Human Associated Microbiota Identification by Using Sequence-Based Methods

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Abstract: The microbiota can be defined as the community of microorganisms that live in a particular environment and as we all know, different types of microbes colonized the human body, derived from bacterial communities, microbial eukaryotes and viruses that are specific for each anatomical sites or environments. The next- generation DNA sequencing has allowed more and more advanced sampling and analysis of these complex systems by methods called culture- independent, these methods are indicating the differences in community structure between individuals, between diseased states and healthy and between anatomical sites. The advent of next generation sequencing (NGS) offered a cost-effective method that eliminated the cloning step by amplifying 16S rRNA genes using primers containing sequencing connecters and barcodes. However, this sequencing may not resolve closely related species at all times and probably miss the intra-species diversity. A shotgun sequencing was developed for direct sequencing of DNA. Metagenomics is the practice of sequencing DNA from the genomes of all organisms present in a particular sample, and has become a common method for the study of microbiota population structure and function. As sequencing-based microbiota analysis continues to be the greatest general technique across the arena, this review aim is to provide a general introduction to the technical opportunities and trials of sequence-based identification of human associated Microbiota and for understanding of the human Microbiota and their effect on human health and diseases.

Keywords: Human, microbiota, sequence based methods.

INTRODUCTION

Our bodies harbor a very large array of microorganisms in and outside. Bacteria are the biggest players, and also archaea, as well as viruses and fungi, these are named the human microbiota, together, these microbiotas are extremely complex and varied, which consist of a wild array of Gram-negative and Gram-positive organisms. Different parts of the body, vagina, gut and the skin have separate and dissimilar microbial communities. Microbial communities that associated with the host, play a critical role for human, animal and plant biology and health, Microbiotas also vary from person to person (Davis, 2018).

The interaction among the human Microbiota and immune system has an influence on several metabolic functions in human, such interaction between humans and microbes could be crucial in determining the human disease status. Techniques that using PCR have been altered the observation of the human Microbiota as well as marked the way for the metagenomics founding (Malla et al., 2019).

It is good to mention in this regard the technology that adopted by Carl Woese, Norman Pace and others to identify environmental bacteria, that used sequencing small subunit ribosomal RNA genes (16S rRNA). This technology become a predominant to figure out human microbiota diversity, for phylogenetic of microbial communities (Weinstock, 2012).
NGS and bioinformatic pipelines methodologies, have resulted in a fundamental change in clinical microbiology and infectious diseases due to complex interactions within the microbiota community (Hollister et al., 2014).

Effect of microbiota on human body

The majority of cells in the body are comprised from commensal microbiota and play a key role in human health (Adler et al., 2013). Microbiota plays an important role in the development of many of diseases such as metabolic disorders, liver diseases, respiratory diseases, gastrointestinal (GI) malignancy, autoimmune diseases and mental diseases (Wen et al., 2008).

As mentioned by Johnson et al., (2017), one of main components of the Microbiota (the Bacteroidetes), their genetic changeability and effect on metabolic diseases such as type II diabetes and obesity. Other study reported by (Yiu et al., 2017) suggested that metabolism, body weight and some diseases such as obesity are influenced by the interaction between the, metabolism and Microbiota, and the immune system.

The relationship between host aging and its Microbiota content

Organisms accumulate molecular damage with age such as in proteins and DNA, dysfunctional organelles and undergo compositional variations in the extracellular section (Vaidya et al., 2014). These functional and molecular changes cause organ and systemic decline, and eventually results in death (Zhou et al., 2014). The microbiota responds to exposed to a changing environment, by altering the bacterial species composition of individual and metabolic functions (Childs et al., 2015). The host immune system shows an important role in determining commensal microbial communities by allowing commensals to thrive and selectively eliminating pathogens (Ewald et al., 2015). The progressive or sudden immune dysfunction and generalized inflammation occur during aging, lead to inappropriate surveillance at the interface between the microbiota and host and this can result in dysbiosis or also called an imbalance in composition of bacterial community.

In young humans, associated microbiota is enriched with bacterial taxa such as Bifidobacterium and Clostridiales, while in old humans, associated bacterial communities are enriched with Enterobacteriaceae and have a higher representation of Proteobacteria (Li H et al., 2016; Elderman et al., 2017). The Microbiota or beneficial bacteria can provide nutrients from food otherwise indigestible to humans an prevent colonization by other harmful bacterial species. Recently, the human Microbiota has been described as a biomarker for hepatocellular carcinoma (Rao et al., 2020).

Figure 1: Human-associated microbiota within age (Ottman et al., 2012)
Microbiota studied previous to next generation sequencing (NGS)

Prior to the arrival of NGS methods, many challenges confronted the precise profiling of microbial communities and for characterizing of human Microbiota, a diverse and highly dense community containing only a minor percentage of microbes that could be cultured (Eckburg et al., 2005). Premature studies of the human Microbiota complicated the culturing of the microbes present in samples and studying the interactions between co-cultured microbial taxa (Gibbons et al., 1964). These techniques offered information on an inadequate set of microbial taxa and interactions and they failed to deliver information about the entire community composition and the dynamics between the taxa comprising the total community.

The appearance of NGS technologies in the past decade, has overwhelmed the limitations of studies created on culturing techniques, advancements in NGS have led to an important decrease in the genome sequencing cost, and these advances have allowed the sequencing of some genomes in a day at a cost (Malla et al., 2019).

The human Microbiota sampling

In a Microbiota identification studies, the first step is the collection of stabilized microbial specimens that will be analyzed in various assays. The gut Microbiota is commonly tested from stool, that represents the microbial community of the colonic lumen and the small intestine (Yasuda et al., 2015).

While the skin sampling is controlled by the little microbial biomass that is found on usual surfaces, the moist or dry and sebaceous sites across the body have different ecologies that are not easily differentiate without specified profiling (Grice and Segre., 2011), swab sampling is simplest but recovers only smallest biomass and microbial adhesion can be affected by the type and substance of used swabs (Aagaard et al., 2013).

The respiratory tract is divided into the lower and upper regions, each region involvements different exposures to the external environment and has different properties of the mucosal- epithelial barrier (Nriagu, 2019). Diverse clinical methods and sampling tools have been used to get material from the nasal passages, oral cavity, sinus cavities, the tracheobronchial tree and pharyngeal region. The upper respiratory tract sample is usually taken by using swab that claim different amounts of material in compared with lavage, aspirates, brushings and sputum, sputum can be collected via generation protocols, such as inhalation of hypertonic saline (Perez-Losada et al., 2016; Huffnagle et al., 2017).

The protocols that have used for human Microbiota sampling are sensitive to a set of technical but not biologic effects and for the differences that result from numerous stages of the sampling and data analysis and generation process (Salter et al., 2014). Samples were stored at 4°C and aliquots made within 36 h of sample collection. Processed samples were stored at −80°C prior to nucleic acid extraction (Stokholm, 2012).

Bacterial samples must culture using standard methods on selective and non- selective media, then, based on the growth on selective media characteristics of colonies and cellular morphology, all distinctive bacterial colonies will have been isolated, and all bacterial isolates will be identified biochemically using many automated identification systems such as VITEK-2 system (Gupta et al., 2019).

The differentiation among active and total microbes

Microbiota studies based on sequencing methods is typically depend on DNA as exclusive indication for the Microbiota presence in a sample. Still, DNA from spores or dying or cell-free DNA in a sample may be indication for microbial contact, but it does not essentially indicate an active microbiota in the sample, such as that the presence of a blood Microbiota remains contentious, in spite of that PCR-based sign for bacterial 16S rRNA genes in blood DNA extracts from non-Septic individuals as tries to culture bacteria from the same samples have typically been unsuccessful (Potgieter et al., 2015).

In addition, the adaptation of bacteria to the sever conditions of the stomach has been illustrated, it is hard to distinguish metabolically between active microbes that found inside the stomach and inactive microbe (Wurm et al., 2018).

The bioinformatic and experimental approaches have lately been planned to detect the metabolically active microbes that reveal a thriving microbiota (Fricker et al., 2019). AS mentioned by Chu et al., (2017), the propidium monoazide (PMA) intercalates into double- stranded DNA and preventing it from actuality amplified by PCR, has been used to remove free DNA from dead microbes previous to the sequencing of 16S rRNA gene amplicon (Chu et al., 2017). Numerous groups have revealed that 16S rRNA-based taxonomic microbiota compositions vary between RNA and DNA segments isolated from the same sample, this has been used to distinguish between transcriptionally active bacteria which are recognized based on the RNA evidence and all other bacteria which are identified based on the DNA evidence (Fricker et al., 2019).
The Microbiota identification by sequencing-based methods

Sequencing of 16S rRNA gene is one of the largely used methods for microbial taxa identification (Kim and Chun, 2014). Different sequencing approaches had been used for studying microbial communities including; the targeted sequencing 16S rRNA and the shotgun sequencing of the metagenome, each of these methods can deliver amazingly different results when used in metagenomic analyses. The second Shotgun sequencing methods are usually considered greater for the identification and characterization of Microbiota and microbial communities, as they typically provide a greater level of diversity compared to amplicon sequencing, amplicon-based sequencing matches the DNA sequence amplified by using universal primers based on the highly-conserved 16S rRNA to sequences of known bacterial taxa (Tessler et al., 2017). 16S rRNA differs for each bacterial species, a bacterial species is difficult to define, but is frequently taken as organisms with 16S rRNA gene sequences having as a minimum 97% identity an operational taxonomic unit (OTU). The 16S rRNA gene sequence of about 1.5 kilobases has nine short hypervariable regions that discriminate bacterial taxa; one or more of these regions sequencing is targeted in a community survey (Weinstock, 2012).

Microbiota identification using nucleotide sequencing

This method is still the main tool used for studying the human Microbiota for many reasons including it increasing accessibility and decreasing cost of nucleotide sequencing that improved studies of human-Microbiota (Franzosa et al., 2015). The amplicon5 sequencing is one of the initial and highly prevalent techniques, the single genomic locus is targeted for polymerase chain reaction PCR amplification and the chosen locus must be largely conserved throughout microorganisms.

Then these products are sequenced and compared with known reference sequences database. Amplicon sequencing usually targets the 16S rRNA gene that is practically universal among bacteria, while the 18S rRNA gene and internal transcribed spacer (ITS) sequence variants are progressively common for eukaryotic profiling (Hamady and Knight, 2009; Findley et al., 2013). The amplicon is a DNA or RNA segment amplified during a replication result in the cell or through a polymerase chain reaction (National Academies of Sciences, 2018).

Microbiota Identification by 16S rRNA sequencing

16S rRNA gene sequencing is the “gold standard” for identification and classification of diverse Microbiota species, amplification and sequencing of 16S amplicons are broadly used for profiling the structure of human body microbiota the bacterial 16S rRNA sequence competition has been appeared as a valued genetic technique and can lead to the recognition of new microbes. The 16S rRNA gene hyper variable regions sequences give species-specific signature sequences valuable for Microbiota identification, it serves as a fast and economy to Microbiota identification, it is also accomplished to reclassifying bacteria into entirely new species or genera (Teng et al., 2018). New species that could not been successfully cultured in laboratories, could be described by sequencing techniques. DNA extraction can be vital to the achievement of Microbiota sequencing. The broad variation of cell compositions and membrane structures can posture an important challenge to the well-organized and bias-free extraction of genomic DNA (Hwang et al., 2012). Cell lysis is the first step in DNA extraction protocol and involve physical, chemical and enzymatic disruption (Abusleme et al., 2014).

Figure 3: The Scheme show human associated Microbiota sequence-based identification steps (in this study)
The choice of 16S rRNA hyper-variable regions targeted for sequencing, which have been the most widely used markers to evaluate the phylogenetic diversity of microbes, 16S sequencing uses PCR to mark and amplify portions of the hyper variable regions (V1-V9) of the bacterial 16S rRNA gene (Laudadio et al., 2019). The V1–V3 region is the frequent choice, but its application has been so far limited to Roche/454 pyrosequencing platform (offering up to 750 bp single-end length reads) (Fouhy et al., 2016).

These V3–V4 hypervariable region has been targeted through the MiSeq platform which can produce single-end reads of 350 bp, which can permit for more precise and commercial characterizations of Microbiota samples (Loman et al., 2012). The V4-V5 region is also employed hypervariable region in 16S rRNA based Microbiota profiling studies (Parada and Fuhrman, 2017). The studies that mentioned previously have indicated that the choice of particular hypervariable region targeted in 16S rRNA sequencing can significantly change the identified structure of microbial community (Tremblay et al., 2015; Walker et al., 2015).

Figure 2: DNA analysis methods for Microbiota (Fiona Stewart, 2014)

Shotgun sequencing of the metagenome

This method allows researchers to broadly sample all genes in all organism’s current in an assumed complex sample, it enables microbiologists to assess bacterial diversity and notice the abundance of microbes in various environments including human body Microbiota (Koslicki et al., 2014). It also delivers an income to study microorganisms that are unculturable and then incredible or difficult to analyze (Sharpton, 2014). Shotgun metagenomic sequencing sequences all given genomic DNA from a sample, unlike 16S sequencing, which targets 16S rRNA genes only.

A distinctive work for taxonomy analysis of shotgun metagenomic data includes quality trimming and comparison to a reference database comprising whole genomes such as Centrifuge (Wood et al., 2014) or selected marker genes such as (MetaPhlAn and mOTU) to generate a taxonomy profile, shotgun metagenomic sequencing coverings all genetic information in a sample, therefore data can be used for extra analyses such as metagenomic compilation, antibiotic resistance gene profiling and metabolic function profiling (Kim et al., 2016; Laudadio et al., 2019).

Probes based method for Microbiota characterization

There are three moieties for chemical probes for activity-based protein profiling (ABPP) method for microbial community applications including: the first is a reactive group that forms an irreversible covalent bond with a target protein both intracellular or extracellular, the second is a binding group such as protein substrate or metabolite that biases the probes toward a target protein or protein family, and the third is a reporter tag for sensitive and rapid measurement of labeled enzymes. There is only so little is known about organism content or overall functional capacity in...
Microbiota. The possible rule of probes to reveal the functional landscape is huge and varying the reactive groups can produce probing of diverse functions (Whidbey and Wright, 2018).

The ABPP is able to characterize the Microbiota functional capacity at both protein and cell measures. when proteins are probe labeled, options exist for directly incorporating a reporter tag such as fluorophore or biotin in the probe or exploiting click chemistry (CC) reactions to connect a reporter to the probe afterward it bound its target, the last option keeps a smaller probe size that can minimize unwanted influences on reactivity with the target protein or permeability of the cell (Anderson et al., 2016).

Probe-assisted fluorescence-activated cell sorting (FACS) is an influential tool for describing the Microbiota functional ability at the cell scale. Fluorophore is choosing to enable FACS is dictated by the background of Microbiota samples fluorescence, the fluorescent signal strength needed to find the preferred functional cell type other fluorescent reagents compatibility and the cells permeability to the fluorophore. Activity-based probe-enabled cell sorting enables the isolation of functional guilds of microbial cells from complex Microbiotas. Also, the probe enabled sorting can give decreased sample complexity to support with proteomics analyses. The Microbiota complexity and the cell sorting can assistance a reduced organism search space for completing proteomic investigations. In the future, probes could be used for the isolation of living organisms’ functional groups from Microbiotas for following cultivation and analytical studies about Microbiota function. Probe-assisted cell sorting can supply the way to improve database about functional Microbiota associations (Whidbey and Wright, 2018).

CONCLUSION

Over the last decades, development of molecular techniques has significantly sped up and improved the identification and analysis of human-associated Microbiota. Metagenome sequencing, metatranscriptomic sequencing and amplicon sequencing have diverse weaknesses and strengths, all are sensitive to specific protocols that have been used for nucleotide extraction from samples and that is requires care to prevent biasing experimental results. The shotgun metagenomic sequencing has a greater genomic coverage and data output. Genome sequencing has shifted the research of human Microbiota from characterized identification, to metagenomics methods that unfold microbial species and mechanism of microbial metabolic activities that associated with human health and disease. We highly recommend to study every community as a whole as many of these communities or organisms could not be cultured independently. Finally, taking these considerations would help selecting an accurate sequencing method in future Microbiota studies and projects. This review was dedicated to provide a pure and scientifically useful overview of the sequence-based identification of human associated Microbiota.

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