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REVIEW

An insight into gut microbiota and its functionalities

Atanu Adak[1] · **Mojibur R. Khan**[1]

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Abstract
Gut microbiota has evolved along with their hosts and is an integral part of the human body. Microbiota acquired at birth develops in parallel as the host develops and maintains its temporal stability and diversity through adulthood until death. Recent developments in genome sequencing technologies, bioinformatics and culturomics have enabled researchers to explore the microbiota and in particular their functions at more detailed level than before. The accumulated evidences suggest that though a part of the microbiota is conserved, the dynamic members vary along the gastrointestinal tract, from infants to elderly, primitive tribes to modern societies and in different health conditions. Though the gut microbiota is dynamic, it performs some basic functions in the immunological, metabolic, structural and neurological landscapes of the human body. Gut microbiota also exerts significant influence on both physical and mental health of an individual. An in-depth understanding of the functioning of gut microbiota has led to some very exciting developments in therapeutics, such as prebiotics, probiotics, drugs and faecal transplantation leading to improved health.

Keywords Gut microbiota · Functions · Health · Therapeutics

Introduction

The life forms on this earth can be clustered into three broad domains: namely Archaea, Bacteria and Eukaryota [1]. All life has evolved from a simple unicellular common ancestor over billion years of evolution giving rise to a complexity of cells within an organism. The human is a superorganism that functions in harmony with trillions of symbiotic bacteria and eukaryotic cells. The host and its symbionts together are called a "holobiont," and their collective genome is known as "hologenome". Variation in the hologenome either by changes in the host genome or the microbiome may occur with reasonable fidelity maintaining plasticity of the holobiont [2]. In 2001, the human genome project was completed after which it was correctly argued that the "crowning achievement" in biology would be incomplete until the synergistic activities between human and microbes are understood [3–5]. Subsequently, several scientific efforts were initiated to understand the relationships between human and human-associated microbial communities. Discoveries of the Human Microbiome Project (HMP) and the Metagenome of Human Intestinal Tract (MetaHIT) opened new horizons in microbiome research for an enhanced understanding of host–microbe interactions at four major colonisation sites of the human body; viz. oral, gut, vagina and skin. Of these four sites, the human gut microbiota has drawn the attention of microbiologists for its clinical significance. Several gut microbiome projects including the Australian Gut Project, the American Gut project, the British gut project, the Canadian Microbiome Initiative, the Human MetaGenome Consortium Japan, the My NewGut project of the European Union and the International Human Microbiome Consortia, etc. were undertaken for a better understanding of the complex gut ecosystem and its role in health and diseases. The human gut (200–300 m^2 of mucosa) is the "secret garden" of ten trillion diverse symbionts (50 bacterial phyla and about 100–1000 bacterial species), collectively known as the 'microbiota'. Microbiota are ten times more abundant than our somatic and germ line cells of the body. The collective genes of microbiota are known as the 'microbiome' which is 150 times larger than the human genome [6, 7]. In an individual, 150–170 bacterial species predominate and get benefits from the warm nutrient rich environment of the gut and perform protective, metabolic and structural functions.

✉ Mojibur R. Khan
mojibur.khan@gmail.com

[1] Molecular Biology and Microbial Biotechnology Laboratory, Life Science Division, Institute of Advanced Study in Science and Technology (IASST), Guwahati, India

In this review, we have summarised (a) the general features of the gut microbiota, the laws of symbiosis and its development along with its distribution in the gastrointestinal (GI) tract; (b) the relationships between the gut microbiota in a metabolic, immunological and structural landscapes of the human body and (c) promising therapeutic approaches including prebiotics, probiotics, drugs and faecal microbiota transplantation that involve in modulation of the gut microbial ecology.

Methods to study gut microbiota

Culture-dependent

In 1881, Robert Koch introduced the plating technique both to culture and identify microorganisms by characterising their biochemical and physiological properties. A culture-based method detects only 30–50% of the bacteria inhabiting in the intestine. Artificial cultural conditions provide a less favourable environment for the growth of uncultivable bacteria. Conventionally, different selective and nutrient agar media are used to isolate and culture the bacteria. Improved methods, such as the enrichment culture technique and pre-incubation of faeces in blood culture bottles, rumen fluid and sterile stool extract were introduced in different culture and physicochemical conditions. These cultural modifications act as natural simulants and facilitate the isolation of previously uncultivable bacteria [8–11]. However, the growth of predominant bacteria in faeces masks the isolation of less dominant ones. Further modifications including the use of antibiotics, bacteriophages and filtration overcome these difficulties. For example, the dominant population of *Escherichia coli* under Proteobacteria phylum hindered the isolation and identification of new bacterial species. The addition of lytic bacteriophages in the culture plate lysed *E. coli* and facilitated growth of an unknown bacterial species (*Enterobacter massiliensis*) of the Enterobacteriaceae family that was not previously detected using classical culture methods. An extensive study on the culturomics approach highlighted the gap between culture-dependent and independent studies and estimated the number of the uncultured microbes from the different microbiome projects [9]. They introduced three modifications to isolate uncultured bacteria with multiple cultural conditions viz. (1) preincubation of a sample in a blood culture bottle, (2) the addition of rumen fluid and (3) the addition of sheep blood that enhanced the isolation of bacterial species by 56, 40 and 25%, respectively. This culture-based approach also used the matrix-assisted laser desorption/ionization based time-of-flight mass spectrometry to discriminate a large number colonies and 16S rRNA sequencing for bacterial identification. This culturing approach had identified 1057 prokaryotic species that include (1) 531 species to the human gut repertoire of which 146 bacteria were previously not reported in the gut, (2) 187 bacteria and 1 archaea (*Haloferax alexandrinus*) not previously isolated from humans and (3) 197 potentially new species. Therefore, the application of culturomics is significant in exploring gut microbial diversity as well as in understanding their causative or curative roles in health and disease.

Culture-independent

With the advent of Sanger sequencing in 1970, Carl Woese and his colleagues introduced 16S rRNA to investigate the bacterial taxa and their phylogeny based on conserved sequences of hypervariable (V1–V9) region [12]. In the following 30 years, polymerase chain reaction (PCR)-based methods, such as denaturing/temperature gradient gel electrophoresis, single-strand-conformation polymorphism, restriction fragment length polymorphism, terminal restriction fragment length polymorphism and quantitative PCR have been developed to study the diversity of microorganisms. Likewise, non-PCR-based molecular techniques, such as microarray and fluorescence in situ hybridization have also been adopted to aid the exploration of microbial diversity [13]. In the 1990s, preparation of a clonal library and sequencing of the metagenome by Sanger's method paved the way to identify taxonomic composition and phylogeny of a microbial community in an ecological niche. In the following years, a number of improvements were made to Sanger's sequencing method which mostly involved the replacement of phosphor tritium-radiolabelling with fluorescently tagged bases and its fluorometric detection through capillary-based electrophoresis. About a decade later in 2005, high-throughput sequencing technique (next generation of sequencing called NGS) was developed to overcome the difficulties identified in Sanger's method in term of ease, cost and time [13]. In this technique, adapter-bracketed DNA molecules are passed over a lawn of complementary oligonucleotides bound to a flow-cell. Thereafter, the bound DNA fragment undergoes a process known as bridge amplification and produces a cluster of clonal populations. During amplification, each fluorescent labelled deoxyribonucleotide triphosphate is detected during sequential cycles of DNA synthesis and identified by fluorophore excitation in a massive and parallel fashion. A typical workflow involved in a culture-independent study of the microbiota is presented in Fig. 1. In 2010, catalogues of 3.3 million non-redundant faecal microbial genes were reported using NGS. This number of genes was 200 times more than any previous studies [6]. Thereafter, a catalogue of 9.8 million non-redundant microbial genes was published from the metagenomic data with 1267 samples; including 760 European from MetaHIT, 139 American from the HMP and 368 from China (large diabetic study). Each sample consisted of 750,000 genes of which 300,000

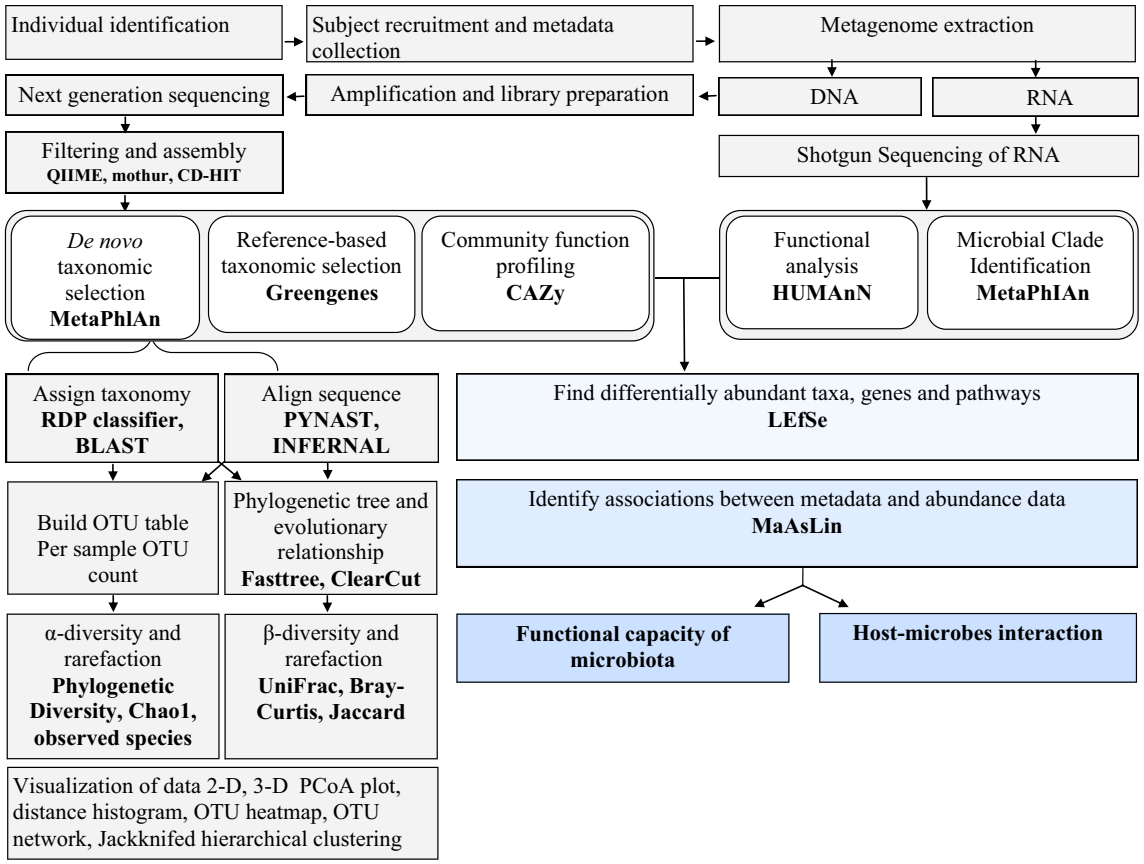

Fig. 1 Bioinformatics work flow of culture independent approach. *QIIME* quantitative insights into microbial ecology, *MG-RAST* metagenomics rapid annotation using subsystem technology, *CAZy* carbohydrate active-enzymes, *MetaPhlAn* metagenomic phylogenetic analysis, *KEGG* Kyoto encyclopaedia for genes and genomics, *COG* clusters of orthologous group, *PICRUst* phylogenetic investigation of communities by reconstruction of unobserved states, *HUMAnN* The Human Microbiome Project Unified Metabolic Analysis Network, *LEfSe* Linear Discriminate Analysis with Effect Size, *MaAsLin* Multivariate Association with Linear Models, *MetaPhlAn* Metagenomic Phylogenetic Analysis, *PICRUSt* Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, *rRNA* ribosomal RNA

genes were similar in more than 50% of the individuals [14]. A Danish study of 540 adults reported the concept of high and low gene counts of the gut microbiome related to obesity [15]. In the recent years, sequencing of the whole genome by NGS involves a fragmentation step before sequencing and then assembling into one long contiguous sequence in silico. Moreover, DNA extraction, library preparation, choice of primer and short read lengths of DNA could be prone to errors [16]. Now, the third generation of DNA sequencing techniques such as PacBio RS II and the MinION nanopore device have shown substantial advancement over second-generation DNA sequencing. In 2011, the PacBio developed a technology based on single molecule real-time sequencing platform using zero-mode waveguides properties. MinION was developed by Oxford Nanopore's technology in which DNA is passed through a nanoscale pore structure to measure the changes of electrical field surrounding the pore which is base dependent. Both technologies can produce longer reads over the NGS and can cover the whole genome. Hence, these techniques can bypass the computational challenges of genome assembly and transcript reconstruction. In addition, epigenetic modifications such as DNA methylation at CpG site and histone modification are detected directly without tagging, breaking and filtering of the DNA. Moreover, the third generation of sequencers are potable and require minimal pre-processing. Therefore, data are collected and analysed in real time for efficient diagnosis and rapid remedial actions. For example, Ebola virus (EBV) was detected within 44 s using Oxford Nanopore's MinION.

Features of GI microbiota

Symbiosis and stability

The gut microbial community is dynamic and has adapted to colonize in the GI. Some specific characteristics are pivotal for colonization, such as an arsenal of enzymes to utilize available nutrients, the right cell-surface molecular pattern

to attach at the "right" habitat, ability to evade bacteriophages and fitness to the reaction-ready immune system of host. A microbe should withstand the physical and chemical stresses in the gut to proliferate rapidly without washing out [17]. Finally, it should survive in the dry and toxic stresses of the "ex-host" environment when making a jump to other hosts [18]. The relationship between host and microbes is commensal (one partner benefits while the other seems unaffected) rather than mutualistic (both partners benefited). The homeostasis of gut microbiota is maintained through feedback mechanisms. Positive feedback disrupts the cooperation of the microbial community in which two microbial species increase in their abundance and can generate a runaway effect [19, 20]. For example, cooperation between two species to increase their abundances in which upsurges of one species increases the abundance of the second and so on. The upsurges of both microbes disrupt the overall diversity and abundance of a community in an ecological niche. Three negative feedback mechanisms have been proposed by which host can create a selective pressure between the cooperating microbes to maintain their homeostasis in the gut. First, the host immune response shapes the microbial community by provoking a specific immune response. Second, spatial segregation of microbial species as these can grow in separate locations as their interaction will be weakened. Finally, supplementation of microbes with alternative carbon sources in such a way that these species no longer rely on their cooperative partners [21]. Mostly, two levels of natural selection of an individual maintain the law of symbiosis and its stability. At the host level, "top-down" selection on the microbial community favours stable societies with a high degree of functional redundancy. An opposing "bottom-up" selection pressure originates from the microbes to allow them to become functionally specialized [17]. Existing relationships between the host and adaptation of the microbial community maintains the gut ecological niche by nurturing its richness and diversity.

Diversity and integrity

The human gut microbiota consists of several types of microbes including bacteria, archaea, eukarya, viruses and parasites [19]. The gut microenvironment mainly favours the growth of bacteria from seven predominant divisions (Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia and Cyanobacteria) [22]. Among these seven divisions, the Bacteroidetes and Firmicutes constitute more than 90% of the total population. Most of the species under the phylum Bacteroidetes belong to the genera of *Bacteroides* and *Prevotella.* Bacterial species under the phylum Firmicutes such as *Clostridium* clusters IV and XIVa which include the genera *Clostridium*, *Eubacterium* and *Ruminococcus* are predominant in gut. *Methanobrevibacter smithii* a hydrogen-consuming methanogen and halophilic *Haloferax alexandrinus* and *Haloferax massiliensis* spp. nov. from Archaea had been reported from human gut [9]. Apart from taxonomic classification, the human microbiome is classified into three discrete enterotypes: *Bacteroides* or *Prevotella* or *Ruminococcus*. Enterotype 1 is characterised by dominance of *Bacteroides* with saccharolytic and proteolytic activities [23]. Enterotype 2 is *Prevotella* dominant and acts as a mucin glycoprotein degrader. Enterotypes 1 involved in synthesis of biotin, riboflavin, pantothenate and ascorbate, while enterotype 2 involved in thiamine and folate synthesis. Enterotype 3 is characterised by dominance of *Ruminococcus* with mucin degrading activities and membrane transportation of sugars. However, the concept enterotypes is debated due to the observation of a high degree of variation between individuals and some data showing more of a continuum rather than three discrete clusters, i.e. the debate is not related to diet differences, though clearly diet drives different composition types [24]. Intake of animal protein and fat for a long period enriched for *Bacteroides* enterotype while carbohydrate-enriched diet encouraged *Prevotella*. Sometimes, the enterotypes *Ruminococcus* and *Bacteroides* are overlapped and found to be indistinguishable [25]. A group of researchers from Taiwan classified enterotypes into *Bacteroides, Prevotella* and *Enterobacteriaceae* and also correlated with their dietary habits. They also claimed that *Enterobacteriaceae* could be a new subenterotypes in the Asian population [26]. Whatever may be the enterotypes, the abundance of bacterial phyla may vary significantly from individual to individual in where some microbial members function as "core microbiota", while others act more like a "flexible pool". The "core microbiota" adapts for reproducibility the "flexible pool" helps in adaptation of a host. The flexible pool is generally acquired from ingested food, water and various components from the environment [27]. The microbial species, *Faecalibacterium prausnitzii, Roseburia intestinalis* and *Bacteroides uniformis* are considered as core bacterial species ($<0.5\%$ relative abundance). Sometimes, exchange of genetic material between core and flexible pool confers the fitness to host for adaptation either to an environment or to a dietary habit. For example, the genes of porphyranases, agarases and its associated proteins were found in the marine bacterium, *Zobellia galactanivorans* living outside the gut. These genes has been transferred to the gut bacterium *Bacteroides plebeius* in Japanese individuals due to consumption of seaweeds that are absent in the North American [28]. In spite of the exchange of genes, different individuals share similar metabolic and functional pathways, including fructose/mannose metabolism, amino-sugar metabolism and N-glycan degradation [29].

Gut microbiota composition

The GI tract is divided functionally and anatomically into the stomach, small and large intestine (LI). The distinct microenvironment and physiochemical barrier of each compartment selects the growth of specific microbiota (Fig. 2).

Stomach

Previously the stomach was thought to be sterile and unreceptive to bacterial growth due to a bactericidal barrier, reflux of bile acids, thickness of the mucus layer and gastric peristalsis. In 1981, the Lancet reported a large number of acid-resistant bacterial strains, such as *Streptococcus, Neisseria* and *Lactobacillus* in the stomach. While in 1982, *Campylobacter pyloridis* was discovered by Robin Warren and Barry Marshall and later in 1984, it was named as *Helicobacter pylori*. In stomach, more than 65% of phylotypes originated from the mouth. These mouth originated bacteria such as *Veillonella*, *Lactobacillus* and *Clostridium* were found to be acid-resistant and transient [30]. Healthy human stomach is normally inhabited by five major phyla viz. Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria and Proteobacteria and the bacterial genera *Prevotella, Streptococcus, Veillonella, Rothia* and *Haemophilus* [31].

Small intestine (SI)

The SI majorly helps in digestion and absorption of foods and nutrients. The SI is divided into three parts: duodenum, jejunum and ileum. Duodenum microenvironment is characterised by bile acids, pancreatic secretions, and antimicrobials agents where faster transit of food and plenty of oxygen limits the bacterial density ($10^{3–4}$ CFU/ml) and diversity. Firmicutes and Actinobacteria are the predominant phyla in the duodenum [32]. The jejunum supports the bacterial colonisation in terms of diversity and density ($10^{3–7}$ CFU/ml) and mostly supports the growth of Gram-positive aerobes and facultative anaerobes ($10^{3–7}$ CFU/ml) including *Lactobacilli, Enterococci* and *Streptococci* [32]. In the transition to the ileum, the bacterial density reaches up to 10^9 CFU/ml with predominance of aerobic species. In contrast, the distal part of ileum close to the ileocecal valve is populated with anaerobes and Gram-negative organisms similar to the colon.

Large intestine (LI)

The LI majorly consists of ascending, transverse and descending colon and cecum. It is a predominant site of water absorption and fermentation of undigested food due to the slower transit of food and its anaerobic condition. In the LI, the number of anaerobes outnumbers the aerobes by a factor of 100–1000. The bacterial density reaches to 10^{12} CFU/ml and is mainly dominated by Firmicutes and Bacteroidetes [33]. The ratio of these two bacterial phyla may alter in different stages of life and even in various pathophysiological conditions. The ratio of Firmicutes and Bacteroidetes is considered as a predictive marker of health and diseases [34]. In the lumen of LI, bacterial genera such

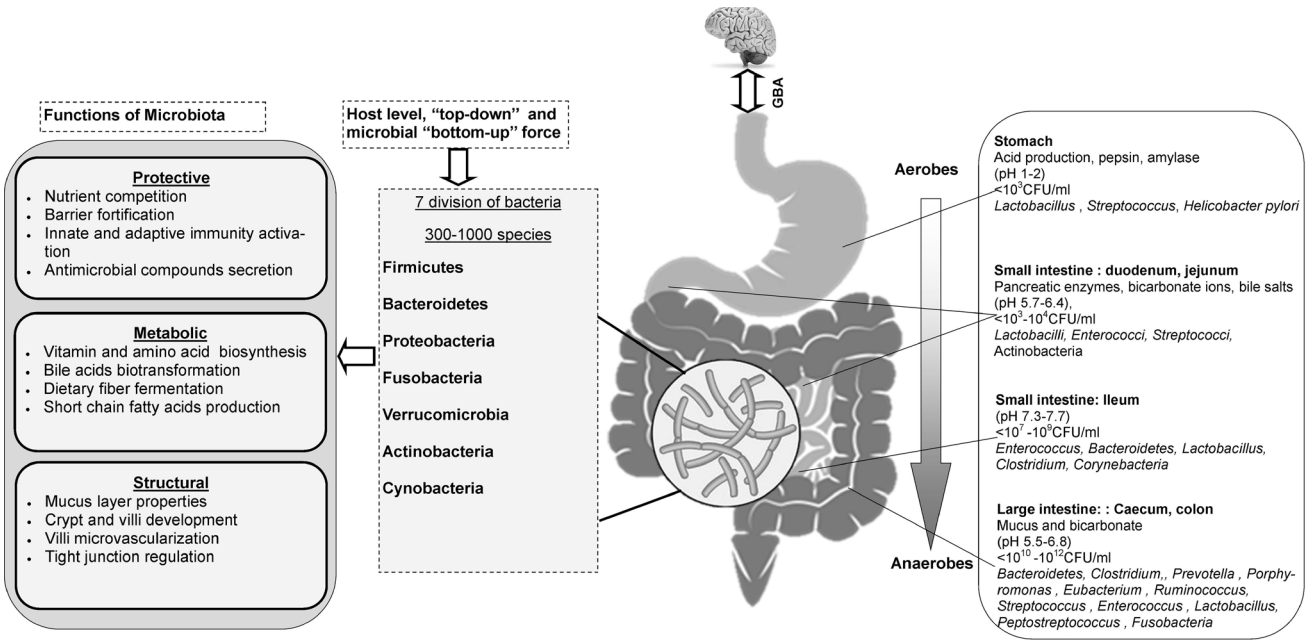

Fig. 2 Distribution of normal gut flora in different parts of intestine and its functional activities and GBA. *GBA* gut brain axis

as *Bacteroides*, *Bifidobacterium, Streptococcus, Enterobacteriaceae, Enterococcus, Clostridium, Lactobacillus* and *Ruminococcus* are predominant, whereas *Clostridium, Lactobacillus, Enterococcus* and *Akkermansia* are associated with the mucosa. In addition, a few pathogens including *Campylobacter jejuni*, *Salmonella enterica, Vibrio cholera*, *E coli* and *Bacteroides fragilis* may present in the LI with lower abundance (0.1%) [35].

Change of microbiota with age

The human microbiota has evolved along with its host and is an integral part of human body. Microbiota is acquired at birth and develops in parallel with the host and plays an important role in the body through adulthood until death. A century of research had claimed that the womb, placenta, amniotic fluid and meconium are sterile and microbiota starts to be acquired after birth. However, recent PCR and DNA sequencing-based methods suggested the presence of bacterial communities in the placenta, amniotic fluid and meconium [36]. Although these advanced techniques overcome the limitations of the cultivable approach, however, they have some inherent limitations. First, the detected bacterial DNA may come from either live or dead bacteria. For example, *Bifidobacterium* and *Lactobacillus* were detected in placenta by the molecular approach; however, the same bacteria were unculturable even though these are readily cultivable organisms [37]. Second, DNA-based assessments of a sample with low microbial biomass are extremely prone to error and contamination in a clinical setting. There is controversy over two opposing hypotheses "sterile womb" and "in utero colonisation" [36]. Apart from this controversy, various maternal factors including prenatal stress, antibiotic therapies and prolong gestation have roles in colonisation of the gut microbiota of a new-born baby. A few studies showed the changes of hormonal profiles during pregnancy that encouraged the growth of *Lactobacillus* (*iners, crispatus, jensenii* and *johnsonii*) and Clostridiales, Bacteroidales and Actinomycetales in the vagina [38, 39]. During vaginal birth, a baby acquires these microbes in their gut. In contrast, a baby born by caesarean section (C-section) acquires microbes from the mother's skin including *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp. Lower microbial diversity and delayed colonisation of *Bacteroidetes* in the C-section baby makes the baby vulnerable to certain pathogens and atopic diseases [40]. After birth, breast-fed babies consume more lysozyme, immunoglobins, lactoferrin, glycans, sialylated and other complex oligosaccharides through mother's milk than formula-fed babies. As a result, gut microflora of breast fed babies are dominated by *Bifidobacterium* and *Lactobacillus* sp. whereas formula fed baby are dominated with *Clostridium, Granulicatella, Citrobacter, Enterobacter* and *Bilophila* [7, 41]. These evidences indicate that vaginal birth and breast-fed babies have healthier gut microbes compared to the formula-fed babies. Thereafter by the age of 3–5 years, the unstable structure and composition of microbiota starts to differentiate and acquires similarity (40–60%) to that of adult [7]. During this period, the gut microbiome also changes in parallel from the earliest lactate utilization to plant polysaccharide digestion, vitamin biosynthesis and xenobiotic degradation [42]. The composition and functions of the established microbiota remain the same if there is no change in long-term dietary habits, antibiotics treatment, stress and pathophysiology in adulthood. In China, research on longevity revealed that the genera *Roseburia* and *E. coli* were significantly higher, whereas *Lactobacillus, Faecalibacterium, Parabacteroides, Butyricimonas, Coprococcus, Megamonas, Mitsuokella*, *Sutterella,* and *Akkermansia* were significantly less in centenarians [43]. In Italy, research on centenarians showed a decline in the abundance of core microbiota (*Ruminococcaceae, Lachnospiraceae,* and *Bacteroidaceae*) with the prevalence of subdominant genera and families (*Eggerthella, Akkermansia, Anaerotruncus, Synergistaceae, Bilophila,* and *Christensenella*) along with age. Abundance of *Akkermansia*, *Bifidobacterium* and *Christensenella* has been identified as a putative signature in the gut ecosystem for healthy ageing and longevity [43–45]. In the centenarians, lifespan decreased due to changes within the phylum Firmicutes and enrichment of 'pathobionts' and proinflammatory response mediated by the cytokines TNF-α, IL-6, MCP-1 and IL-8 [46]. In contrast, microbial secreted colanic acid, a polysaccharide that has been found to regulate fission and fusion dynamics of mitochondria for tuning longevity of *Caenorhabditis elegans* [47].

Genetic variation

Host genetics can influence gut microbiota and its metabolic phenotype. A study in the UK analysed > 1000 faecal samples from 416 twin pairs and showed that host genetics shapes the gut microbiome and obesity phenotype. They found that Christensenellaceae family is the heritable taxon and formed a co-occurrence network with other heritable bacteria and methanogenic Archaea. Transplantation of *Christensenella minuta* in germ-free mice altered the gut microbiota and reduced adiposity and weight in the recipient mice [48]. In a transcriptional analysis, it was found that the expression of 6000 genes of the colonic epithelia was linked to gut microbiota. They identified 12 allele-specific single-nucleotide polymorphism (SNPs) linked to gut microbiota and 8 were found to be associated with diseases including colorectal cancer, Type 2 diabetes (T2D) and obesity [49]. A large-scale cohort study on 1561 healthy individuals

indicated that one-third of the faecal bacterial taxa were heritable and 58 SNPs in 1098 subjects were associated with the relative abundance of 33 taxa. Among these, four loci were replicated in the second cohort of 463 subjects related to *Rikenellaceae, Faecalibacterium, Lachnospira,* and *Eubacterium* [50]. A genome-wide study on Hutterites, a religiously isolated group of people in North America showed that at least eight bacterial taxa were associated with SNPs of the host. The bacterial genus *Akkermansia* was found to be related to obesity and body mass index. Besides, a gender wise variation of gut microbiota was also observed due to difference of their social practices and daily activities [51]. Gut microbial composition also depends on secretor status of ABH antigen in mucosa. The enzyme fucosyltransferase 2 encoded by FUT2 gene that converts type 1 *N*-acetyllactosamine glycan into H antigen which functions as a precursor for the A, B and Lewis b antigens. Non-secretor individual is unable to secrete ABH antigen in their mucus due to a nonsense mutation in the FUT2 gene. Bifidobacterial diversity and richness, particularly *B. bifidum, B. adolescentis* and *B. catenulatum/pseudocatenulatum* was found to be reduced in the non-secretor individual [52]. Research from Israel showed that environmental factors dominate over genetics in shaping the gut microbiota. A cohort of 1046 healthy individuals showed that genetically unrelated individuals had similar gut microbiota composition rather than their relatives who did not have a history of household sharing. They also analysed the previous data of 2252 twins from the UK and found that only 1.9–8.1% of microbiome taxa was heritable. In contrast, 20% of the inter-person microbiome variability is associated with factors such as diet, drugs, anthropometric parameters and lifestyle [53].

Geographical location

The 'Baas-Becking' hypothesis proposed that all microbes distributed everywhere and the local environment is the determinant of the microbial biodiversity [54]. For example, the diet and foraging lifestyle of the hunter-gatherer group (Hadza of Tanzania) determined microbial richness and diversity in the gut in comparison with urban Italian and the African rural farming groups [57]. The dominance of *Prevotella*, *Treponema* and unclassified *Bacteroidetes* in the Hadza aided in the digestion of plant originated fibrous foods [55]. The functionalities study of microbiome on the same group showed that metabolic pathways involved in digestion of a broad-spectrum of carbohydrates, branched-chain amino acid degradation and aromatic amino acid biosynthesis. In contrast, microbiomes of the urban Italians had resistome functionality that was similar to antibiotic resistance genes found in their environment [56]. In the Western lifestyle, the intake of more amino acids, lipids, cholesterol and dairy supports the growth of *Faecalibacterium, Ruminococcus, Bifidobacterium, Bacteroides, Blautia, Bilophila* and *Alistipes* while consumption of sugar and complex carbohydrates by the non-industrialized ethnic societies supports the growth of *Prevotella* [57]. Microbial profiling of rural and urban cohorts from seven ethnic groups of 9 provinces in China reported nine core bacteria (*Balutia, Clostridium, Ruminococcus, Faecalibacterium, Subdoligranulum, Roseburia, Coprocus, Bacteroides* and *Phascolarctobacterium*) linked to their ethnicities/geographies and lifestyles [58]. In India, we have reported six core bacteria (*Faecalibacterium, Eubacterium, Clostridium, Blautia, Ruminococcus,* and *Roseburia*) from 15 ethnicities of four geographical locations. The gut bacterial profiles of Indian ethnicities were similar to the Mongolian population [59]. However, faecal metabolites were dissimilar due to a degree of association of gut microbiota across the ethnicities [60]. Exposure to extreme environments such as high altitude (above 1493 m) is also a factor responsible for altering the gut microbiota due to lower pressure of atmospheric oxygen. As a result, high-altitude visitors like pilgrims, trekkers, climbers and military personnel suffer from non-specific GI complications [61–63]. The cold environmental stress is also a factor for changing of the gut microbiota and energy homeostasis. The transplantation of gut microbiota from cold stress mice to the germ-free mice promoted the conversion of the white fat into more brown fat. In addition, gut microbiota enhanced the absorption of nutrients during stress by increasing the length of villi and microvilli of the intestine to satisfy the demand of the energy in cold condition [64].

Functions of microbiota

Gut microbiota perform their function on four different landscapes in the human body: metabolic, protective structural and neurological.

Metabolic

Dietary fibres

The GI of humans digests about 85% of carbohydrates, 66–95% of proteins and all fats. In the colon, gut microbiota scavenge ~ 10–30% of energy and the rest is excreted as faeces [65]. Dietary fibres including lignin, non-starch polysaccharides, resistant starch (RS) and oligosaccharides [e.g. fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS)] are resistant to digestion by host digestive enzymes [66]. Gut microbes have an array of enzymes for utilisation of these diverse carbohydrates. carbohydrate active enzyme (CAZy) a database (http://www.cazy.org/) documented all of the enzymes and categorised these into four families of

glycosidases such as glycoside hydrolases (153 subfamilies), glycosyltransferases (106 subfamilies), polysaccharide lyases (28 subfamilies) and carbohydrate esterases (16 subfamilies) [67]. Members of the phyla Bacteroidetes and Firmicutes have the largest set of GH encoding genes in their genomes for utilization of different polysaccharides as carbon sources. Gut microbiota of the LI ferments all of the dietary fibres, resulting in the releases of gases (methane, hydrogen and carbon dioxide), SCFAs (formate, acetate, propionate, butyrate, valerate, isovalerate and hexanoate), smaller amounts of organic acids (lactate and succinate) and alcohols (methanol and ethanol). Gut microbial species such as *Roseburia* spp., *Eubacterium rectale* and *Faecalibacterium prausnitzii* and *Clostridium* groups IV and XIVa are the main producers SCFAs [68]. Among the SCFAs acetate, propionate and butyrate are prevalent and present in cecum in the molar ratio of 40:40:20 to 75:15:10 depending on diet. SCFAs (90–95%) are absorbed in the colon through passive diffusion or by ion exchange process and meets about 10% of caloric demand of the human [69]. Acetate is found in the blood (0.10–0.15 mM) and serves as an energy source to peripheral tissues and lipogenesis and cholesterol biosynthesis in liver. Butyrate serves as an energy source of colonocytes and produces ketone bodies and carbon dioxide. Apart from being energy source, butyrate also regulates energy homeostasis by stimulating gut enteroendocrine cells for the production of leptin from adipocytes including the production Glucagon-like peptide-1 in L cells [70, 71]. It also reduces the effect of harmful metabolites of bile acids and phenols. Propionate generally traverses the colonocytes and transports to the liver where it functions as acetate [72].

Protein and amino acids

In the LI, undigested protein is broken into peptides, amino acids and other metabolites via extracellular bacterial proteases and peptidases. These metabolites can be broadly classified into neuroactive compounds (nitric oxide, the tryptamine and phenethylamine), sulphide-containing metabolites (H_2S), aromatic compounds (phenol, *p*-cresol and indole), polyamines (spermine, spermidine and cadaverine), SCFAs (isobutyrate, 2-methylbutyrate and isovalerate from branch chain amino acids) and ammonia. Some of the metabolites such as phenol, *p*-cresol and indole have been shown to induce IBD, colorectal cancer and kidney dysfunction [70]. The MEROPS database documented the peptidase and protease enzymes of the gut bacteria [73]. According to this database *Clostridium* spp. *Bacteroides* spp., *Lactobacillus* spp., etc. have a large diversity of proteases. [74]. The sources of dietary protein have a distinct effect on gut microbial composition. For example, a beef meat rich diet increased the population of *Bacteroides* and *Clostridia* but had a lower abundance of *Bifidobacterium adolescentis* [75]. In contrast, pea and whey proteins encouraged the growth of both *Bifidobacterium* and *Lactobacillus*, while whey additionally suppressed the pathogenic *Bacteroides fragilis* and *Clostridium perfringens* [76, 77]. Interestingly, protein and amino acids in association with the commensals also influence food choice, behaviour and reproduction. In *Drosophila melanogaster*, commensal bacteria elicited specific appetites for amino acid rich food. It was observed that two gut bacteria, *Acetobacter pomorum* and *Lactobacilli,* influenced the preference of flies for sucrose in absence of isoleucine in a food [78]. In addition, availability of dietary protein to carbohydrate ratio determines the catabolic pathways of the gut microbiota. Catabolism resulted in the production of GABA (*Lactobacillus* spp., *Bifidobacterium* spp. and *Lactococcus lactis*), norepinephrine (*Escherichia* spp. and *Bacillus* spp.), dopamine (*Bacillus* spp.), histamine and serotonin (*Streptococcus* spp., *Escherichia* spp. and *Enterococcus* spp.) [74]. These catabolic products may have an influential role in modulating the gut–brain axis or in maintaining the nitrogen balance of a host.

Lipid

A high-fat diet (HFD) induces microbial dysbiosis, enhances adiposity and low-grade inflammation in the adipose tissue. In rodents, feeding of HFD promoted an increase in *Bilophila wadsworthia*, a sulphite-reducing pathobiont. Further, increase in the population of *B. wadsworthia* exerted a pro-inflammatory response mediated by TH1 cell of genetically susceptible Il10(−/−), but not in wild-type mice [79]. Experimental evidences suggested that consumption of HFD caused a decrease in Bacteroidetes and an increase in both Firmicutes and Proteobacteria [80]. Gut microbiota transplantation from obese mice to germ-free mice induced some obesity-associated phenotypes, i.e. increased intestinal permeability and inflammation enhanced lipogenesis and adipogenesis. Diets rich in saturated fat increased the population of *Bacteroides, Bilophila* and *F. prausnitzii,* while unsaturated fatty acid increased the Lactic acid bacteria, *Bifidobacteria* and *A. muciniphila* [81, 82]. African Americans consume higher animal protein and fat and lower fibre diet than rural Africans. This dietary practice elevated the level of colonic secondary bile acids, lowered colonic short-chain fatty acid and increased the risk of colon cancer in the African Americans [83].

Bile salt

Bile acid is a steroid acid that is synthesized by at least 17 different enzymes in the liver for the excretion of excess cholesterol. Hydroxylation of cholesterol initiates synthesis of primary bile via the action of cholesterol 7α hydroxylase and produces chenodeoxycholic acid (45%)

and cholic acid (31%). Before secretion of primary bile acids into the canalicular lumen, they are conjugated via an amide bond at the terminal carboxyl group of glycine or taurine. The glycoconjugates and tauroconjugates are more amphipathic in nature to facilitate the metabolism of dietary fat, fat-soluble vitamins and cholesterol [84]. The released conjugated bile salt in the duodenum is reabsorbed (95%) from the distal ileum via sodium-dependent bile transporter present in the brush border membrane of the enterocytes. A small portion of the unabsorbed bile salt undergoes deconjugation by the action of bile salt hydrolase of gut microbiota and produces deoxycholate, ursodeoxycholate and lithocholate [70, 85]. These secondary bile acids are passively absorbed in the colon and are transported back to the liver through enterohepatic circulation and the rest is excreted through faeces. The transformation of bile acids is mainly performed by anaerobic bacteria of the genera *Bacteroides, Eubacterium* and *Clostridium* (Clusters XIVa and XI) and a minor fraction of aerobic bacteria such as Actinobacteria and Proteobacteria. Bile salt hydrolase is also found in the human gut including members of *Lactobacilli* and *Bifidobacteria*. Deconjugation by 7αβ-dehydroxylation of bile salts increases their hydrophobicity and lowers its absorption and showed pathological effects including obesity and cancer [86, 87].

Choline

Choline is an essential water-soluble nutrient and a constituent of lecithin, which is present in many plants and animal organs. Choline and its metabolites help to maintain the structural integrity and signalling roles of cell membranes, cholinergic neurotransmission (acetylcholine synthesis), and a source for methyl groups via its metabolite, trimethylglycine (betaine), which participates in the biosynthesis of S-adenosylmethionine. The gut microbiota metabolizes choline into trimethylamine (TMA) which is absorbed in the gut. Moreover, TMA is converted to trimethylamine-N-oxide (TMAO) by flavin monooxygenases 1 and 3 (FMO1 and FMO3) in the liver. The formation of TMAO exacerbates hepatic insulin signalling and glucose tolerance and also promotes adipose tissue inflammation, atherosclerosis and cardiovascular diseases [88]. Now, the microbial conversion of dietary choline is an emerging metabolic hallmark of cardiovascular diseases. Eight gut bacteria including *C. sporogenes, Anaerococcus hydrogenalis, Providencia rettgeri, C. asparagiforme, C. hathewayi, E. fergusonii, Proteus penneri* and *Edwardsiella tarda* have been identified to produce TMA due to the consumption of a choline-rich diet [89]. Two enzymes (CutC and CutD) were reported in *Desulfovibrio desulfuricans* that can convert the choline into TMA and acetaldehyde [90].

Polyphenol

Polyphenols are secondary metabolites formed in a variety of plants through shikimate/phenylpropanoid and/or polyketide pathways which contain more than one phenolic unit and are broadly classified into two main groups, flavonoids and non-flavonoids. The majority of the polyphenols are present as glycosides with conjugation of different sugars such as glucose, galactose, rhamnose, ribulose, arabinopyrinose and arabinofuranose units. Inactive polyphenols including flavones, flavonols, flavanones, flavanols, catechins, anthocyanins, isoflavones, hydroxycinnamic acids, lignans, etc. are transformed into active compounds after removal of the sugar by the gut microbiota in the SI [91]. The polyphenols such as hydroxycinnamic acids are commonly esterified to sugars, organic acids and lipids which are transformed into aglycone by microbial esterases. The aglycones are found to be absorbed in the SI or colon and metabolized into hydroxyphenylacetic acids. Another dietary polyphenol such as ellagic acid and ellagitannins are transformed into urolithin A (an anti-inflammatory metabolite) by *Gordonibacter* spp. [92]. Polyphenols can also detoxify gut metabolites and repress the growth of pathogenic bacteria, such as *Clostridium perfringens, Clostridium difficile* and *Bacteroides* spp. [70]. Experimental evidence suggested that supplementation of epigallocatechin-3-gallate (GCG) and resveratrol (RES) altered the human gut microbial composition. Supplementation with GCG and RES increased oxidation of fat with reduced abundance of *Bacteroidetes* and *F. prausnitzii* in men but not in women [93].

Protective

The GI tract is the interactional scaffold of gut microbiota and gut immune system [94]. The first layer of the gut immune system including gut-associated lymphoid and Peyer's patches developed due to fundamental intertwining with gut commensal. The developed immune barrier and a set of immune mechanisms limit the direct contact of commensal to the epithelial cell surface. A second layer of immunity rapidly detects and kills the bacteria without penetration to intestinal tissue. A third set of immune responses localized within the mucosa without activating the systemic immune system. The innate immune barrier consists of mucus, antimicrobial peptides and secretory IgA.

Mucus layer

The mucus layer consists of mucin glycoprotein secreted from goblet cell and assembled into a viscous gel-like layer on the intestinal epithelia that protects from the attachment of microbes directly to epithelia. It also acts as a lubricant and transports the luminal contents without hampering the

epithelial lining. Chemically, mucin backbone consists of a peptide with alternating O-linked glycosylated (70–80%) and non-glycosylated domains. *N*-Acetylglucosamine, *N*-acetylgalactosamine, fucose and galactose are the four primary mucin oligosaccharides with either terminal sialic acid or sulfate groups. The sulfated (sulfomucins) or nonsulfated (sialomucins) mucins are acidic in nature, expressed throughout the LI epithelia. The mucus layer is about 150 µm thick epithelia and forms two distinct strata of alternating sialo- and sulphomucins. The acidic sulfated mucin is proximal to epithelia and is less degradable by bacterial glycosidases and host proteases. The acidic mucin prevents direct adherence of commensal to colonic epithelial cells [95]. The mucin not only acts as a barrier but also provides a direct source of carbohydrates and peptides for commensals. For example, *Bacteroides thetaiotaomicron* produces multiple fucosidases that cleave fucose from glycans and activate the goblet cells in secretion of mucin. Besides, *Bacteroides fragilis* synthesizes fucosylated capsular polysaccharides, a component of the bacterial outer membrane which confers advantages for competitive and preferential colonisation [96]. *Bifidobacterium bifidum* can produce endo-α-*N*-acetylgalactosaminidase and 1, 2-α-L-fucosidase to use mucins as an energy source in vitro [97]. In contrast, mucolytic bacteria such as *Ruminococcus torques, Ruminococcus gnavus* may also increase in intestinal bowel disease (IBD) and provide the substrate to other non-mucolytic bacteria [98]. Some of the other pathogenic bacteria are genetically equipped and try to cross the mucus barrier to induce the infection. For example, *H. pylori* produce urease to increase the pH and to lower viscosity of mucus for the penetration of epithelial cell surface [99]. Pathogens such as *Campylobacter jejuni* and *Salmonella* spp. use their flagella to cross the mucus barrier. *E. coli* and *Shigella flexneri* do so by secreting a mucin-binding serine protease which rapidly digests mucus and also induce its hyper secretion to compete against indigenous bacteria [100].

Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMP) are secreted by intestinal epithelial cells including enterocytes, Goblet and Paneth cells which restrict the access of commensals as well as pathogens to epithelia. The AMPs including defensins, cathelicidins and C-type lectins are a group of conserved small (20–40 amino acids) cationic proteins virtually retained in the mucus layer. These can bind either with negatively charged microbial cell membranes or enzymatically attack the cell wall to disrupt the outer or inner membrane. Defensins are major antimicrobial peptide classified into α, β and θ defensins. α-Defensin expressed constitutively as pro-cryptdin irrespective of a microbial signal (Fig. 3). Later it is converted into mature-cryptdin by matrix metalloproteinase-7 (MMP-7) to defend microbial adherence to mucosa [101]. Mice lacking of the MMP-7 gene are unable to produce mature cryptdin and are highly susceptible to *Salmonella typhimurium* infection [102]. β-Defensin is expressed both in SI and LI epithelia through the signalling of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and depends on a microbial signal derived from the lumen. NOD2 deficiency in mice resulted in an increased susceptibility to *Listeria monocytogenes* infection due to lower expression of defensins [103]. θ-Defensin has only been reported in the leukocytes of rhesus macaques. Cathelicidin-related antimicrobial peptide (mCRAMP) is encoded by the gene Cnlp and its expression is restricted to surface colonic epithelia. Colonic epithelial cell extracts from $Cnlp^{+/+}$ and mutant $Cnlp^{-/-}$ mice showed that lack of mCRAMP expression increased the adherence of enteric pathogen *Citrobacter rodentium* to intestinal epithelial cells [104]. The C-type lectins are a superfamily consisting of more than 1000 proteins having one or more characteristic C-type lectin-like domains. C-type lectins play an important role in innate and adaptive immunity to control the gut bacterial infections. Microorganism-associated molecular patterns activate toll-like receptors (TLRs) recruiting cytosolic adaptor myeloid differentiation primary-response protein 88 (MYD88) to restrict the penetration of bacteria through epithelial layer (Fig. 3). For example, RegIIIγ, a secreted antibacterial lectin effective against Gram-positive bacteria and maintaining a ~ 50 µm zone that physically separate the microbiota from the epithelial surface of SI [105].

IgA

Secretory IgA (SIgA) serves as the third line of defence and protects the intestinal epithelia from enteric toxins and pathogenic microorganisms. Differential immune recognition of pathogens and commensals maintains immune tolerance and activation of the cells present in Pyers' patches, lymphoid follicle or lamina propia. The macrophage-like non-migratory $CX3CR1^{+}$ dendritic cells (DCs) of Peyer's patches randomly capture luminal antigens through transepithelial transport. The captured antigen is transferred to migratory $CD103^{+}$ DCs which move to the interfollicular area of Payer's patches [106, 107]. The $CD103^{+}$ DCs interacts with Treg cells to promote B cells for IgA-induction with the assistance of retinoic acid, transforming growth factor-β (TGF-β), IL-10 and cytokine thymic stromal lymphopoietin. Activation of Treg cells by commensals induces B cell to differentiate into plasma B cells for producing of IgA. Thereafter, the secreted IgA translocate from lymphoid sites to the intestinal lamina propria and transcytosis across the epithelial cell layer. Transcytosis of IgA in the lumen entraps bacteria in mucus to neutralize toxins and pathogens as blocking their access to epithelial receptors. It

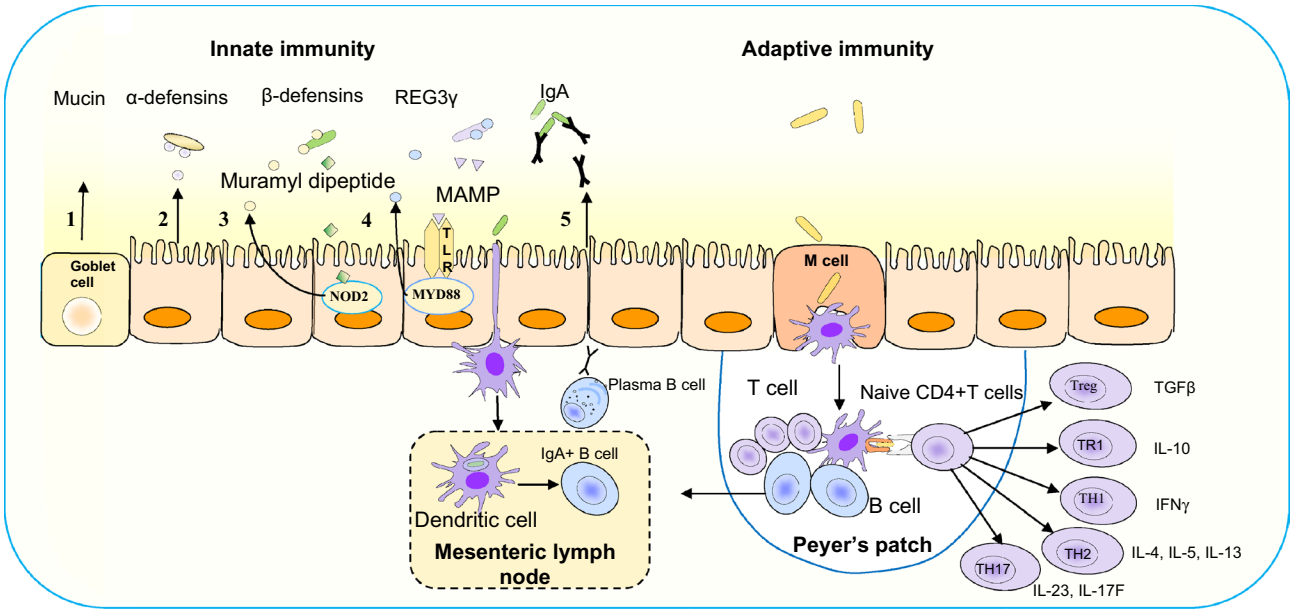

Fig. 3 Immune mechanisms for limiting the commensals within the epithelial layer. Innate immunity: (1) mucin glycoproteins of the goblet cells form a layer over the epithelia to restrict bacterial adhesion; (2) constitutive expression of α-defensins from epithelial cells without microbial signal; (3_ β-defensin expression by muramyl dipeptide of Gram positive bacteria mediated by cytosolic nucleotide-binding oligomerization domain-containing protein 2 (NOD2); (4) C-type lectin regenerating islet-derived protein 3γ (REG3γ) via microorganism-associated molecular patterns (MAMP) activates toll-like receptors (TLRs) recruiting cytosolic adaptor myeloid differentiation primary-response protein 88 (MYD88) to restrict bacterial penetration through epithelial layer; (5) dendritic cells (DC) located beneath the epithelial dome of Peyer's patches take up bacteria and migrate to mesenteric lymph node and induce B cells to differentiate into IgA^+ plasma cells for secretion of IgA. Transcytosis of IgA across the epithelial cell layer limits bacterial association with the epithelium. Adaptive immunity: transcytosis of bacteria through M cells and luminal antigen activates DC in the Peyer′s patch and helps to differentiate the naive $CD4^+$ T cells into Treg and TR1 characterized by expression of anti-inflammatory cytokines transforming growth factor (TGFβ) and IL10. The TH1, TH2, and TH17 cells are characterized by proinflammatory cytokines including (1) IFNγ, (2) IL-4, 5 and 13 and (3) IL-23 and IL-17F, respectively

also facilitates their removal by peristaltic and mucociliary activities [108]. The population density of commensals vs. pathobionts or its associated endogenous molecules determine the IgA secretion in the gut. For example, endogenous polysaccharide A from a commensal, *Bacteroides fragilis* mediated an anti-inflammatory response characterized by induction of Treg cells and IL-10 and TGF-β. In addition, metabolites such as butyrate made by commensals can also activate the same anti-inflammatory response mediated by Treg cells. In dysbiotic condition, a certain subsets of commensal are increased in their abundance and act as "pathobionts" [94]. The bloom of pathobionts and breakdown of tolerance to immune system activate the pro-inflammatory response. For example, in experimental colitis *H. hepaticus* activated the TH17 cell which is mediated by inflammatory cytokines such as IL-17, IL-23 and TNF-α. The malnourishment of children is also a factor to alter the gut microbial composition and IgA responses. Malnourishment phenotype of Malawian infants can be transferred after the transplantation of 11 IgA(+) bacterial consortium to gnotobiotic mice. The transplantation of the bacterial consortium caused a rapid disruption of the intestinal epithelial barrier, weight loss and sepsis. These sepsis conditions were prevented by the administration of two IgA-targeted bacterial species from a healthy microbiota [109].

Innate and adaptive immunity

The innate immune response can discriminate between commensals and pathogens via germline-encoded pattern-recognition receptors. Toll like receptors (TLRs) identify the conserved microbial-associated molecular patterns including bacterial-derived lipopolysaccharide (LPS), lipoprotein, flagellin and unmethylated CpG-containing DNA of pathogens. The subfamilies of TLR1, TLR2 and TLR6 recognise lipids, whereas TLR7, TLR8 and TLR9 recognise nucleic acids. However, TLR5 identify flagellin and TLR4 can recognise several structurally unrelated ligands such as lipopolysaccharide and plant diterpene paclitaxel [110]. The cytosolic antigens including γ-D-glutamyl-meso-diaminopimelic acid and muramyl dipeptide of bacterial peptidoglycan layer are recognised by the NOD like receptors [111, 112]. Antigen-presenting cells like DCs and macrophages also express TLRs which ingest and degrade pathogens and express

co-stimulatory molecules and cytokines. These cytokines help to differentiate the naive $CD4^+$ T into T helper 1 (TH1), TH2, TH17 and Treg and TR1 cells (Fig. 3). A balance of Treg cells and the $CD4^+$ effector T cells in the intestinal mucosa discriminate commensal and pathogenic microbial constituents. Microbial dysbiosis may induce inflammatory response mediated by TH1, TH2 and TH17 cells. The subset of TH1 and TH2 cell activation is characterized by the expression of pro-inflammatory cytokines including IL-4, IL-5, IFN-γ and IL-13. TH17 cell is characterized by the synthesis of IL-17 which stimulates stromal cells to express the pro-inflammatory cytokines including IL-6 and IL-8 and IL-22 [113, 114].

Microbiota produced metabolites in immunity

Short chain fatty acids (SCFAs) The carbohydrates that escape from the digestion and absorption in SI are utilized by a number of microbes present in LI. The saccharolytic activities of the microbes present in LI ferment the undigested carbohydrates resulting in SCFAs production, viz. acetate, propionate and butyrate. SCFAs are either transported or diffused into host cells and sensed by binding with G protein-coupled receptors such as GPR41, GPR43 and GPR109A on epithelial and immune cells [71]. Butyrate binds with GPR43 and activates anti-inflammatory cytokine production such as TGFβ and IL-10 as well as upregulates the FOXP3 of Treg cells. Butyrate also inhibits histone deacetylase activity and down regulates the nuclear factor-κβ mediated inflammatory response. Experimental evidence indicated that acetate had greater binding affinity to GPR43 than butyrate. Feeding of acetate stimulated in secretion of IgA in the intestine of wild type mice but not in $GPR43^{-/-}$ knockout mice. Acetate induced expression of Aldh1a2 in DC which converts vitamin A into retinoic acid mediating the signalling of B and goblet cells in secretion of mucins and IgA to fortify the barrier function [115]. The knockout of the receptor GPR43 in mice showed an increased susceptibility to dextran sodium sulfate-induced colitis by increasing chemotaxis of neutrophils and inflammatory gene expression. Propionate and butyrate in combination also effective to inhibit LPS induced inflammation by activating Treg cell and reduced production of inflammatory cytokines such as IL-6 and IL-12 [116].

Aryl hydrocarbon receptor (AHR) AHR is a cytoplasmic ligand-induced receptor expressed by epithelial and some tumour cells. Kynurenine, a metabolite of tryptophan is a ligand of AHR. Three groups of microbial metabolites, viz. (1) tryptophan derived (indole, indole-3-acetate and tryptamine), (2) bacterial virulence factors (phenazine, naphthoquinone and phthiocol) and (3) short chain fatty acids also binds to AHR. Some dietary components including flavonoids, stilbenes, carotenoids and indoles from plants are also ligands of AHR [71, 117]. AHR ligands prevent the infection of *Citrobacter rodentium* and *Candida albicans* by snatching of metal ions. Supplementation of dietary synthetic AHR ligands and transfusion of intraepithelial lymphocytes to $Ahr^{-/-}$ mice help to restore the epithelial barrier function and normalized dysbiotic bacterial population [118].

Polyamines The polyamines are polycationic in nature and found in all the life forms. There are three major sources of polyamines, viz. food, cellular biosynthesis and microbial biosynthesis in the gut. The major polyamines are putrescine, spermidine and spermine synthesized from arginine by the enzyme arginase 1 (which converts arginine to ornithine). The ornithine decarboxylase (which synthesizes putrescine from ornithine) and other enzymes sequentially interconverts putrescine to spermidine and spermine [71]. Arginase 1 and nitric oxide synthase compete for arginine to produce either polyamines or nitric oxide to balance immune responses. Administration of arginine in combination with *B. animalis* subsp. *lactis* LKM512 was shown to increase colonic polyamines which lowered the colonic and circulatory TNF-α and IL-6 [119]. Apart from arginine supplementation, spermine can also inhibit the activation of M1 macrophage by suppressing the expression of ornithine decarboxylase in the synthesis of pro-inflammatory cytokines without modifying the expression anti-inflammatory mediators TGFβ and IL-10 [120]. Polyamine enrichment to breast feed rat pups accelerated maturation of intraepithelial $CD8^+$ and $CD4^+$ T cells with early appearance of B cells in spleen [121]. Higher production of mucus and secretory IgA was also observed in the SI that closely resembled to that of breast-fed mice. These findings indicated that polyamines in the diet provide immunological benefits to the host.

Structural

Hippocrates (400 BC) stated that "bad digestion is at the root of all evil" and "death sits in the bowels" suggesting that the gut may act as a "motor" of critical illness. This hypothesis states that overgrowth and imbalance of commensal flora increases gut permeability which may cause systemic sepsis that may lead to death [122]. The intestinal epithelium is a single layer of columnar cells which are tightly bound together by intercellular junctional complexes that regulate the paracellular permeability. The junctional complexes consist of tight junction (ZO; zonula occludens), adherens junction (zonula adherens) and desmosome. The adherens junctions are located beneath the tight junction and both together make the apical junctional complexes that are associated with actin cytoskeleton. A bridging between

apical junctional complexes and actin filaments involved in cell–cell adhesion and intracellular signalling whereas adherens junctions and desmosomes provide the adhesive forces necessary for the maintenance of intercellular interactions [123]. The tight junction barrier exhibits both size and charge selectivity with two distinct routes across an intact epithelial monolayer, termed as the 'pore' and 'leak' pathways. The pore pathway refers to a high-capacity, size-selective and charge-selective route, whereas the leak pathway is a low-capacity pathway that has more limited selectivity [124]. The enterotoxins of some bacterial pathogens such as enteropathogenic *E. coli, C. difficile* and *C. perfringens* are capable of weakening the barrier functions of the tight junctions [123, 125, 126]. Cytokines are produced in the intestine including IL-4, IL-6, IL-13, IL-1β, TNF-α and IFN-γ promote to lose the tight junctions and increase the permeability. Cell culture and animal model based studies revealed that cytokines including IL-10, IL-17 and TGF-β renovate the intestinal barrier to decrease permeability [124, 127]. Nutrients and food factors such as glutamine deprivation, fatty acid molecules (eicosapentaenoic, docosahexaenoic, c-linoleic acids, capric acid and lauric acid), ethanol and acetaldehyde increase intestinal permeability. The amino acids glutamine and tryptophan and other nutritional factors including casein peptide, SCFAs vitamin A and D and polyphenolic molecules (quercetin and curcumin) restrict the infiltration of luminal content [128]. Furthermore, treatments of probiotics such as *L. plantarum* DSM 2648 and *E. coli* Nissle 1917 on Caco-2 cells have been shown to upregulate the expression of tight junction proteins [129, 130].

Neurological

The bidirectional communication between the gut and the brain is known as gut-brain axis (GBA). The gut is connected to the brain through the enteric nervous system (ENS) involving parallel outflow of the parasympathetic (vagus nerve) and sympathetic (prevertebral ganglia) nervous systems and hypothalamic- pituitary- adrenal axis (HPA) (Fig. 2) [131, 132]. The ENS is regarded as the "second brain" of the gut and consists of millions of neurons and is divided into two types of ganglia (myenteric and submucosal plexuses). Activities of gut microbiota can control the ENS as well as CNS via (1) production, expression and turnover of neurotransmitters and neurotrophic factors, (2) maintaining the intestinal barrier and tight junction integrity, (3) modulating the enteric sensory afferents, (4) production of bacterial metabolites and (5) mucosal immune regulation. The ENS can sense more than 30 neurotransmitters most of which are found in the CNS, such as acetylcholine, dopamine and serotonin. More than 90% of serotonin and 50% of dopamine originate in the gut which are mainly produced by the gut microbiota. These two neurotransmitters play an important role in transmission of "fight to flight" message to the brain in controlling of mood, happiness and pleasure of an individual [133]. Bacterial metabolites, particularly SCFAs were shown to induce tryptophan hydroxylase 1 in enterochromaffin cells to release serotonin in the gut. Serotonin can stimulate the sympathetic nervous system to influence memory and learning process. Emerging data supports the fact that dysbiosis in gut microbiota during functional GI disorders disrupts the GBA and leads to mood disorders. Experimental evidences also suggested that probiotic can induce brain derived neurotrophic factors in the hippocampus and cerebral cortex and regulate cognitive functions as well as muscle repair, regeneration, and differentiation [131]. In parallel, probiotics can modulate the HPA to reduce the release of cortisol from the adrenal glands. The attenuated level of cortisol and a complex interaction with the amygdala, hippocampus and hypothalamus to lower anxiety-like behaviour and stress reactivity [131]. A study on IBS grouped the IBS population into two subgroups, IBS1 (microbial profile distinct from healthy control) and healthy control like-IBS (microbial profile indistinguishable from healthy control). The microbial genera *Blautia, Streptococcus* and *Bacteroides* differentiated gut microbiota of the IBS1 patients and healthy controls. Such dysbiosis in gut microbiota was associated with the volume of the brain region including sensory- and salience-related regions and with a history of early life trauma [134]. A study from China showed a relationship between the gut microbiota with major depressive-like disorder (MDD). They found that abundance of Bacteroidetes, Proteobacteria and Actinobacteria and inadequacy of Firmicutes was linked to "active major depressive disorder" (high score of clinically significant depression) and "responded MDD" (reduction of MDD after 4 weeks of treatment) [135]. Treatment with efficacious probiotics like *Bifidobacterium longum* NCC3001 reduced depression in IBS patients and improved their quality of life. Although the probiotic bacterium had no effect on IBS symptoms, they however reduce the response of negative emotional stimuli in multiple regions of brain, including amygdala and fronto-limbic region [136]. The neurodegenerative disease Alzheimer's is characterized by extracellular amyloid beta (Aβ) deposits and plaque formation in the brain. Gut microbial profiling of Aβ-precursor protein (APP) of transgenic mice exhibited a remarkable shift as compared to non-transgenic wild-type mice. There was a reduced abundance of *Allobaculum, Akkermansia* and unclassified genera in *Rikenellaceae* and S24-7 was found as compared to wild-type mice. Subsequently, they found that transplantation of gut microbiota from conventionally raised APP transgenic mice to germ-free APP transgenic mice increased the cerebral Aβ pathology unlike the transplantation of microbiota from wild-type mice [137].

Therapeutic interventions

Probiotics

Élie Metchnikoff (1905) believed that some Bulgarian peasants who consumed a fermented milk product called 'kefir' had better health and longer lives. Subsequently, the bacterium *Lactobacillus delbrueckii* ssp. subsp. *bulgaricus* was isolated from 'kefir' and has since been used in commercial curd preparations. During the First World War, the physician Alfred Nissle isolated a strain of *E. coli* (*E. coli* Nissle 1917) from a healthy soldier from an army suffering from infectious diarrhoea and has since been used as a commercial probiotic. Such empirical observations introduced the concept of probiotics, presently defined as "live microorganisms which, when administered in adequate amounts confer health benefit to the host" [138, 139]. Commercially available probiotics are commonly of *Bifidobacterium, Lactobacillus* and *Streptococcus* genera as well as yeasts of the *Saccharomyces* genus either singly or in combinations. To date, the mechanisms of action of probiotics are ascribed to their adhesion to the intestinal–lumen interface, competition with pathogens for nutrients and receptor binding, fortification of mucosal barrier, promotion of innate and adaptive immune responses of host, production of bacteriocins, production of signalling molecules via CNS and modulation of cell kinetics [140]. For adhesion to the mucosal surface of the intestine, probiotics such as *Lactococcus lactis* ssp. *lactis* BGKP1 exhibit auto-aggregation and its mucin binding surface protein attaches to the gastric mucin protein MUC5AC [141]. The protein "Transaldolase" of *B. bifidum* has also been reported to act as an important colonisation factor. Recently, a group of proteins called moonlighting proteins including elongation factor Tu (EF-Tu), α-enolase, glyceraldehyde-3-phosphate dehydrogenase, etc. have been shown to play an important role in colonisation of human gut by degradation of extracellular matrix or by facilitating a close contact with the epithelium [142, 143]. Directly, probiotics exhibit antimicrobial effect against pathogens by secreting an array of antibacterial substances which includes organic acids, hydrogen peroxide and bacteriocins. These compounds mainly help to reduce the viable count of pathogens as well as their metabolisms and toxin production through the reduction of luminal pH and production of SCFAs. Over 60% of the human population suffers from lactose intolerance due to lower level and activity of lactase enzyme. The lactose intolerance depends on several factors, such as age, race and integrity of the SI membrane and transit time. The probiotics can produce lactase which promote the digestion of unabsorbed lactose and prevent acid and gas formation [144]. Probiotics also prevent the cardiovascular disease by regulating the metabolism of lipid, viz. (1) decreasing the absorption of cholesterol through co-precipitation with the deconjugated bile salts, (2) incorporating and assimilating the cholesterol in the cell membrane, (3) converting the cholesterol into coprostanol and (4) inhibiting the expression of cholesterol transporter in the enterocytes [145]. Recently, researchers are exploring the role of probiotics in modulating of inflammatory disease, obesity, type 2 diabetes, cardiovascular disease, autoimmuno psoriasis, arthritis and cancer. A randomized double-blind placebo-controlled study of a formulation of probiotics with fermented milk containing (*S. salivarius* subsp. *thermophilus*, *E. faecium*, *L. rhamnosus* GG, *L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii* subsp. *Bulgaricus*, *B. breve* and *B. animalis* subsp. *lactis*) and fructooligosaccharides (FOS) was found to be effective in management of constipation associated with Parkinson's disease [146]. Another formulation of probiotics in which consumption of milk (200 ml/day) containing *L. acidophilus, L. casei, B. bifidum* and *L. fermentum* (2×10^9 CFU/g for each) for 12 weeks was shown to improve cognitive function in elderly Alzheimer's patients without effecting the biomarkers of oxidative stress and inflammation, fasting plasma glucose and lipid profiles [147]. Based on more than 38 studies, the probiotic bacteria *Bifidobacteria* and *Lactobacilli* have been reported to improve psychiatric disorder including anxiety, depression, autism spectrum disorder, obsessive–compulsive disorder and memory abilities (spatial and non-spatial) [148]. A large-scale trial in India has shown that administration of *L. plantarum* and FOS to new-borns within 7 days of birth reduced neonatal sepsis and morbidity rate to 5.4% as compared to 9% in their first two months of life [149]. In Finland, results of 4 trials conducted between 1997 and 2012 (with 10–15 years of follow-up) showed that perinatal administration of probiotic *L. rhamnosus* GG singly or in combinations with *B. lactis* Bb-12, *L. paracasei* ST11 and *B. longum* BL999 reduced allergic diseases [150]. Treatment with *Saccharomyces boulardii* for 12 weeks in HIV patients decreased population of *Clostridiaceae* family and reduced chronic inflammation [151].

Prebiotics

Gibson and Roberfroid (1995) defined prebiotics as a class of compounds that stimulate the growth and/or activity of a limited number of beneficial bacteria (*Lactobacilli* and/or *Bifidobacteria*) in the colon to improve host health [152]. However, this definition has now been broadened and included carbohydrate and non-carbohydrate substances that confer microbiota-mediated health benefits [153]. The European Union has approved prebiotics (inulin, FOS and

GOS) as safe food ingredients whereas the FDA has not yet recognised these. In the GI tract, prebiotics can resist gastric acidity and can reach in LI where their selective utilization by microbiota produces SCFAs. Prebiotics such as FOS and GOS including the patented GOS produced by Bimuno (B-GOS) modulate neural growth factors such as brain-derived neurotrophic factor, neurotransmitters and synaptic proteins including synaptophysin and NMDA receptor subunits [154, 155]. Hence, prebiotic utilization by gut microbiota may confer health benefits elsewhere in the body. For example, GOS stimulated growth of *Bifidobacteria* in the mouse gut that led to modulation of cortical IL-1β and 5-HT2A receptor expression that reduced anxiety levels and increased brain barrier function in obese mice [156, 157]. Experimental evidence also suggested that prebiotic supplementation was effective against stunted memory, Alzheimer's disease and dementia. In rats, improved memory was observed due to administration of polydextrose/GOS mixture and oligofructose-enriched inulin [158, 159]. Previously, it was reported that the metabolism of sialic acid of sialyllactose enhanced memory [160]. Recently, deficiency of sialylated human milk oligosaccharides of Malawian mothers have shown to cause stunted infants. Transplantation of faecal microbiota of a 6-month-old stunted baby to germ-free animal model on Malawian diet prototype with sialylated bovine milk oligosaccharides promoted quality composition of gut microbiota for anabolic process, lean body mass gain and brain metabolism [161]. A similar study on infant formula showed that bovine milk-derived oligosaccharides and *B. animalis* ssp. *lactis* (CNCM I-3446) improved the GI health markers (microbiota pattern, faecal IgA and stool pH) as compared to breastfed infants. No difference has been found in diarrhoea and febrile infection across the studied population [162].

Faecal microbiota transplantation (FMT)

Faecal microbiota transplantation (FMT) refers to a "stool transplant" into the GI tract from a healthy individual to a patient for the treatment of specific diseases. During Dongjin dynasty in the fourth century, the benefits of FMT were recognised in Chinese medicine. The doctor prescribed the use of faecal suspension orally for patients who suffered from food poisoning or severe diarrhoea. In the sixteenth century, FMT was known as "yellow soup" and a medical miracle that brought patients back from the brink of death. In the seventeenth century, an Italian anatomist, Fabricius Aquapendente, introduced faecal therapy in veterinary medicine for compelling rumination. In United States, the FDA has considered human faeces as an experimental drug since 2013. Now, FMT has been used experimentally to treat GI diseases including colitis, constipation, IBD, and chronic fatigue syndrome, Parkinson's disease, multiple sclerosis [163, 164]. In modern medicine, FMT has been shown to be more effective against pseudomembranous colitis and *C. difficile* infection (CDI) than neurological and other complications. The success rate of FMT on CDI patients was found to be over 90% who had failed to respond to antibiotic treatment [164, 165]. In recent techniques of FMT, enemas, nasogastric intubation, enteric intubation, colonoscopes and gastroscopes are used for faecal transplantation. The treatment of CDI with antibiotics including metronidazole, vancomycin, or fidaxomicin may cause recurrent infection, antibiotics resistance and dysbiosis of normal gut microbiota [166]. A study on CDI patients showed profound dysbiosis commonly characterized by complete disappearance of Bacteroidetes with a marked reduction in Firmicutes and massive increases in the relative abundances of Proteobacteria [167]. FMT increased the abundance of the families Bacteroidaceae, Rikenellaceae and Porphyromonadaceae under Bacteroidetes and Ruminococcaceae, Lachnospiraceae, Verrucomicrobiaceae and unclassified Clostridiales under Firmicutes and reduced the population of Proteobacteria in CDI patients. In addition to dysbiosis of normal microbiota in CDI, presence of primary bile salts such as taurocholate, cholate and chenodeoxycholic acid stimulate the germination of *C. difficile* spore. In contrast, post-FMT increased secondary biles (lithocholic acid and deoxycholate) of donor samples that inhibit spore germination as well as vegetative growth of *C. difficile* [168]. A systematic review on FMT based on 18 studies with 611 patients highlighted that primary cure, overall recurrence, early and late recurrence rates of CDI were 91.2, 5.5, 2.7 and 1.7%, respectively. The adverse events such as IBD flare, infectious and autoimmune diseases did not have any significant relation with FMT [169]. Moreover, in-depth study on understanding of the mechanisms of FMT, optimisation of methodologies for FMT including donor screening, stool preparation and routes of administration may improve the efficacy and safety for a better clinical application.

Drugs

The gut microbial enzymes process pharmaceutical compounds and alter pharmacokinetics, absorption, distribution, metabolism and elimination [170, 171]. Drug efficacy may vary from individual to individual due to variation in the microbial composition of their gut. In 1971, a preclinical study in rats showed that oral administration of prontosil or Neoprontosil transformed to inactive sulphanilamide by gut microbiota before absorption [172]. Similarly, in the 1980s, researchers discovered that the bacterium *Eggerthella lenta* (*Actinobacteria* phyla) inactivated digoxin (a cardiac glycoside) and its bioavailability, therapeutic index and induced toxicity [173]. Cardiac glycoside reductase is the homolog of FAD-dependent

fumarate reductases responsible for the reduction and inactivation of digoxin [174]. Paracetamol, a common analgesic and antipyretic drug is detoxified in the liver by glucuronidation and sulfation. The microbial metabolite *p*-cresol produced by *C. difficile* acts as competitive inhibitor of hepatic sulfotransferases for sulfonation that elevate the risk of hepatotoxicity [175]. Irinotecan (CPT-11) is a chemotherapeutic drug that is transformed into the active form (SN-38) by carboxylesterases in the host tissues and acts as an inhibitor of topoisomerase I in colon tumor cells. Hepatic UDP-glucuronosyltransferases conjugate SN-38 into nontoxic SN-38-G before secreting into the SI. However, conversion of the nontoxic SN-38-G to SN-38 by β-glucuronidase activity of *E. coli*, *Bacteroides vulgatus* and *C. ramosum* leads to diarrhoea, inflammation and anorexia [171, 176]. Levodopa, a dopamine precursor undergoes decarboxylation within the central nervous system and exerts dopaminergic effect against Parkinson's disease. Gut microbiota can transform levodopa into *m*-tyramine and *m*-hydroxyphenylacetic by dehydroxylation that leads to decreased bioavailability and induces *H. pylori* infection [176]. Metformin is a popular drug and used in controlling T2D. The treatment of metformin increased the abundance of butyrate producers such as *Roseburia* spp., *Subdoligranulum* spp. and a cluster of butyrate-producing *Clostridiales* spp. as compared to a metformin-untreated group. Microbial shifts under metformin treatment enhanced its efficacy to control glucose level in plasma [177]. Similarly, Gegen Qinlian Decoction is a traditional Chinese herbal formula used for treatment of T2D patients that increased *F. prausnitzii* and short-chain fatty acid production [178]. Berberine is a Chinese herb (*Coptis chinensis*) that also induces the growth of *Allobaculum* and *Blautia*, as well as SCFA levels in HFD-fed rats [179]. These findings suggest a role for the gut microbiota in drug metabolism that needs to be considered as a new parameter for assessment of a drug's therapeutic index to treat diseases.

Future perspective

Modern lifestyle and the growing environmental stresses are bringing new challenges to the human race to sustain on this planet. The human race cannot evolve quickly to adapt to such changes. However, the hidden design in the "hologenome" of human body may help to adapt to the changing environment. Nowadays, there is evidence that diets with smart microbial communities may assist in human-microbe symbiosis for better health. An in-depth understanding of the functioning of microbiota in different health and disease conditions may further help to formulate a strategy for targeted intervention as and when required.

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