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Exploring the Potential of Metagenomics and Metabolomics to Understand the Fresh Water Microbial Ecosystem

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Abstract— **Freshwater ecosystems are important for ecological balance because they contain a diverse range of physical and biological elements, including aquatic microorganisms, flora, and fauna. They serve as vital diversity reservoirs, supporting a diverse range of life forms which provide essential ecological functions such as nutrient cycling, water purification, and habitat provision. Apart from their environmental benefits, freshwater ecosystems are critical to human survival by providing water for consumption, agriculture and for commercial use. They also make significant contributions to biodiversity by providing habitat for a wide range of endemic and endangered species. The complex interdependence of freshwater and terrestrial ecosystems highlights their importance in promoting environmental stability, local habitat management, and long term natural resource sustainability. Integrated Omics technologies like Metagenomics and metabolomics have emerged as a powerful tool to investigate the taxonomic and functional diversity of microbial communities in freshwater environments that not only provide their prolonged existence but also helps to understand their contribution in surrounding and global ecosystem and associated stress. Metagenomic analysis can disclose the actual microbial composition of freshwater revealing the presence of ecologically important taxa and functional abundance. It is vital for understanding the ecosystem's health and identifying potential threats, such as the occurrence of opportunistic human, animal and plant pathogens. Metabolomics can provide major insights into the chemical diversity and dynamics of dissolved organic matter, which is essential for characterizing toxicity and toxicogenomic effects. These advanced omics techniques offer vast potential for exploring industrially relevant secondary metabolites and developing novel bioremediation strategies to support sustainable management of freshwater resources. This study explores the interaction between these omics technologies to analyze microbial communities and metabolic constituents with activities in freshwater environments and their effect on human health. By employing high-throughput sequencing and advanced omics profiling, we can reveal the complex relationships between microbial diversity and ecosystem functions with their global industrial importance. This comprehensive review provides insights into the role of omics technologies, anthropogenic impact, and ecosystem resilience, facilitating well-equipped water management practices.**

Keywords— Metagenomics; Metabolomics; Freshwater; Microbial ecosystem, Sustainable Development; Microbial Diversity; Bioremediation; Chemical diversity.

Introduction

Metagenomics can provide valuable information about the taxonomic diversity and functional capabilities of microbial communities in freshwater lakes and rivers. Metagenomics may be used to reveal the actual microbial community and identify ecologically significant taxa such as *Proteobacteria* and *Firmicutes* [1]. It can also detect the presence of infectious diseases that affect humans and plants [1]. This knowledge is vital for comprehending the overall health and functioning of the freshwater environment. Metabolomics, however, can reveal the intricate variety and changes in dissolved organic matter in these aquatic habitats [2]. Characterizing carbon transport and microbial-driven biogeochemical cycling is crucial for understanding the sustainability of freshwater resources [3]. The utilization of both metagenomics and metabolomics can also result in the identification of industrially significant secondary metabolites generated by the local microbiome [2]. These novel compounds could have applications in bioremediation, food, and pharmaceutical industries, thereby contributing to sustainable development goals related to responsible consumption, clean water, and life on land. Furthermore, these advanced omics techniques can help identify microbial genes and pathways involved in the degradation of xenobiotics and other pollutants [3]. Metagenomics approach can play a major role in targeted bioremediation strategies to support the sustainable management of freshwater ecosystems impacted by anthropogenic activities. Metagenomics is transforming our understanding of microbial ecosystems by allowing researchers to analyze genetic material directly from environmental samples rather than culturing microorganisms using conventional methods. This approach provides insights on microbial diversity, ecological dynamics, and bioremediation strategies, transforming environmental science and remediation processes.

Metagenomics has become a powerful tool for understanding the taxonomic composition and functional potential of microbial communities in freshwater environments. By directly sequencing the collective genomes of all microorganisms present in a sample, metagenomics provides insights into the diversity and roles of both culturable and unculturable microbes [3]. Some key applications of metagenomics in freshwater microbial ecology include:

Profiling the taxonomic structure of microbial communities: By analyzing the 16S rRNA gene sequences that assist to reveal the diversity and relative abundances of different bacterial and archaeal taxa [4].

Identifying the functional genes and metabolic pathways that is encoded in the metagenome: This elucidates the functional potential of the community and how it may change in response to environmental conditions.

Assembling draft genomes of uncultured microbes from metagenomic data: This enables studying the biology of important but elusive members of the community.

Tracking shifts in microbial community: Revealing the microbial composition and function along environmental gradients or in response to disturbances [5,6]. This provides insights into the ecological drivers shaping the microbiome. However, challenges remain in fully capturing the diversity of freshwater microbiomes, assembling complete genomes from complex metagenomes, and accurately annotating the functions of genes [7]. Continued method development and integration with other omics approaches will further enhance the power of metagenomics to elucidate the roles of microbes in freshwater ecosystems.

Metagenomics can significantly improve bioremediation techniques in polluted freshwater lakes in several ways:

1. Identifying key microbial taxa involved in pollutant degradation: Metagenomic analysis can reveal the taxonomic composition of microbial communities in polluted lakes, highlighting the presence of ecologically important taxa like Proteobacteria and Firmicutes that may play crucial roles in degrading organic pollutants [8,9]. This information can guide the selection of appropriate microbial strains for targeted bioremediation.

2. Discovering novel genes and enzymes for bioremediation: By analyzing the functional potential of microbial communities, metagenomics can uncover novel genes, proteins, and enzymes involved in the degradation of xenobiotics and other pollutants [10]. These discoveries can lead to the development of more efficient bioremediation strategies and the engineering of microbes with enhanced pollutant-degrading capabilities.

3. Monitoring the success of bioremediation efforts: Metagenomic techniques can be used to track changes in microbial community composition and functional potential before, during, and after bioremediation interventions [11]. This allows for the evaluation of the effectiveness of bioremediation strategies and the optimization of treatment approaches based on the observed responses of the microbial community.

4. Identifying potential bioindicators of ecosystem health: Certain microbial taxa or functional genes detected through metagenomics may serve as bioindicators of the overall health and recovery of polluted freshwater ecosystems. Monitoring these bioindicators can provide valuable insights into the long-term success of bioremediation efforts and guide adaptive management strategies.

5. Exploring the potential of microbiome engineering: Metagenomic data can inform the design of synthetic microbial consortia or the targeted manipulation of native microbial communities to enhance their pollutant-degrading capabilities [10]. This approach, known as microbiome engineering, holds promise for developing more effective and sustainable bioremediation solutions for polluted freshwater lakes.

II. METAGENOMICS REVEALS THE DIVERSE MICROBIAL LANDSCAPE OF FRESHWATER ECOSYSTEMS

Freshwater lakes and rivers are vital ecosystems that provide essential services to human societies, such as drinking water, food, and recreation. Understanding the microbial communities inhabiting these aquatic environments is crucial, as they play pivotal roles in nutrient cycling, carbon sequestration, and overall ecosystem health [8,9]. Conventional culturing methods have limitations in capturing the full diversity of microbes, as a majority of them remain unculturable. However, the advent of metagenomics, which involves the direct sequencing of genetic material from environmental samples, has revolutionized our ability to explore the taxonomic and functional diversity of these microbial communities [10,11].

Recent metagenomic studies have provided valuable insights into the microbial composition and functional potential of various freshwater lakes and rivers around the world. In an analysis of microorganisms in the freshwater lakes of the Amazon basin, researchers found that the microbial communities were dominated by Proteobacteria and Firmicutes, indicating these taxa play important ecological roles in these aquatic environments [12,13]. Similarly, a study on the sediment microbiome of three lakes in China revealed the prevalence of metabolic pathways involved in nutrient cycling, xenobiotic degradation, and secondary metabolite production, highlighting the functional versatility of these microbial communities [14]. Metagenomic investigations have also shed light on the impacts of anthropogenic activities on freshwater ecosystems. A study on the Dal Lake in India, a freshwater urban lake, found the presence of opportunistic human and plant pathogens, likely due to the indiscriminate disposal of waste and other pollutants [15]. This underscores the need for regular monitoring and bioremediation efforts to maintain the health of these sensitive environments. Furthermore, metagenomic analyses have the potential to identify novel microbial taxa and functional capabilities with biotechnological applications, such as the production of valuable secondary metabolites or the development of bioremediation strategies.

Beyond taxonomic profiling, metagenomics also enables the exploration of microbial community dynamics in response to environmental changes. For instance, a study on Lake Lanier, a temperate freshwater ecosystem in the United States, revealed shifts in the planktonic microbial community structure and functional potential driven by seasonal variations in temperature and other abiotic factors [16]. Such insights can inform our understanding of how freshwater microbiomes may respond to the impacts of climate change. In addition to providing a comprehensive view of microbial diversity, metagenomics also offers a powerful tool for water quality monitoring and management. By identifying the presence of pathogenic microorganisms or detecting changes in the functional capabilities of microbial communities, metagenomic approaches can help inform decision-making processes and guide the implementation of appropriate remediation strategies [15,17]. As the field of metagenomics continues to evolve, with advancements in sequencing technologies and bioinformatics tools, the potential for unlocking the secrets of freshwater microbial communities grows ever stronger. By combining metagenomics with other complementary techniques, such as metabolomics and proteomics, researchers can gain an even more holistic understanding of the complex interactions and functions within these vital aquatic ecosystems [15].

Here are the key steps for performing whole metagenome sequencing on freshwater samples:

Sample Collection and Preservation

Collect replicate water samples (6 replicates of 3 L per site) and filter through 0.22 µm membrane filters to capture microbial cells. For biofilm samples, brush the entire surface area of submerged coupons/slides and suspend in phosphate buffered saline (PBS), then concentrate by 0.22 µm filtration. Immediately preserve filtered samples by freezing at -80°C in the dark until DNA extraction.

DNA Extraction and Quantification

Extract total community DNA using a method based on proteinase K digestion followed by chloroform/isoamyl alcohol extraction. Assess DNA quantity and purity using a spectrophotometer like Nano-Drop. Pooling replicates may be necessary to obtain sufficient DNA for sequencing. If DNA concentration is low (<2 ng/µL), perform whole genome amplification to increase DNA yield prior to library preparation.

Library Preparation and Sequencing

Shear extracted DNA to the desired fragment size (300-500 bp) using a sonicator or enzymatic method. Prepare sequencing libraries using a kit like Illumina TruSeq, including steps for end-repair, A-tailing, adapter ligation and PCR amplification. Quantify libraries using a fluorometric method like Qubit and assess fragment size distribution on a Bioanalyzer. Pool libraries in equimolar ratios and sequence on an Illumina platform (HiSeq, NovaSeq) to generate paired-end reads (2 × 150 bp).

Bioinformatic Analysis

Quality control raw sequencing reads using tools like Trimmomatic to remove adapters, low quality bases and short reads. Assemble high-quality reads into longer contigs using a metagenome assembler like metaSPAdes or MEGAHIT. Predict and annotate genes on assembled contigs using tools like Prodigal and databases like KEGG, COG, Pfam. Taxonomically classify contigs and genes using reference databases and tools like Kraken2, Bracken, MEGAN. Bin contigs into metagenome-assembled genomes (MAGs) based on tetranucleotide frequency and coverage using tools like MetaBAT2 or MaxBin2. Assess MAG quality metrics like completeness and contamination using CheckM or BUSCO. Functionally annotate MAGs by mapping to databases like KEGG, COG, CARD, CAZy to infer metabolic potential [17].

These methods allow researchers to comprehensively profile the taxonomic composition, functional capabilities, and population genomes of microbial communities in freshwater ecosystems. The resulting insights can inform our understanding of microbial ecology, biogeochemical cycling, and responses to environmental perturbations in these vital aquatic habitats.

Fig 1. Illustration of the basic workflow of Environmental Metagenomics

III. METABOLOMICS: QUALITATIVE or QUANTITATIVE STUDY OF COMPOUNDS PRESENTS IN FRESH WATER

Metabolomics plays a crucial role in exploring and understanding freshwater ecosystems. It provides valuable insights into the metabolic processes and adaptations of organisms living in these dynamic environments.

One key application of metabolomics in freshwater ecosystems is assessing the health and stress responses of aquatic organisms. For example, studies have used metabolomics to evaluate the metabolic profiles of freshwater bivalves, such as mussels, in response to various stressors. By analyzing the changes in metabolite levels, researchers can gain insights into the physiological responses of these organisms to factors like pollution, temperature fluctuations, or changes in water quality [18,20]. This information can help in monitoring the overall health and functioning of freshwater ecosystems.

Metabolomics can also be employed to study the microbial communities in freshwater environments. Microorganisms, such as bacteria and algae, play a vital role in nutrient cycling, water purification, and the overall functioning of aquatic ecosystems. Toxicogenomics is a multidisciplinary area of study that integrates toxicology, genomics, and bioinformatics to examine how hazardous compounds impact the expression of genes and the genetic material within living organisms. The objective is to comprehend the impact of exposure to toxic substances on biological systems at the molecular scale. By analyzing the metabolic profiles of these microbial communities, researchers can gain insights into their interactions, activity, and response to environmental changes [20]. This information can be valuable for understanding the dynamics of freshwater ecosystems and developing strategies for their management and conservation. Metabolomics can be used to investigate the toxicogenomic responses of organisms in freshwater environments. Toxicogenomics is the study of the genomic and metabolomic responses of organisms to various toxicants or pollutants. By combining metabolomics with other "omics" approaches, such as genomics and transcriptomics, researchers can gain a more comprehensive understanding of the molecular mechanisms underlying the toxicological effects of contaminants on aquatic organisms [19]. This knowledge can inform risk assessment and the development of strategies for mitigating the impact of pollutants on freshwater ecosystems.

One example of the application of metabolomics in freshwater toxicogenomics is the study of the metabolic responses of the freshwater fly, Chironomus riparius, to exposure to the pesticide imidacloprid [19]. The researchers used metabolomics to analyze the changes in the metabolite profiles of the midges, which revealed alterations in various metabolic pathways, including energy metabolism, amino acid metabolism, and lipid metabolism. These findings provided insights into the underlying mechanisms of toxicity and the adaptive responses of the midges to the pesticide exposure. Such information can be valuable for understanding the impacts of contaminants on aquatic organisms and developing strategies for their protection. Another example is the use of metabolomics to study the metabolic adaptations of freshwater fireflies (*Coleoptera: Lampyridae*) to the aquatic environment [18]. The researchers compared the metabolic profiles of aquatic firefly larvae and terrestrial firefly adults, identifying a suite of metabolites associated with freshwater adaptation. These included changes in amino acid metabolism, energy production, and osmoregulation, which help the aquatic larvae cope with the challenges of the freshwater environment. This study demonstrates the power of metabolomics in elucidating the metabolic mechanisms underlying the adaptation of organisms to specific environmental conditions. Thus, metabolomics is a powerful tool for exploring and understanding freshwater ecosystems. By analyzing the metabolic profiles of aquatic organisms and microbial communities, researchers can gain insights into the health, stress responses, and adaptations of these systems. When combined with other "omics" approaches, such as toxicogenomic, metabolomics can provide a comprehensive understanding of the molecular mechanisms underlying the impacts of environmental stressors and pollutants on freshwater ecosystems. This knowledge can inform management and conservation efforts, as well as the development of strategies for mitigating the effects of human activities on these vital natural resources.

IV. Gas Chromatography – Mass Spectrometry: Important tool in Metabolomics

GC-MS is playing a crucial role in metabolomics studies of freshwater ecosystems. Metabolomics, the comprehensive analysis of small molecules or metabolites in biological systems, has become a powerful tool for understanding the complex interactions between organisms and their environment [21,22]. In the context of freshwater ecosystems, GC-MS based metabolomics has been employed to identify a wide range of toxic and potentially carcinogenic compounds in river water. These compounds can originate from various sources, such as agricultural runoff, industrial effluents, and urban wastewater discharge [23].

One example of a GC-MS based metabolomics study in freshwater systems is the analysis of "blue-green water" from a copper-contaminated river. Researchers used a combination of fluorescence spectroscopy and GC-MS to characterize the metabolite profile of these water samples [25]. The study identified several potentially toxic compounds, including phenol, benzaldehyde, and various fatty acids, which were likely derived from the degradation of organic matter in the presence of copper.

Another study used a targeted metabolomics approach with liquid chromatography-high resolution mass spectrometry (LC-HRMS) to investigate the metabolic response of the freshwater crustacean Gammarus pulex to controlled exposures of the pesticide imidacloprid [24]. The researchers were able to detect and quantify a range of metabolites, including amino acids, organic acids, and lipids, which showed significant changes in response to the pesticide exposure. This type of targeted metabolomics approach can be valuable for identifying specific biomarkers of exposure to environmental contaminants.

In a more comprehensive GC-MS metabolomics study, researchers investigated the metabolic differences between *Siniperca chuatsi*, a commercially important freshwater fish, raised in recirculating aquaculture systems (RAS) and traditional pond-based systems [21]. The study identified 33 metabolites that were significantly different between the two farming systems, including several potentially toxic compounds such as uric acid and xanthurenic acid. These findings highlight the utility of GC-MS based metabolomics in understanding the impact of different aquaculture practices on the health and quality of farmed fish.

Some of the key toxic and potentially carcinogenic compounds that have been identified in freshwater ecosystems using GC-MS based metabolomics include:

1. Polycyclic aromatic hydrocarbons (PAHs): PAHs are a class of persistent organic pollutants that are known to have carcinogenic and mutagenic properties. They can be derived from the incomplete combustion of fossil fuels and are often found in urban and industrial wastewater [23].

2. Pesticides and herbicides: These compounds, commonly used in agriculture, can contaminate freshwater systems and have been linked to various health effects, including cancer [24].

3. Phthalates: Phthalates are a group of chemicals used in the production of plastics and personal care products. They have been detected in river water and have been associated with endocrine disruption and other health concerns [23].

4. Phenols and phenolic compounds: Phenols and their derivatives can be generated from the degradation of organic matter and have been found to have toxic and carcinogenic properties [23].

5. Heavy metals: Heavy metals, such as mercury, lead, and cadmium, can accumulate in freshwater ecosystems and pose a significant threat to aquatic life and human health, including the potential for carcinogenic effects [24].

The identification of these toxic and potentially carcinogenic compounds in freshwater systems using GC-MS based metabolomics is crucial for understanding the impact of human activities on the environment and for developing strategies to mitigate these threats. By providing a comprehensive analysis of the metabolite profiles in freshwater, GC-MS can help researchers and policymakers identify the sources of contamination, monitor the effectiveness of remediation efforts, and inform the development of water quality standards and regulations. GC-MS based metabolomics has become an invaluable tool in the study of freshwater ecosystems, enabling the detection and quantification of a wide range of toxic and potentially carcinogenic compounds. The examples highlighted in this response demonstrate the power of this analytical technique in providing a holistic understanding of the complex interactions between organisms and their aquatic environment, ultimately contributing to the protection of freshwater resources and public health.

Fig 2. Illustration of the basic workflow of Environmental Metabolomics

V. CONCLUSION

Metagenomics and metabolomics are powerful tools for exploring the complex microbial communities in freshwater ecosystems, which play a crucial role in achieving sustainable development goals. These approaches have enabled researchers to unravel the microbial diversity, metabolic potential, and ecological interactions within aquatic environments, ultimately contributing to the sustainable management and conservation of freshwater resources. Metagenomics has revolutionized our understanding of microbial diversity in rivers and other freshwater habitats. By directly sequencing the genetic material from environmental samples, researchers have discovered a vast array of previously unknown microorganisms, including bacteria, archaea, and viruses. This knowledge is crucial for assessing the ecological health and functioning of freshwater ecosystems, as microbes play pivotal roles in nutrient cycling, energy flow, and ecosystem resilience.

Metabolomics is a field of study that examines the tiny chemicals or metabolites produced by organisms. It has yielded vital information about the functional abilities of microbial communities in freshwater habitats. Researchers have discovered new enzymes and pathways that are involved in breaking down toxins and xenobiotics by studying the metabolic profiles of bacteria. This understanding can be utilized for bioremediation procedures, in which microbes are utilized to eliminate or convert detrimental compounds, such as pesticides, heavy metals, and industrial waste, from water bodies. Metagenomics and metabolomics have been utilized in the field of toxicogenomics, which seeks to comprehend the impact of environmental pollutants on organisms. Researchers can create early warning systems for monitoring the health of freshwater ecosystems by examining the genetic and metabolic reactions of microorganisms to different contaminants. This information is vital for policymakers and water management authorities to make well-informed decisions and implement efficient policies for safeguarding and rejuvenating aquatic habitats. Although metagenomics and metabolomics have provided valuable insights into freshwater ecosystems, there remain unresolved difficulties that must be confronted. An important obstacle is the incorporation of multi-omics data, necessitating the use of sophisticated bioinformatics tools and computing resources. Furthermore, the process of cultivating newly discovered microorganisms using metagenomics techniques is still difficult due to the fact that many of these organisms are not easily grown in lab conditions. Although there are difficulties, the potential for metagenomics and metabolomics in freshwater research is encouraging. With the progression of technology, scientists will have the capability to produce more extensive and precise datasets, facilitating a more profound comprehension of microbial communities and their interactions with the environment. Acquiring this knowledge is essential for creating sustainable management plans for freshwater resources, ensuring their availability and quality for both current and future generations.

In conclusion, metagenomics and metabolomics have revolutionized our understanding of freshwater ecosystems by providing insights into microbial diversity, metabolic potential, and ecological interactions. These tools have applications in bioremediation, toxicogenomics, and freshwater monitoring, contributing to the achievement of sustainable development goals related to clean water and sanitation. As these technologies continue to evolve, they will play an increasingly important role in the sustainable management and conservation of freshwater resources worldwide.

VI. CONFLICT OF INTEREST

The authors declare no conflict of interest

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