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Metagenomics and Single-Cell Technologies for Microbiome Big-Data Mining: Precision Medicine in the Making

Sreenivasa Rao Veeranki and Manish Varshney

Department of Computer Science and Engineering, School of Engg. & Tech., Maharishi university of Information Technology, Lucknow, INDIA

Abstract

The microbiome is no longer "microbial black matter" thanks to advances in high-throughput sequencing and analytics. First, we described the existing big-data mining technologies, such as single-cell sequencing and metagenomics, that are currently being applied. Finally, we discussed the integration of different approaches, demonstrating the huge benefits of characterising the microbiome in its entirety from many angles at the same time. The usefulness of big-data mining in clinical practice was further shown by our discussion of the relationship between gut microbiota and host organs and illnesses, as well. New thoughts on the trend of big data mining in the microbiome employing multi-omics techniques and single-cell sequencing were suggested at the end of the study Multi-omics techniques and single-cell sequencing may give a complete knowledge of the microbiome at both the macroscopic and microscopic levels, therefore helping to personalised therapy.

INTRODUCTION

Strategies for Big-Data Mining

According to several studies, the microbiome in the human gut has a strong influence on a person's overall health and well-being, affecting everything from metabolism to nutrition to physiology to immune function. Because of this, the study of the microbiome in the human digestive tract and its connection to illness has enthralled a large number of researchers. When it comes to bacterial species, the human gut microbiome contains 15,000 to 36,000 strains of bacteria (Frank and colleagues, 2007), with an estimated total cell count of 1013 to 1014, which is comparable to that of human cells (3.0 1013). (Sender et al., 2016). More than 100 times as many genes are found in the gut microbiota compared to the 25,000 genes found in humans (Gill et al., 2006). The gut microbiome has a wealth of information that may be mined through sequencing, rather than conventional cultural techniques. Sequencing is a prerequisite for getting gut microbiome raw genetic materials, which are then assembled and annotated for taxonomy and function. Data mining in microbiome communities is now being approached in several ways, including the following (Table 1).

Methods	Advantages	Disadvantages	Solution
Amplicon sequencing	(1) Relatively low cost; (2) Taxonomic annotations of uncultured microbial communities.	(1) Low resolution: cannot identify microbes at species or stain level; (2) Cannot realize functional annotations of microbial communities.	(1) Combined with metagenomics; (2) Use PICRUSt to obtain predicted metagenomics and functional annotations.
Metagenomic sequencing	(1) Taxonomic and functional annotations of uncultured microbial communities; (2) Obtain the full genetic repertoire of the microbial communities.	(1) Difficulties in metagenome assembly and taxonomically and functionally assign accurately; (2) Lack of high genome coverage; (3) Cannot link all the functional genes of one microbe to its phylogeny.	(1) Long-read sequencing and improved algorithms for assembly; (2) Combined with single-cell sequencing.
Single-cell sequencing	(1) Taxonomic and functional annotations of uncultured microbes at cell level; (2) Generate a high-quality genome for microbes with low abundance; (3) Dissect virus-host interactions of uncultured microbes.	(1) Difficulties in cell sorting; (2) Easily influenced by contaminated DNA; (3) Uneven read coverage, chimeric reads caused by MDA.	(1) Combined with metagenomics; (2) Improved experimental operation and various computational approaches to control DNA contamination and errors caused by MDA.

Amplicon Sequencing

Amplicon sequencing employs microbe-specific marker genes, such as 16S ribosomal RNA for bacteria and Internal Transcribed Spacer (ITS) for fungus, to identify microbial species. In an uncultured microbiome, assigning reads to reference reads helps to answer the question "who is there?" This sequencing approach primarily addresses that question. However, amplicon sequencing has a poor resolution level (cannot reach species or strain level) and a lack of functional annotation, which mainly restricts its use. To address these issues, amplicon sequencing and metagenomic sequencing are now being used together. Researchers may first gain a sense of the microbial community's makeup via amplicon sequencing, which is very inexpensive. They may then use metagenomic sequencing to verify the concept from both a phylogenetic and functional standpoint.

Metagenomic Sequencing

All cells in a community are extracted, DNA fragmented, sequenced, and the taxonomic makeup of the community is determined using a variety of techniques such as marker gene analysis, binning, or contig assembly. At a high degree of precision to the strain level, metagenomic sequencing may reveal "who is there" and "what are they doing." Predicting the function of metagenomic reads encoding proteins may be done by many methods such as the recruitment of gene fragments, categorization of protein families, and de novo gene predictions (Sharpton, 2014). Metagenomics sequencing has the following drawbacks. In the first place, the short reads generated by next-generation sequencing and the difficulty of sequence assembly, particularly when numerous species are involved, restrict the usefulness of this technology (Sczyrba et al., 2017).

Single-Cell Sequencing

Serial dilutions, microfluidics, flow cytometric, micromanipulation, or encapsulation in droplets are used to separate individual cells in single-cell sequencing. Whole-genome amplification and DNA sequencing are the next stages, followed by alignment and assembly of the sequences. The little quantities of DNA contained inside a bacterial cell must be amplified to meet the micrograms minimum need for high-throughput sequencing, which is more than the femtograms (Xu and Zhao, 2018). As an alternative to the traditional polymerase chain reaction (PCR), multiple displacement amplification (MDA) (Dean and colleagues, 2002) employs random hexamer primers annealed on the template and high-fidelity polymerase of the Bacillus subtilis virus phi29 (Blanco et al., 1989). To provide enough genome coverage and decrease amplification error for the subsequent sequencing study, the Phi29 DNA polymerase may function at a moderate isothermal state with a strong strand displacement activity and an inherent 3′–5′ proofreading exonuclease activity.

Single-cell sequencing has a key benefit over metagenomic sequencing in that it can provide a high-quality genome for species with low abundance. These functions may be linked to particular species by distinguishing and validating individual functions within the community. In addition, single-cell sequencing may concurrently recover bacterial genomes and extrachromosomal genetic elements in a cell, allowing researchers to study virus-host interactions at the cell level (Yoon et al., 2011). Since its inception, single-cell sequencing has yielded numerous groundbreaking discoveries, including the identification of bacteria with an alternative genetic code (Campbell et al., 2013), the discovery of gut microbiome cells that utilise substances originating from the hosts (Berry et al., 2013), and the ability to quantify the absolute taxon abundances within the gut microbiome (Props et al., 2017).

The Integration of Single-Cell Genomics and Metagenomics

Individual cell genomes may or may not include the whole genetic repertoire of all bacteria in the environment, but metagenomics is a comprehensive view of the microbiota's genome. Because of this, the combination of these two technologies may compensate for each other's inadequacies (Figure 1). For example, metagenomic reads and contigs may help in the single-cell genomic genome assembly (Mende et al., 2016). When reference genomes are lacking, single-cell genomics may serve as a scaffold for comparison or recruitment of metagenomics (Swan et al., 2013; Roux et al., 2014). Several research has used the merging of single-cell genomics and metagenomics to produce much better microbe genome assemblies from a range of microbial

communities (Dupont et al., 2012; Nobu et al., 2015). The downside of combining these two procedures is that more advanced methods would be required to cope with the accumulated potential faults of each.

FIGURE 1

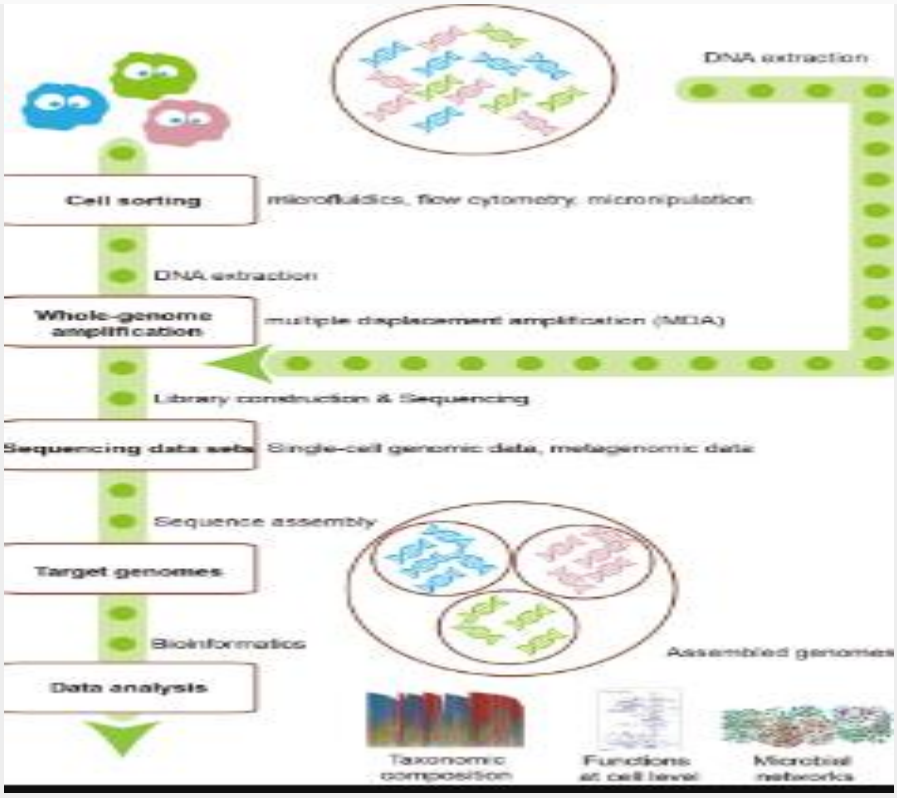

Figure 1 Single-cell sequencing and metagenomics work better together because of the integration. While single-cell sequencing might supply metagenomics with reference scaffolds, it could also improve single-cell sequencing's genome assembly.

The Integration of Metagenomics and Three-Dimensional Genomics

Hi-C sequencing can identify all of the community's chromatin interactions, resulting in a three-dimensional (3D) genome that reflects both the genetic content and the topological chromatin structures as digital information through metagenomics, which can quantify the genetic materials of the community (Belaghzal et al., 2017). The genetic structure and composition of a microbial community may be seen in complete detail thanks to the combination of metagenomics and 3D genomics. Hi-C for single-cell analysis was also used in recent work to get 3D genomes of individual cells (Nagano et al., 2017).

Microbial Multi-Omics Analysis

Research on microbial communities, known as "multi-omics analysis," is possible because of breakthroughs in sequencing technology and bioinformatics. The metagenome, metatranscriptome, metaproteome, and metabolome are all included in this study. An organism's metagenome provides information on its taxonomic makeup as well as its expected functional expression. The expected roles of the metatranscriptome, metaproteome, and metabolome may be confirmed, revealing even more about how bacteria behave as a group. There are a variety of omics approaches that may give valuable insight into a microorganism's ecology.

The Connection Between Microbiota and the Human Body

Food consumption and environmental exposure, such as the use of antibiotics (Pérez-Cobas et al., 2012; Raymond et al., 2016), may have a significant impact on the microbiota of the human digestive system. Those factors would then be interpreted by the gut microbiota, which would produce signals affecting human distant organs including the liver, the brain, and the lungs, as seen in Figure 2. It is possible to use both microorganisms' structural components and the metabolites they make as a signal molecules. Both directly and through neurons or hormones from the stomach, these signals can influence metabolism in distant organs. (Schroeder and Bäckhed, 2016)

FIGURE 2

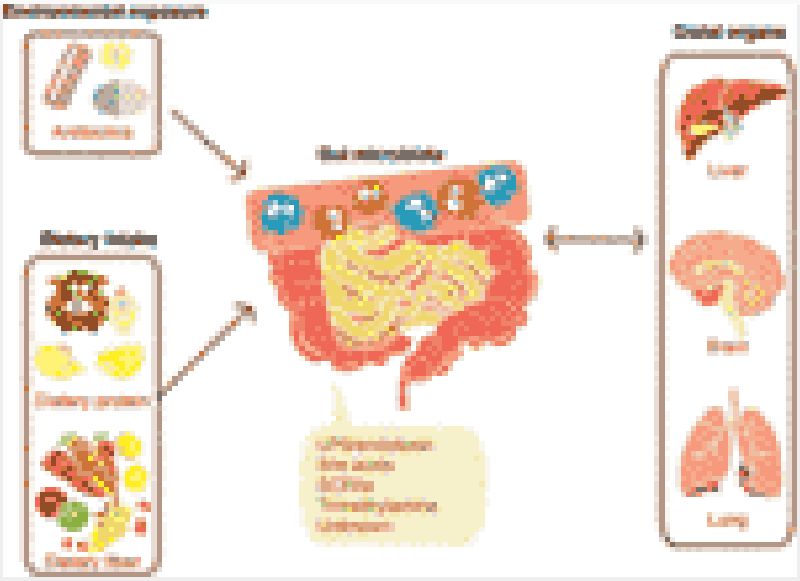

Figure 2 Gut-to-distal organ communication. Gut microbiota may be modulated by a variety of variables, including nutrition and exposure to the environment. Distal organs will be affected by changes in gut microbiota through signal molecules consisting of their structural components, such as lipopolysaccharide (LPS), and their metabolites, such as short-chain fatty acids (SCFAs).

Gut–Liver Axis

The role of the gut microbiota in regulating liver metabolism has been established (Kim et al., 2007; Khalsa et al., 2017). Microbiota in the distal small intestine and colon, for example, may modify BAs produced from liver cholesterol (Schroeder and Bäckhed, 2016). Deconjugation by ileal microbiota after they enter the small intestine allows primary BAs to avoid absorption and be further chemically modified by colonic microbiota, which further enhances their bioavailability (Midtvedt, 1974; Swann et al., 2011). To affect the metabolism of the host, BAs may activate nuclear receptors such as the farnesoid X receptor and the G-protein–coupled receptor (Fiorucci et al., 2009).

Gut-Brain Axis

The dual autonomic nervous system and the endocrine system work together to link the brain to other organs. It is described as the interplay between the central nervous system and the digestive system, which includes afferent and efferent neuronal, endocrine and nutritional signals (Romijn et al., 2008). According to several studies, the gut microbiota has an impact on how our brains develop and respond to stress, and it may even be the cause of stroke (Schroeder and Bäckhed, 2016). Due to the difficulty of conducting human studies on brain shape, most research has been conducted on mice. The gut microbiota has been observed to change the structural

integrity of the amygdala and hippocampus in contrast to germ-free mice (Luczynski et al., 2016). The prefrontal cortex was hypermyelinated in germ-free animals, increasing hippocampal neurogenesis (Hoban et al., 2016).

Gut–Lung Axis

In recent years, the gut-lung axis theory has gained traction, although more research is needed to elucidate its workings. To begin, food intake has the potential to influence both the flora in the stomach and the flora in the lungs (Marsland et al., 2015). Short-chain fatty acids (SCFAs), a kind of lipid found in higher concentrations in the diet, are linked to changes in gut and airway microbiota (Trompette et al., 2014). A high-fat diet, on the other hand, is linked to changes in the makeup of the gut microbiota and increased sensitivity to allergens in the airways (Myles et al., 2013). There are several interactions between microbes, metabolites, immune cells, and the lung in the gut–lung system. The anti-inflammatory actions of bacterial metabolites such as SCFAs may be exerted in other organs through circulation.

Microbiota and Clinical Medicine

Gastrointestinal Disease

The absorption of nutrients and water is a primary function of the gut in humans. Figure 3 shows the intestinal barrier, a vital barrier in the gut, which blocks the transmission of hazardous chemicals and infections. Increased intestinal permeability may come from the breaking of this barrier by pathogenic microorganisms. As an example, enteropathogenic E. coli (EPEC), for example, induces the loss of enterocyte microvilli and the creation of an elevated pedestal structure for the establishment of solid bacterial attachment (Lapointe et al., 2009). Enterohemorrhagic E. coli also has an attachment and effacement locus, although it has less of an impact on the barrier (Kaper and Nataro, 2004). Additionally, diarrhoea may be caused by enteroaggregative and enterotoxigenic E. coli, which affect the intestinal epithelium's chloride secretion (Dubreuil, 2012). Pathogenic microorganisms in the intestinal lumen may be identified via single-cell sequencing.

FIGURE 3

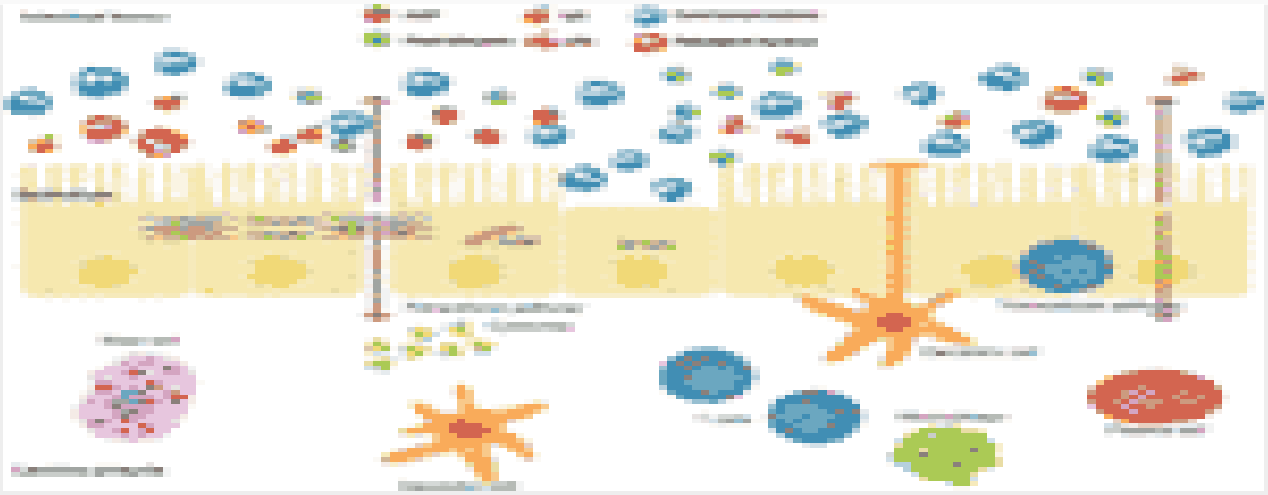

Figure 3 Factors influencing the intestinal barrier. The mucus layer, the epithelial layer, and the underlying lamina propria make up the intestinal barrier, which protects the gut from hazardous bacteria and chemicals. Antimicrobial peptides (AMPs), secretory IgA, and commensal bacteria all reside in the intestinal lumen and work to keep pathogens at bay. Intestinal surfaces are protected by a coating of mucus. Tight junction proteins like occludin and claudin prevent paracellular transit in the epithelium's single layer of cells. This layer also contains M cells and intraepithelial lymphocytes. There are many immune cells located in the lamina propria. gut barrier function may be affected by a variety of things including allergens in food and lipopolysaccharides (LPS), as well as pathogenic bacteria like EPEC.

Thrombosis

Researchers have found a link between human plasma levels of trimethylamine–N-oxide (TMAO) and an increased risk of thrombosis (Zhu et al., 2016). The microbiome of the gut is particularly important in the production of TMAO (Tang et al., 2013). hepatic flavin-containing monooxygenases convert TMA, which is absorbed in the gut and transformed in the liver into TMAO by gut microbiota, to TMAO by processing phosphatidylcholine, choline, and carnitine (Tilg, 2016). The use of animal products including beef and eggs in persons with pre-existing coronary heart disease has been linked to an elevated risk of catastrophic cardiovascular events (Tang et al., 2013). Antibiotics may also significantly lower plasma levels of TMAO.

Hepatitis B Virus

The gut microbiome has been linked to hepatitis B virus (HBV), one of the most frequent infectious pathogens in the world (Chou et al., 2015). There is a strong correlation between viral clearance and the age at which a person was exposed. The findings of control studies on adult and young mice demonstrated that young mice with immature gut microbiota had an immune-tolerating route to HBV. To speed up the clearance of HBV after the formation of gut bacteria, adult mice's mature gut microbiota enhanced hepatic immunity (Chou et al., 2015). Understanding the virus-host interaction might aid in the development of new HBV therapies. It is possible to study the virus-host relationship using single-cell sequencing (Labonte et al., 2015).

Depression

Hypothalamic–pituitary–adrenal (HPA) axis dysregulation has been linked to depressive episodes (Barden, 2004), whereas normalisation of this axis results in the resolution of depressive systems (Heuser et al., 1996; Nickel et al., 2003). The HPA axis' early programming and lifelong stress reactivity are both influenced by the bacteria in the gut (Foster and Neufeld, 2013). An immature mechanism, the stress response develops in the neonatal period in tandem with the colonisation of the intestines by bacteria. Stress may lead to an increase in intestinal permeability, allowing germs to pass through the mucosa and directly contact both immune cells and neurons in the enteric nervous system (Gareau et al., 2008; Teitelbaum et al., 2008).

AIDS

The human immunodeficiency virus (HIV) disease progression has recently been linked to the gut microbiome (Vujkovic-Cvijin et al., 2013). Mucosal-adherent bacteria enriched in Proteobacteria and low in Bacteroidia were shown to be related to indicators of mucosal immunological disruption, heightened T-cell activation, and chronic inflammation in HIV-infected individuals. Among HIV-infected patients receiving very aggressive antiretroviral medication, a dysbiotic community was observed (Vujkovic-Cvijin et al., 2013). The amount of dysbiosis was also linked to two recognised indicators of illness development, including the activity of the kynurenine pathway of tryptophan catabolism and plasma concentrations of the cytokine interleukin 6. (Vujkovic-Cvijin et al., 2013). This suggests that more research is needed into a possible relationship between colonic bacteria that attach to the mucosa and HIV-related immunopathogenesis.

Cancer

A number of the human stomach (Helicobacter pylori), liver (Opisthorchis viverrini, Clonorchis sinensis), and bladder (Schistosoma haematobium) malignancies have been linked to gut bacteria (Bhatt et al., 2017). For example, H. pylori may cause gastritis and stomach ulcers, which are precursors to gastric cancer (Marshall et al., 1984). H. pylori, on the other hand, has been shown to reduce acid reflux and lower the risk of esophageal adenocarcinoma (Vaezi et al., 2000). As a result, the oncogenicity of microorganisms should be studied and assessed using multi-omics techniques because of their involvement in numerous biological processes.

The Trend of Big-Data Mining for Microbiome

In the past, scientists were unable to get a complete knowledge of the microbiota because of constraints in their ability to gather and analyse microbial big data. The great dimensional complexity of the gut microbiota cannot be met by sequencing methods or analytical tools. It is now possible to study the microbiota from various angles thanks to high-throughput sequencing technologies like MDA (Dean et al. 2002) for single-cell sequencing and numerous statistical analysis tools, such as QIIME for 16S sequencing data (Caporaso et al., 2010) and MetaPhlAn (Segata et al. 2012) for metagenomics data. There will be no complete research of microbiota if current sequencing technologies are not included. First, amplicon sequencing and metagenomic sequencing may be used to collect taxonomic information at different levels. Metatranscriptomics, metaproteomics, and metabolomics may all be used to anticipate and validate the functional annotation predicted by metagenomics. Third, single-cell sequencing may be used to establish the link between a microbe's functions and its phylogeny. Finally, Hi-C sequencing can reveal all chromosomal connections. As a result of integrating these methodologies, we can answer questions such as "who is there?" and "what are they doing?" as well as "how are they doing?" at the microscopic and macroscopic levels. It is necessary to establish the link between clinical illness and microorganisms via extensive big data analysis and rigorous in vivo and in vitro tests before any particular treatment can be developed. In addition, a common pipeline for the future integration of various technologies may create a large number of data sets. The geographical qualities come from large data sets collected across continents, while the temporal aspects come from large data sets collected over extended periods.

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