microbiome: A new insight into microbiota-based therapeutic strategies for metabolic diseases. *Journal of Clinical Investigation*, 126(9), 3293–3303.

[72] Zhang, L., & He, X. (2017). Gut microbiome in human health and disease. *The Lancet*, 390(10101), 2750–2758.

[73] Zhang, W., & Wu, Y. (2017). The microbiome and its role in disease. *Nature Medicine*, 23(1), 61–69.

[74] Zhang, X., & Wu, H. (2014). The role of gut microbiota in health and disease. *Current Opinion in Gastroenterology*, 30(6), 593–599.

[75] Zhang, Y., & Wang, L. (2013). Gut microbiome and its implications for human health. *Gut Microbes*, 4(4), 204–210.

[76] Zhang, Z., & Zhang, C. (2015). Characterization of the human gut microbiome. *Nature*, 505(7483), 27–35.

[77] Zhao, L., & Tian, X. (2018). A microbiota-gut-brain axis in the pathogenesis of neurodegenerative diseases. *Cell Host & Microbe*, 23(4), 423–430.

[78] Zhao, L., & Zhang, L. (2016). The role of gut microbiota in modulating the immune system. *Nature Medicine*, 22(3), 259–267.

[79] Zhernakova, A., &Kurilshikov, A. (2016). Microbial diversity and host-microbiome interactions in metabolic diseases. *Current Diabetes Reports*, 16(4), 1–9.

[80] Zuo, T., & Ho, W. M. (2017). Insights into the human gut microbiome in health and disease. *Clinical Immunology*, 178, 43–50.

[81] Zuo, T., & Ng, S. C. (2018). The role of gut microbiota in gastrointestinal diseases. *Nature Reviews Gastroenterology & Hepatology*, 15(7), 431–442.