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EC MICROBIOLOGY
Opinion

Next in Human Microbiome Research

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Received: February 15, 2017; **Published:** February 28, 2017

Microbes play a vital role in human life; there are more than 10^{14} microbes that are associated with the human body. This fact means that the number of microbes within a human body exceeds the number of human cells present [1]. The gastrointestinal tract harbors the majority of the microorganisms. Microbes in the gastrointestinal tract perform many vital roles in the digestion of complex nutrient, production of micronutrients, protection from the pathogens and regulating the immune system. Dysbioses on these microbiota has been associated with many diseases [1]. For this reason, it is always important to have a stable microbiome in order to maintain human health. Analysis of these microorganisms structural and functional diversity is inevitable in modern medical research. Though there are several biotechnological and molecular biology technological advances used in the analysis of the microbiome, refined and specific techniques to explore its function in human health are still needed. However, uncultivable microorganisms are very difficult to analyze; the fact is that about 99% of gut microbiota are uncultivable. Early molecular techniques such as restriction fragment length polymorphism and gradient gel electrophoresis techniques provided very little information on microbiome research.

When DNA sequencing technologies were introduced, it opened the preliminary gateway to the analysis of the microbiome. Metagenomics approaches of 16S rRNA gene analysis helped in analyzing complexity in the microbiome. Polymerase chain reaction (PCR) based methods with targeted probes then led to another way of characterizing the microbiome. However, these technologies had many limitations in analysis, e.g. 16S technologies require previously reported bacterial sequences as a reference to identify unknown bacterial species, unfortunately all the references were created from cultivable bacterial sequences. In recent years, scientists using a PCR based high throughput sequencing method with the help of next generation sequencing (NSG) techniques, and for analysis of the robust results were improved with the help of new bioinformatics software. This bioinformatics softwares are capable of handling an enormous amount of data and has created several models for interpreting results. Also, given different dimensions in the process of analyzing uncultured microorganisms, these advances only allow for identification of the diversity of the microbes present in a microbiome. In most cases open reading frame (ORF) based sequence alignment method is only able to categorize the microbial diversity in genus or family level.

As a next step, NSG technology results compared with metabolomics where the structural diversity of the bacteria is then compared to its functional diversity. It is hard to predict which individual microbe is involved in the production of specific metabolites. This means that even these advanced methods are limited in analysis or interpretation of uncultured microorganisms. Very recently single cell genomics and metabolomics have successfully been used in human cells to analyse the gene expression level related to given conditions [2,3]. This approach has also been used in the analysis of bacterial cells. However, these techniques are still in early stages and have not been used in microbiome analysis.

Several methodologies have been used for the purpose of separating single bacterial cells such as fluorescence-activated cell sorting (FACS), micromanipulation, microfluidics and optofluidics [2]. This single cell separation has paved the way for analyzing single cell genomics, transcriptomics, metabolomics, and proteomics [2]. While single cell genomics has proved to be an established technique, there

are few reports that have showed the success in analyzing the single cell genomics of uncultivable bacteria. This informations gives way to increased confidence in analyzing human microbiome with targeted identification.

Using these single cell omics approaches with the human microbiome will create large amounts of data, which can then be used as reference data for targeted analysis in the microbiome. However, creating this big data is a daunting task and involves a lot of necessary technological advancements needs with enormous amounts research funding. This is how the human whole genome sequencing started, which went on to produce a tremendous impact on medical advancements and the comprehension and curing of many challenging diseases. Initially, yes this going to be random and a half and half success. However, once this kind of data has been generated, this will change the entire perception of the microbiome research.

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Volume 6 Issue 3 February 2017