Review Article

Insights into The Human Gut Microbiome - A Review

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ABSTRACT

Various microbial communities and their genes collectively known as microbiome exist throughout the human body. The microbiome endows us with physiologic capacities that we have not had to evolve on our own and thus is both a manifestation of who we are genetically and metabolically, and a reflection of our state of well-being. Our distal gut is the known ecosystem with highest density of microbial population and the most comprehensively surveyed to date. The gut microbiome is a complex ecosystem that affects the development, immunological responses and nutritional status of the host. This review briefly discusses the significance of the gut microbiome in human health and wellness.

Keywords:

Microbiome, Gut, Health, Probiotics, Metagenomics

INTRODUCTION

DNA is what makes us what we are, but many of us don't realize that the DNA that defines us is not just that of our own human cells, but also that of the millions of microbes living in/on the human body.[1] There are over 100 trillion microorganisms residing in the human gut, including bacteria, fungi, algae, and protozoa, making up what are known as the "Human Microbiome". As Hippocrates quoted "Death sits in the bowels" and "bad digestion is the root of all evil", it is known that health is incomplete without a healthy gut.[2] DNA based technologies expanded our knowledge by generating adequate information on the composition and functional microbial communities. Human Microbiome Project (HMP) by the US National Institutes of Health and the European project, MetaHIT pioneered to answer fundamental questions like their function, composition, pathways of microbes, biology and medical significance of the human microbiome and its collective genes.[3] The gut microbiome and its byproducts play a vital role in overall health and/or disease depending on the strains of microorganisms that predominate.[4] As the implications for human health and disease are wide ranging, the study of humans and model animal systems with strong phenotypes is essential for making focus not only on bacteria, but also other microbes. The microbial composition is determined mostly by environmental factors with limited input from host genetics.[5] The principle of ancient associations between hosts and their microbial communities is evident in the present day effects that the gut microbiome exerts on host biology, ranging from the structure and functions of the gut and the innate and adaptive immune systems, to host energy metabolism.[6,7] In this review we address the significance of gut microbiome in human health and wellness.

IMPORTANCE OF MICROBIOME

Human Microbiome is essential for human biology as they facilitate the metabolism, produce essential vitamins, confer protection against invasion by opportunistic pathogens, plays a key role in maintaining tissue homeostasis, as well as, required for the development and differentiation of the immune system.[6,8] In addition to playing a critical role in human health, changes in microbiome

composition is associated with a number of human disease such as Crohn's disease, chronic periodontitis, inflammatory bowel disease, irritable bowel syndrome, tropical enteropathy, antibiotic-associated diarrhea, and bacterial vaginosis. For each disease a microbial community has been proposed, leading to stereotypic interactions between community members associated with some of these pathologies.[3,9,10]

DIVERSITY IN GUT MICROBIOME

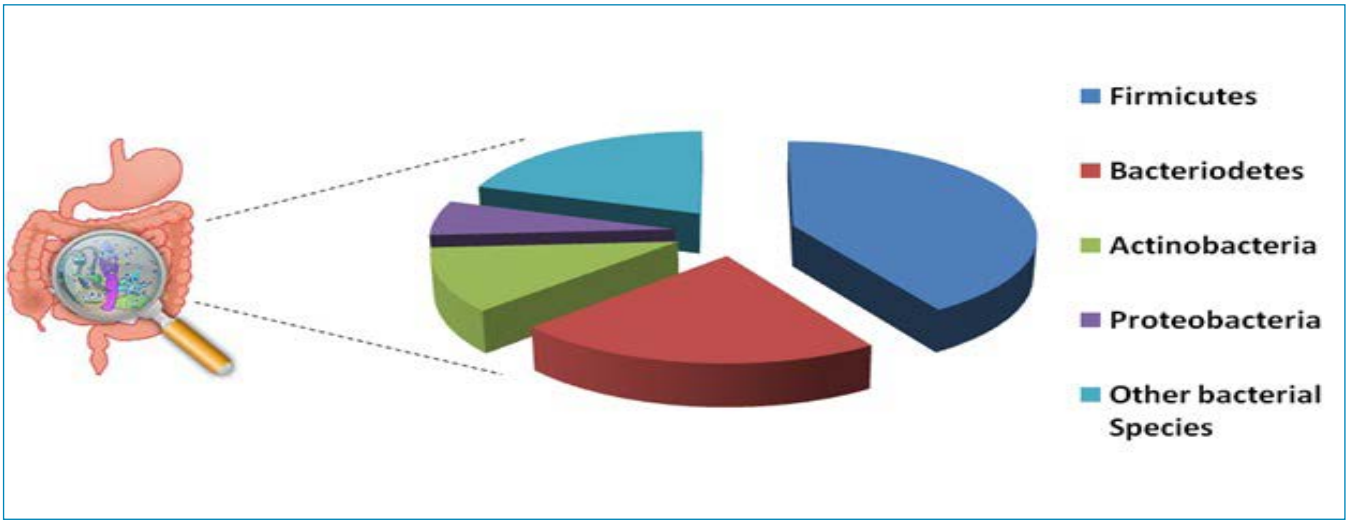
A human harbors a population of ~10^{14} bacterial cells, the composition of which varies by anatomical site such as skin, mucosal surfaces and intestinal lumen. Each area consists of certain families and genera, which can be of a unique, fingerprint.[11,12] These characteristics indicate that mankind has co-evolved with their microbial partners. The microbiome in the colon is the most biodiverse ecosystem in the human body [13,14] representing more than 70% of all microbes in the body. Colon is the largest organ with majority of microbial colonization. The normal gut microbiome is predominated by anaerobic bacteria, which outnumber the aerobic and facultative bacteria by thousand fold. Indeed, out of the 100 different bacterial phyla detected on our planet, only seven are found in human gut – Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, Tenericutes, and Fusobacteria[15] – of which Firmicutes and Bacteroidetes represent together up to 90% of the ecosystem[16]. (Figure 1) Altogether, the human microbiome is similar to that of other mammals at the phylum level, but most bacterial families and genera seem to be distinct.[17]

Mainly two gradients of microbial population are found in the gut. First, microbial density increases from proximal to the distal gut: stomach contains 10^1 microbial cells/gram, duodenum 10^3 cells/gram, jejunum 10^4 cells/gram, ileum 10^7 cells/gram and colon up to 10^{12} cells/gram and along the tissue lumen axis with few bacteria adhering to the tissue or mucus but a large number being present in the lumen. Second, bacterial diversity increases in the same axes and manner as microbial density.[2] Many bacterial species are present in the lumen, whereas fewer, but well adapted species, including several proteobacteria and *Akkermansia muciniphila*, adhere and reside within the mucus layer close to the tissue.[18] In fact, the metabolic activity of the gut microbiome equals that of the liver, and the intestinal microbiome can therefore be considered as an additional organ.[16]

Next generation sequencing-based approaches have detected an impressive biodiversity at lower phylogenetic levels with up to1000 different species among people, and each subject harbors a very unique subset of microorganisms. The total genome of these microorganisms provides the host with essential functional traits that human beings have not evolved on their own.[19] For instance, the carbohydrate-active enzymes encoded in the microbial glycobiome allow the host to extract energy from otherwise indigestible polysaccharides,[20] complementing the poor human glycobiome diversity.[21]

Various factors such as mother's microbiome, mode of delivery, feeding type, the environment, including weaning and use of antibiotics influences gut

Figure 1 : Major bacterial Phyla and their diversity in the human gut microbiome.



microbiome among individuals. Babies microbiome, born through C-section, showed similar skin microbial communities of mothers, whereas vaginally delivered infants acquired bacterial communities resembling the vaginal microbiome of their mothers.[22] Scientists believe that microbiome-fetus-in utero relationships co-exists even before birth determining the course of pregnancy and development of the fetus.[23] The neonate gut is dominated by bifidobacteria, especially in milk fed infants. Genome analysis of *Bifidobacterium longum subsp. infantis* revealed that feeding pattern like breast milk or formula, determined the types of bifidobacteria found. Breast fed infants have high composition of *Bifidobacterium breve*, formula fed infants lacked *B. breve* but contained *Bifidobacteria longum*. Infants fed with breast milk and later a prebiotic formula consisting of standard formula milk containing a mixture of specific galacto and fructo oligosaccharides, has a *Bifidobacteria breve* fecal population.[24]

Compared to early stages of a human life, microbiome composition undergoes significant changes as the human ages, largely determined by lifestyle modifications, diet change, increase in infection rates and inflammatory diseases and use of antibiotics. Study of gut microbiome of elderly people revealed high levels of *Escherichia coli* and Bacteroidetes as well as a significant difference in the Firmicutes to Bacteroidetes ratio for adults and elderly individuals.[25] Using the Human Intestinal Tract Chip (HITChip) and qPCR, the Firmicutes/Bacteroidetes ratio was observed for the centenarians, elderly and young adults to be 3.6, 5.1, and 3.9 respectively. However, there was a decrease in the Firmicutes subgroup Clostridium cluster XIV and an increase in Bacilli. Furthermore, there was a significant increase in several facultative anaerobes, members of the Proteobacteria phylum, many of which constitute opportunistic pathogens. These were inversely associated with bacteria belonging to Clostridium cluster XIV and Clostridium cluster IV.[26,27]

IDENTIFICATION AND CHARACTERIZATION OF MICROBIOME

Identification and characterization of bacteria were done by the cultivation and isolation that have long been considered as the gold standard. After the isolation of single colonies, bacterial identification was achieved by observation of the bacterial cells and their morphology, biochemical testing, differential staining, and the use of enrichment cultures. On several occasions, it was noted that the number and diversity of cells observed microscopically far exceeded those of cells grown in culture. Gram staining was the earlier technique that was used to broadly identify the physiological character of the microbial community but lacked lower taxonomic levels.[28] As the culture based approaches led to bias, isolation of full diversity of microorganisms remained largely underexplored. The uncultivable microbes identified by the DNA based methods lead to identification of taxonomic diversity of the microbial community at a broad level and detect the presence or absence of individual biochemical functions.[29] Fluorescent in situ hybridization (FISH) was the one of the earliest targeted metagenomic assays to study the unculturable microbial community from species to taxonomy.

Nevertheless, DNA sequencing of cloned libraries are expensive and laborious. High-throughput DNA sequencing in combination with genome-scale platforms such as proteomics or metabolomics are currently used to study microbiome. With the advent of bacterial phylogeny, based on the small-subunit 16S ribosomal RNA (rRNA) sequence, the work of Woese *et al*[30] set the stage for wider genomic identification and analysis of microbial communities. Later, Pace *et al* developed a method to circumvent culture based approaches in identifying bacteria[31] based on isolation of rRNA genes through polymerase chain reaction (PCR). The 16S rRNA gene, which is 1,500 base pairs in length, contains species-specific hypervariable regions but is conserved enough for PCR amplification using broad-range primers.[32] The 16S rRNA gene sequences are compared with the phylogenetic reference tree to assign taxonomy. The Ribosomal Database Project (RDP) contains more 16S rRNA sequences and phyla that can be referred to identify bacterial diversity in a sample.

Measurement of gases dissolved in bloodstream and exhaled in breath is used as a mechanism of measuring bacterial activity.[33] However, non-specificity of its indication has resulted in trial development of techniques like ingestible electronic capsule capable of sensing different gases in the gut. The sensing capsule was designed to report oxygen, hydrogen and carbon dioxide gas levels in different parts of the gut directly to a handheld receiver linked via Bluetooth to a smartphone application.[34]

GUT MICROBIOME AS POTENTIAL THERAPEUTICS

Human gut microbiome is involved in the regular physiology and host metabolism.[35] Microbial metabolism products act as signaling molecules and influence the host's metabolism, the physiological

Table 1: List of Computational methods used in microbiome analysis

Method	Description	References
Assembly		
Genovo	Generative probabilistic model of reads	53
khmer	Probabilistic de Bruijn graphs	54
Meta-IDBA	De Bruijn graph multiple alignments	55
metAMOS	A Modular Open-Source Assembler component for metagenomes	56
MetaVelvet	De Brujin graph coverage and connectivity	57
MetaORFA	Gene-targeted assembly approach	58
MOCAT	Assembly and gene prediction toolkit	59
SOAPdenovo	Single-genome assembler commonly tuned for metagenomes	60
Functional profiling		
HUMAnN	Presence/absence and abundance of microbial pathways in meta'omic data	61
PRMT	Predicted Relative Metabolomic Turnover	62
RAMMCAP	Rapid analysis of Multiple Metagenomes with Clustering and Annotation	63
General tool kit		
CAMERA	Dashboard for environmental metagenomic and comparative analysis tools	64
IMG/M	Integrated metagenome data management and comparative analysis system	65
MEGAN	Software for metagenomic, metatranscriptomic, metaproteomic, and rRNA	66
METAREP	Online storage and analysis environment for meta'omic data	67
MG - RAST	Storage, quality control, annotation and comparison of meta'omic samples	68
Smash Community	Stand-alone annotation and analysis pipeline suitable for meta'omic data	12
STAMP	Comparative meta'omics software package	69
Interaction networks		
SparCC	Estimates correlation values from compositional data for network inference	70
CCREPE	Predicts microbial relationships within and between microbial habitats	71
Simulators		
GemSIM	Error-model based simulator of next-generation sequencing data	72
MetaSim	A sequencing simulator for genomics and metagenomics	73
Statistical tests		
Metastats	Statistical analysis software for comparing metagenomic samples	74
LefSe	Nonparametric test for biomarker discovery in proportional microbial community data	75
Shotgun FunctionalizeR	A statistical test based on a Poisson model for metagenomic functional comparisons	76
SourceTracker	A Bayesian approach to identify and quantify contaminants in a given community	77
Single cell sequencing		
IDBA-UD	Assembler for single-cell or metagenomic sequencing with uneven depths	78
SmashCell	Software framework for the analysis of single-cell amplified genome sequences	79

Method	Description	References
Taxonomic profiling		
Amphora, Amphora2	Automated pipeline for Phylogenomic Analysis	80
CARMA3	Taxonomic classification of metagenomic shotgun sequences	81
ClaMS	Classifier for Metagenomic Sequences	82
DiScRIBinATE	Distance Score Ratio for Improved Binning and Taxonomic Estimation	83
INDUS	Composition-based approach for rapid and accurate taxonomic classification	84
MARTA	Suite of Java-based tools for assigning taxonomic status to DNA sequences	85
MetaCluster	Binning algorithm for high-throughput sequencing reads	86
MetaPhlAn	Analysis of microbial communities from metagenomic shotgun sequencing data	87
MetaPhyler	Taxonomic classifier for metagenomic shotgun reads	88
MTR	Taxonomic annotation of short metagenomic reads	89
NBC	Naive Bayes Classification tool for taxonomic assignment	90
PaPaRa	Aligning short reads to reference alignments and trees	91
Metataxa2	Identification and taxonomic classification of small and large subunit rRNA	92

function of liver, brain, adipose and muscle tissue. These microbial metabolites play an important role in host nutrition,[6] childhood allergies,[36] inflammatory responses[37] and immune system modulation.[38] These metabolites also modulate the function of the gut-brain axis affecting behavior and pain, in addition to depression, anxiety, other disorders belonging to the central nervous system.[39] Gut-Brain axis is well established to changes in the functional composition of gut microbial activity. For example, bacterial amyloid, even from commensal gut microbiome, can cause protein misfolding, increase oxidative toxicity triggering neuro inflammation followed by slow neuro degeneration or changes in serotonin, tryptophan metabolism resulting in Parkinson's disease.[40] Gut-brain axis is also involved in irritable bowel syndrome,[41] autism,[42] insulin secretion,[43] obesity.[44]

On this understanding, gut microbiome can be considered as a valuable drug target for complex human pathophysiology. For example, Wei *et al* (2008) have stated two strategies for the development of gut microbiome targeted therapies: first, direct elimination or modification of a specific gut microorganism, or certain species of bacteria that influences the condition as disease target; secondly, Vaccination with an antigenic epitope of a bacterium or toxin of interest to evoke an immune response capable of interacting with the gut microbial flora upon microbe proliferation in the gut.[45]

META-ANALYSES OF MICROBIOME

Technological advances now make sequencing a single bacterial genome to sequencing thousands a reality. National Institutes of Health (NIH) Common Fund's Human Microbiome Project (HMP) is involved in sequencing 3,000 cultivated and uncultivated bacterial reference strains. This catalog of reference genomes is intended as a scaffold for the assembly of metagenomic sequences and as a reference for 16S rRNA gene sequences.

Parallel DNA sequencing technology, produces ~1 million ~400-nucleotide reads per run, by multiplexing samples into a single sequencing run enabling researchers to amplify hundreds of thousands sequences present in the sample.[46] Although the next generation sequencing technologies discard cloning biases, 16S rRNA gene diversity surveys still rely upon limited PCR amplification, which can potentially introduce bias. By Sanger sequencing, introducing whole-genome random sequencing, *Haemophilus influenzae* in 1995 was the first bacterial genome to be sequenced.[47] At 1.8 Mb, the *H. influenzae* genome was 10 times larger than previously sequenced viral genomes. The elimination of cloning DNA into bacterial genomes for shotgun sequencing may be one of the reasons. Promoter regions of bacterial genes are to some degree uncloneable, and as a result, these regions are typically underrepresented in initial data sets generated from cloning-based Sanger sequencing. Since 2012, the Genomes Online Database

(GOLD; http://www.genomesonline.org) provided nearly 3,000 finished bacterial genomes, with many thousands more underway.

Initially, the lack of reference genome sequence was a major setback in analyzing whole genome sequencing. To generate reference genome databases, it is crucial that new methods be developed to cultivate and isolate previously uncultivable organisms. Nichols *et al*., developed a technology known an ichip (isolation chip) composed of hundreds of miniature diffusion chambers, each inoculated with a single microbial cell.[26] This method can be useful only for seawater environment, for human samples, dissociation into single non-adherent bacterial cells would be a required to employ this method. Some technologies are overcoming the cultivation step like microfluidic isolation of bacteria from the subgingival crevice followed by single cell genome amplification and sequencing resulted in the phylum TM7, for which no isolate or reference genome sequence data was needed.[48] Various bioinformatics analytical tools used for the microbiome analysis have been listed in Table 1.

PROBIOTICS AND IMPLICATION ON HEALTH

Probiotics are live microorganisms that provide health benefits to human beings when consumed. Probiotics act through diverse mechanisms positively affecting the composition and function of the host microbiome.[49] The most common probiotic bacteria are Lactobacillus, Bifidobacterium, Enterococcus, and also some Propionibacterium strains. For the past several decades, studies have been done to know the beneficiary role of probiotics, using different strategy to prevent or treat diseases. Modulation of an unbalanced indigenous microbiome would be the aim of probiotic therapy and opens new prevention and treatment possibilities of diseases in which alteration of the microbiome plays relevant roles. Recommendation of probiotics to pregnant women, nursing mothers, or newborns can influence the establishment and composition of infant gut microbiome impacting later life health status.[50] There are also controversial studies that showed prenatal consumption of *L. rhamnosus* failed to modulate the diversity of early infant gut microbiome despite promoting a beneficial bifidobacteria profile.[51] Prenatal maternal supplementation with probiotics may have important preventive effects over allergic diseases among children such as atopic eczema, allergic rhinitis and asthma.[52] The host factors such as genetic susceptibility, microbiome composition and environmental factors, such as geographic region and diet, may also influence the above results.

FUTURE PERSPECTIVES

The main perspective of the human microbiome study is to improve our understanding of microbial communities in each habitat of the human body and its temporal variation among individuals with respect to variables such as diet, host genotype and health status. Most recent studies highlight how a co-evolved microbiome can markedly affect host biology at the molecular level, and these findings call for a complete reanalysis of human physiology and immunology. The future of the microbiome study should focus on gaining knowledge of the taxonomical variation in microbial composition and its inheritance related to host microenvironment.

CONFLICTS OF INTEREST

None

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