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Microbiome Research: Application of "Omics" Technology

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ABSTRACT

Microbiome research is a thriving field focused on characterizing the composition and functionality of microbial populations or microbiomes from a wide array of ecological niches. Microbiomes occupy living organisms, soil, the atmosphere, and bodies of water and exist in moderate and extreme climates. Understanding the intractable microbial universes in various environments is challenging and potentially rewarding to humankind. Historically, elucidating pathogenic microbes and their impact on host species has dominated microbiome-based studies. Moreover, a tiny percentage of microbes can be cultured using classical culturing methods. With advancements in high throughput experimentation and computational tools derived from microbial ecology, there is a driving force to gain insight into the entire microbial consortium from various environmental and biological locations. Metagenomics, the study of all the microbial genomes in a sample using sequencing techniques (e.g., 16s rRNA amplicon sequencing and shotgun sequencing), has so far dominated the types of investigations conducted in the field of microbiome research. More recently, however, researchers are becoming increasingly interested in better understanding the complex microbe-associated molecular network and specific protein and metabolite functions associated with microbial genetic potential. Metaproteomic, meta transcriptomics, and metabolomics are three potent methods to accumulate information about microbial proteins, messenger RNA, and metabolites in a microbial community. These methods are currently being applied in laboratory settings to address our general lack of understanding of microbe-microbe interactions and microbe-environment interactions.

Keywords: Metagenomics; Metaproteomics; Metatranscriptomics; Metabolomics

Introduction

Over the last two decades, there has been a swift transition in our understanding of the microbiome. The microbiome refers to the entire collection of microorganisms in a particular ecosystem. Historically, the apparent focus was to investigate pathogenic microorganisms that cause human disease and to develop methods to reduce or eliminate them from the body. While profuse studies have been conducted on pathogenic organisms, the scientific community is becoming increasingly interested in understanding the non-pathogenic members of the microbial community associated with humans. It is becoming clear that microbiomes are predominantly advantageous to their host or resident environment and help maintain a highly evolved ecological balance that, when disrupted, could have negative consequences. Today, high throughput protocols have fast-tracked our understanding of the extensiveness of the microbial diversity inhabiting the environment and living

organisms [1,2]. In the past, a significant roadblock in cataloging microorganisms inhabiting the planet has been a lack of molecular technology and computational tools to identify and classify the taxonomic members of a particular microbial community. For many decades we have known that there were massive gaps in our understanding of the abundance and types of microbial taxa in the biosphere. Before the advent of high throughput protocols and analytical software, our knowledge of microbial composition in a particular environment relied on growing microbes in the lab and conducting morphotype analysis. In 1985 Staley and Konopka highlighted the insufficiency of culture-based methods to effectively verify the existence of a microorganism in environmental samples [3]. The "great plate count anomaly" asserted by Staley and Konopka characterizes the inability of microbiologists to culture bacterial and fungal species using traditional laboratory media and culture techniques.

Millions of bacterial species grow in extremely inhospitable ecological niches; thus, it is challenging to formulate media based on their unique physical and chemical propagation requirements. Additionally, bacteria growth, for instance, relies on individual polyfactorial interactions with highly evolved molecular communication and response mechanisms that may not exist on a culture plate. Moreover, plate counts do not accurately reflect the assortment of microbial species present; plate cultures typically mispresent diversity and reflect dominant species that respond more favorably to the culture conditions. Based on the longstanding limitations of the utilization of culturing techniques to examine the biodiversity of microorganisms, it was recommended that molecular extraction and sequencing methods or culture-free approaches would generate a more precise assessment of the microbial taxonomy present in a sample [4]. Rhoads and colleagues [5] examined bacterial composition in chronic wounds using culturing and molecular sequencing strategies. They found that by employing 16S rRNA sequencing, they could identify 338 bacterial taxonomic groups compared to only 17 bacterial taxonomic groups using exclusively aerobic culture methods. This study and similar studies suggest amplicon and shotgun sequencing yields much higher bacterial resolution than classical culturing approaches. Current techniques to understand microbial composition still utilize basic culture methods and culture enrichment protocols; however, often, next-generation sequencing methods precede the culture of microbes of interest or culturing, and sequencing are performed in parallel to counteract bias that results from the utilization of sequencing-based methods alone [6,7]. The focus of this review is to discuss how metaproteomics, metatranscriptomics, and metabolomics are driving microbiome research using recent experimental evidence to document their interdisciplinary significance.

The field of microbiome research has seen an explosion in the last few years. The amount of microbiome publications has increased at a phenomenal rate over the last two decades. As is the case for any discipline, the rate of comprehension and experimental breakthroughs depends largely on the development of technological advancements. In the early stages, microbiome researchers focused on high throughput metagenomic studies [8,9]. Metagenomics using 16s rRNA sequencing or shotgun sequencing explores the genetic information of uncultured microorganisms following DNA extraction and DNA sequencing of various samples. These types of studies have significantly improved our understanding of the diversity of microorganisms in insects [10], plants [11], soil [12], and aquatic environments [13]. Since the introduction of metagenomic protocols into the research landscape scientists have pursued strategies to improve taxonomic resolution potential [14]. Adopted an approach that targets several variable regions of the prokaryotic 16S rRNA gene to improve performance and reduce primer bias. Compared to a 16s rRNA sequencing scheme that only focused on one variable region which only produced 44%-61% predictive values, targeting multiple regions on the 16s rRNA gene yielded 65%-91% predictive values. Metagenomics facilitates the unearthing of fastidious microorganisms from myriad environments that are challenging to culture.

Metaproteomics

Metagenomics studies have established a link between microbiome modulations and colorectal cancer [15]. However, recent work has also explored the potential impact of microbial proteins in the development and progression of colorectal cancer. While metagenomics is instrumental in identifying the microbial taxa present, metaproteomics helps provide information about microbial function. Metaproteomics refers to the characterization and quantification of microbial proteins within a complex microbial community and provides insights into microbial phenotypes. Characterizing the microbial proteins associated with human diseases opens the door for a deeper understanding of how the microbiome contributes to health and disease at the protein level [16]. For example, [17] compared the intestinal microbiome proteome of colorectal cancer patients and healthy individuals and identified 341 microbial proteins associated with colorectal cancer. The human fecal metaproteome was also analyzed to determine the nature of the high abundance of proteins observed with the transition to a healthier lifestyle and reduced body mass.

Metaproteome analysis revealed an enhancement of microbial proteins associated with the hydrolysis of carbohydrates [18]. This result is significant considering that a reduction in carbohydrate breakdown has been related to several metabolic disorders. Recently, [19] designed an experiment to assess the types of microbial proteins in the sputum of cystic fibrosis patients. This approach involved microbial enrichment steps to increase the

number of microbial components in the highly heterogenous sputum sample. Utilizing their enrichment protocol, the sample rose from 199-425 bacterial proteins and protein groups in nonenriched samples to 392-868 bacterial proteins and protein groups in enriched samples. It was determined that the arginine deaminase pathway and additional proteases might play a role in adverse clinical outcomes in cystic fibrosis patients. These studies are critical because they allow for a broader understanding of the impacts of pathogens and opportunistic microorganisms in disease progression. It also points to establishing more effective therapeutic options to treat respiratory diseases. The development of proteomic-based databases, such as ProteoClade, will facilitate our ability to assign specific microbiome-based proteins to appropriate taxonomic groups to assess the functional relevance of various microbiome members more efficiently [20]. Recent reviews pontificate the importance of microbiome-based proteomics data on individualized medical approaches and the potential problems associated with microbial proteomics findings [21].

Metatranscriptomics

Metatranscriptomics is an essential tool to evaluate gene expression profiles of specific microbial communities [22,23]. Additionally, metatranscriptomics allows scientists to compare changes in gene expression patterns due to environmental variations such as changes in healthy and unhealthy individuals, polluted and non-polluted environments, anthropogenic and nonanthropogenic factors, and many other different conditions to examine microbial function at the gene level. Metatranscriptomics also offers insight into gene regulation mechanisms which may provide clues as to potential effects associated with clinical issues, climate change, diet alterations, environmental perturbations, and pharmacological intervention. Recently, scientists conducted metatranscriptomic studies to examine the gut microbiome gene expression changes. For example, a metatranscriptome study was performed to investigate gene expression profiles of the duodenale microbiome in obese and lean humans [24]. They found that the human and microbial gene expression landscape differed for the two groups. Specifically, pathways associated with catabolic and anabolic processing of proteins, lipids, and carbohydrates in obese study participants were distorted compared to lean study participants. Additional studies are necessary to explore the genetic crosstalk between human and microbial gene expression. During disease development, do microbial gene products activate human genes, or do human genes activate or suppress microbial genes? Answers to these questions will provide necessary details that are currently unclear.

Inflammatory Bowel Diseases (IBD), such as ulcerative colitis and Crohn's disease, was examined using metatranscriptomics. In one study, [25] compared global microbial gene expression ulcerative colitis and Crohn's disease patients with non-inflammatory bowel disease controls. Evidence showed that specific species exhibited differential transcription levels in diseased patients, and some bacterial species exhibited undetectable gene expression levels. This suggests that immune responses observed in IBD are particular to a subset of the gut microbiome. This type of data can inform bacterial targeting or bacterial restoration treatment strategies. [26] evaluated the transcriptome of the salivary microbiome to identify potential diagnostic biomarkers of oral cancer. The significance of this study is that the investigators examined gene expression results at different stages of oral cancer pathogenesis to determine if unique gene expression signatures existed. Microbial transcriptional profiling of environmental samples such as soil and aquatic bodies is paramount to evaluate the ecological health and response to environmental pollutants. For example, the soil microbiome was evaluated to examine the effects of phenanthrene, an organic pollutant, on soil microbial gene signatures. As expected, genes involved in aromatic compound metabolism, detoxification, and the stress response were upregulated [27]. These types of sequencing studies may reveal new molecules beneficial in bioremediation approaches.

Metabolomics

Metabolomics is another form of microbial community profiling that produces accurate qualitative and quantitative assessments of the metabolites produced in a particular host or environmental setting [28]. This type of molecular assessment involving mass spectrometry, nuclear magnetic resonance, and high-performance liquid chromatography has existed for many years. It is primarily used in medical and clinical applications to diagnose and prevent certain diseases [29]. Metabolomics supports the identification of microbial metabolites responsible for conferring the phenotype of the host organism. Moreover, compared to metagenomics, metabolomics provides a more robust interrogation of how the microbiome mediates various outcomes at the molecular level. Metabolites are essential molecules that mediate metabolic processes and may be products of metabolic pathways. Primary and secondary metabolites have several cellular and extracellular functions and play roles in metabolic regulation, toxicity, defense, cell stimulation, cell communication, and cell signaling. Metabolite estimation enables the comprehension of the central molecules that facilitate functional outcomes caused by the microbiome or alterations to the normal microbiome. Microbiome-based metabolites control microbial and non-microbial responses and regulate homeostasis, animal and environmental health, physiology, and disease.

This experimental investigation provides further insight into the microbiome's specific molecules and the molecular causes of disease outcomes, biological mechanisms, and environmental

responses to changing conditions. Current studies explore the role of metabolites in an entire microbial community or specific taxonomic unit. The metabolic pathways that utilize or produce metabolites can be identified, organized, and visualized using computational integrated pathway analysis software. Metabolomics-based studies have uncovered valuable data about the microbial metabolome associated with human disease. One study showed that the gut microbiome directly impacts the progression of chronic kidney disease. For example, Feng et al. [30] investigated gut microbial metabolites using a nephrectomized rat model. They identified glycine-conjugated and polyamine metabolites as primary pathophysiologic mediators of chronic kidney disease. Treatment with poricoic acid A and Poria cocos was sufficient to counteract the overabundance of glycine-conjugated and polyamine metabolites and decelerate disease progression. In addition to microbial-derived metabolites altering the trajectory of chronic kidney disease, metabolites produced by the fecal microbiome play a role in colorectal cancer (CRC). [31] isolated fecal microbiota and subjected samples (CRC patients and healthy subjects) to metagenomic and metabolomic examinations. Reduced species diversity detected in the CRC samples was coupled with increased levels of cadaverine and putrescine, clinical biomarkers for colorectal carcinoma [32]. Microbe-associated metabolites were investigated to explore their potential association with allergic and non-allergic asthma in children [33]. Not surprisingly, compared to healthy participants, dissimilar microbial populations were detected in allergic and non-allergic asthma patients. Forty-two microbial-derived metabolites were seen for the allergic asthma group, while there were fifty-eight microbial-derived metabolites for the non-allergic asthma group. This data provides a connection between gut microbial metabolites and childhood asthma and suggests that modulation or suppression of regulatory metabolites may serve as a promising clinical strategy to alleviate asthma symptoms. Microbiome metabolite mining may lead to biotechnologically and medically relevant molecules.

Conclusion

The United States federal government has spent over two billion dollars to complete the Human Microbiome Project and additional microbiome research extrapolation projects. Taken as a whole, microbiome research can fundamentally impact a wide array of disciplines, including microbiology, crop science, bioinformatics, immunology, soil science, biotechnology, neuroscience, and environmental science. In addition, to providing answers to unresolved research questions, microbiome explorations can have important ramifications in tackling global warming, human disease, renewable energy, crop output, and sustainability of ecosystems. Undoubtedly, microbiomes contain hundreds of unknown microbes that produce thousands of biological products of immense commercial value for various industries, including agriculture, biotechnology, and medicine. Combinations of metagenomics, metaproteomics, metatranscriptomics, and metabolomics techniques can be employed to address a variety of biological and environmental research questions [34]. For example, using the methods described in this article, scientists can examine the structure and physiology of prokaryotic and eukaryotic microbes to assess the effects of climate change on beneficial soil, ocean, and plant microbiomes. These techniques also provide excellent opportunities for incorporation in academic settings and integration into the curricula as student research projects and course-based undergraduate research experiences (CUREs) [10].

Microbiome studies focus on three main issues or questions from a research perspective. The three main activity areas of microbiome research include microbial compositional assessment (What microbes are present?), functional assessment (What is the ecological role of the microbial communities present?), and coordination assessment (What are the pathways, mechanisms, and processes that mediate microbial function?). The high throughput technologies described in this article have catalyzed our rapid understanding of the complex microbial communities found in diverse environments. A complete picture of the microbiome's impact on biology and human and environmental health will rely on the integration of metagenomics, metaproteomics, metatranscriptomics, and metabolomics data forming detailed networks of genes and gene products and how they contribute to promoting a healthy individual and a healthy planet. Moreover, the data generated from these molecular techniques can be utilized to diagnose diseases and identify biomarkers. Using this immense source of cell-based and biomolecular data, we can understand how microorganisms within a particular ecosystem work together to promote the health of animals and environmental health. Cross study comparisons in which scientists utilize the same equipment, protocols, computational software, and analytical techniques are needed to enhance knowledge harvest. We are still at the beginning stages of deciphering the uses of metaomics technology. Based on the studies conducted worldwide, metaomics technology will continue to expand and increase our understanding of the composition and functionality of microbial communities.

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