

Metagenomics in Microbial Diversity Studies: Advances and Challenges

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Abstract:

The advent of metagenomics has proven to be an invaluable tool in studying microbial diversity in an effort to understand the collective genetic information of microbial communities without having to culture them. In this review, I will highlight some of the developments, applications, and challenges in using metagenomics to understand human microbial diversity with regards to taxonomic and functional diversity, geographic and demographic variation, uncultured microorganisms, metagenome-assembled genomes, strain-level diversity, sequencing technology, and bioinformatics. With the emergence of metagenomics, human-based studies such as the Human Microbiome Project and gut microbiome investigations have shown great microbial diversity among anatomical regions, individuals, and different populations and have led to the discovery of many unknown microorganisms and microbial genes. Further advancements in shotgun metagenomics, long-read sequencing, genome assembly, and metatranscriptomics have provided even better tools for characterizing microbial genomes and their metabolisms and functions. However, there are still important challenges such as incomplete databases, host DNA contamination, geographic and demographic biases, sequencing and computational needs, methodology variations, and differentiating between microbial genetic capacity and biological activities. The review highlights the need for standardized methodologies, diverse and longitudinal human-based datasets, improved microbial genome resources, strain-resolved analysis, and integration of multi-omics approaches. Overall, metagenomics provides an essential framework for advancing the comprehensive understanding of human microbial diversity and its functional significance.

Keywords: Metagenomics; Human Microbiome; Microbial Diversity; Shotgun Metagenomics; Metagenome-Assembled Genomes; Microbial Genomics

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1. INTRODUCTION

Earth is blessed with amazing variety of living organisms consisting of both micro and macroorganisms such as bacteria, fungi, plants and animals¹. Metagenomics, has opened new horizons in the development of biotechnology based on the exploitation of uncultivated microbial species². Metagenomics has proven to be one of the most important techniques in modern microbial ecology due to the ability to study the genomic content of complex microbial communities without the necessity to grow each microorganism separately³. The development of technologies such as high-throughput sequencing, shotgun metagenomics, metagenomic assembly, and bioinformatics made possible the research of microbial diversity linked to humans. Human-associated microbes reside at various locations within the human body like gastrointestinal tract, mouth, skin, respiratory tract, and reproductive system and perform different tasks related to metabolism, utilization of nutrients, regulation of the immune system, protection of epithelia, and physiological homeostasis. With the use of metagenomic techniques, one can study the composition of microbial community, identify the genes and metabolic functions of microorganisms, assemble genomes, and detect uncultivated species of microorganisms, which is why metagenomics provides a broader knowledge of human microbial diversity in comparison with traditional culture-based methods. Human Microbiome Project and other large-scale studies on human microbiome showed that microbial communities vary greatly depending on the location and the person and share certain functional features.

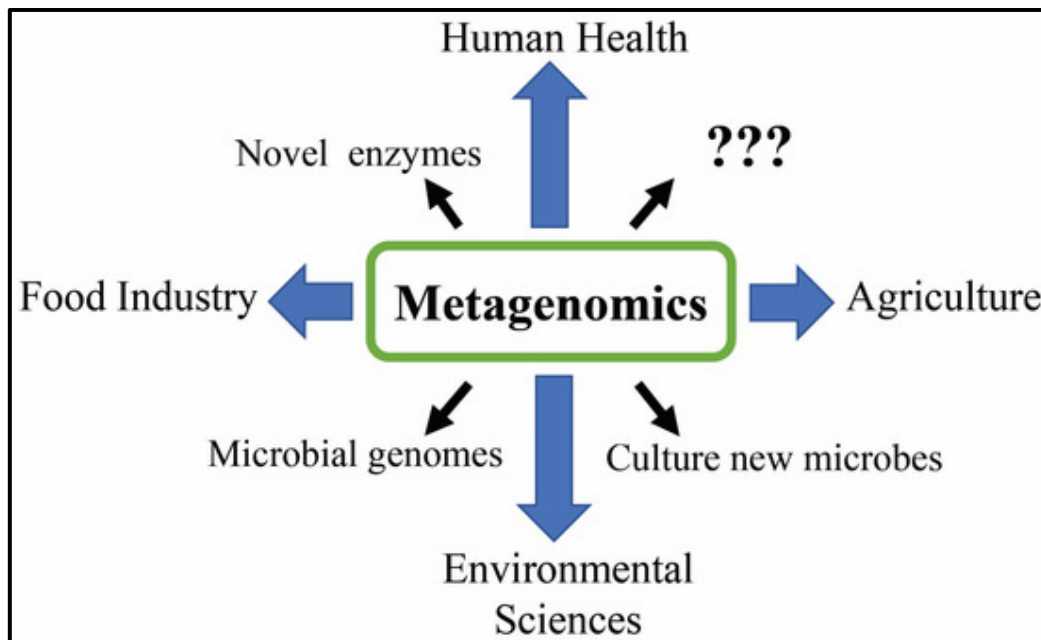


Figure 1: Metagenomics⁴

By utilising vast data sets to address the challenges of early human disease detection, notably in cancer, advances in artificial intelligence and machine learning have paved the way for autonomous illness diagnosis tools⁵. Researchers are continuously investigating the

gastroprotective properties of a wide variety of medicinal plants in an effort to find new ways to enhance currently available anti-ulcer drugs⁶. The recent improvements in metagenomic techniques have allowed its use not only for the mere identification of microbes but also for taxonomic, functional, genomic, and strain-level profiling of microbial communities. Shotgun metagenomics may provide the information about microbial genes and pathways, metagenome-assembled genomes enable scientists to reconstruct the genomes of human-associated yet uncultivated microorganisms, long-read sequencing has advanced genome assembly and strain-level resolution, and metatranscriptomics offers additional insights into microbial gene expression⁷. Nonetheless, several issues remain unaddressed in human metagenomic research – these include insufficient reference database, contamination with host DNA, geographical and populational biases, sequencing depth, computational issues, methodology, and problems in discrimination between genetic potential and actual function. Thus, an assessment of existing evidence is required.

1.1 Background Information and Context

The traditional approach to microbial diversity studies has largely relied on culture-based techniques, microscopic examination, biochemical testing, and targeted molecular methodologies. Despite making important contributions to microbial identifications, these methods do not fully reflect microorganisms that are either hard or impossible to grow in standard laboratory conditions⁸. Metagenomics overcame this obstacle by making it possible to directly analyze the DNA of microbes present in human-derived samples. The pioneering metagenomic study of the human gut by Qin et al. showed that there are about 3.3 million non-redundant microbial genes, thus emphasizing the rich diversity of the human microbiota. In turn, the Human Microbiome Project took the study of microbial diversity to the next level by describing communities of microbes residing in different parts of the human body and their significant diversity among different individuals and body regions⁹. Furthermore, it is well known that germs that are resistant to several drugs provide a significant danger to the health of humans in the 21st century¹⁰. Increased human activities like deforestation, urbanization, agricultural intensification, grazing and mining were creating serious problem to the flora and fauna of many terrestrial ecosystem across the world¹¹. Somatic embryogenesis has the potential for the improvement of plants of commercial importance¹². Subsequent metagenomic research allowed scientists to discover many new uncultivable species and gain insights into microbial functions, metabolic pathways, strain-level variations, and population-specific diversity.

1.2 Objectives of the Review

The main objectives of this review include:

- To give a brief introduction of the evolution of metagenomics as well as its key applications in human microbiome studies.

- To give an overview of significant studies performed by humans on the microbial taxonomy and functional diversity.
- To review the advancements in shotgun metagenomics, MAGs, strain analysis, long read sequencing, and meta transcriptomics.
- To critically analyze the methods, key findings, strengths, and weaknesses of the metagenomics techniques in human microbiome research.
- To analyze the impact of geographical and environmental factors on human-associated microbial diversity.

1.3 Importance of the Topic

Research in recent years has shown that the gut microbiota plays a significant role in human health¹³. Microorganisms that grow in saltwater environments are an important source of bioactive compounds¹⁴. The knowledge of human microbial diversity is significant since the microorganisms that live in the human body have an effect on the metabolism of nutrients, immune regulation, defense against pathogens, and physiological balance¹⁵. Culture-dependent technologies cannot completely characterize microbial diversity; however, metagenomics provides the means of studying the community comprehensively through identification of both known and unknown microorganisms, as well as their genetic and functional capacities. Metagenomic techniques also offer significant data on variability of populations, functional capacity of microbes, diversity of strains, and their changes related to human health and diseases. However, some issues, including geographic sampling biases, inadequate reference sequences databases, host-DNA contaminations, strain-level diversity, and the absence of functional characterization may influence results. Transcription factors are master regulators of gene expression¹⁶. Thus, standardization of procedures, diversity of human populations, and the use of multi-omics technologies are key factors.

2. ADVANCES IN METAGENOMICS FOR HUMAN MICROBIAL DIVERSITY STUDIES

Biodiversity is the variability among living organisms and is important for all of us¹⁷. Human-associated metagenomics has become an increasingly valuable culture-free technique for investigating the diversity of microbes linked to humans. In contrast to traditional microbiology techniques that involve microbial isolation and culturing, metagenomics is capable of allowing the study of microbial DNA obtained directly from human-associated samples⁶. Metagenomics gives information on microbial taxon composition, microbial genes, metabolic pathways, uncultivable microbes, and genomic diversity. Human microbiome investigations have shown tremendous diversity both taxonomically and functionally, and technological developments in shotgun sequencing, genome assembly, MAGs, and bioinformatics have improved microbial profiling. Nevertheless, challenges regarding references, host-DNA contamination, sampling bias, informatics difficulties, and strain differentiation still persist. The following part will focus on the progress in human metagenomics studies.

2.1 Development of Metagenomics in Microbial Diversity Research

The use of metagenomics was a significant change from the cultivation-dependent study of microbes to the culture-independent study of microbes¹⁸. The problem with earlier techniques was that it was necessary to cultivate microorganisms for studying them, which was not possible with many microbes found on humans. Metagenomics overcomes this problem.

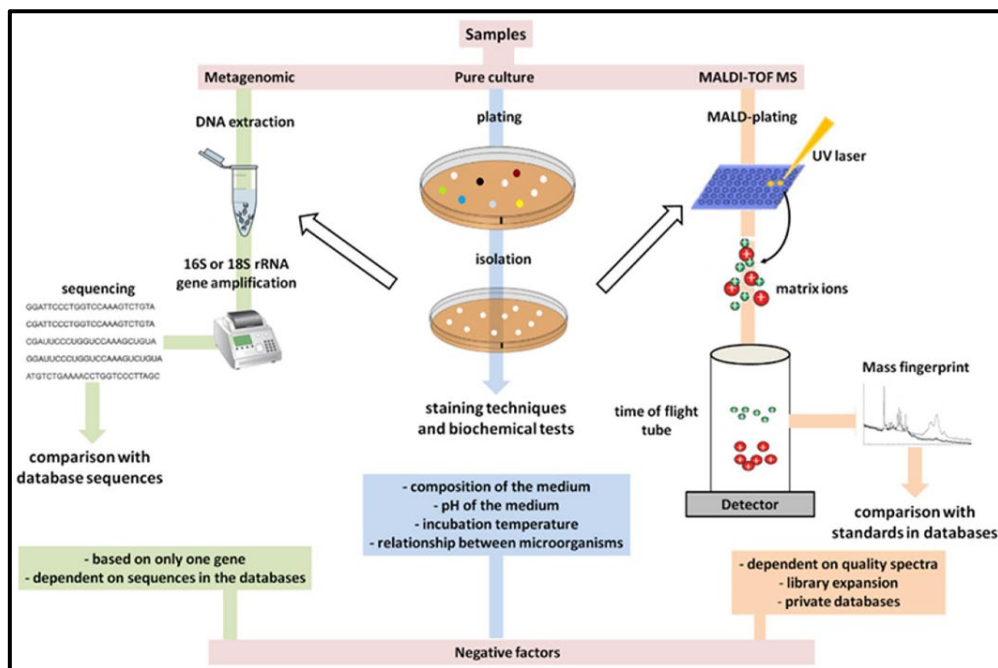


Figure 2: Microbial Diversity¹⁹

The first human metagenomics studies were mostly confined to the gut microbiome. According to a study carried out by Qin et al., about 3.3 million genes have been identified from the human gut, which revealed the high level of genetic diversity of human microbiome⁹. In subsequent metagenomics studies, different body sites of human beings have been explored²⁰.

Strengths

- Culture-independent identification of microorganisms
- Detection of hard-to-cultivate microbes
- Taxonomic and functional profiling
- Discovery of genomes and genes
- Applicable to large-scale human microbiome projects

Weaknesses

- Necessitates sophisticated sequencing and computing technology
- Possible contamination with host genome in human samples
- Incomplete databases for reference

- Sequencing does not automatically imply microbial activity
- Interpretations depend on methodology used

2.2 Human Microbiome Project and Microbial Diversity

Human Microbiome Project served as a landmark achievement in human microbial diversity studies²¹. This project included the investigation of several different body sites of 242 healthy adults and provided valuable references for the study of the human microbiome. This project illustrated that microorganism populations were highly diverse among body sites and individuals.

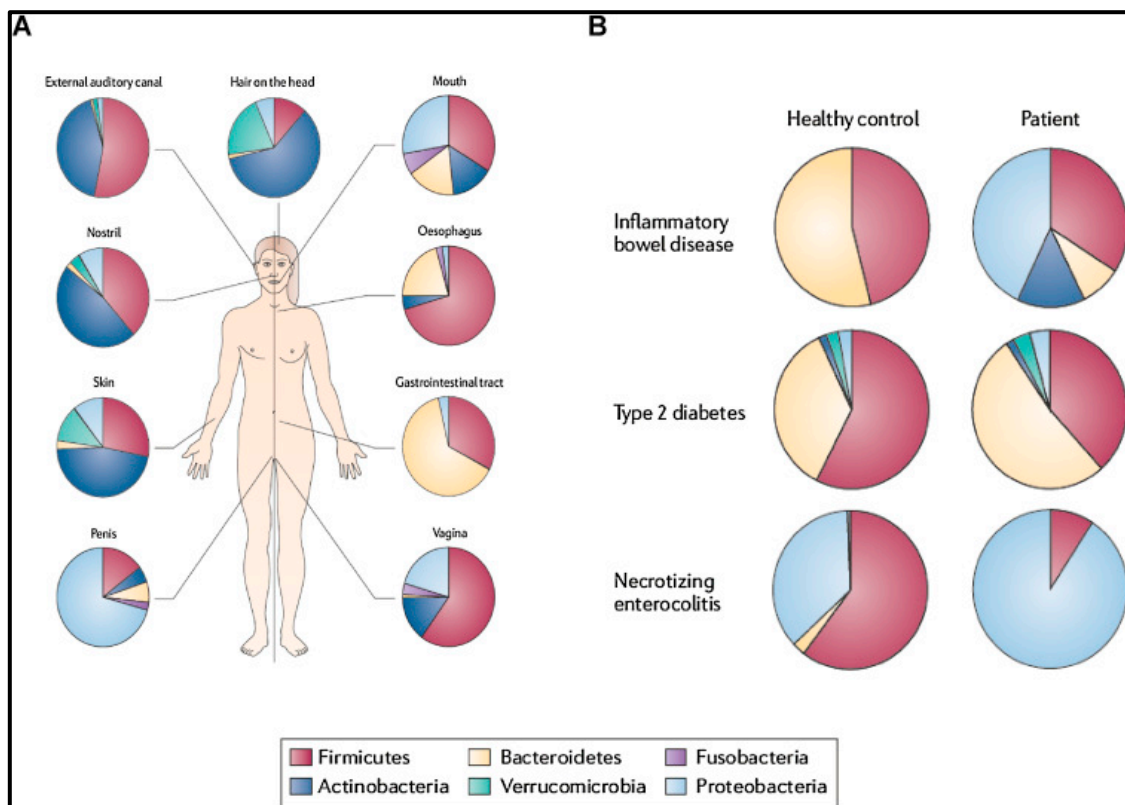


Figure 3: Human Microbiome Project²²

There were specific microbial communities present in the gut, mouth, skin, and other body regions, depending on their respective environments. Nevertheless, despite the differences in the microbial composition, there may be individuals who have similar functions. It became apparent that one had to study not just the presence but also the function of microbes in the body.

Strengths

- Created a significant human microbiome database system
- Had a number of human body sites

- Bettered knowledge about variation within microbes
- Gave information on taxonomy and function

Weaknesses

- Inadequate representation of some global populations
- Mainly aimed at healthy subjects
- Reference sequences for uncultured species were scarce
- The cross-sectional data provided inadequate information on changes over time

Therefore, the Human Microbiome Project created a significant basis for later research in human metagenomics.

2.3 Taxonomic Diversity of Human-Associated Microbiota

Metagenomics provides an opportunity for comprehensive description of microorganisms existing in human communities. First gut microbiome investigations have revealed that people contain hundreds of different microorganisms. In their research, Qin et al. found that there were around 1,000-1,150 prevalent bacterial species among investigated people, at least 160 prevalent species in each individual²³.

Nevertheless, these early estimates were restricted by the lack of complete reference genome databases. Metagenomic assembly and reconstruction contributed to the advancement of microbial detection methods and uncovered many new unknown microorganisms.

A study on human gut metagenome assembled more than thousands of genomes and found a considerable number of candidate bacterial species which had no culture counterparts. The results indicated that the real diversity of the human microbiome was higher than traditional reference-based methods.

Strengths

- Offers comprehensive microbial classification
- Recognizes a variety of microbes
- Allows for the comparison of individuals and populations
- Helps to discover new groups of microbes

Weaknesses

- Depends on the quality of reference database
- Similar microorganisms could be hard to differentiate
- Less abundant microorganisms might be overlooked
- Depth of sequencing could affect diversity estimation

2.4 Functional Diversity of Human Microbiomes

Taxonomic diversity on its own will not be able to describe the functions of microbial ecosystems. Microbes carry genes for metabolic pathways related to the metabolism of carbohydrates, amino acids, vitamins, and other biochemical reactions.

Research on human microbiomes has proved that two people can possess distinct microbes but still share the same metabolic abilities. Metagenomics is able to provide data on functional potentials along with the taxonomic information²⁴.

Strengths

- Gene identification of microbial species and metabolic pathway identification
- Provides insight into functional capacity
- Explains functional similarities among people
- Can be combined with transcriptomics and metabolomics research

Weaknesses

- Gene presence doesn't imply gene expression
- Prediction of function depends upon the reference database
- Genetic capability doesn't necessarily mean functional capability of microorganism
- Function results require further verification

2.5 Geographical and Population-Level Diversity

Human microbes show variability depending on the geography, age, diet, environment, exposure to drugs, and many other factors. Comparison studies of humans have shown remarkable differences in microbes and in their functional genes²⁵.

In the work of Yatsunenکو et al., there were significant differences in the gut microbes in humans of the United States, Malawi, and the Venezuelan Amazon. This indicated that human microbes can be affected by population-specific factors and common biological factors.

Strengths

- Microbial features specific to each population are identified.
- The diversity of microbes varies around the world.
- It aids in understanding the impact of environment/lifestyle on microbes.
- Facilitates the creation of reference databases for a wider range of samples.

Weaknesses

- Many populations are underrepresented
- Differences in diet and lifestyle may be confounding variables
- The differences in sampling may affect comparison
- The global diversity of microbes is not fully known

Greater representation of geographically diverse human populations is therefore required.

3. THEMATIC ADVANCES IN HUMAN METAGENOMICS: POPULATION DIVERSITY, UNCULTIVATED MICROORGANISMS, STRAIN RESOLUTION, AND COMPUTATIONAL ANALYSIS

In terms of the human microbiome, metagenomics has increased knowledge regarding the microbial diversity of humans through the study of microbes in an uncultured manner. Some of the advancements in the field include geographic differences, uncultured microbes, genomic reconstruction, strains diversity, sequencing methods, and computation²⁶.

3.1 Geographical and Population-Level Diversity

There is huge variability in human microbial populations between different geographical and demographically distinct populations. Diet, age, lifestyle, use of drugs, environment, economic status, and cultural traditions may affect the microbial community. A large number of insect pests cause considerable damage to the crop²⁷. Yatsunen et al. compared gut microbial populations of 531 people from the US, rural Malawi, and the Venezuelan Amazon and found large variations in bacterial populations and the functional gene pool between different populations. But there are also similarities in development between different populations especially during childhood period²⁸.

These observations have shown that human microbial diversity is affected by both universal biological mechanisms as well as population specific influences. This variability also shows that it is important to create metagenomic databases with geographic variability. Populations in Asia, Africa, Latin America and other underrepresented regions need to be represented more.

3.2 Uncultivated Human-Associated Microorganisms

Another contribution of metagenomics that can be highlighted is that it enables scientists to discover microorganisms that cannot be grown using traditional laboratory methods²⁹. This is because certain microorganisms associated with the human body need special nutritional or environmental conditions for survival. Through metagenomics, genetic material of such microorganisms can be obtained directly from the human body without their cultivation.

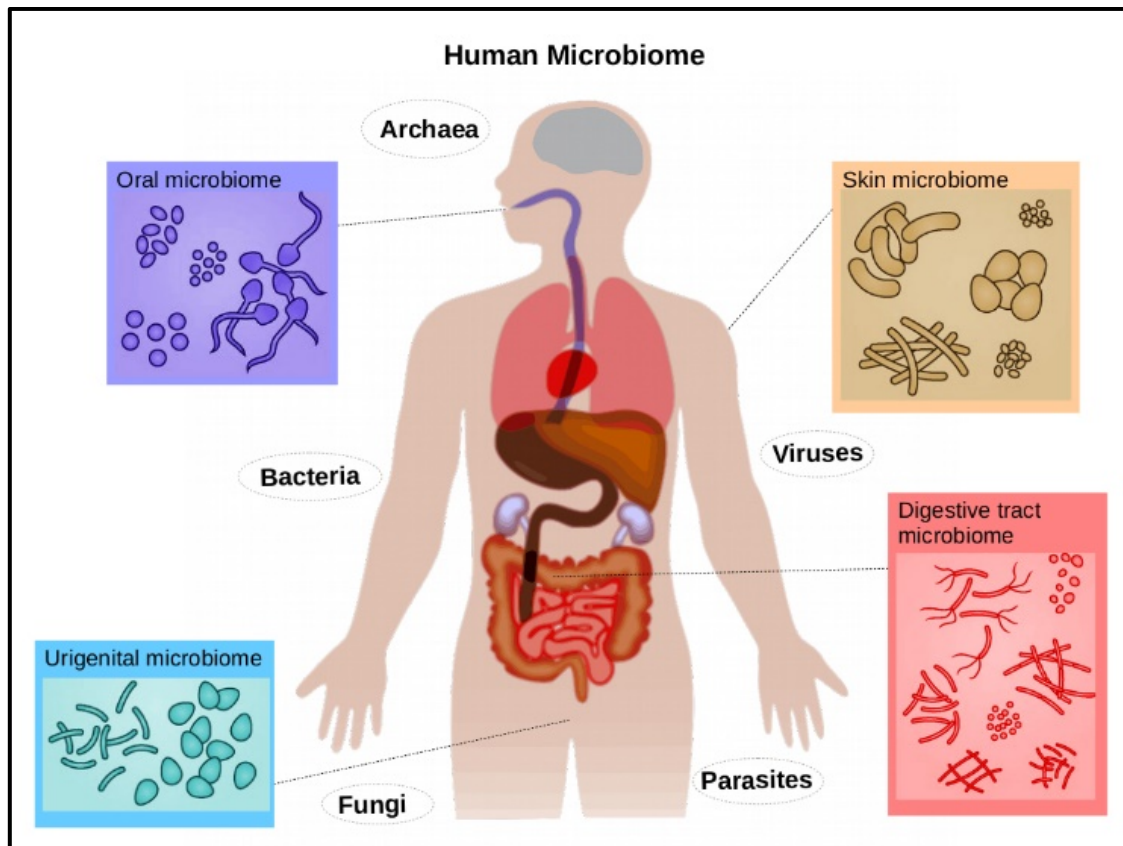


Figure 4: Human Microorganisms³⁰

Human gut metagenomic studies have provided knowledge about thousands of new microbes that were previously unknown. Nayfach et al. managed to assemble 60,664 genomes of prokaryotes based on human gut metagenomes, while Almeida et al. managed to assemble 92,143 metagenomic assemblies and found many new uncultured candidates for microbial species. This proves that metagenomics is not only useful in finding known microbes but is a powerful discovery tool as well³¹.

3.3 Metagenome-Assembled Genomes and Strain-Level Diversity

The process of metagenome-assembled genomes (MAGs) is one where the MAGs are assembled from sequencing data generated directly from microbial communities³². The process usually includes steps such as sequence filtering, genome assembly, binning of the genome, assessment of genome quality, and taxonomic and functional annotation. MAGs have proven very useful in the study of uncultivable microorganisms.

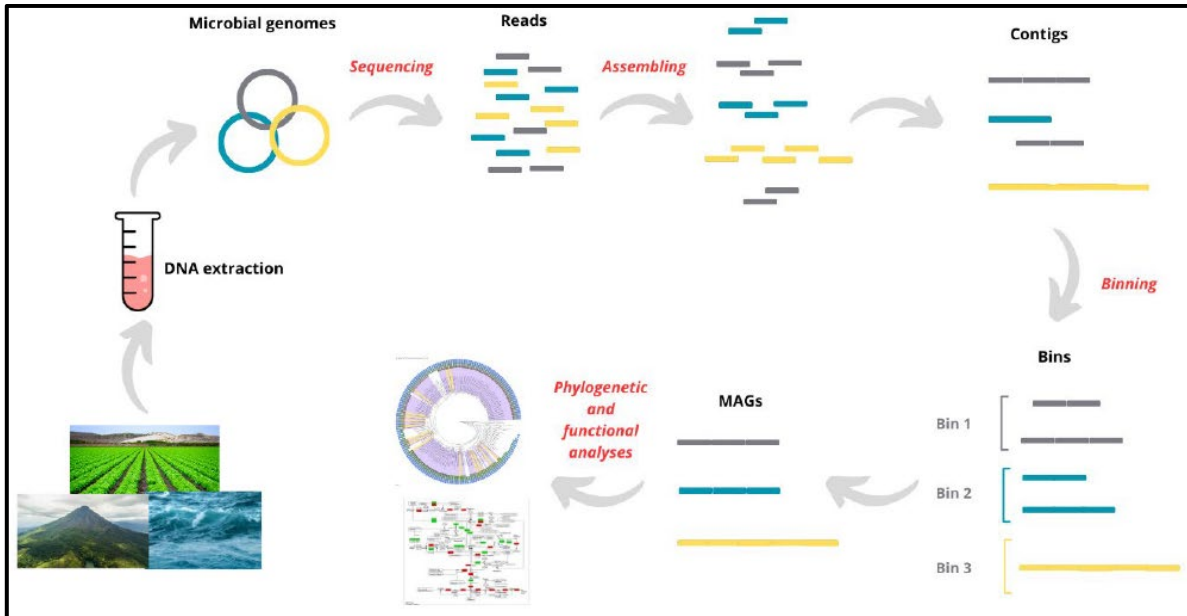


Figure 5: Metagenome-Assembled Genomes (MAGs)³³

Metagenomics has not only contributed to genome reconstruction but also helped in understanding the diversity at the strain level. The various strains of the same species have different genes, different metabolisms, and different ecologies. This means that two people can be carrying the same species of microorganisms but of different genetic strains. Strain-level analysis gives more insight into the microbial diversity of humans than species-level analysis.

3.4 Advances in Sequencing Technologies

Sequencing of 16S rRNA genes has been traditionally used for the identification of bacteria and archaea due to their cost-effective nature and ability to be applied at a high throughput scale. However, such analysis offers little information about microbial genes and functions and lacks resolution for further investigation at the strain level. Metagenomic shotgun sequencing represents an advanced approach due to the fact that it sequences all DNA in a whole microbial community³⁴.

Long-read sequencing technology has helped make metagenomics studies even better because it has made it possible to sequence long stretches of DNA, which would be helpful in assembling genomes and strain differentiation. Short-read sequencing can make fragmented assemblies due to the presence of repetitive sequences or because of similar strains, but long reads have the ability to link difficult to assemble genomic regions. This should help in getting more complete microbial genomes.

3.5 Metatranscriptomics and Functional Microbial Activity

Metagenomics analyses the microbial genes, thereby giving information about the genetic potential of the microbes in question. However, the presence of the gene does not always mean its expression. This gap is filled by metatranscriptomics since it involves the analysis of RNA of the microbes to find out what genes are expressed³⁵.

Metagenomics and metatranscriptomics combination will therefore help differentiate between the organisms and their genes and the functional expression of those microbes. Such an approach can be helpful in understanding how human microbiome reacts to diet, disease, medicine, and environmental factors. Metatranscriptomics analysis is however much more complicated compared to metagenomics due to the instabilities of RNA.

3.6 Bioinformatics and Computational Analysis

Bioinformatics is very important in order to process and analyze huge amounts of data that have been obtained during metagenomic sequencing³⁶. The common pipeline includes such stages as sequencing, quality control, removing of DNA of human origin, taxonomy, metagenome assembly, gene finding, functional annotation, genome binning, MAG evaluation, and statistics. Such approaches allow analyzing a lot of human samples.

Despite all this, however, disparities in software, reference databases, sequencing depth, statistical methods, and quality control practices can lead to inconsistencies. Partial reference databases might also cause some human-associated microbes not to be identified. It is thus very important to have standardized bioinformatics pipelines and quality control practices when conducting human metagenomics research³⁷.

4. APPLICATIONS OF METAGENOMICS IN HUMAN MICROBIAL DIVERSITY STUDIES

Metagenomics has developed into a valuable technique for studying the make-up and diversity of microorganisms found on various parts of the human body. It allows the study of the microbial communities in the human body without culturing them by analyzing the microbial DNA extracted from human samples. Metagenomics allows the identification of microbes that are not easily cultivable, both those which are highly abundant as well as those which are present in low abundance. This technique has allowed a better understanding of the differences in microbial communities among various body parts and individual human beings³⁸.

Metagenomics is also used in studying microbial diversity in connection to human health and disease. Metagenomics allows researchers to find out any differences in microbial diversity, abundance of certain genes, and metabolic pathways in healthy versus diseased patients. For example, comparative metagenomics could reveal microbial signature that is connected to gastrointestinal, dental, metabolic, respiratory diseases, and other human health problems. Yet, care must be taken when interpreting the associations of the microbiome with certain diseases, because presence of different microbes does not mean that there is any causative effect on health³⁹.

Another growing application of metagenomics is in the identification of new species of microorganisms in association with humans. The use of methods such as genome reconstruction and the metagenome-assembled genomes method has allowed researchers to gain genomic information about microorganisms that cannot easily be cultivated. This has helped in expanding the database of microbial species in relation to humans and has improved their taxonomic and functional classification. Strain-level metagenomics can further identify genetic differences between closely related microorganisms, providing greater resolution for understanding individual and population-level microbial diversity⁴⁰.

Lastly, the technique of metagenomics has practical uses in studying the human microbiome on a personalized or population level scale. Metagenomics is suitable for the study of how elements like age, diet, geographic location, lifestyle, drug treatment, and any other factors associated with humans affect the microbes⁴¹. With the combination of metagenomics with other techniques such as metatranscriptomics, metabolomics, etc., information on both the potential and functioning of microbes can be obtained. Consequently, there is significant potential for the advancement of human microbiome research using metagenomics.

Table 1: Summary of Selected Metagenomic Studies⁴²

Author(s) & Year	Study Focus	Methodology/Model	Key Findings
Nwachukwu & Babalola (2022)⁴³	Agricultural microbial diversity	Metagenomic review	Metagenomics improved characterization of microbial diversity and functional potential in agricultural systems.
Grossart et al. (2020)⁴⁴	Aquatic microbial ecology	Metagenomic review	Metagenomics linked microbial community composition with metabolic and biogeochemical processes.
Saito et al. (2019)⁴⁵	Ocean microbial communities	Metaproteomic review	Metaproteomics provided insights into microbial functions and emphasized standardized data sharing.
Kim et al. (2024)⁴⁶	Human microbiome medicine	Genome-resolved metagenomics	Genome-resolved approaches improved identification of microbial genomes, diversity, and functional potential in human microbiomes.

Chen et al. (2022)⁴⁷	Wastewater microbial communities	Metagenomic review	Metagenomics characterized microbial communities, functional genes, and metabolic pathways involved in wastewater treatment.
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5. DISUSSION

This is an evaluation of the results discussed in the literature review of metagenomics in the study of microbial diversity in humans. The results clearly show that there have been significant improvements in the understanding of the microbial community in humans using metagenomics since it has made it possible to gain culture-independent information on the microbial composition, functional genes, metabolic pathways, uncultivable microbes, and strains⁴⁸. There have been considerable variations in the microbial composition found in different parts of the body, different individuals, and different population groups in humans. However, metagenomic findings have been affected by several challenges.

5.1 Interpretation and Analysis of Current Findings

From the results, it is clear that the use of metagenomics in studying human microbial diversity has become important. Early investigations on the human gut microbiome were able to reveal the genetic diversity in the microbiome, and the Human Microbiome Project was able to illustrate high variability in microbial communities between body sites and individuals. In genome-reconstruction analyses that followed, many unknown and uncultivated microorganisms were found to exist⁴⁹. This proves that traditional methods have only been able to identify some aspects of human microbial diversity. The results also demonstrate that microbial diversity needs to be studied at both functional and taxonomic levels since individuals may have different microbial species but retain similar functions.

5.2 Significance and Implications of the Findings

These results are of significant importance to the study of human-associated microbial communities. Metagenomics can help identify microorganisms from various human body habitats and analyze microbial variability based on factors like geography, age, diet, lifestyle, medicine usage, and diseases. Moreover, metagenomics is a powerful tool for finding new microbial species that cannot be cultivated and for enriching the human microbial genome library⁵⁰. The development of shotgun sequencing and long-read sequencing technologies made it possible to improve taxonomic, functional, genomic, and strain analysis, and metatranscriptomics can add valuable data about microbial activity. But the results obtained through metagenomics should be carefully analyzed since the presence of a microbial gene means its potential but not always its activity.

5.3 Research Gaps and Limitations

Notwithstanding the many advances made, there are a number of challenges in the study of human metagenomics. First of all, there are still many geographical and demographic populations whose representation is inadequate, which might lead to an underestimation of global microbial diversity. Furthermore, partial reference databases might hinder the discovery of microorganisms present in humans, and closely related or rare microorganisms might be challenging to characterize⁵¹. Variations in the sequencing depth, in software, in reference databases, and in quality control methods might also contribute to inconsistencies in the results of different studies. Another important limitation is that metagenomics tends to reveal the potential genetic capacity of microbes, but not their activity, and association does not mean causation.

5.4 Future Research Directions

Future studies need to focus on large-scale, longitudinal, and geographically dispersed human samples for enhanced knowledge about the global diversity of microbes as well as the changes in microbes over time⁵². The increased use of strain-resolved metagenomics and long read sequencing can help to better characterize similar microbes and construct complete microbe genomes. Using metagenomics together with metatranscriptomics and metabolomics can help to differentiate between the genetic potential of microorganisms and the biological role played by these microorganisms. There is a need for standardized sampling, sequencing, quality control, and bioinformatics procedures for enhanced reproducibility.

6. CONCLUSION

Metagenomic studies conducted in the current study conclude that metagenomics has become an efficient culture-independent tool for investigating the microbial diversity of humans, due to the ability to identify and characterize the microorganisms that are poorly investigated using traditional culturing techniques. It can be seen from the discussed findings that human microbiomes contain a great number of diverse taxa and genes. Moreover, advancements in the field of shotgun metagenomics, metagenome-assembled genomes, long-read sequencing, and strain-level analysis allowed to discover many new microorganisms that have not been characterized earlier, as well as to expand human microbial genomes databases. At the same time, the problem of incomplete references, sampling bias, contamination with host DNA, computational demands, methodology variations, and lack of functional analysis is quite critical. For this reason, future research will need to consider human populations of different ages and locations, standardization of the methodologies, improvement of the microbial reference databases, functional validation, and incorporation of the multi-omics analysis.

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