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Review Article

Need for identification of gut microbiota with the use of 16s rDNA Sequencing Technique

Ayushi Goyal*

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan, India

***Corresponding e-mail:** angelgoel16@gmail.com

ABSTRACT

Human gut is inhabited by millions of identified and unidentified microbes. It forms an intricate and active, lively and effective population known as the gut microbiota that directly or indirectly affects the hosts' normal life cycle. It is observed that each organism has a unique microbiota that is in direct interface with the environment. Its composition and diversity is dependent on several factors, that may be any of the, i.e., age, surrounding, life style, food we eat, even with any past medical crisis that we have encountered directly affects the microbiota. It is observed that the microbiota effects the immunological cycle of host. Any change or alteration in the microbiota can led to multiple diseases and can also be the factor for the future related problems, so, it becomes essential to identify the diversity of microbe within the host. As much of the microbes resides in the large intestine, so the human fecal sample being the most appropriate selection for isolation. For identification the 16S rDNA technique has emerged as the most significant one the work. This review summarises our current understanding of the development and composition of the human GI microbiota, its impact on host health aiming to isolate and identify the microbiota using the 16S rDNA sequencing technique.

Keywords: Gastrointestinal tract, dysbiosis, gut microbiota, microbiome

INTRODUCTION

The human gut is inhabited by a collection of bacteria, archae and eukarya that has been then co-evolved with the one forming a complex and mutually beneficial relationship with the organism (Backhed, 2005; Neish, 2009). It has been estimated that the number of microbes inhabiting the human gastrointestinal tract is approximately 10 times more than the number of human cells and 100 times more than the amount of genomic content of human genome (Backhed, 2005; Gill et al., 2006). The microbiota provides several advantages to the host like strengthening the gut integrity or shaping the intestinal epithelium (Natividad et al., 2013), harvesting energy (Besten et al., 2013), protecting against pathogens (Вдумler et al., 2016) and regulating host immunity (Gensollen et al, 2016). Any disruption or change in these mechanisms can lead to dysbiosis. Due to such cases it has become important to understand the host-microbe interaction within the human gut and for this the 16S rDNA has resulted as a boom for the identification of the "n" no. of microbes residing the human gut. 16S rDNA sequencing is generally useful in the case of bacteria with different phenotypic profiles, rare bacteria, slow-growing bacteria, uncultivable bacteria and culture-negative infections. With the use of such technique novel bacterial species can be identified that can be helpful in understanding the host specific microbial interactions (Moore et al., 1974). It provides the species-specific signature sequences for the identification of the novel bacterium from the human gut microbiome.

Conventionally, bacterial identification was performed by using phenotypic tests, using Gram staining and various biochemical tests, taking into account culture requirements and growth characteristics. However, these methods of bacterial identification have major limitations. Initially, organisms showing different biochemical characteristics that do not match with the patterns of any known genus and species were encountered occasionally and secondly, it is not applicable with the unculturable bacteria and lastly, identification of some bacteria would require special

equipment and expertise that was not present in clinical laboratories. These all problems can be overcome by using a single technique i.e., the 16S rDNA technique. By using this technique we can identify the genus in ≥ 90% of cases and species in ≥ 63-80% of cases. Although there is difficulty in identifying the intraspecific species.

FLORA COMPOSITION

Most microbes residing the human gut are primarily the obligate anaerobes and also the facultative aerobes. Apart from these aerobes and facultative aerobes are also present. Human intestine is inhabited by the largest number of bacterial community, of which the more is observed within the large intestine. In small intestine the proximal portion is in influence with the stomach and is slightly acidic so the bacterial communities are slightly similar and with the distal portion the conditions are alkaline that is inhabited by different microbial groups. The bacterial communities observed within the acidic conditions are basically the gram-positive ones and the one within the alkaline conditions are the gram-negative ones.

The microbiome provides the regulatory signals that enable the development and utility of the gut. Overgrowth of bacteria in the small intestine can lead to intestinal failure (Moore et al., 1974). The bacteria make up the 60% of the dry mass of the faeces, so, this makes the feces as the most ideal source for the identification of the gut flora (Quigley et al., 2006). Most of the bacterial communities within the gut is resided within the colon part of the large intestine and the 99% of these are the anaerobes i.e., the Bacteroides and the Bifidobacterium. The composition and diversity varies with the age, diet, geographical conditions and even with any past medical problem if experienced.

ROLE OF GI MICROBIOTA ON HEALTH

Presence of a large diversity of microbiota within the human gut is beneficial for the host as it maintains the integrity of the mucosal barrier, provides the various vitamins and nutrients to the host body cells an even prevents from the invasion of the foreign microorganisms (Stephen et al., 1980). The relationship between the commensal bacteria and the mucosal immune system plays a very crucial role in the proper functioning of the host immune system (Arbique et al., 2004; Poretsky et al., 2014). Microbial metabolites are reported to have an impact on intestinal barrier functions, epithelium proliferation and the immune system (Suau et al., 1999).

Any potent disruption or change within this colonisation will result into dysbiosis that can lead to several health problems. It may have same future related issues regarding the host health (Hugon et al., 2015; Li et al., 2014). Recently, it has been observed that some bacterial colonies (*F*aecalibacterium prausnitzii) are even involoved with the occurrence of the colorectal cancer (CRC). Their involvement is seen in several other health related problems like diabetes, obesity, digestive disorders etc. (Arumugam et al., 2011).

INVOLVEMENT OF 16S rDNA SEQUENCING IN CLINICAL MICROBIOLOGY LABORATORIES

Conventionally used techniques in some manner are inexpensive and can be used for the identification of same of the common bacterial colonies but these are incapable for the identification of the rare bacterial colonies even these technique requires the pure culture for the identification, so, these cannot be used for the identification of the unculturable bacterial colonies (Jeffery et al., 2012). 16S rDNA technique has emerged universal solution for these limitations that can be helpful for the identification of rare, unculturable, slow growing and even unusual bacteria, often within 48 hr, which are reproducible among laboratories. In many situations it is the ultimate solution for the identification of the aetiological agents of infectious diseases (Hooper et al., 2010).

For the isolation and identification of the novel bacterium the most suitable sample being the fecal matter is used from which the different bacterial colonies have been identified that are isolated using the PCR and ELECTROPHORESIS technique (David et al., 2013). The sample used for the sequencing is the PCR sample with the pure DNA content i.e., within the ratio of 1.8-1.9.

USES

- Identification of bacteria
- Identification of bacteria with unusual phenotypic profiles
- Identification of slow growing bacteria
- Discovery of novel bacterial genus and species
- Detection of uncultivable bacteria
- Diagnosis of culture negative infection

CONCLUSION

This has been definitely proved that there is a close association and symbiotic relationship between the gut microbiota and the host that has a great impact and influence in host life. The presence of good and balanced microbiota is shown with the presence of beneficial properties with the host otherwise it shows the dysbiosis condition within the host. The dysbiotic microbiota can either be a cause or a consequence this can be taken in account with the properties of the gut microbiome. So the identification of such communities has become very important and for this the 16S rDNA

sequencing technique has emerged as a most fundamental answer to all the questions regarding its isolation and characterisation. For proper assistance to patients and for providing them with the best treatment, accurate and objective identification of the isolates, rarely identifiable bacterias, unculturable bacterias, bacterias that have a slow growth has provided assistance to clinicians with providing the patients with the more accurate antibiotics, and facilitate with more accurate treatment. So with the use of the 16S rDNA sequencing technique it has become possible to identify and discover new bacterial genera and species to facilitate much more in this direction.

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