

The Human Microbiome: An Acquired Organ?*

Rajkumar Dhanaraju and Desirazu N Rao

A diverse milieu of harmless microbes thrives on the surface of the human body. These human-associated microbes comprise an enormous collection of prokaryotes (archaea and bacteria), eukaryotes (fungi and protozoa), and viruses. The discovery of universal phylogenetic taxonomic molecular markers and the availability of robust deoxyribonucleic acid (DNA) sequencing tools have enabled the identification of several previously unknown human-associated microbes. Consequently, the Human Microbiome Project (HMP)—the first comprehensive survey of the human-associated microbes—has determined the microbial diversity and its abundance in health and disease. HMP study shows that human adults have a similar microbial composition as that of higher taxonomic level (phylum), although uniquely differing from lower taxonomic level (genus and species). Bacteria are the predominant microbial constituent of the human body, and the large intestine (the lower gut), especially, is the most densely populated microbial niche. The human gut is estimated to have over 100 trillion microbes encompassing over 1000 bacterial species, outnumbering the total human body cells by a factor of ten. Gut microbes have a significant impact on human physiology through their role in protection against gut infections, expanding nutrient harvest, educating the infant immune system, modulating drug efficacy, and so forth. The gut microbial communities are collectively recognized as an ‘organ’ for their indispensable contribution to health. Gut microbes supplement human biology with numerous functional genes, metabolic pathways, bioactive metabolites, etc. The perturbation of gut microbiota composition has a pathologi-



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cal impact on human physiology. Hence, the ensemble of the microbial genetic material associated with us represents ‘our acquired genome’. Overall, the human-microbial synergistic interaction is an evolutionary amalgamation of three domains of cellular life—Archaea, Bacteria, and Eukarya—mutually co-existing with acellular viruses, and collectively referred to as a ‘superorganism’.

Introduction

“Life would not long remain possible in the absence of microbes”.

—Louis Pasteur

For over 3.5 billion years, Earth has been a thriving ecosystem, home to innumerable, diverse, and invisible microbes. Microbial life has made Earth’s ecosystem conducive to the evolution of complex higher life forms. Insightfully, Louis Pasteur advocated: “*Life would not long remain possible in the absence of microbes*”, alluding to the microbes’ quintessential role in the sustenance of life on Earth. In recent times, modern tools have provided us with unprecedented access to the microbial world, leading to the discovery of new human microbial inhabitants. The human-microbe symbiotic association is an outcome of a profound mutual inter-dependency, evolved over the ages to establish a sustainable ecosystem. The entire human-associated microbial inhabitants are collectively known as ‘microbiota’, and their total genetic content is the ‘microbiome’. The constituents of human microbiota and its physiological functional role remained an enigma until the advent of molecular taxonomy, metagenomics, meta-omics, and germ-free animal models.

Beyond Microbial Pathogens: Who Are They?

From time immemorial, human history is shrouded with numerous accounts of deadly microbial pandemics, such as the black plague (*Yersinia pestis*), Spanish flu (H1N1 influenza A virus), cholera (*Vibrio cholerae*), AIDS (Human Immunodeficiency Virus), and COVID-19 (SARS-CoV-2). Microbial pathogens continue to drastically change the demography around the world. Herculean efforts to develop antibiotics, vaccines, and aseptic techniques



have curtailed microbial pathogens' impact on human and domesticated animals. Despite the sophisticated antimicrobial interventions, a barrage of emerging new microbial pathogens continues to challenge public health. However, surprisingly only a fraction of all known microbes are pathogenic, whereas most are commensal or mutual in their interactions.

Discovery of Gut Commensals

In the early days of microbiology research, scientists and physicians began to hypothesize and experiment with gut microbiota to unravel its potential role in health. In 1683, Antonie van Leeuwenhoek first demonstrated the existence of microscopic motile bacterial cells—'animalcules'—in his teeth and faeces [1]. Astoundingly, the microbial examination of fecal matter has led to the discovery of several gut commensals. For example, in 1886, Theodor Escherich isolated the gut bacterium *Escherichia coli* (*E. coli*) from a healthy infant [1]. Subsequently, *E. coli* emerged as the laboratory model organism, 'workhorse' to understand the central dogma processes of molecular biology. Despite the ubiquitous presence of commensals across the mammalian gut, their functional role in health remained obscure. In 1905, Ilya Mechnikov, an immunologist, theorized that gut commensals likely play an essential role in excluding gut pathogens.

In 1906, Hendri Tissier, a pediatrician, isolated a 'Y-shaped' gut bacterium, *Bifidobacterium bifidum* (*B. bifidum*), from the feces of healthy breastfed infants [1]. He postulated the beneficial role of *B. bifidum* in protecting against gut pathogens, and proposed the therapeutic use of this bacterium in treating dysentery. Similarly, in 1917, physician Alfred Nissle demonstrated the antagonistic property of a gut commensal against pathogens [1]. Nissle isolated an *E. coli* strain named '*Escherichia coli* Nissle 1917' (EcN) from the stool of a German soldier. Intriguingly, this soldier had returned from a shigellosis endemic battle region and remained free from diarrhoeal symptoms, unlike his colleagues. On a petri dish, Nissle demonstrated the growth inhibitory ef-

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fect of EcN on co-cultured gut pathogens such as *Salmonella enterica* serovar Typhimurium and *Shigella flexneri*. EcN is a widely prescribed beneficial microbe (probiotic), marketed under the tradename ‘Mutaflor’. Likewise, in the 1920s, Minoru Shirota, a physician, identified the therapeutic potential of the lactic acid-producing bacterium ‘lactobacilli’ in suppressing gut pathogens in his patients. He isolated a strain of lactobacilli (*Lactobacillus casei* strain Shirota) and formulated a lactobacilli-based milk beverage under the brand name ‘Yakult’ in 1935. Furthermore, anecdotes from ancient human civilizations indicate fermented milk intake to treat gut disorders such as diarrhea and constipation.

The Great Plate Anomaly: Why Don’t They Grow in the Laboratory?

Attempts to culture human commensals in the laboratory have shown that only a fraction of the total commensals present in the microbiota sample is culturable; this bottle-neck conundrum is referred to as “the great plate count anomaly” [2]. Cumbersome nutritional requirements (fastidious) of unculturable commensals and the limitations in reconstituting their respective *in vivo* growth conditions under a laboratory environment underscores the great plate anomaly. Conventional microbiology investigation necessitates a pure monoculture to understand the microbial phenotype, metabolism, and genotype. However, a high-throughput cultivation method—‘culturomics’—is employed in the modern laboratory to determine the commensals’ esoteric growth conditions [2]. Although, so far, only 20–30% of human commensals have been cultured [2]. Hence, an alternative culture-independent DNA sequence-based approach is widely applied to determine unknown commensals’ identities and genetic makeup.

Molecular Taxonomy and Metagenomics

The seminal contribution by Carl Woese during the 1970s, in the conserved ribosomal ribonucleic acid (rRNA)-based phyloge-



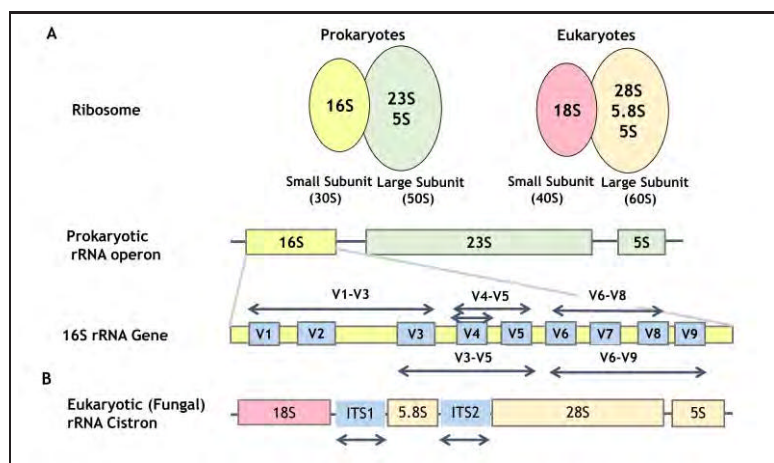


Figure 1. Molecular taxonomic markers. The evolutionarily conserved prokaryotic 16S rRNA gene, the eukaryotic 18S rRNA gene and ITS represent the taxonomic specific molecular markers. **(A)** The schematic diagram shows the prokaryotic and eukaryotic ribosomal subunits (coloured ovals) and their (numbered) respective rRNA components. The prokaryotic operon shows the linear arrangement of ribosomal rRNA genes: 16S, 23S, and 5S. The microbiome studies target the underlined variable (V) window regions of the 16S rRNA gene: V1-V3, V3-V4, V4, V3-V5, V4-V5, V6-V8 and V6-V9. **(B)** The eukaryotic fungal rRNA cistron shows the linear organization of ribosomal rRNA genes: 18S, 5.8S, 28S and 5S; and the underlined ITS (ITS1 and ITS2) region.

netic classification of cellular life enabled the field of molecular taxonomy to precisely establish microbial identity. Prokaryotes and eukaryotes are identified based on their 16S rRNA gene and 18S rRNA gene, respectively (*Figure 1*). The rRNA taxonomic molecular markers have enabled the discovery of several unculturable microbes. Subsequently, Norman Pace's work in targeted sequencing of the 16S rRNA gene has revolutionized the culture-independent identification of prokaryotes. The 16S rRNA is one of the components of the small subunit (the 30S) of the prokaryotic ribosome (*Figure 1A*) [3]. The 16S rRNA gene is 1500 bp in length, which contains nine individual variable regions (V1-V9) interspersed by conserved regions (*Figure 1A*) [3]. A combination of single nucleotide polymorphisms (SNPs) found within the respective variable regions of the 16S rRNA gene is used to establish the taxonomic identity of prokaryotes [3]. Similarly, the 18S rRNA gene and the internal transcribed spacer (ITS) sequences located within the eukaryotic rRNA cistrons are together used to determine the taxonomic identity of fungal commensals (*Figure 1B*).

On the contrary, the taxonomic identification of human-associated viruses and bacteriophages poses a significant challenge due to the lack of a conserved virus-specific universal taxonomic marker and limitations in the reliable sampling of entire virus-like par-

ticles (VLPs) within a niche [4]. Primarily, VLPs are isolated through (0.2 μ m) filtration and ultracentrifugation, based on their size and density, respectively. VLPs are either identified or predicted based on their similarity to the known family of viruses available in the viral metagenomic database, such as Virus Orthologous Groups (VOGDB), Human Virome Protein Cluster (HVPC), and vContact2 [4].

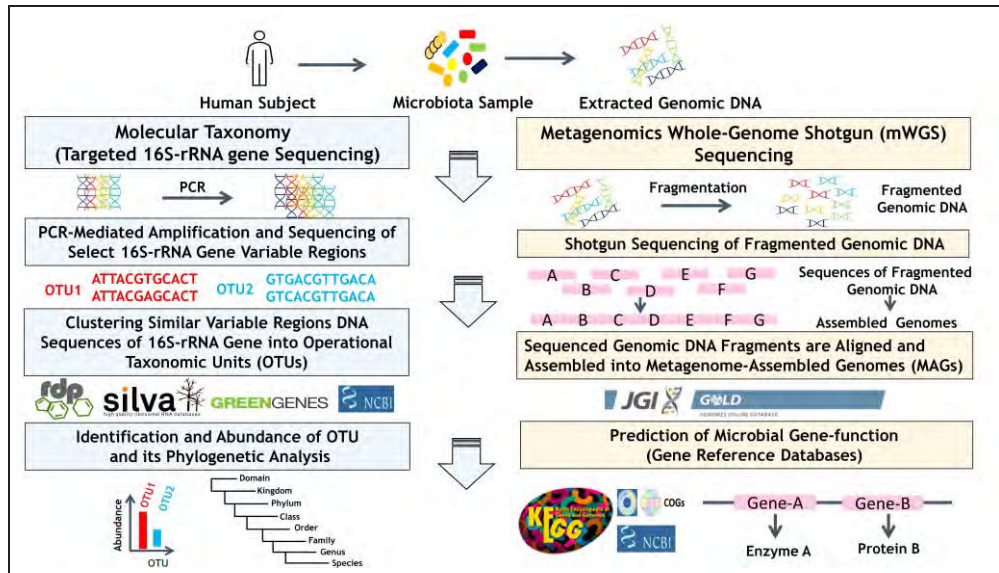
A typical prokaryotic taxonomic profiling study involves three key steps: first, the extraction of microbial genomic DNA; second, targeted amplification of 16S rRNA gene variable region by polymerase chain reaction (PCR); and third, sequencing the amplified 16S rRNA gene variable regions (*Figure 2*) [5]. Parallel to the taxonomic determination, the extracted microbial genomic DNA is fragmented and randomly sequenced by the metagenomic whole-genome shotgun sequencing (mWGS) method. Subsequently, the sequences of the fragmented DNA are aligned and assembled into metagenome-assembled genomes (MAGs) based on the overlapping sequences between the fragmented DNAs (*Figure 2*) [5]. Moreover, the metagenomic information helps to authenticate the targeted 16S rRNA gene-based taxonomic identity [5]. Hence, the DNA sequence-based culture-independent taxonomic and metagenomics approach together surmounts the great plate anomaly.

Human microbiota's functional aspects are assessed based on the nature of microbial genes hosted by the commensals. The annotation of commensals' genes and their associated metabolic pathways are performed based on their match to the existing reference gene databases such as the Kyoto Encyclopaedia of Genes and Genomes and Cluster of Orthologous Groups.

Meta-omics

Human microbiota's functional aspects are assessed based on the nature of microbial genes hosted by the commensals. The annotation of commensals' genes and their associated metabolic pathways are performed based on their match to the existing reference gene databases such as the Kyoto Encyclopaedia of Genes and Genomes (KEGG) and Cluster of Orthologous Groups (COGs) (*Figure 2*) [6]. Furthermore, the high throughput 'meta-omics' approaches such as the meta-transcriptomics (profile of actively transcribed genes), meta-proteomics (profile of expressed-proteins), and meta-metabolomics (profile of cellular-metabolites), are col-





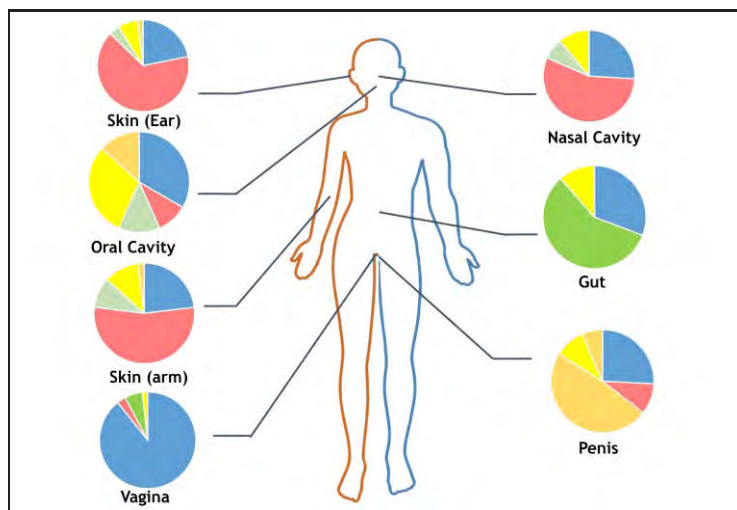
lectively used to define the biochemical and metabolic potential of commensals [5, 6]. Thus, the combined metagenomics and meta-omics approach enable the researcher to distinguish between the causation and correlation associated with the microbiota composition in the state of health and disease.

Human Microbiome Project (HMP)

Large-cohort-based human microbiome studies were possible with the advent of the low-cost, high-throughput next-generation (DNA) sequencing (NGS) platforms and the necessary computational tools to process and analyze the extensive DNA sequencing data. Notably, DNA sequencing technology and computational tools have undergone phenomenal development during the decade-long Human-Genome Project (HGP) (1990–2003). The first comprehensive study of the human microbiota, known as the Human Microbiome Project (HMP), was initiated by the National Institute of Health (NIH), USA, to determine the microbial composition of healthy adults [7]. HMP study was held in two phases during 2007–2016 with a budget of 215 million US dollars [7]. The first phase of HMP primarily established the clinical sampling protocols and

Figure 2. Molecular taxonomy and metagenomics. The graphical illustration shows the key steps involved in the 16S rRNA gene-based taxonomic profiling of prokaryotes and metagenomic analysis.

Figure 3. Human microbiome. The commensals' composition differs in its abundance and proportion across the human body sites. The Human Microbiome Project (HMP) study shows that human adults share similar bacterial microbiota composition at the phyla level, except at the genital region [8, 16]. A representative model pie-chart (not drawn to scale) shown in different colors represents different bacterial phyla shared between the adult males (blue) and females (brown). For detailed information on the specific bacterial (phyla) constituents found in the human body, please refer to the following cited articles [8, 16, 40].



computational tools, which enabled the metagenomic and taxonomic profiling of human microbiota in healthy adults [8]. In contrast, the second phase of HMP addressed the microbiota composition associated with clinical conditions such as pregnancy, preterm birth, and chronic diseases such as inflammatory bowel disease (IBD) and type-2 diabetes [6].

During the first phase of the HMP study, 242 healthy adults (129 males and 113 females) enrolled within the 18–40 age group, representing a cohort of healthy adults [8]. The enrolled subjects were sampled thrice during the two-year sampling period. A total of 4,788 specimen samples were collected during the study, where 15–18 samples were collected per subject from the five primary body sites: mouth, skin, nose, gut, and vagina (Figure 3) [8]. To avert drug-mediated perturbation of microbiota composition in the enrolled subjects, medications such as antibiotics and immunomodulators were restricted [8].

HMP study employed Roche 454 pyrosequencing method, an NGS sequencing platform, to perform the taxonomic specific targeted sequencing of the 16S rRNA gene. The short sequence coverage limitation of the Roche 454 sequencing method restricted the accessibility to a short portion of the 16S rRNA gene [8]. Thus, the



HMP study targeted three variable windows regions spanning the nine variable (V1-V9) regions of the 16S rRNA gene: (V1-V3), (V3-V5), and (V6-V9) (*Figure 1A*) [3, 8, 9]. The accuracy and resolution of diverse microbial taxa are dependent on the degree of confidence and reliability of a chosen variable or its window regions. The DNA sequencing primers (oligonucleotides) targeting the V1-V3 and V3-V5 window regions yielded high-quality sequencing data. However, only V3-V5 window region sequencing data has shown high taxonomic accuracy and sensitivity, enabling the detection of diverse bacterial taxa compared to V1-V3 and V6-V9 window regions [3, 8]. Therefore, probing all three window regions of the 16S rRNA gene enabled the HMP study to describe bacterial diversity accurately [3, 7]. In addition, metagenomic studies widely target individual or combination of variable regions such as V3-V4, V4, V4-V5, and V6-V8 for their high reliability and taxonomic accuracy (*Figure 1A*) [10].

The ensemble of DNA sequences that resulted during the 16S rRNA gene-targeted sequencing was clustered and classified into operational taxonomic units (OTUs) depending on their degree of sequence similarities. In metagenomic studies, OTUs serve as a substitute for conventional taxonomic units (*Figure 2*) [8]. A sequence identity threshold of 97% and 99% are typically applied as a cut-off to define the OTU clusters as the genus and species, respectively [8, 10]. Furthermore, the OTU clusters are annotated under the conventional taxonomic units based on their closeness to the known 16S rRNA gene reference (ribosomal) databases, namely Greengenes, SILVA, and Ribosomal Database Project (RDP) (*Figure 2*) [8]. Finally, the OTUs and their respective sequenced copy numbers account for the commensals' taxonomic diversity and abundance in the sampled body site (*Figure 2*) [8].

Taxonomic Diversity and Metagenomics

HMP study shows that the human body sites differ in their microbial composition, varying in their distribution and ratio (*Fig-*



ure 3) [8]. For example, gut bacterial microbiota is composed of major phyla such as Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, and Verrucomicrobia. However, Firmicutes and Bacteroidetes alone represent about 80–90% of the gut bacteria [8, 11–14]. The human gut microbiota composition also undergoes gradual and subtle changes with aging [11, 15]. The interpersonal variation in microbial composition is restricted to the lower taxonomic levels (species and genus); in contrast, they share a similarity in the higher taxonomic level (phyla) composition, except differing between the male and female genital regions (*Figure 3*) [8, 16].

Around 4.5 trillion bases of microbial DNA were sequenced during the first phase of the HMP study amounting to 14.23 terabytes (TB) electronic storage data. Over 2200 bacterial reference metagenome-assembled genomes (MAGs) were deposited by HMP in the Genomes Online Database (GOLD).

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Gut Microbiota: What Are They Doing in Our Gut?

The mechanistic understanding of gut microbiota’s role in human health and disease remains an active area of biomedical research. The evolutionary pressure to acquire gut microbiota is primarily attributed to the commensals’ essential role in expanding our digestive capability, enabling protection against gut pathogens, maturing the immune system, etc., [18]. Remarkably, the perturbation of the initial gut microbiota acquisition process during infancy is associated with the increased susceptibility of developing chronic disorders such as autoimmune diseases, metabolic disorders, recurring gut infections, and so forth [18]. Hence, the initial acquisition of gut commensals and their complex assem-



bly into a stable microbial community remains a prerequisite to its functionality as a physiological organ and support the infant's growth and development.

How Do We Acquire Our Gut Microbiota?

Our foremost colonizing gut commensals resemble either that of the mothers' skin or vaginal microbiota, depending on the type of birth delivery—Caesarean-section or vaginal, respectively [18]. The womb's sterile (microbe-free) environment is long-held to be critical for fetal gestational growth; however, this assumption is currently challenged [18]. Strikingly, the analysis of the infant's first stool 'meconium' shows the presence of bacterial DNA, which resembles that of the mother's placental and oral microbiota, suggesting the commensals' transmission from mother to the fetus occur before birth [18].

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Fetal Development and Maternal Microbiota

Our interaction with microbes and their metabolites begins right from conception in the fallopian tube [18]. The mother's vaginal and gut microbiota profoundly impacts the prenatal developmental stages—germinal, embryonic, and fetal [18]. The infant's low birth weight, stunted growth, and preterm birth are attributed to the maternal microbiota imbalance [18]. For example, the deficiency of *Lactobacillus* spp. in the mother's gut and vaginal microbiota has been associated with reduced fetal developmental growth and shortened fetal gestation period [18]. Therefore, maternal microbiota balance (homeostasis) is vital during fetal development and growth.

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Initial Gut Microbial Colonization

The initial gut microbial colonization during infancy impacts the growth and maturation of the immune, neural, and endocrine organs [18, 19]. Poor nutrition and antibiotics perturb an infant's initial gut colonization process [18]. For instance, contrary to



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formula-fed infants, breastfed infants have a lower risk of developing autoimmune disorders [18]. Mainly, breast milk contains microbial growth substrates (prebiotic) used by gut commensals, thus facilitating selective microbial colonization of the infant's gut. Also, the mucus secreted within the gut lumen serves as a growth substrate for mucin-degrading bacterial gut commensals, namely *Akkermansia muciniphila* [18]. Furthermore, the maternal antibodies transferred to the infant via breast milk specifically target the gut pathogens (passive immunity), ensuring selective gut microbial colonization [19]. Thus, the initial gut microbial colonization is a critical facilitated event for establishing functional gut microbiota and maintaining long-term health.

Maternal Microbiota and Infant Gut Colonisation

Breast milk contains over 200 human-indigestible short oligosaccharides, known as human milk oligosaccharides (HMOs). A select bacterial gut commensals' (*Bifidobacterium* spp. and *Bacteroides* spp.) can metabolize the HMOs, thus enabling their selective gut colonization [18]. During the initial gut microbial colonization onset, the active microbial consumption of HMOs correlates with the declining levels of HMOs in the infant stool [18]. The succession of the infant gut microbiota composition occurs during the three significant dietary transitions: a breastfed diet, followed by the introduction of solid food (dietary fiber) with breastfeeding (weaning), and finally the solid food without breastfeeding (weaned) (Figure 4) [18]. Therefore, HMOs and dietary fibers, together, serve as prebiotics, fostering the initial gut microbial colonization.

The initial colonizing gut commensals are primarily acquired from the mother. The maternal bacterial commensals such as *Lactobacillus* spp., *Prevotella* spp., *Staphylococcus* spp., and *Corynebacterium* spp., present in the vaginal cavity and the skin colonize the infant's gut [18]. The breastfed infant further acquire commensals found in the mother's mammary gland and on the surface of the nipple [18, 20]. Strikingly, breast milk contains over $\sim 10^9$ bac-



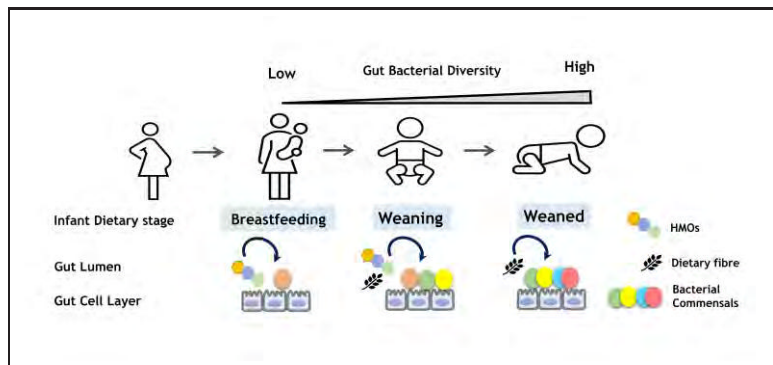


Figure 4. Infant gut colonisation. Infant's initial dietary transitions facilitate gut microbial colonization. The initial gut colonizing commensals are naturally selected based on their ability to use the HMOs present in breast milk. During the infant's initial breastfed state, the gut has a low bacterial diversity. However, the subsequent transition to a dietary fiber-containing solid food during the weaning and weaned states increases the gut bacterial diversity significantly.

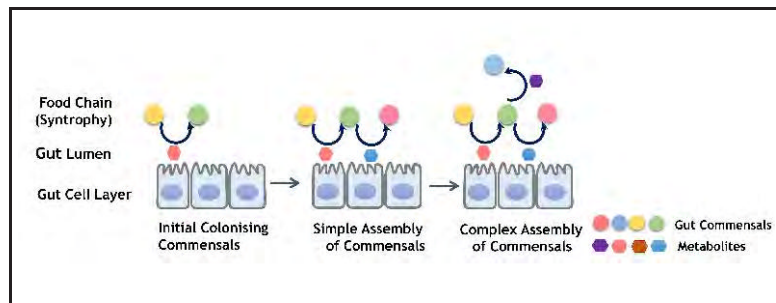
teria per liter, facilitating commensals' direct transfer from the mother to the infant [18, 21]. The bacterial microbiota of the breast milk mainly contains Proteobacteria (*Enterobacteriaceae* spp. and *Pseudomonadaceae* spp.), and the rest are Bacteroides (*Bifidobacterium* spp.) and Firmicutes (*Lactobacillus* spp.) [20]. In contrast, the surface of the nipple is mainly populated by Firmicutes (*Staphylococaceae* spp. and *Streptococaceae* spp.) [20]. Thus, vaginal delivery and breastfeeding provide a natural passage to acquire maternal commensals during birth and infancy.

Gut Microbiota Assembly and Syntrophy

During the initial three years of infant life, the gut microbiota composition undergoes a series of successions until an adult-like microbiota composition (stable community of diverse commensals) is attained (Figures 4 and 5) [18]. Intriguingly, a web of interconnected food chains sustains the complex assembly of diverse gut microbial communities. The metabolic byproducts (metabolites) released by commensals serve as a growth substrate for other commensals; this interdependent cross-feeding food chain is called syntrophy (Figure 5). Dietary preferences profoundly impact gut microbial diversity. For example, African children fed on a high-fiber diet show a high abundance of gut Bacteroidetes; in contrast, Italian children on a low-fiber Western diet have gut Firmicutes [22]. Therefore, diet and microbial syntrophy together enable the assembly of diverse gut commensals.

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Figure 5. Gut microbiota and syntrophy. During the infant's initial gut microbial colonization, the gut microbiota undergoes a gradual transition from fewer gut commensals (simple assembly) to an enormous collection of diverse commensals (complex assembly) sustained by their nutritional interdependency (syntrophy). The cross-feeding of metabolites (colored hexagons) between the gut commensals (colored circles) enables a stable gut microbial community structure.



Gut Immunity Maturation and Tolerance

Gut microbiota plays a vital role in maintaining a healthy immune system. In the absence of gut microbiota, maturation of the infant immune system—distinguishing the commensal (friend) from a pathogen (foe)—gets compromised [19]. For example, germ-free mice (mice lacking microbiota) show stunted growth of immunological organs: spleen, lymph node, and Peyer patch, and reduced counts of B (Bursa) and T (Thymus) specialized immune cells of the adaptive immune system [19]. Also, germ-free mice suffer from vitamin K and B12 deficiency [23]. Notably, most of the immune system workforce guards the gut alone [19]. Gut immune cells undergo maturation to maintain restraint (tolerance) towards trillions of diverse gut commensals [19]. The immunosuppressive signals presented by the gut commensals and the breast milk components during infancy programme the infant immune cells to maintain tolerance towards gut commensals [19]. Thus, during infancy, gut commensals and breast milk play a critical role in the maturation of the infant's immune system.

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Gut Colonization Resistance

The presence of anatomical gut mucosal barrier averts the incursion of food antigens, pathogens, and commensals present in the gut lumen from entering the bloodstream (*Figure 7*). Besides, gut microbiota plays a crucial role in protecting the gut from the invasion of gut pathogens, commonly referred to as gut colonization resistance. Gut commensals compete and curtail the access of

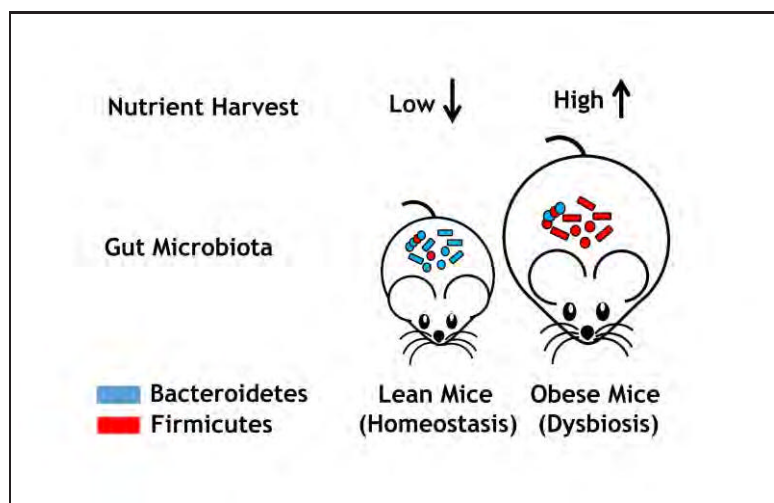


Figure 6. Obesity and gut microbiota. Lean and obese mice significantly differ in their gut microbiota composition, particularly in the proportion of bacterial phyla: Firmicutes and Bacteroidetes [13, 28, 41]. The higher proportion of gut Firmicutes over Bacteroidetes observed in obese mice (gut dysbiosis) is attributed to their enhanced nutrient harvesting capability, leading to the increased body fat build-up.

gut pathogens to their niche, essential nutrients, and trace metals [24]. For example, gut commensals specifically target their competitor bacteria through their weaponry type-VI secretion system (T6SS) to inject a toxin into its target cell (*Figure 7*) [24]. Also, the fermentation end-products released by the gut bacterial commensals, such as the short-chain fatty acids (SCFAs), secondary bile salts, and antibacterial peptides (bacteriocins), suppress the growth of bacterial gut pathogens (*Figure 7*) [24]. Bacteriophages further control the overall gut bacterial load and pathogens by their bacteriolytic infections [24]. Gut colonization resistance and the gut immune system fortify the gut mucosal barrier function [24]. Therefore, gut colonization resistance works as a frontline defense system against pathogens, alleviating the burden on the mucosal barrier and the gut immune system.

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Faecal Microbiota Transplantation (FMT)

Antibiotics impair the gut microbiota composition and its homeostasis, leading to a pathological state known as dysbiosis. Rampant antibiotic usage has led to the emergence of antibiotic-resistant bacterial pathogens, posing severe health challenges. Gut dysbiosis compromises the gut's colonization resistance function, caus-

their primary energy source [26]. Besides, gut microbiota also provides water-soluble B-vitamins and essential fat-soluble vitamins (vitamin-K), required for the biosynthetic pathways and blood coagulation. Therefore, gut commensals fulfill our nutritional requirements.

Diet plays a major determining factor in shaping gut microbiota composition. Adequate dietary soluble fiber (prebiotic) intake is needed to maintain high SCFAs levels to support healthy gut cells and gut microbiota [26]. A plant-based high-fiber diet has gained prominence in Western countries due to its association with lower cardio-metabolic risks, such as type-2 diabetes, obesity, and coronary heart disease [27]. Moreover, commonly consumed substances such as ultra-processed foods, antibiotics, artificial sweeteners, food preservatives and dyes, chlorinated water, and so forth are known to alter gut microbiota, triggering gut dysbiosis. In essence, a low-fiber diet and synthetic chemicals with antimicrobial activity (xenobiotics) contribute to gut dysbiosis.

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Obesity and Gut Microbiota

Obesity—often referred to as a lifestyle disorder—is the most prevalent health challenge faced by the modern urban civilization. The role of gut microbiota in obesity has been experimentally demonstrated in animal models. For example, gut microbiota transplantation from obese to lean mice led to transforming the recipient mice's phenotype from lean to obese [28]. Furthermore, the enhanced nutrient harvesting capability of the gut microbiota in obese mice is attributed to the abundance of gut Firmicutes over Bacteroidetes, thus contributing to increased body fat deposition (*Figure 6*) [28]. Interestingly, obese mice transplanted with the gut microbiota of lean mice led to its phenotype transformation from obese to lean [29]. In addition, the consumption of low (sub-therapeutic) doses of antibiotics is seen to cause obesity in pigs by altering their gut microbiota. The plasticity of gut microbiota composition and its associated phenotype suggests a potential intervention strategy to treat human metabolic disorders.



Microbiota-Gut-Brain Axis

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The gut has over 500 million neurons and is often referred to as our ‘second brain’ [30]. The gut-brain bidirectional communication, known as the ‘gut-brain axis’, regulates mood, hunger, cognition, memory, sleep, and so forth [30]. The gut and brain communication occur through neurotransmitter-mediated signaling via the vagus nerve (*Figure 7*). Neurotransmitters such as serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), and histamines, modulate the gut-brain axis [30, 31]. Some of these neurotransmitters are produced in the brain, adrenal gland, and gut (endocrine, immune and neural cells). Serotonin (synthesized from amino acid precursor tryptophan) regulates mood and happiness, sleep-wake cycle, bowel movement (peristalsis), and respiration [31]. Dopamine (produced from amino acid precursor tyrosine) regulates arousal, reward, and reinforcement behavior [31]. Norepinephrine (made from neurotransmitter precursor dopamine) is involved in alertness, behavior, and cognition (memory and learning) [31]. Histamine (made from amino acid precursor histidine) controls the sleep-wake cycle. Histamines have neuromodulator activity, and thus regulate the release of neurotransmitters such as serotonin and norepinephrine [31].

In contrast, GABA (produced from amino acid precursor glutamate) acts as an inhibitory neurotransmitter in the brain and spinal cord by suppressing neural transmission [30, 31]. Remarkably, several bacterial gut commensals are shown to either produce these neurotransmitters or consume host-made neurotransmitters. Thus, gut microbiota exerts influence over the host physiology and behavior. For example, gut bacteria such as *E. coli* (serotonin), *Bacillus* spp. (dopamine and norepinephrine), *Bifidobacterium* spp. and *Lactobacillus* spp. (GABA and histamine), are known for their synthesis of neurotransmitters [31]. In addition, SCFAs produced by gut commensals modulate gut neurotransmitter (serotonin) production (*Figure 7*) [31].

Interestingly, neurotransmitters modulate virulence, growth, and biofilm formation in gut bacteria through a quorum sensing mech-



anism [31]. The neurotransmitter levels found in the gut lumen and serum directly correlate with the gut bacteria's presence. Unlike conventional mice, germ-free mice have lower neurotransmitters in the gut. Gut commensals mediated excess production of neurotransmitters, and the depletion of the host neurotransmitters by gut commensals are likely to have physiological consequences by modulating the gut-brain axis. Thus, gut commensals regulate the gut-brain axis by modulating the levels of gut neurotransmitters.

Gut microbiota also modulates gut hormones production. For example, the elevation of gut hormone (ghrelin) by gut commensals results in overeating behavior through the overstimulation of the vagus nerve [30]. Besides, gut commensals-derived peptides alter our satiety and hunger by mimicking the gut hormones [30]. Numerous gut microbial metabolites play a vital role in health. Hence, qualitative and quantitative profiling of gut metabolites likely serves as a diagnostic marker to assess gut health. For example, the gut archaeon, *Methanobrevibacter smithii*, mediated excessive production of methane is associated with constipation and obesity. Thus, gut commensals play a crucial role in regulating our behavior and physiology through the gut-brain axis.

Drug Efficacy and Gut Microbiota

Orally administered drugs are the most widely prescribed pharmacological drugs. Oral drugs undergo modification by the liver's detoxification process, known as the 'first-pass effect', determining the drug efficacy and toxicity. In addition, gut microbiota-drug interaction modulates the effectiveness and toxicity of orally taken drugs [32]. For example, the patients' response to chemotherapy and immunotherapy is also attributed to the gut microbiota-drug interaction [32]. The plethora of drug-interacting microbial proteins and enzymes associated with the gut microbiota potentially alter the active form of the drug and regulate its bioavailability [32]. The current drug development process assesses the potential gut microbiota-drug interaction.

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Human Microbiome: Treasure Mine for Novel Antimicrobials

In the 1920s, Alexander Fleming's accidental discovery of the first antibiotic, 'penicillin' produced by fungi, *Penicillium rubens*, revolutionized the antibiotics as a formidable antibacterial arsenal of modern human civilization. However, antibiotic-resistant microbial pathogens are on the rise, namely, methicillin-resistant *Staphylococcus aureus* (MRSA), while discovering new natural antibiotics classes has plummeted since the 1970s. Several novel molecules produced by the human gut microbiota were found to have antibiotic properties; for example, lugdunin produced by *Staphylococcus lugdunensis*, a bacterium found in the human nose, is shown to kill MRSA [33]. The field exploring novel antimicrobial molecules and their associated biosynthetic gene clusters (BGCs) is referred to as bioprospecting [34]. Hence, discovering novel antibiotics will enable us to counter the current and future challenges of antibiotic-resistant pathogens.

Microbiota of the reproductive tract plays a crucial role in fertility health. Interestingly, the female genital (vaginal) microbiota has a low microbial diversity, mainly dominated by the lactic acid-producing bacteria (*Lactobacillus* spp.), which survives primarily on the glycogen released by the vaginal epithelial cells.

Reproductive Tract Microbiota

Microbiota of the reproductive tract plays a crucial role in fertility health. Interestingly, the female genital (vaginal) microbiota has a low microbial diversity, mainly dominated by the lactic acid-producing bacteria (*Lactobacillus* spp.), which survives primarily on the glycogen released by the vaginal epithelial cells [35]. The female sex hormone estrogen regulates the availability of vaginal glycogen during the menstrual cycle and menopause; correspondingly, the vaginal pH and microbiota compositions alter with the levels of vaginal glycogen [35]. Low vaginal pH serves as a hostile environment for the growth of pathogens.

The urethra and glans harbor the microbiota of the male genital (penile). Bacterial commensals, namely *Corynebacterium* spp., *Staphylococcus* spp., and *Anaerococcus* spp., populate the glans, while circumcised men show enrichment of *Staphylococcus* spp. [36]. Notably, the high and low-quality sperms are associated with the characteristic microbiota composition of the se-



men. While high-quality sperm is related to *Lactobacillus* and *Gardnerella* spp., *Prevotella* and *Bordetella* spp. are associated with low-quality sperm [37]. Therefore, the correlation between the genital microbiota composition and the reproductive health outcome necessitates a novel therapeutic intervention to treat infertility.

Skin Microbiota

Skin—the largest human organ—functions as an anatomical barrier and protects from the external environment. The skin surface is acidic (pH 5), although it varies across the body in its moisture, salt, and oil content. Predominantly, Firmicutes harbor moist skin surfaces, whereas Actinobacteria and Proteobacteria colonize oily skin [38]. In contrast, dry skin surface harbors diverse bacterial microbiota comprising Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes [38]. Basidiomycetes, namely *Malassezia* spp., are the only dominant fungal skin commensals [38]. Strikingly, archaea are sparingly found on young adults' skin, in contrast to their significant presence in young children and aged adults, suggesting a strong correlation between the microbiota composition and age-related physiological state of the skin.

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Human Microbiome Research in India

From a relatively slow start, human microbiome research is picking up pace in India. Given the complex ethnic diversity in India, there is a need to take it up in a more comprehensive manner to develop specific solutions for the Indian population. India's first metagenomics study compared the gut microbiota of a malnourished urban child with a healthy control [19]. The malnourished child's gut microbiota shows enrichment of enteric pathogens (*Campylobacter jejuni* and *Helicobacter pylori*) [39]. The malabsorption of nutrients in malnourished children is attributed to the gut inflammation induced by enteric pathogens [39]. A three-year comprehensive Indian microbiome project (2019–



2022) has been initiated by the National Centre for Microbial Resource (NCMR) to profile around 20,600 Indians covering 103 endogamous communities across India to study the impact of environment, diet, and genetics on their microbiome.

Conclusion

Metagenomics and meta-omics approaches have bolstered researchers to explore the human microbiota and its physiological role. A plethora of correlational studies implicate the role of distinct gut commensals in the state of health and disease; however, their causation and mechanism of action are still unclear. Insightfully, Hippocrates advocated: “*All disease begins in the gut*”, highlighting the lynchpin role of the gut in maintaining overall health. Hence, understanding our hardwired physiological nexus with our gut microbiota has the potential to revolutionize the future course of medicine. Earth has innumerable undocumented microbial species. Therefore, 21st century will be an era of reckoning for the field of metagenomics in unraveling the entirety of Earth’s microbiome and tracing our cellular lineage to the last universal common ancestor (LUCA). Besides revealing novel insights regarding the impact of microbial communities on human health, this knowledge will also have far-reaching implications. So, with a complete understanding of the human microbiota, one can envision creating a synthetic ecosystem for human colonization on the moon and beyond.

“*All disease begins in the gut.*”

–Hippocrates



Box 1. Glossary

Archaea: A taxonomical domain of life that exhibits prokaryotic cellular organization but shares evolutionary closeness to the eukarya domain.

Autoimmune disease: A chronic disorder manifested by abnormal immune reaction misdirected against one's body tissue.

Bacteriocin: An bactericidal peptide secreted by certain bacterial strains to target closely related bacteria.

Bacteriophages: An acellular entity (virus) that specifically infects and kills its host bacteria.

Commensal: Organisms that benefit from a biological interaction without harming their partner.

LUCA: A common ancestor to all cellular life on the Earth.

Metagenomics: Studying the collective microbial genomes (metagenome) using the DNA sequencing approach.

Mutual: A biological interaction in which both the interacting partners get benefits.

Neurotransmitter: A chemical molecule secreted by the neuron that diffuses across the interneuron gap to transmit a neural signal to a target neuron.

NGS: A high-throughput DNA sequencing platform that superseded the first-generation Sanger sequencing.

Operon: An array of genes controlled by a single promoter.

Passive immunity: A transient acquired immunity obtained following the administration of antibodies from an immunized individual to a non-immunized individual.

PCR: A method to exponentially duplicate a linear duplex template.

rRNA: Ribosomal ribonucleic acid (rRNA) is the catalytic component of the ribosome (protein-synthesizing cellular organelle).

Shotgun sequencing: A sequencing approach that involves fragmentation of a large genome into shorter DNA fragments. The sequences of the short DNA fragments are orderly assembled into their original genome size based on their contiguous overlapping sequences.

SNP: A genetic variation found within a population where individuals differ at a single-nucleotide position within a conserved gene sequence.

Syntrophy: A nutritional interdependency of one species on the product of another species.

T6SS: A secretory system present in gram-negative bacteria that enables injecting a toxin directly into a target bacterium.

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Suggested Reading

- [1] E Farré-Maduell, and C Casals-Pascual, The origins of gut microbiome research in Europe: From Escherich to Nissle, *Human Microbiome Journal*, 14: p.100065, 2019.
- [2] J C Lagier et al., Culturing the human microbiota and culturomics, *Nat. Rev. Microbiol.*, 16, pp.540–550, 2018.
- [3] Jumpstart Consortium Human Microbiome Project Data Generation Working Group, Evaluation of 16S rDNA-based community profiling for human microbiome research, *PLoS One*, Vol.7, No.6, p.e39315.
- [4] M Khan Mirzaei et al., Challenges of studying the human virome - Relevant emerging technologies, *Trends Microbiol.*, 2020.
- [5] F N New, and I L Brito, What is metagenomics teaching us, and what is missed? *Annu. Rev. Microbiol.*, Vol.74, pp.117–135, 2020.
- [6] Integrative HMP (iHMP) Research Network Consortium, The Integrative Human Microbiome Project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease, *Cell Host Microbe*, Vol.16, No.3, pp.276–289, 2014.
- [7] Team N.I.H.H.M.P.A., A review of 10 years of human microbiome research activities at the US National Institutes of Health, Fiscal Years 2007–2016, *Microbiome*, Vol.7, No.1, p.31, 2019.
- [8] Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome, *Nature*, Vol.486, No.7402, pp.207–214, 2012.
- [9] Human Microbiome Project Consortium, A framework for human microbiome research, *Nature*, Vol.486, No.7402, pp.215–221, 2012.
- [10] J S Johnson et al., Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis, *Nat. Commun.*, Vol.10, No.1, p.5029, 2019.
- [11] A Vaiserman et al., Differences in the gut Firmicutes to Bacteroidetes ratio across age groups in healthy Ukrainian population, *BMC Microbiol.*, Vol.20, No.1, p.221, 2020.
- [12] M Arumugam et al., Enterotypes of the human gut microbiome, *Nature*, Vol.473, No.7346, pp.174–180, 2011.
- [13] F Magne et al., The Firmicutes/Bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? *Nutrients*, Vol.12, No.5, p.1474, 2020.
- [14] L Qian et al., Association analysis of dietary habits with gut microbiota of a native Chinese community, *Exp. Ther. Med.*, Vol.16, No.2, pp.856–866, 2018.
- [15] E B Hollister et al., Structure and function of the healthy pre-adolescent pediatric gut microbiome, *Microbiome*, Vol.3, p.36, 2015.
- [16] D E Nelson et al., Bacterial communities of the coronal sulcus and distal urethra of adolescent males, *PLoS One*, Vol.7, No.5, p.e36298, 2012.



- [17] B T Tierney et al., The landscape of genetic content in the gut and oral human microbiome, *Cell Host Microbe*, Vol.26, No.2, pp.283–295 e8, 2019.
- [18] R C Robertson et al., The human microbiome and child growth – First 1000 days and beyond, *Trends Microbiol.*, Vol.27, No.2, pp.131–147, 2019.
- [19] M Dzidic et al., Gut microbiota and mucosal immunity in the neonate, *Med. Sci.*, (Basel), Vol.6, No.3, 2018.
- [20] P S Pannaraj et al., Association between breast milk bacterial communities and establishment and development of the infant gut microbiome, *JAMA Pediatr.*, Vol.171, No.7, pp.647–654, 2017.
- [21] L Fernandez et al., The human milk microbiota: Origin and potential roles in health and disease, *Pharmacol. Res.*, Vol.69, No.1, pp.1–10, 2013.
- [22] C De Filippo et al., Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa, *Proc. Natl. Acad. Sci., USA*, Vol.107, No.33, pp.14691–14696, 2010.
- [23] E Rackaityte and S V Lynch, The human microbiome in the 21(st) century, *Nat. Commun.*, Vol.11, No.1, p.5256, 2020.
- [24] M T Sorbara and E G Pamer, Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them, *Mucosal Immunol.*, Vol.12, No.1, p.1–9, 2019.
- [25] R E Ooijsaar et al., Clinical application and potential of fecal microbiota transplantation, *Annu. Rev. Med.*, Vol.70, pp.335–351, 2019.
- [26] G den Besten et al., The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism, *J. Lipid Res.*, Vol.54, No.9, pp.2325–2340, 2013.
- [27] H Kahleova, S Levin and N Barnard, Cardio-metabolic benefits of plant-based diets, *Nutrients*, Vol.9, No.8, p.848, 2017.
- [28] P J Turnbaugh et al., An obesity-associated gut microbiome with increased capacity for energy harvest, *Nature*, Vol.444, No.7122, pp.1027–1031, 2006.
- [29] Z L Lai et al., Fecal microbiota transplantation confers beneficial metabolic effects of diet and exercise on diet-induced obese mice, *Sci. Rep.*, Vol.8, No.1, p.15625, 2018.
- [30] J F Cryan et al., The microbiota-gut-brain axis, *Physiol. Rev.*, Vol.99, No.4, pp.1877–2013, 2019.
- [31] P Strandwitz, Neurotransmitter modulation by the gut microbiota, *Brain Res.*, 1693(Pt B), pp.128–133, 2018.
- [32] Y Vazquez-Baeza et al., Impacts of the human gut microbiome on therapeutics, *Annu. Rev. Pharmacol. Toxicol.*, Vol.58, pp.253–270, 2018.
- [33] A Zipperer et al., Human commensals producing a novel antibiotic impair pathogen colonization, *Nature*, Vol.535, No.7613, pp.511–516, 2016.
- [34] Y Sugimoto et al., A metagenomic strategy for harnessing the chemical repertoire of the human microbiome, *Science*, Vol.366, No.6471, p.eaax9176, 2019.
- [35] D H Martín, The microbiota of the vagina and its influence on women's health and disease, *Am. J. Med. Sci.*, Vol.343, No.1, pp.2–9, 2012.
- [36] R Mandar, Microbiota of male genital tract: impact on the health of man and his partner, *Pharmacol. Res.*, Vol.69, No.1, pp.32–41, 2013.
- [37] M Rowe et al., The reproductive microbiome: An emerging driver of sexual



GENERAL ARTICLE

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selection, sexual conflict, mating systems, and reproductive isolation, *Trends Ecol. Evol.*, Vol.35, No.3, pp.220–234, 2020.

- [38] E A Grice and J A Segre, The skin microbiome, *Nat. Rev. Microbiol.*, Vol.9, No.4, pp.244–253, 2011.
- [39] S S Gupta et al., Metagenome of the gut of a malnourished child, *Gut Pathog.*, Vol.3, p.7, 2011.
- [40] H E Blum, The human microbiome, *Adv. Med. Sci.*, Vol.62, No.2, p.414–420, 2017.
- [41] P J Turnbaugh et al., Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome, *Cell Host Microbe*, Vol.3, No.4, pp. 213–223, 2008.

