Identify the bacteria within and on your-self

The human intestine (gut) and other body sites harbor numerous microorganisms (bacteria, viruses, archaea, including fungi), collectively referred to as the microbiome or microbiota. The genome of these microbes encodes for metabolic functions and contribute significantly to the host physiology. The microbiome is unique, and performs day to day activities such as – regulate energy harvest from food, perform metabolic functions, synthesize amino acids, maintain immune system and intestine health. But, on the contrary if there is an imbalance or dysbiosis in the microbiome it leads to diseases such as obesity, inflammatory bowel disease, kidney disease, pulmonary disorders, cardiovascular diseases etc. The microbiome also affects the survival and function of organ transplants such as in kidney transplants recipients. The field of microbiome and its role in human health and diseases is relatively new and burgeoning.

Biomedical researchers routinely use laboratory culture-dependent techniques to identify these bacteria based on their morphology, appearance, enzyme degrading properties and DNA (genome) analysis. However the culture-dependent techniques are limited in identifying the bacteria as majority (more than 95 %) cannot be grown in laboratory. On the other hand the culture-independent techniques involves isolation of the total microbial community DNA (called metagenome) without growing the microbes. The organization of metagenome is deciphered by using a high-throughput next-generation DNA sequencer, and bioinformatics to identify the bacterial species in the sample.

The genome or the genetic material (which is hereditary) and transferred from the parents to offspring, consist of four letters – A, T, G and C, called nucleotides. These four letters are joined together in multiple combinations and in various length and is termed as ‘genome’. The genome length can range from thousands in viruses and bacteria, to billion in animals and humans. This genome organization is specific to each species on earth, and are responsible for the genetic, morphological and physiological makeup of the organism. The organization of the DNA of any organisms is inferred by DNA sequencing. Once this information is obtained, then bioinformatics is used to know which bacterial species are present. The DNA information (called ‘DNA sequences read’ in sequencing world), is then compared with the DNA databases (repository of previously known sequences), and the organism is identified. Also the smaller reads can be assembled to generate larger fragments called contig. The larger the length of DNA sequences and the high similarity score with known sequences in the repository, the more confidence of correctly identifying the organism in the samples.

The most common sequencing approach to analyze the microbiome, is based on the sequence analysis of the conserved bacterial 16S ribosomal RNA (rRNA) gene called amplicon sequencing. However this technique has multiple experimental biases and limited ability to precisely identify the bacterial species, which are major drawbacks.

The aim of this research was to perform a comparative study of an alternative approach to identify

the microbial species in the microbiome using shotgun metagenomics sequencing, with multiple sequencing platforms and strategies. We performed the following comparative study – 1) amplicon versus the shotgun metagenomics method, 2) multiple DNA sequencing platforms, 3) the analysis of DNA sequences read versus *de novo* assembled contigs, and 4) the effect of shorter versus longer DNA sequence reads. Our findings from the study, demonstrates that shotgun metagenome sequencing has multiple advantages compared with the amplicon method, which includes enhanced detection of bacterial species, increased detection of microbial diversity and increased prediction of microbial functional genes. The findings from our study will be very useful for the biomedical researchers working in the field of microbiome, as they can plan the experimental work for a better identification of microbial species. We're at the tip of really understanding how the microbial community affects us both negatively and positively and a precise identification, of microbial community can lead to health benefits, and has application in personalized and precision medicine.

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