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Metagenomics Approach as New Strategy for Elucidate Microbiome of Wastewater

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Highlights:

Metagenomic Sequencing: Enhanced understanding of biodiversity.
Public Health Benefits: Early drug-resistant variant detection, efficient pathogen spread monitoring.

Keywords: Metagenomics; Genetic diversity; Environmental monitoring.

INTRODUCTION

The metagenomic sequencing approach is a powerful and advanced technique used to analyze microbiomes present in environmental samples. This method allows for comprehensive and unbiased characterization of microbial communities without the need for culturing microorganisms in the laboratory. Metagenomics efficiently identifies new genes, microbial metabolic pathways, studies disease-host relationships, microbial diversity, host-microbiota interactions, and potential coevolution. With new sequencing platforms, the technique has been refined into metabarcoding, revolutionizing our understanding of biodiversity in aquatic ecosystems (Taberlet et al., 2012; Leray & Knowlton, 2016). Metabarcoding, a high-throughput sequencing-based approach, allows for simultaneous analysis of thousands of DNA sequences from different organisms from various environmental sources, including wastewater (Leray et al., 2013). It also helps monitor the spread of pathogens within communities.

Wastewater is a valuable source of information, reflecting the presence of infectious agents in the population. Sequencing the genomes of these microorganisms allows for mapping transmission patterns, identifying high-risk geographical areas, and implementing more effective control measures. Additionally, expanding genomic sequencing contributes to real-time epidemiological surveillance. This approach enables a proactive stance, where health authorities can make informed decisions based on the latest genomic data, aiding in the prevention and containment of outbreaks.

METHODOLOGY

The methodology implemented involved weekly collection of raw sewage samples at the wastewater treatment plants (WWTPs) in the city of Curitiba/Paraná. Total DNA was extracted from the concentrated samples and prepared for shotgun sequencing. DNA libraries were sequenced using a next-generation sequencer (NGS), and the raw data underwent bioinformatic analyses for the identification and characterization of microorganisms present in the samples.

RESULTS AND CONCLUSIONS

The results obtained revealed significant genetic diversity in the samples. Multiple species of bacteria, archaea, viruses, and eukaryotes were found. Among the most abundant prokaryotes, representatives of *Prevotella copri* (8%), *Moraxella osioensis* (6%), and *Escherichia coli* (6%) stood out. The remaining 80% of representatives showed a frequency lower than 5% (Figure 1A). In the composition of Eukaryotes (Fungi Kingdom), the results identified the highest prevalence of *Pichia kudriavzevii* – 35%, *Botrytis cinerea* – 21%, *Candida dubliniensis* – 15%, *Malassezia restricta* – 10%, *Thermothelomyces thermophilus* – 9%, *Talaromyces rugulosus* – 3%. The other 7% of identified organisms represent organisms with a frequency lower than 3% of occurrence (Figure 1B).

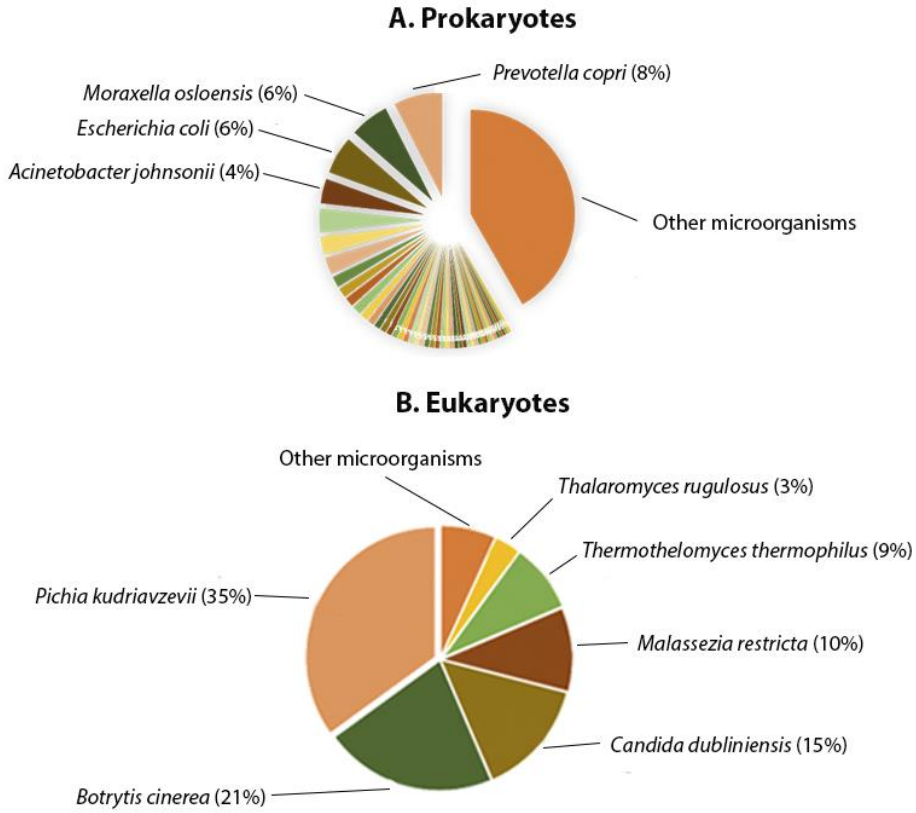

Figure 1: Composition of prokaryotes (A) and eukaryotes (B) found in the microbiome analysis of sewage samples carried out in the study.

These analyses provided a detailed insight into the genetic composition of the samples and indicated the presence of a variety of organisms and molecules in the analyzed samples. This knowledge can be crucial for understanding the health of the studied aquatic ecosystem and assisting in decision-making regarding control measures and environmental monitoring. Moreover, by identifying pathogenic or potentially harmful microorganisms, such as *Escherichia coli* and *Candida dubliniensis*, this study contributes to a more targeted monitoring approach, allowing early detection of pathogens that may lead to disease outbreaks. Proactive identification of these microorganisms can enable health authorities and environmental agencies to act swiftly, implementing preventive measures, such as containment or eradication, before pathogens reach critical levels capable of causing widespread infections.

Furthermore, understanding the genetic diversity of pathogens, particularly in viruses and bacteria, can help track mutations and emerging variants, which is vital for assessing their potential to become more virulent or resistant to existing treatments. Continuous monitoring of such genetic variability enables better predictions about the likelihood of an epidemic or pandemic outbreak, aiding in the development of diagnostic tools, vaccines, or other therapeutic measures. In this way, the data generated through this research can serve as an early warning system for public health and environmental surveillance, facilitating the anticipation and mitigation of future outbreaks.

However, it is important to highlight that the identification of genes and organisms through the approach used relies on comparisons with reference databases and may present some limitations. For example, there may be a lack of reference sequences for certain species or genes, making it difficult to precisely identify all organisms present in the samples. This caveat is relevant as we recognize that metagenomic analysis is a complex and evolving process.

Therefore, while the approach through metagenomic sequencing is a powerful tool for exploring genetic diversity in complex biological environments, it is necessary to interpret the results cautiously, considering the possible limitations. Although the limited availability of reference sequences may influence the results and be a limiting factor, studies like this one can generate updates or improvements to reference databases in the future.

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