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Review on Airborne Metagenomics: Challenges and Opportunities

Dr Md Mazhar Alam, Assistant Professor,
Department of T.B. & Chest, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India
Email Id- dr.mazhar.alam@gmail.com

ABSTRACT: ***Recent metagenomic investigations of settings such as marine and soil have greatly improved our knowledge of the various microbial communities that live in these habitats including their vital roles in the maintenance of large ecosystems. The rise in publications related to soil and marine metagenomics contrasts sharply with those pertaining to airborne microbes, despite the fact that airborne bacteria are thought to have a substantial impact on many aspects of life, from their potential roles in atmospheric events including such cloud formation, precipitation, and atmospheric chemistry to their major impact on human health. We will address recent advances in airborne metagenomics in this review, with a particular emphasis on the difficulties and possibilities of doing such investigations. The following are the major obstacles to performing metagenomic investigations of airborne microbes: Low microbe density in the air, effective recovery of microorganisms from the air, heterogeneity in airborne microbial community, lack of defined procedures and methodologies, including DNA sequencing or bioinformatics-related difficulties are all factors to consider. Novel genes and metabolic pathways important to meteorological as well as industrial applications, environmental bioremediation, including biogeochemical cycles may be discovered as a result of airborne metagenomic research.***

KEYWORDS: ***Airborne Microorganisms, Culture-Independent Studies, Microbial Diversity, Metagenomics, Metabolic Potential.***

1`. INTRODUCTION

Despite the fact that microorganisms are widespread in the air, the effect of airborne microbes and their total contributions to the global environment remain understudied. Airborne microorganisms were formerly thought to be passive inhabitants that moved with the wind; nevertheless, numerous investigations have shown that they are metabolically active. Microorganisms in cloud water digest organic materials and may contribute to the earth's biogeochemical cycles. Some writers claim that microorganisms influence atmospheric chemistry and the earth's precipitation cycles, implying that airborne bacteria have a role in atmospheric processes like ice nucleation and cloud formation. The rise in cloud formation may be to blame for the planet's climatic shifts.

Aside from these research, there is a scarcity of information on the possible effect of airborne microorganisms on our planet. Culture-based research have contributed to our present knowledge of airborne microorganisms; nevertheless, the majority of atmospheric microbes cannot be grown in this manner. Furthermore, because pure cultures of microorganisms only contain one type of microbe, culture-based approaches miss out on studying interactions between different microbes and their surroundings; as a result, they are unable to uncover the genomic variations and biological functions associated with such interactions. The capacity to grow microorganisms in bulk and examine them at both the molecular and cellular levels is the

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primary benefit of culture-dependent research. Their major drawback is that they prefer certain bacteria more than others, giving an incorrect picture of the microbial population as a whole[1].

Through molecular studies of microbe genetic material and culture-independent investigations of environmental samples, our understanding of airborne microbial diversity including their potential metabolic effect has grown considerably in recent years (Figure 1). The polymerase chain reaction (PCR)-based rRNA (16S or 18S) gene sequencing approach for assessment of microbial diversity, wherein the single rRNA gene is used as a phylogenetic marker to make comparisons relatedness between microbes the whole-genome shotgun metagenomics approach for evaluation of microbial diversity, where a single rRNA gene is being used as a phylogenetic marker to compare relatedness between microorganisms[2].

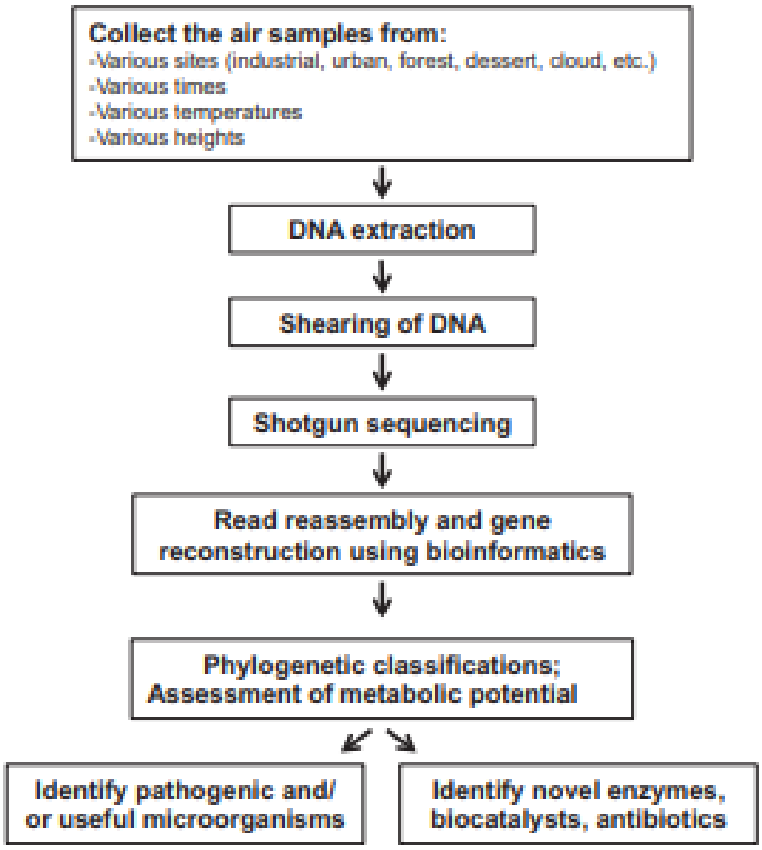

Figure 1: From the collecting of air samples through the analysis of their microbial composition, the process of shotgun metagenomic sequencing of airborne microorganisms is shown in this flow chart[3]**.**

Airborne microorganisms play an active role in the atmosphere; exploring this ostensibly unexplored frontier has enormous potential. A number of studies in culture indicate that airborne microorganisms are metabolically active and/or perform active roles in atmospheric events. Bacteria in the air, for example, have been proven to metabolize glucose. The absorption of tetrazolium dye by bacteria as an indication of metabolic activity in cells was recently discovered to be active in 76 percent of bacteria in cloud water. Cloud waters containing nitrifying bacteria had high amounts of inorganic and dissolved organic nitrogen,

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indicating that the bacteria in cloud water play a role in the cycling of organic nitrogen in the atmosphere.

The rise in ATP content in cloud water served as a marker of cell metabolic activity, indicating that the bacteria in the cloud were expanding exponentially and metabolically active. The bacteria isolated from cloud water metabolized the main carboxylic compounds in cloud water, and the end products of these metabolic activities were found in abundance in cloud water, implying that these bacteria were actively involved in the transformation of organic compounds in the atmosphere. By participating in ice-nucleating activities, cloud formation, or precipitation, airborne bacteria may have an impact on atmospheric conditions. Plant-associated Gram-negative bacteria such as Pseudomonas syringe, Pseudomonas fluorescens, and Erwinia herb cola have ice-nucleating proteins on their outer membranes[4].

1.1.Exploring Microorganism Diversity in the Atmosphere Using Culture-Independent rRNA Gene Analysis:

Hundreds of thousands of individual microorganisms with a variety of taxa equal to that found in soil may be discovered in a cubic meter of air. "Who" are these bacteria and "what" do they do? We have been able to answer the first question regarding who these organisms are via their phylogenetic diversity in recent years using culture-independent techniques and, in particular, PCR-based applications such as rRNA gene sequencing. According to research into the origins of airborne microorganisms, the bulk of these bacteria come from local sources (soil, plants, and marine), but others are unique to the local environment. These microorganisms may have come from far away and been carried in by powerful winds, sandstorms, or hurricanes. The composition of atmospheric sand-associated bacteria was altered during sandstorm occurrences in China and South Korea, according to a recent research utilizing amplicon-based 16S rRNA gene sequencing[5].

1.2.Current Advances in Airborne Metagenomics and Their Possibilities:

Although development in air metagenomics has been sluggish in comparison to other settings, the little success achieved so far is encouraging. During a severe smog occurrence in China, researchers used metagenomics to examine the microbial makeup of air samples and successfully discovered a variety of airborne microorganisms, including double-stranded DNA viruses. Their research uncovered the sequences of numerous respiratory infections and allergens in the air, demonstrating that their relative abundance increased as air pollution rose. a metagenomic framework for investigating the microbial composition of air samples from a variety of indoor and outdoor settings, and found extremely diversified microbial communities containing genes involved in metabolism, transport, translation, and signal transduction[6].

1.3.The Difficulties of Airborne Metagenomics:

1.3.1. Microorganisms in the Air Have a Low Density:

Bacteria, viruses, and fungus are among the microorganisms that may be found in the atmosphere. The concentration of bacteria in air is estimated to be between 104 and 106 microbes/m3, considerably lower than in marine and soil settings, where 1 g of topsoil contains about prokaryotes and 1 ml of sea water has approximately microorganisms.

1.3.2. Microorganisms from the Air Can Be Collected Effortlessly:

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Because microorganisms in the air have a lower density, it is critical that air-sampling equipment capture microorganisms with high efficiency. Air samplers typically pull particles-laden air through an airflow nozzle, which then directs the air toward a collecting surface. Intake air velocity, the form and diameter of the airflow nozzles, the distance between the nozzle and the collecting surface, the characteristics of the collection surface, the particle cut-off diameter, and collection durations all influence the sampling effectiveness of such devices. The inertial characteristics of microorganisms, which are linked to their size and density, also affect sampling efficiency[7].

1.4.Variability in Microbial Community Composition in the Air:

Due to climatic, geographical, and temporal trends, the composition of airborne microorganisms varies throughout time. The geographic variability in airborne microbial composition in the near-surface atmosphere, and discovered that bacterial composition differed substantially depending on land-use type, implying that bacteria from nearby land sources contributed to this variability. In an urban environment, temporal variability in airborne bacterial community composition revealed that bacterial composition changed substantially depending on the season. Even within the same season, the bacterial makeup varied considerably from day to day[8].

1.5.Sample collection and processing methods are not standardized:

The methods and techniques used to extract nucleic acids from the air have a big impact on the results of metagenomic research. Because metagenomic sequencing is done on nucleic acids collected from the environment, the amount and quality of these nucleic acids influence the study's result. The metagenomic data will not adequately reflect the sampled population if sufficient DNA is not collected. Similarly, if the extracted DNA does not adequately reflect the makeup of the sampled population, neither will the metagenomic data.

1.6.Challenges in DNA Sequencing or Bioinformatics:

Metagenomics has become one of the fastest growing areas in the field of environmental microbiology, because to rapid advancements in NGS technology. Despite its well-deserved success, metagenomics still confronts a number of obstacles that must be overcome. Despite the fact that metagenomics has the ability to discover and describe whole microbial communities and their genomes from the environment, identifying and characterizing potentially millions of genes in a soup of nucleic acids remains a difficult job. Environmental samples usually include various communities of unknown species of high and low abundance whose genomes must be sorted and identified, in contrast to culture-based research that investigate the genomic contents of single recognized bacteria[9].

Environmental DNA must be sheared into tiny pieces before metagenomic sequencing since existing DNA sequencing methods are incapable of sequencing lengthy complete genomes. The resulting millions of tiny reads must be reconstructed from scratch using sophisticated bioinformatics techniques and software. For metagenomics, reassembling these reads into continuous pieces (contigs) is a significant computational problem. Despite significant advances in the development of bioinformatics software and techniques, full genome reconstruction of microorganisms in environmental samples, particularly those from complicated settings, remains a challenge. Depending on the platform, read lengths acquired from contemporary NGS systems usually vary from 75 to 1,000 bp. When read durations are

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short or coverage depth is shallow, significant gaps in the assembled contigs are introduced. Accurate assembly of the contigs, and therefore reconstruction of the complete gene sequences, may be difficult or impossible depending on the length and quantity of these gaps. When numerous lengthy repeat sequences are present in the genes, as they typically are in bacterial and archaeal genomes, assembling short reads becomes much more difficult. Reconstructing the complete genome of microbial communities is frequently difficult[10].

2. DISCUSSION

Metagenomics is a powerful approach to screening microbial diversity and metabolic capabilities in any environment. The approach has proven effective in characterizing the genomic diversity and metabolic potential of many environments, including soil, marine, and the human guts, and has unraveled the vast impact of microorganisms on these ecosystems. The air harbors vastly diverse microorganisms with a potentially diverse range of metabolic activities. This frontier of microbial discoveries has largely been unexplored due to a number of challenges, which are due to the lower density of microorganisms in the air; inefficient retrieval of microbes and their nucleic acids from the air; lack of standardized approaches and methodologies; and bioinformatics-related challenges of de novo genome reconstruction. Overcoming these challenges could potentially pave the way to discoveries of novel pathways and genes important in meteorological and industrial applications, and environmental bioremediation. Metagenomic studies can additionally facilitate monitoring of airborne microorganisms through identifying pathogenic microbes and their distribution patterns and involvement in disease outbreaks that impact plant, animal, and human health.

3. CONCLUSION

Metagenomics is a useful tool for identifying microbial diversity and metabolic capacities in any setting. The method has been successful in assessing the genetic diversity and metabolic capacity of a variety of habitats, including soil, marine, and human intestines, as well as elucidating the enormous influence of microbes on these ecosystems. The air is home to a wide variety of microorganisms with a wide range of metabolic activities. We propose a consortium of interested parties to establish uniform approaches and methodologies designed to improve the collection efficiency, protocol reproducibility, and subsequent comparative analysis of airborne microbes. Conferences or meetings specifically designed for airborne metagenomics would provide the necessary forums for interested parties to discuss potential solutions to the present challenges. Overcoming these obstacles may pave the way for a more thorough investigation of airborne microorganisms and their potential effect on the environment, global climate, and human health. Metagenomic studies provide a unique opportunity to examine viral and bacterial diversity in the air and monitor their spread locally or globally, including dangerous microorganism concerns. Novel genes and metabolic pathways important to meteorological or industrial applications, environmental bioremediation, including biogeochemical cycles may be discovered as a result of airborne metagenomic research.

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