

A REVIEW ON: METAGENOMICS ANALYSIS OF MICROBIAL DIVERSITY

**Mrs. Narayanamma Degala, Satya Sai. Puppala , Sushma. Samsani ,
Vyshnavi. Puritigadda , Devi Harshitha .Rudraraju, Varshitha.satti,Dr.N.V.V.
Jagan Mohan Reddy, Dr.D.Narendra**

ABSTRACT

Metagenomics is a revolutionary molecular approach that enables the direct analysis of genetic material recovered from environmental samples without the need for microbial cultivation. Traditional microbiological techniques are limited because the majority of microorganisms present in nature cannot be cultured under laboratory conditions. Metagenomics overcomes this limitation by utilizing high-throughput sequencing technologies and advanced bioinformatics tools to characterize microbial communities and their functional potential. The approach has transformed microbial ecology by revealing previously unknown microbial taxa, metabolic pathways, and ecological interactions in diverse habitats including soil, aquatic ecosystems, extreme environments, and the human microbiome. Metagenomic studies contribute significantly to environmental monitoring, agriculture, biotechnology, industrial microbiology, and medical sciences. This review discusses the principles of metagenomics, methodologies, sequencing technologies, bioinformatics approaches, applications, challenges, and future perspectives in microbial diversity exploration. The integration of next-generation sequencing, artificial intelligence, and systems biology is expected to further enhance the understanding of microbial ecosystems and their contributions to global sustainability.

Keywords: Metagenomics, Microbial Diversity, Next-Generation Sequencing, Microbiome, Bioinformatics, Environmental Microbiology

1.INTRODUCTION

Microorganisms constitute the largest and most diverse group of living organisms on Earth and are present in virtually every habitat, including soil, oceans, freshwater systems, the atmosphere, extreme environments, and the human body. These microscopic organisms perform essential ecological functions such as nutrient cycling, decomposition of organic matter, carbon sequestration, nitrogen fixation, and maintenance of ecosystem stability^[1]. Despite their ecological significance, the vast majority of microorganisms remain poorly understood because traditional microbiological techniques rely on laboratory cultivation, which can recover only a small fraction of naturally occurring microbial species^[2].

For many decades, microbial diversity studies were limited to culture-based methods, microscopic observations, and biochemical characterization. Although these approaches contributed significantly to microbiology, they failed to reveal the true extent of microbial diversity present in natural environments. It has been estimated that more than 99% of environmental microorganisms cannot be cultivated using standard laboratory techniques, creating a substantial gap in our understanding of microbial ecosystems^[2]. The development of molecular biology techniques, particularly the use of 16S ribosomal RNA (rRNA) gene sequencing, provided a breakthrough in microbial

identification and phylogenetic analysis, enabling scientists to detect previously unknown microorganisms without cultivation ^[3].

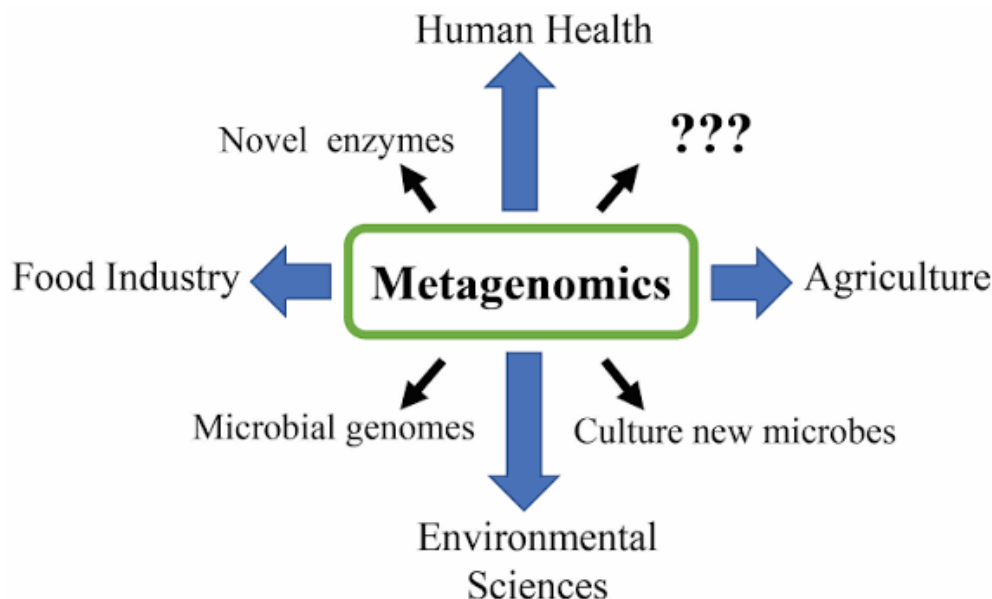
The emergence of metagenomics revolutionized microbial ecology by allowing direct extraction and analysis of genetic material from environmental samples. The term “metagenomics” was first introduced by Handelsman and colleagues in 1998 to describe the culture-independent study of microbial genomes obtained directly from environmental communities ^[4]. Unlike conventional microbiology, metagenomics investigates the collective genome, or “metagenome,” of all microorganisms present within a given habitat. This approach provides comprehensive information about microbial community composition, genetic diversity, evolutionary relationships, and functional capabilities ^[5].

Advances in DNA sequencing technologies have significantly accelerated the growth of metagenomics. High-throughput sequencing platforms such as Illumina, PacBio, and Oxford Nanopore enable rapid and cost-effective analysis of millions of DNA sequences from environmental samples ^[6]. Combined with powerful bioinformatics tools, these technologies allow researchers to reconstruct microbial genomes, identify novel genes, and predict metabolic pathways involved in ecosystem functioning ^[7]. Consequently, metagenomics has become one of the most important approaches for studying complex microbial communities in diverse environments.

Microbial diversity is generally categorized into taxonomic diversity, genetic diversity, and functional diversity. Taxonomic diversity refers to the variety and abundance of microbial species within an ecosystem, while genetic diversity reflects variations in gene content among microorganisms. Functional diversity describes the range of metabolic activities and ecological roles performed by microbial communities ^[8]. Understanding these dimensions of diversity is essential for elucidating ecosystem processes, environmental adaptation, and microbial interactions.

Metagenomic investigations have revealed an extraordinary diversity of microorganisms in terrestrial, aquatic, and host-associated ecosystems. Soil ecosystems, for example, contain billions of microbial cells and thousands of species per gram of soil, contributing to nutrient cycling and plant productivity ^[9]. Similarly, marine microbial communities play crucial roles in global carbon cycling, oxygen production, and climate regulation ^[10]. In recent years, human microbiome research has demonstrated that microbial communities inhabiting the human body significantly influence digestion, immunity, metabolism, and disease susceptibility ^[12].

The applications of metagenomics extend beyond ecological studies. In agriculture, metagenomic approaches facilitate the identification of beneficial microorganisms that enhance crop productivity and soil health ^[13]. In biotechnology, metagenomic libraries serve as valuable resources for discovering novel enzymes, antibiotics, and industrially important biomolecules ^[14]. In medicine, metagenomics supports pathogen detection, antimicrobial resistance monitoring, and personalized therapeutic strategies ^[15]. Environmental scientists also employ metagenomic tools for pollution monitoring, bioremediation, and assessment of ecosystem health ^[16].



Despite its remarkable advantages, metagenomics faces several challenges. DNA extraction biases, sequencing errors, assembly difficulties, and the management of massive datasets can affect the accuracy of results ^[16]. Furthermore, the interpretation of metagenomic data requires advanced computational resources and specialized bioinformatics expertise ^[18]. Nevertheless, continuous improvements in sequencing technologies, computational methods, and artificial intelligence are helping overcome these limitations and expanding the scope of metagenomic research.

Today, metagenomics represents one of the most powerful tools for exploring microbial diversity and understanding the ecological and functional significance of microorganisms in natural and engineered environments. By providing unprecedented insights into the hidden microbial world, metagenomics is transforming environmental science, biotechnology, agriculture, and medicine, ultimately contributing to sustainable development and human well-being ^[24,25].

2.HISTORY AND DEVELOPMENT OF METAGENOMICS

The development of metagenomics represents a major milestone in microbiology and microbial ecology. Before the advent of molecular techniques, microorganisms were primarily studied through culture-based methods. Scientists relied on growing microbes in laboratory media to identify and characterize them. However, these methods were limited because only a small fraction of environmental microorganisms could be cultivated under laboratory conditions. This discrepancy between microscopic observations and culturable microbial populations became known as the “great plate count anomaly,” highlighting the need for new approaches to study microbial diversity ^[26].

The foundations of microbial ecology were established by pioneering scientists such as Louis Pasteur and Robert Koch during the nineteenth century. Their work demonstrated the importance of microorganisms in disease, fermentation, and environmental processes. Despite these achievements, studies remained restricted to microorganisms that could be isolated and cultured, leaving the majority of microbial life unexplored ^[27].

A significant breakthrough occurred in the late twentieth century with the emergence of molecular biology techniques. The development of DNA sequencing technologies allowed scientists to investigate microorganisms based on their genetic material rather than cultivation characteristics. Carl Woese and colleagues introduced the use

of 16S ribosomal RNA (rRNA) gene sequencing as a molecular marker for microbial identification and phylogenetic analysis. This approach revolutionized microbial taxonomy and led to the discovery of a new domain of life, Archaea [28].

During the 1990s, polymerase chain reaction (PCR) technology enabled researchers to amplify and analyze microbial DNA directly from environmental samples. PCR-based studies revealed an extraordinary diversity of microorganisms in soil, aquatic environments, and extreme habitats. These findings demonstrated that traditional cultivation methods significantly underestimated microbial diversity in nature [29].

The term “metagenomics” was first introduced in 1998 by Handelsman and colleagues. They described metagenomics as the study of collective genomes recovered directly from environmental samples. This culture-independent approach enabled researchers to access the genetic resources of entire microbial communities, including uncultivable organisms. The introduction of environmental DNA cloning and library construction marked the beginning of modern metagenomic research [30].

A major advancement occurred in 2004 when Venter and colleagues performed environmental genome shotgun sequencing of microbial communities from the Sargasso Sea. Their study generated millions of DNA sequences and revealed thousands of previously unknown microbial genes, demonstrating the vast genetic diversity present in marine ecosystems [31].

In the same year, Tyson and colleagues reconstructed microbial genomes directly from environmental samples collected from an acid mine drainage system. This study provided one of the first successful examples of recovering near-complete genomes from uncultured microorganisms, opening new opportunities for studying microbial physiology and evolution [32].

The rapid development of next-generation sequencing (NGS) technologies during the mid-2000s dramatically expanded the scope of metagenomic research. Sequencing platforms such as 454 Pyrosequencing, Illumina, Solid, and later PacBio and Oxford Nanopore technologies enabled researchers to generate millions of DNA sequences quickly and cost-effectively. These technological advancements made it possible to study complex microbial communities with unprecedented depth and accuracy [33].

The launch of major international projects further accelerated metagenomic investigations. Initiatives such as the Human Microbiome Project (HMP) and the Earth Microbiome Project (EMP) aimed to characterize microbial diversity across diverse habitats and understand the relationship between microbial communities and environmental or human health. These projects generated enormous datasets and established standardized protocols for metagenomic analysis [34]. In recent years, metagenomics has evolved beyond taxonomic identification to include functional and comparative analyses. Researchers now investigate metabolic pathways, gene expression patterns, microbial interactions, and ecosystem functions using integrated multi-omics approaches. Advances in bioinformatics, machine learning, and artificial intelligence have further enhanced the ability to process and interpret complex metagenomic dataset

Today, metagenomics is recognized as one of the most powerful tools for s [35]. studying microbial diversity and ecosystem functioning. It has transformed environmental microbiology, biotechnology, agriculture, medicine, and industrial research by providing direct access to the vast genetic resources of uncultured microorganisms. Continued improvements in sequencing technologies and computational methods are expected to drive further discoveries and expand our understanding of the microbial world [36].

3. PRINCIPLES OF METAGENOMICS

Metagenomics is a culture-independent approach that involves the direct extraction and analysis of genetic material from environmental samples. Unlike conventional microbiological methods, which require isolation and cultivation of microorganisms, metagenomics studies the collective genomes of entire microbial communities. This approach enables researchers to investigate microbial diversity, community structure, ecological interactions, and functional potential without the limitations associated with laboratory cultivation ^[37].

The fundamental principle of metagenomics is based on the recovery of environmental DNA (eDNA) from a sample. Environmental samples such as soil, water, sediments, wastewater, plant surfaces, and animal tissues contain genetic material from diverse microorganisms. DNA is extracted directly from these samples, ensuring that both culturable and unculturable microorganisms are represented in the analysis ^[38]. This provides a more comprehensive understanding of microbial communities than traditional microbiological techniques.

Another important principle of metagenomics is unbiased community analysis. Since microbial populations are examined collectively, researchers can assess the diversity and abundance of microorganisms present in a particular habitat. This allows the identification of rare, novel, and previously unknown microorganisms that may play important ecological roles ^[39]. Consequently, metagenomics has significantly expanded knowledge of microbial diversity across terrestrial, aquatic, and host-associated environments.

DNA sequencing is a central component of metagenomic analysis. Following DNA extraction, sequencing technologies are used to determine the nucleotide sequences present in the sample. Two major sequencing approaches are commonly employed: amplicon sequencing and shotgun metagenomic sequencing. Amplicon sequencing targets specific genetic markers such as the 16S rRNA gene for bacterial identification, while shotgun sequencing analyzes all DNA fragments present in a sample, providing information on both taxonomic composition and functional genes ^[40]. Sequence assembly and annotation represent another important principle of metagenomics. After sequencing, millions of short DNA reads are assembled into longer contiguous sequences using bioinformatics tools. These assembled sequences are then compared with reference databases to identify microbial taxa and predict gene functions. Functional annotation helps researchers determine the metabolic capabilities and ecological roles of microbial communities ^[41].

Taxonomic classification is a key objective of metagenomic studies. By comparing DNA sequences with established microbial databases, researchers can identify bacteria, archaea, fungi, viruses, and protists present in a sample. This enables the characterization of microbial community structure and provides insights into species richness, abundance, and phylogenetic relationships ^[42].

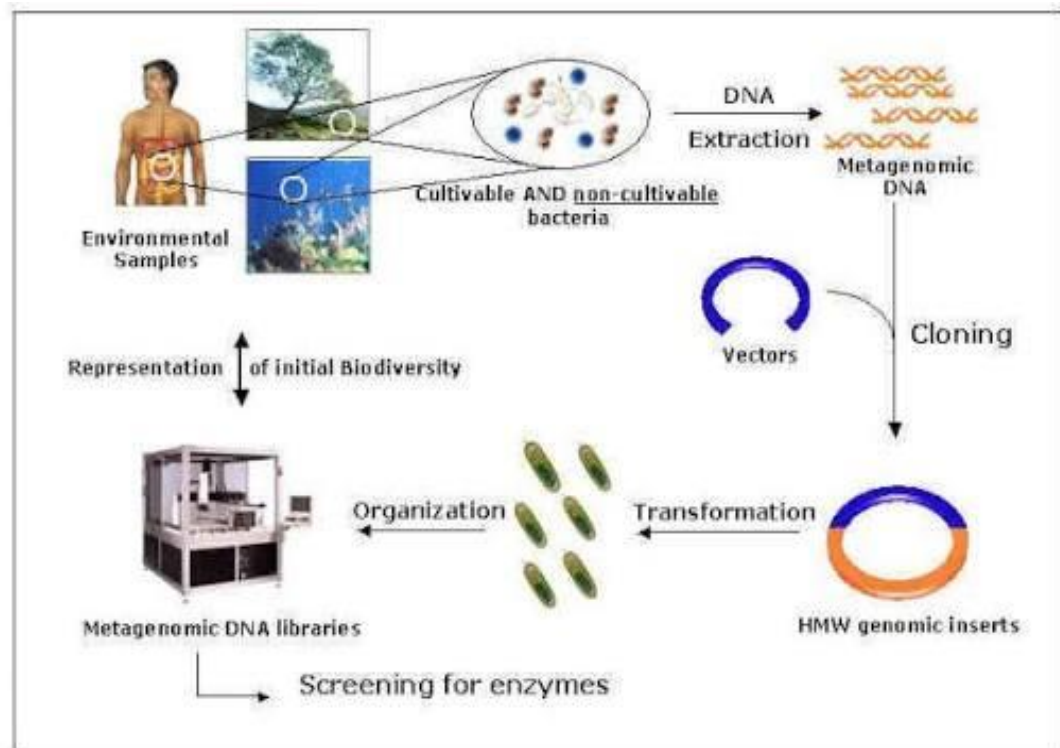


Figure 1 : Principle of metagenomic DNA libraries creation.

Functional Metagenomics is based on the principle that microbial communities can be studied. not only for their composition but also for their biological activities. Analysis of genes involved in nutrient cycling, biodegradation, antibiotic production, and environmental adaptation allows researchers to understand ecosystem functions and discover novel biomolecules with industrial and medical applications ^[43].

Bioinformatics plays a crucial role in metagenomic research. The large volume of sequencing data generated requires specialized computational tools for quality control, sequence alignment,

taxonomic classification, gene prediction, and statistical analysis. Modern metagenomic workflows integrate databases such as KEGG, SILVA, Greengenes, and NCBI to facilitate comprehensive interpretation of microbial community data ^[44].

Comparative metagenomics is another important principle that involves comparing microbial communities from different environments or conditions. Such comparisons help identify variations in microbial diversity, community structure, and functional potential. Comparative studies have been widely used to investigate environmental changes, disease-associated microbiomes, agricultural systems, and ecosystem responses to stress factors ^[45].

One of the most significant principles of metagenomics is the integration of ecological and functional information. By combining taxonomic and functional analyses, researchers can understand how microbial communities contribute to nutrient cycling, ecosystem stability, environmental adaptation, and host health. This systems-level perspective has transformed microbial ecology and enabled a deeper understanding of microbial interactions in natural and engineered environments ^[46].

Overall, the principles of metagenomics provide a comprehensive framework for studying microbial communities directly from environmental samples. Through advances in DNA sequencing, bioinformatics, and functional

analysis, metagenomics continues to uncover previously hidden microbial diversity and offers valuable insights into the ecological and biotechnological significance of microorganism ^[47]

4.Types of Metagenomic Approaches

Metagenomics employs different approaches to study microbial communities and their genetic potential. The choice of method depends on the research objective, available resources, and the level of information required. The three major approaches are amplicon sequencing, shotgun metagenomics, and functional metagenomics ^[48].

4.1Amplicon Sequencing

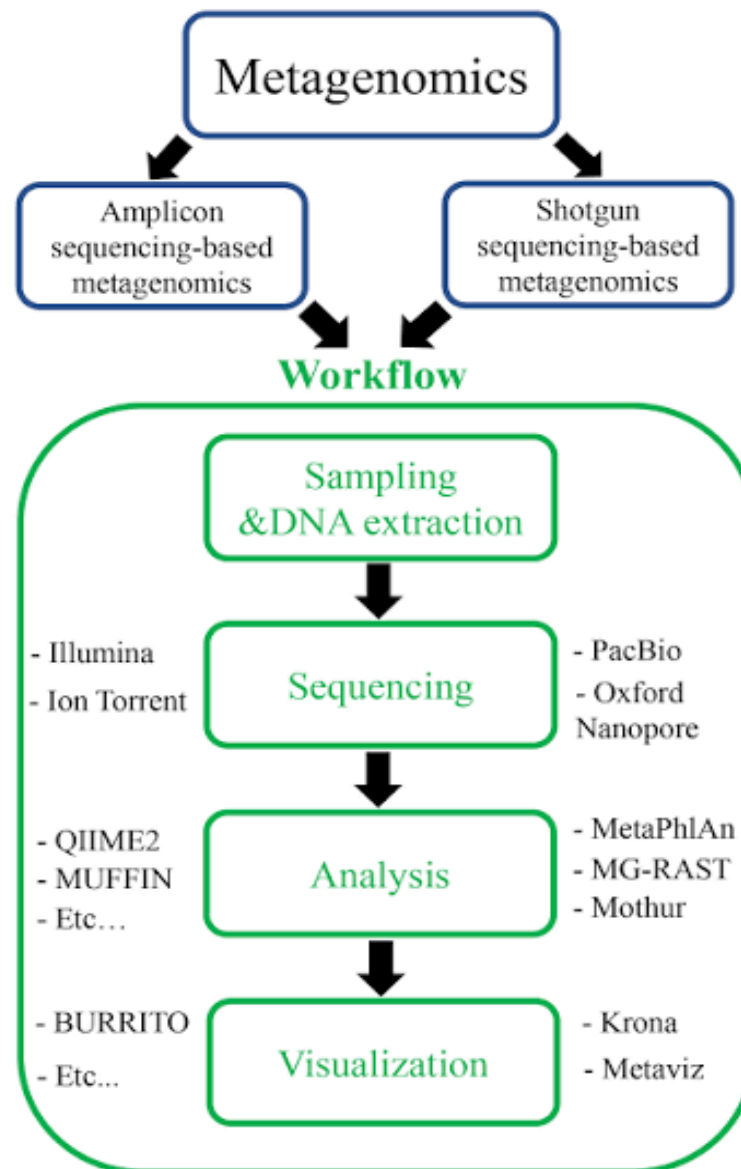
Amplicon sequencing is a targeted metagenomic approach that focuses on specific genetic marker genes, such as the 16S rRNA gene in bacteria and archaea or the ITS region in fungi. The selected gene regions are amplified using PCR and then sequenced. This method is widely used for identifying microbial taxa and determining community composition because it is cost-effective and relatively simple ^[49].

4.2Shotgun Metagenomics

Shotgun metagenomics involves sequencing all DNA present in an environmental sample without targeting specific genes. This approach provides detailed information about both the taxonomic diversity and functional capabilities of microbial communities. It enables the identification of novel organisms, metabolic pathways, and functional genes, making it one of the most comprehensive metagenomic techniques available ^[50].

4.3 Functional Metagenomics

Functional metagenomics focuses on identifying genes that encode specific biological functions. Environmental DNA fragments are cloned into suitable host organisms and screened for desired traits such as enzyme production, antibiotic synthesis, or pollutant degradation. This approach is particularly valuable for discovering novel biomolecules with applications in biotechnology, agriculture, and medicine ^[51].



5. SEQUENCING TECHNOLOGIES AND BIOINFORMATICS TOOLS

Sequencing technologies and bioinformatics tools are essential components of metagenomic research. They enable the identification, classification, and functional analysis of microorganisms present in environmental samples. Modern sequencing platforms generate large amounts of DNA data rapidly and accurately, making microbial diversity studies more efficient ^[52].

Common sequencing technologies used in metagenomics include Illumina, PacBio, and Oxford Nanopore sequencing. Illumina sequencing provides high accuracy and throughput, while PacBio and Oxford Nanopore technologies produce longer DNA reads that improve genome assembly and analysis ^[53].

Bioinformatics tools are used to process and interpret sequencing data. Popular software platforms such as QIIME, Mothur, Kraken, and MetaPhlAn help in taxonomic classification, diversity analysis, and functional annotation of microbial communities ^[54].

6. MICROBIAL DIVERSITY In DIFFERENT ENVIRONMENTS

Microbial communities inhabit a wide range of environments and play essential roles in ecosystem functioning. Metagenomic studies have revealed that microbial diversity varies significantly across habitats due to differences in physical, chemical, and biological conditions. Understanding these microbial communities helps in exploring their ecological importance and functional capabilities ^[55].

6.1 Soil Microbiome

Soil is one of the most diverse microbial habitats on Earth, containing bacteria, archaea, fungi, and viruses. Soil microorganisms are involved in nutrient cycling, organic matter decomposition, nitrogen fixation, and plant growth promotion. Metagenomic analyses have uncovered thousands of previously unknown microbial species in agricultural and natural soils ^[56].

6.2 Marine Microbiome

Marine ecosystems harbor highly diverse microbial communities that contribute to global biogeochemical cycles. Marine microorganisms participate in carbon fixation, nutrient recycling, and oxygen production. Metagenomic studies have revealed extensive microbial diversity in oceans, including many uncultured and novel species ^[57].

6.3 Freshwater Microbiome

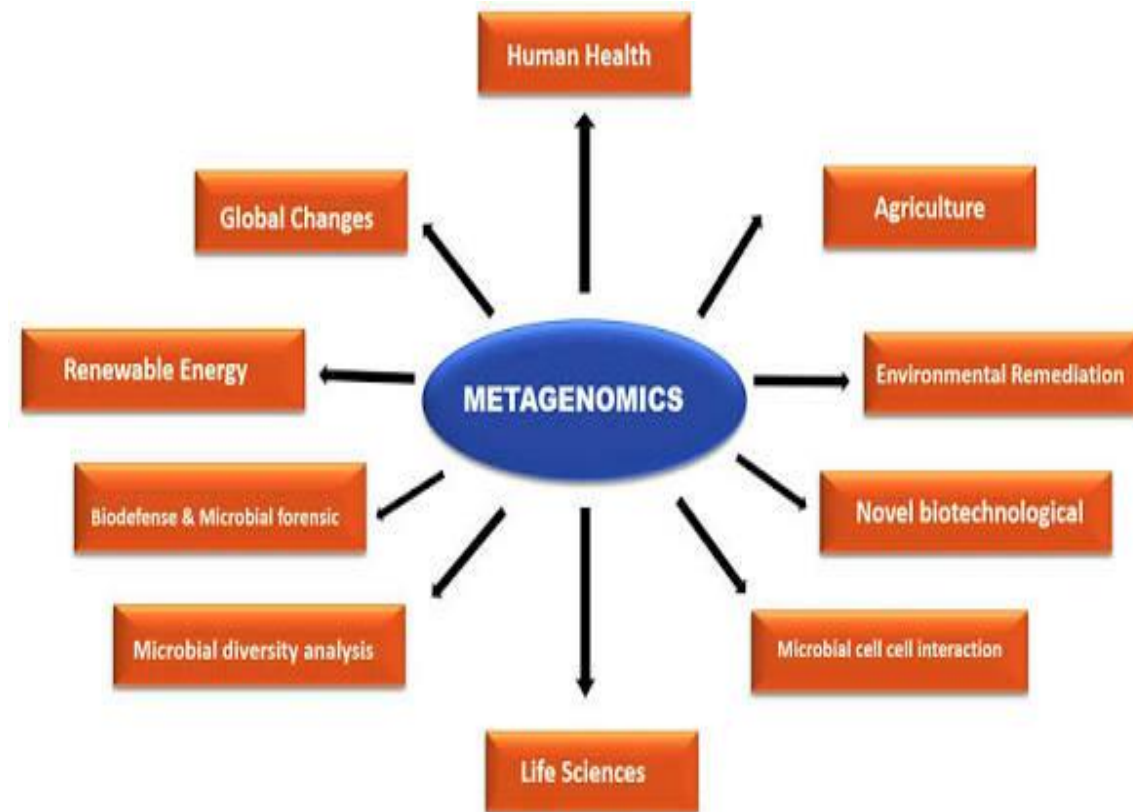
Freshwater environments such as rivers, lakes, and reservoirs contain dynamic microbial communities that influence water quality and ecosystem productivity. These microorganisms play important roles in nutrient transformation, organic matter degradation, and maintenance of aquatic ecosystem health ^[58].

6.4 Human Microbiome

The human body hosts trillions of microorganisms collectively known as the human microbiome. These microbes inhabit the gut, skin, oral cavity, and respiratory tract, where they contribute to digestion, immunity, and overall health. Metagenomic research has demonstrated strong associations between microbial community composition and various human diseases ^[59].

7. APPLICATIONS OF METAGENOMICS

Metagenomics has become an important tool in various scientific fields because it enables the study of microbial communities without cultivation. The information obtained from metagenomic analysis has numerous applications in agriculture, biotechnology, medicine, and environmental monitoring. By revealing the diversity and functional potential of microorganisms, metagenomics contributes to sustainable development and improved human well-being ^[60].



7.1 Agriculture

In agriculture, metagenomics is used to study soil microbial communities that influence soil fertility and plant growth. It helps identify beneficial microorganisms involved in nutrient cycling, nitrogen fixation, and disease suppression. Metagenomic approaches also support the development of biofertilizers and sustainable farming practices ^[61].

7.2 Biotechnology

Metagenomics provides access to a vast reservoir of novel genes and enzymes from uncultured microorganisms. These biomolecules have applications in industrial processes, food production, biofuel generation, and pharmaceutical development. Functional metagenomics has facilitated the discovery of new enzymes with unique properties and commercial value ^[62].

7.3 Medicine

Metagenomics plays a significant role in medical research and healthcare. It is used for studying the human microbiome, detecting pathogens, monitoring antimicrobial resistance, and understanding disease mechanisms. Metagenomic sequencing has improved the diagnosis of infectious diseases and supports the development of personalized medicine approaches ^[63].

7.4 Environmental Monitoring

Environmental metagenomics helps assess ecosystem health and monitor environmental changes. It is widely used for detecting pollution, studying microbial responses to climate change, and evaluating biodiversity in terrestrial and aquatic ecosystems. Metagenomic analysis also contributes to bioremediation by identifying microorganisms capable of degrading environmental contaminants ^[64].

8. ADVANTAGES AND LIMITATIONS OF METAGENOMICS

Metagenomics has revolutionized microbial ecology by enabling the direct study of microbial communities from environmental samples without the need for cultivation. This approach provides comprehensive insights into microbial diversity, genetic composition, and functional potential. However, despite its numerous advantages, metagenomics also faces several technical and analytical challenges ^[65].

One of the major advantages of metagenomics is its culture-independent nature. It allows researchers to study both culturable and unculturable microorganisms, thereby revealing a much broader spectrum of microbial diversity than traditional methods ^[66]. Metagenomics also enables the discovery of novel genes, enzymes, and metabolic pathways that may have applications in biotechnology, agriculture, and medicine. Furthermore, it provides valuable information about microbial interactions, community structure, and ecosystem functions ^[67].

Another important advantage is the ability to analyze entire microbial communities simultaneously. High-throughput sequencing technologies generate large amounts of genetic data, allowing comprehensive taxonomic and functional characterization of microorganisms present in a sample ^[68].

Despite these benefits, metagenomics has certain limitations. DNA extraction methods may introduce biases that affect the representation of microbial populations. Sequencing errors, incomplete genome assembly, and difficulties in data interpretation can also influence the accuracy of results ^[69]. In addition, metagenomic studies generate massive datasets that require advanced computational resources, specialized software, and bioinformatics expertise for analysis ^[70].

The cost of sequencing and data processing remains another challenge, particularly for large-scale studies. Although sequencing technologies have become more affordable, comprehensive metagenomic investigations can still be expensive and time-consuming ^[71].

9. FUTURE PERSPECTIVES OF METAGENOMICS

Metagenomics continues to evolve rapidly with advancements in sequencing technologies, bioinformatics, and computational biology. Future developments are expected to provide deeper insights into microbial diversity, ecological interactions, and functional capabilities. The integration of metagenomics with other omics technologies, such as meta transcriptomics, metaproteomics, and metabolomics, will enable a more comprehensive understanding of microbial communities and their activities in different environments ^[72].

One of the most promising areas is the development of long-read sequencing technologies, including PacBio and Oxford Nanopore platforms. These technologies improve genome assembly and facilitate the recovery of complete microbial genomes from complex environmental samples ^[73]. Advances in single-cell genomics are also expected to complement metagenomics by allowing detailed analysis of individual microbial cells that are difficult to culture.

Artificial intelligence (AI) and machine learning are increasingly being applied to metagenomic data analysis. These tools can process large datasets efficiently, identify hidden patterns, and predict microbial functions with greater accuracy. Such innovations will enhance the speed and reliability of metagenomic studies ^[74].

Future metagenomic research is likely to have significant applications in healthcare, agriculture, biotechnology, and environmental management. Personalized medicine based on human microbiome analysis, precision agriculture through microbiome engineering, and improved strategies for pollution control and bioremediation are among the anticipated outcomes ^[75]. As sequencing costs continue to decrease and computational tools become more accessible, metagenomics will remain a powerful approach for exploring the vast and largely unknown microbial world.

10.CONCLUSION

Metagenomics has transformed the study of microbial diversity by enabling the direct analysis of genetic material from environmental samples without the need for cultivation. This powerful approach has revealed the immense diversity of microorganisms present in soil, aquatic ecosystems, the human body, and extreme environments, many of which were previously unknown. Through advanced sequencing technologies and bioinformatics tools, metagenomics provides valuable information about microbial community structure, genetic composition, and functional potential ^[76]. The application of metagenomics has significantly contributed to various fields, including agriculture, biotechnology, medicine, and environmental science. It has facilitated the discovery of novel microorganisms, enzymes, and metabolic pathways while improving our understanding of ecosystem processes and human health. Despite challenges such as data complexity, sequencing biases, and computational requirements, continuous technological advancements are enhancing the accuracy and efficiency of metagenomic analyses ^[77].

11.REFERENCES

- (1) Whitman, W.B., Coleman, D.C., & Wiebe, W.J. (1998). Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, 95(12), 6578–6583.
- [2] Amann, R.L., Ludwig, W., & Schleifer, K.H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, 59(1), 143–169.
- [3] Woese, C.R. (1987). Bacterial evolution. *Microbiological Reviews*, 51(2), 221–271.
- [4] Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J., & Goodman, R.M. (1998). Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chemistry & Biology*, 5(10), R245–R249.
- [5] Handelsman, J. (2004). Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*, 68(4), 669–685.
- [6] Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., et al. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428(6978), 37–43.
- [7] Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, 304(5667), 66–74.

- [8] Riesenfeld, C.S., Schloss, P.D., & Handelsman, J. (2004). Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics*, 38, 525–552.
- [9] Tringe, S.G., & Rubin, E.M. (2005). Metagenomics: DNA sequencing of environmental samples. *Nature Reviews Genetics*, 6(11), 805–814.
- [10] Simon, C., & Daniel, R. (2011). Metagenomic analyses: Past and future trends. *Applied and Environmental Microbiology*, 77(4), 1153–1161.
- [11] Thomas, T., Gilbert, J., & Meyer, F. (2012). Metagenomics – A guide from sampling to data analysis. *Microbial Informatics and Experimentation*, 2(1), 3.
- [12] Human Microbiome Project Consortium. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, 486(7402), 207–214.
- [13] Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbrück, L., Reeder, J., Temperton, B., et al. (2012). Defining seasonal marine microbial community dynamics. *The ISME Journal*, 6(2), 298–308.
- [14] Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6(8), 1621–1624.
- [15] Knight, R., Callewaert, C., Marotz, C., Hyde, E.R., Debelius, J.W., McDonald, D., & Sogin, M.L. (2017). The microbiome and human biology. *Annual Review of Genomics and Human Genetics*, 18, 65–86.
- [16] Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, 35(9), 833–844.
- [17] Escobar-Zepeda, A., Vera-Ponce de León, A., & Sanchez-Flores, A. (2018). The road to metagenomics: From microbiology to DNA sequencing technologies and bioinformatics. *Frontiers in Genetics*, 9, 324.
- [18] Knight, R., Vrbanac, A., Taylor, B.C., Aksenov, A., Callewaert, C., Debelius, J., et al. (2018). Best practices for analysing microbiomes. *Nature Reviews Microbiology*, 16(7), 410–422.
- [19] Chistoserdova, L. (2019). Applications of metagenomics in biotechnology and industrial microbiology. *Applied Microbiology and Biotechnology*, 103(6), 2363–2374.
- [20] Nayfach, S., Roux, S., Seshadri, R., Udworthy, D., Varghese, N., Schulz, F., et al. (2021). A genomic catalog of Earth's microbiomes. *Nature Biotechnology*, 39(4), 499–509.
- [21] Almeida, A., Nayfach, S., Boland, M., Strozzi, F., Beracochea, M., Shi, Z.J., et al. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, 39(1), 105–114.
- [22] Parks, D.H., Chuvochina, M., Rinke, C., Mussig, A.J., Chaumeil, P.A., & Hugenholtz, P. (2022). GTDB: An ongoing census of bacterial and archaeal diversity through a phylogenetically consistent taxonomy. *Nature Biotechnology*, 40(9), 1347–1355.

- [23] Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., et al. (2023). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, 613, 794–803.
- [24] Rodriguez-R, L.M., Konstantinidis, K.T., & Colleagues. (2024). Advances in environmental metagenomics and microbial ecology. *Trends in Microbiology*, 32(2), 101–118.
- [25] Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., et al. (2024). Structure and function of the global ocean microbiome. *Science*, 385, 450–459.
- [26] Staley JT, Konopka A. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology*.1985;39:321–346.
- [27] Brock TD. Robert Koch: A Life in Medicine and Bacteriology. ASM Press. 1999.
- [28] Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences*.1990;87:4576–4579.
- [29] Pace NR. A molecular view of microbial diversity and the biosphere. *Science*.1997;276:734–740.
- [30] Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM. Molecular biological access to the chemistry of unknown soil microbes. *Chemistry & Biology*. 1998;5–R249.
- [31] Venter JC, Remington K, Heidelberg JF, et al. Environmental genome shotgun sequencing of the Sargasso Sea. *Science*.2004;304:66–74.
- [32] Tyson GW, Chapman J, Hugenholtz P, et al. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*.2004;428:37–43.
- [33] Metzker ML. Sequencing technologies: The next generation. *Nature Reviews Genetics*.2010;11:31–46.
- [34] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*.2012;486:207–214.
- [35] Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. *Nature Reviews Microbiology*.2018;16:410–422.
- [36] Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A. The road to metagenomics: From microbiology to DNA sequencing technologies and bioinformatics. *Frontiers in Genetics*.2018;9:324.
- [37] Handelsman J. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*. 2004;68(4):669–685.
- [38] Tringe SG, Rubin EM. Metagenomics: DNA sequencing of environmental samples. *Nature Reviews Genetics*. 2005;6(11):805–814.
- [39] Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics*.2004;38:525–552.

- [40] Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*. 2017;35(9):833–844.
- [41] Thomas T, Gilbert J, Meyer F. Metagenomics: A guide from sampling to data analysis. *Microbial Informatics and Experimentation*. 2012;2(1):3.
- [42] Wooley JC, Godzik A, Friedberg I. A primer on metagenomics. *PLoS Computational Biology*. 2010;6(2).
- [43] Simon C, Daniel R. Metagenomic analyses: Past and future trends. *Applied and Environmental Microbiology*. 2011;77(4):1153–1161.
- [44] Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. *Nature Reviews Microbiology*. 2018;16(7):410–422.
- [45] Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A. The road to metagenomics: From microbiology to DNA sequencing technologies and bioinformatics. *Frontiers in Genetics*. 2018;9:324.
- [46] Gilbert JA, Jansson JK, Knight R. The Earth Microbiome Project: Successes and aspirations. *BMC Biology*. 2014;12:69.
- [47] Nayfach S, Roux S, Seshadri R, et al. A genomic catalog of Earth’s microbiomes. *Nature Biotechnology*. 2021;39(4):499–509.
- [48] Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics*. 2004;38:525–552.
- [49] Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*. 2012;6(8):1621–1624.
- [50] Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*. 2017;35(9):833–844.
- [51] Simon C, Daniel R. Metagenomic analyses: Past and future trends. *Applied and Environmental Microbiology*. 2011;77(4):1153–1161.
- [52] Metzker ML. Sequencing technologies: The next generation. *Nature Reviews Genetics*. 2010;11:31–46.
- [53] Goodwin S, McPherson JD, McCombie WR. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics*. 2016;17(6):333–351.
- [54] Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. *Nature Reviews Microbiology*. 2018;16(7):410–422.
- [55] Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences*. 1998;95(12):6578–6583.

- [56] Fierer N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*. 2017;15(10):579–590.
- [57] Sunagawa S, Coelho LP, Chaffron S, et al. Structure and function of the global ocean microbiome. *Science*. 2015;348(6237):1261359.
- [58] Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S. A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews*. 2011;75(1):14–49.
- [59] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207–214.
- [60] Handelsman J. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*. 2004;68(4):669–685.
- [61] Berg G, Rybakova D, Grube M, Köberl M. The plant microbiome explored: Implications for experimental botany. *Journal of Experimental Botany*. 2016;67(4):995–1002.
- [62] Simon C, Daniel R. Metagenomic analyses: Past and future trends. *Applied and Environmental Microbiology*. 2011;77(4):1153–1161.
- [63] Integrative HMP (iHMP) Research Network Consortium. The Integrative Human Microbiome Project. *Nature*. 2019;569(7758):641–648.
- [64] Thompson LR, Sanders JG, McDonald D, et al. A communal catalogue reveals Earth’s multiscale microbial diversity. *Nature*. 2017;551(7681):457–463.
- [65] Handelsman J. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*. 2004;68(4):669–685.
- [66] Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics*. 2004;38:525–552.
- [67] Simon C, Daniel R. Metagenomic analyses: Past and future trends. *Applied and Environmental Microbiology*. 2011;77(4):1153–1161.
- [68] Thomas T, Gilbert J, Meyer F. Metagenomics: A guide from sampling to data analysis. *Microbial Informatics and Experimentation*. 2012;2(1):3.
- [69] Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*. 2017;35(9):833–844.
- [70] Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. *Nature Reviews Microbiology*. 2018;16(7):410–422.
- [71] Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A. The road to metagenomics: From microbiology to DNA sequencing technologies and bioinformatics. *FrReferences*

- [72] Gilbert JA, Jansson JK, Knight R. The Earth Microbiome Project: Successes and aspirations. *BMC Biology*. 2014;12:69.
- [73] Goodwin S, McPherson JD, McCombie WR. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics*. 2016;17(6):333–351.
- [74] Knight R, Vrbanac A, Taylor BC, et al. Best practices for analysing microbiomes. *Nature Reviews Microbiology*. 2018;16(7):410–422.
- [75] Nayfach S, Roux S, Seshadri R, et al. A genomic catalog of Earth's microbiomes. *Nature Biotechnology*. 2021;39(4):499–509. *Frontiers in Genetics*. 2018;9:324.
- [76] Handelsman J. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*. 2004;68(4):669–685.
- [77] Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*. 2017;35(9):833–843.

**Copyright & License:**

© Authors retain the copyright of this article. This work is published under the Creative Commons Attribution 4.0 International License (CC BY 4.0), permitting unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.