

Chapter 19

Metagenomic Analysis of Freshwater Bacterial and Viral Biodiversity Using Shotgun Sequencing

Immanuel Suresh Jayasingh

 <https://orcid.org/0000-0002-9709-3164>

The American College, India

Iswareya Lakshmi V

The American College, India

ABSTRACT

The metagenomic analysis is used to investigate complex microbial communities directly from the environment, without culturing or isolating organisms. It is used to identify taxonomic diversity and functional metagenomics. Shotgun metagenomic sequencing is an environmental sequencing approach that can examine thousands of organisms simultaneously and comprehensively for community function and biodiversity. It also detects the low abundance members of microbial communities. It is advantageous over 16S sequencing as it has the cross-domain coverage, greater taxonomy resolution, and functional profiling. Several studies have been carried out using the shotgun sequencing metagenomic analysis to explore the freshwater diversity especially bacterial and viral diversity in different regions. Freshwater metagenomic analysis shows the presence of unknown single-stranded DNA in arctic freshwater, an abundance of actinobacterial in Japan lakes, Virophage and Mimiviridae community in Canadian freshwater. Metagenomic analysis of Himalayan freshwater lake was conducted using non-chimeric sequence.

DOI: 10.4018/978-1-7998-7356-3.ch019

INTRODUCTION

The microorganisms are ubiquitous and are found in almost all environments ranging from the gut of an animal to the geothermal hot springs. Microbial diversity refers to the variety of unicellular organisms, bacteria, archaea, protists, and fungi that exist. The microbes thrive all across the biosphere, defining the boundaries of life and fostering the survival and evolution of other living things. The microbes are distinguished by their distinct cellular metabolism, physiology, and morphology, as well as their diverse ecological distributions and activities, as well as their distinct genomic structure, expression, and evolution. Although the diversity of microorganisms now existing on the Earth is well recognized and assumed to be immense, the exact amount of the microbial diversity is mostly unknown. New molecular technologies are allowing researchers to quickly investigate the diversity of microorganisms, as well as their evolutionary links and history. The major difficulty in studying these microbes is that only a few of them are culturable using artificial media, whereas in the conventional culturing methods most of the bacterial community remains unidentified. Bacterial and viral communities in the freshwater bodies are important components of the freshwater ecosystem. They play a major role in maintaining the balance of the ecosystem and based on their diversity and richness, various parameters of the freshwater including metabolism, water quality can be studied. A study of the complete profile of bacterial biodiversity is difficult using the conventional methods, there is a need for other methods to study the biodiversity associated with the freshwater ecosystem. So, the metagenomics-based study has been found as a boon to study the environmental samples like freshwater and identify the microbial biodiversity based on their genomic data. Since this method is based on genomic information of the microbes in the sample, almost all the microbes can be identified by metagenomic analysis.

The sustainable development of freshwater environments is a worldwide concern. Bacteria and viruses are not only the essential components of food webs, but they also play a critical role in the freshwater ecosystem water quality control and regulation. The most important bacterial groups include Proteobacteria, Cyanobacteria, Bacteroidetes, Actinobacteria, and Verrucomicrobia. Freshwater bacteria and virus research has been primarily focused on the processes in which they are involved, such as water quality and elemental biogeochemical cycles. Despite the efforts over the last ten years, knowledge of the diversity and function of freshwater bacterial and viral communities is still quite restricted. Freshwater biodiversity is still a relatively new field of study, which limits people's ability to learn more about the microbial communities in freshwater settings.

Metagenomics is a technology for extracting the entire genomic DNA from microorganisms straight from the environment, which will cover the vast majority of microorganisms on the planet. With its ability to sequence thousands of organisms in parallel, the latest generation of sequencing technology has shown to be ideal for this application. Metagenomics is a breakthrough strategy for studying the microbial community that is unapproachable by existing conventional methodologies, and it can also detect all of the genomes present in a community of interest. Metagenomics is based on the advancements in microbial genomics as well as gene amplification and cloning using the polymerase chain reaction (PCR). Over the last 5 to 10 years, the area of metagenomics has played a critical role in important advances in microbial ecology, evolution, and diversity. This innovation has provided new insights into the diversity and functions of microbes (Noor et al., 2017). In microbiological research, the introduction of metagenomics-based techniques has overcome prior culture-based prejudices. This also made it possible to execute a more detailed analysis of the microbial ecology of environmental samples. Following the development of next-generation sequencing technologies, which can produce hundreds of millions

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