

Metagenomics to Unveil Microbiome of Human Body

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Microorganisms dwell in almost every habitat, and are present in communities where they work together depending on the environment of the niche. Most of these microorganisms cannot be cultured using standard cultivation methods [1]. Metagenomics offers a solution to study such microbes by using culture independent techniques. Metagenomics (beyond genomics), also called community genomics, deals with the study related to DNA (RNA in case of meta-transcriptomics) of microbial communities. This approach has been widely used in recent years to study microbial community compositions as well as its functional potential, identification of new enzymes, identification of new bacteria and much more. Metagenomics has served a lot in field of environmental microbiology [2,3].

Community DNA extracted (also called metagenome) is fragmented and cloned into host bacteria (usually *Escherichia coli*). The clones are then screened either by function-based analysis or sequence-based analysis. Metagenomic libraries thus constructed can also be analysed completely to get a complete genome or even complete metagenome [4]. Instead of cloning the whole metagenome, genes of interest can also be amplified using Polymerase Chain Reaction and resulting amplicons can be cloned and analysed. Both of these approaches create a large number of clones. Such cumbersome work can be avoided by reducing the number of clones for functional screening, for example, by use of Substrate Induced Gene Expression (SIGEX) [5]. High throughput sequencing technologies also enable metagenomic studies without having need to create clone libraries [6]. Extensive 16S rDNA sequencing has led to creation of large reference databases such as Ribosomal Database Project (RDP) [7], SILVA [8] and Greengenes [9]. Moreover, different types of microarrays are also available to analyse phylogenetic and functional structure of the microbial communities [10].

Metagenomic analysis has also been used to study complex composition of microbial communities that inhabit human body. Grice et al. [11] investigated microbial community composition of human skin microbiota by taking samples from elbow using swab, scrap and punch biopsy methods in order to analyse all depths of skin. They amplified 16S rDNA, cloned the amplicons and sequenced them. They found out that the Proteobacteria were dominant in all the samples. They also reported significant similarities between human and mouse skin microbiota. Gill et al. [12] used metagenomics to analyse microbiome of human distal gut and reported that the microbiome is rich in genes involved in vital metabolic processes. Ley et al. [13] found out that not only Bacteriodes and Firmicutes are dominant microbes in microbiota of human intestine, but their relative proportion is also linked to human obesity. Many researchers have also used pyrosequencing to estimate Single Nucleotide Polymorphism (SNP) allele frequencies [14]. Metagenomic approach was also used for children diarrheal samples, and apart from known viruses, Finkbeiner et al. [15] were also able to identify many novel viral sequences. Allander et al. [16] used clone library approach to identify a third human Polyomavirus. Metatranscriptomics and metaproteomics have also been used to study human microbiota [17]. Belda-Ferre et al. [18] did metagenomic analysis of oral cavity microbiome of persons with and without microbiome. Besides differences in the proportion of different bacterial groups in the

cases and healthy controls, Belda also reported that healthy individuals had many commensal bacteria that when cultured showed inhibition to the growth of cariogenic bacteria, suggesting their roles as probiotics.

Though a decent amount of work has already been done, the understanding of the human microbiota is still in its infancy. With the advances in next generation sequencing technologies along with the availability of new strategies to screen clones, the structure of the human microbiota and the roles that it plays can become more and more vivid in upcoming years.

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