Human Microbiome: Implications on Health and Disease

8

Princy Hira, Utkarsh Sood, Vipin Gupta, Namita Nayyar, Nitish Kumar Mahato, Yogendra Singh, Rup Lal, and Mallikarjun Shakarad

8.1 Introduction

> The way to a man's heart is through his stomach.
>
> John Adams, 1814

The state of our gut not only governs the health of our body but also our mental health and emotional status. Although, this notion is more than a century old and sporadic interest was visible from the 1970s, serious research studies on gut microbiome and its implications on our health have just begun (Schmidt 2015). The human body consists of about 40 trillion cells (Bianconi et al. 2013) with about 22,000 human genes in each cell (Pertea and Salzberg 2010). However, with the association of microbes immediately after birth, the human body contains about 100 trillion cells and more than 2 million genes. The microbiota that gets associated with the human body makes up about 1–3% of the human body mass amounting to 2–6 pounds of microorganisms in a 200-pound adult (Turnbaugh et al. 2007, HMP 2007–2012). The additional cells as mentioned above are the microorganisms that, apart from the gut, also reside on the skin surface, in the deep skin layers, in the mouth, digestive tract and other human organ systems. The sum total of microorganisms that colonize the human body are collectively referred to as 'human microbiome or human microbiota'. The microbiome is central to human biology (Schnorr 2015). With so much of microbiota getting associated with the human body, it

Princy Hira and Utkarsh Sood contributed equally with all other contributors.

P. Hira • U. Sood • V. Gupta • N. Nayyar • N.K. Mahato • R. Lal (✉) • M. Shakarad (✉)
Department of Zoology, University of Delhi, Delhi 110007, India
e-mail: ruplal@gmail.com; beelab.ms@gmail.com

Y. Singh
CSIR Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India

L. Rawal, S. Ali (eds.), *Genome Analysis and Human Health*,
DOI 10.1007/978-981-10-4298-0_8

became imperative to understand the role of microbes that colonize the human body to fully understand and appreciate the human physiology and behaviour under healthy and diseased conditions. With the progress of research in this field, it is proposed that better understanding of human microbiome would pave the way for successful treatment of not only lifestyle diseases but also life-threatening diseases as well as non-genetic behavioural disorders. With the completion of phase 1 of the Human Microbiome Project (HMP 2008–2012) and the researches that have been carried out subsequently, it has become clear that the human microbiome is associated with obesity, cancer, mental health disorders, asthma and autism. While many other aspects of these associations are yet to be investigated, we are not clear whether the differential microbiome composition among the diseased individuals is a consequence of the disease itself or the differing microbiome causes the disease.

8.2 Inception of Microbiota with Human Body

It will be pertinent to mention here that the human foetus grows in an absolutely sterile environment of the uterus for about 266 days from the time of conception till parturition. The first encounter of human with the microbes is during the passage of the infant through the birth canal, specifically the vaginal tract and the vulva (Ravel et al. 2011). Perhaps skin surface microbes are the first colonizers followed by nasal (respiratory) and oral (digestive) tracts, brought about by the processes of breathing and external feeding. Establishment of an unwavering flora on the skin, oral cavity and intestinal tract occurs with handling and feeding of the foetus within the first 48 h. Mode of birth whether normal or caesarean also suggested to influence the microbial colonization of the human infant to a greater extent (Mackie et al. 1999; Dominguez-Bello et al. 2010).

8.3 Diversity of Human Microbiota

Recent investigations revealed that the human microflora is exceedingly intricate and includes more than 200 species of bacteria (Todar 2012). Various factors like genetics, age, sex, stress, nutrition and dietary habit of the individuals greatly influence the diversity and abundance of microflora. The estimated number of bacteria present on the human skin, inside the mouth and the gastrointestinal tract, is 10^{14}, 10^{10} and 10^{14}, respectively (Mikelsaar and Zilmer 2009). The number of bacteria in the human gut alone far exceeds the total number of human cells (Gerritsen et al. 2011). The digestive system alone accounts for 55% of the total human microbiota, followed by skin, respiratory system and urogenital system. Surprisingly, blood contains just about 1% of the total human microbiota, while the conjunctiva has negligible quantity of microbiota (Table 8.1). The microbiota of the human intestine

Table 8.1 Microbiota (prevalent genera) that colonize different human organ systems

S. no.	Body niche	No. of bacteria (as a % of the total microbiota in humans)	Prevalent genus
1	Skin	21	*Staphylococcus*, *Propionibacterium* and *Corynebacterium*
2	Gut	29	*Bacteroides*, *Clostridium*, *Fusobacterium*, *Enterococcus*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, *Bifidobacterium*, *Escherichia* and *Lactobacillus*
3	Oral cavity	26	*Streptococci*, *Lactobacilli*, *Staphylococci*, *Corynebacterium* and *Bacteroides*
4	Vagina (urinogenital)	9	*Lactobacillus*, *Atopobium*, *Peptostreptococcus* and *Staphylococcus*
5	Conjunctiva	0	*Staphylococcus*, *Propionibacterium* and *Haemophilus*
6	Respiratory region	14	*Prevotella*, *Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Fusobacterium*, *Megasphaera*, *Veillonella*, *Staphylococcus and Streptococcus*
7	Blood	1	*Staphylococcus*

Instability of human microbiome (Adopted from Peterson et al. 2009 (NIH Human Microbiome Project))

is suggested to not only help in digestion, produce vitamins and promote gastrointestinal motility but balance the immune system as well (Berg 1996), suggesting the larger implications on human health and diseases. The disturbance of microbiota–host relationship is associated with numerous chronic inflammatory diseases and metabolic syndrome (Chassaing et al. 2015).

8.4 Human Microbiome and Human Health

In order to better understand the impact of the human microbiome on human health and diseases, it is important to understand not only the microbial density/load but also to know the diversity of microbes colonizing different organ systems. Among the different organ systems that were assessed for the microbial diversity, the gut was found to have the highest diversity followed by the mouth and skin. Vaginal region had the least microbial diversity (Li et al. 2012). The highly diverse microflora of the digestive system are perhaps due to variable food habits of individuals, while the diversity of the skin microbiota might be related to the geographical differences (Kau et al. 2011). Shannon–Wiener index

(H′), Simpson's index (D), Brillouin index (HB), richness and evenness are some of the standard diversity measures adopted in elucidating community diversity (Zar 2010). Each of these diversity indices has their own limitations and advantages and is often used in combination for a better understanding. Even the use of these indices in combinations falls short of expectations when one has to understand the microbial communities across human body habitats, specifically the failure to capture low abundant taxa. Tail statistic, τ—a rank-based diversity measure that is similar to standard deviation statistic, σ—is suggested to best suit the 16S profiles that tend to exhibit a long-tailed distribution (Li et al. 2012).

The microbial community colonizing a healthy human body is dominated by four major phyla, viz. *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. At the genus level, the most predominant genera are *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus* and *Ruminococcus*. *Bacteroides* alone constitutes about 30% of the total gut microflora (Sears 2005). *Escherichia* and *Lactobacillus* are the other two genera present to a lesser extent (Khanna and Tosh 2014). Apart from Bacteria, Archaea and Fungi are the other group of microorganisms that are found in variable numbers in the human body. The common fungi include species of the genera *Candida*, *Saccharomyces*, *Aspergillus* and *Penicillium* (Hoffmann et al. 2013).

In addition to the characteristic and systematic differences in the microbial diversity in different human body habitats, differences among individuals were also reported (Li et al. 2012). It is now becoming clear that the microbial community differences among individuals hold a key to human health, diseases and treatment. Introduction (through dietary change) and/or extinction (due to antibiotic treatment) of particular microbial groups would alter the community and population structure of the microbiota that potentially bring about functional variation.

The developments of new sequencing technologies, computational algorithms and bioinformatic tools have made the exploration of the human microbiome a frontier enterprise. The main focus in the recent past has been to elucidate the 'core' microbiome occupying specific human body niches and to ascertain interindividual differences of healthy humans. However, it is critically important to discern the differences between healthy and diseased individuals. Although association of specific microbial communities under physiologically different conditions of healthy subjects was being sporadically reported from the late 1970s, there is a steady stream of publications reporting the microbial communities predominant in human subjects affected by different diseases. In this chapter we have restricted our discussion to the diseases manifested as a consequence of altered gut microbial community structure (Fig. 8.1) due to medication. The specific diseases are *Clostridium difficile* infection (CDI), autism spectrum disorder (ASD), diabetes, gastric cancer, obesity and inflammatory bowel disease (IBD).

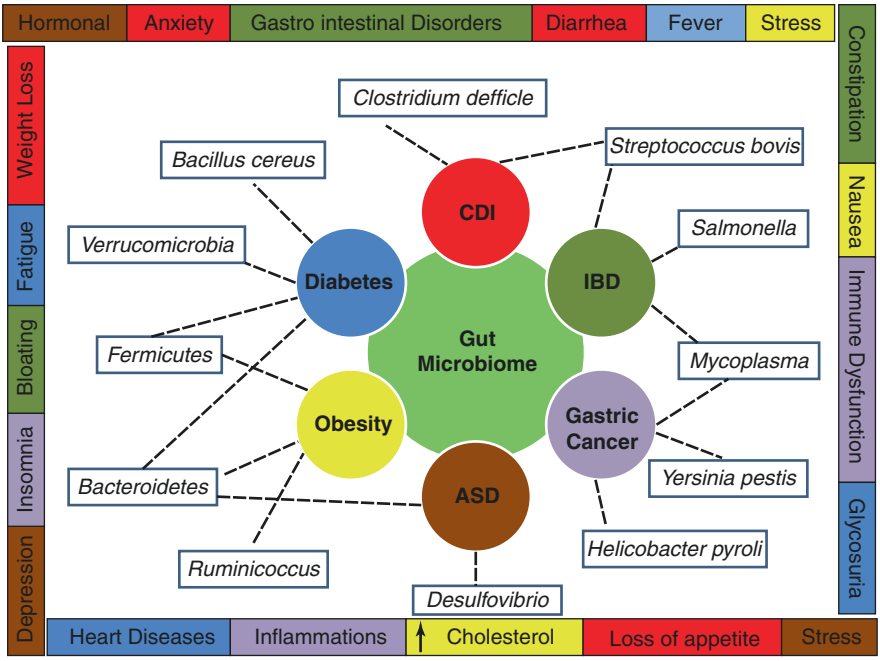

Fig. 8.1 Complex interplay between gut microbes, diseases and their symptoms. Colour of each microbial-associated disease corresponds to its coloured phenotype. The *dotted line* between the causative microbial lineages represents the cross talk through nonlinear signalling interdependence

8.5 Microbiome and *Clostridium difficile* Infection (CDI)

Clostridium difficile is a pathogenic native gut microorganism, found in 3 out of 100 adults and 7 out of 10 babies. In healthy individuals the population numbers of *C. difficile* are maintained at negligible level that is insufficient to cause disease. However, with administration of broad-spectrum antibiotics, the patients develop gastrointestinal illness, due to a toxin produced by *C. difficile* (Buss et al. 2015). The disease is referred to as *C. difficile* infection (CDI). Although our knowledge of CDI pathogenesis is still rudimentary (Britton and Young 2012), CDI is one of the most ubiquitous and expensive nosocomial infections. CDI occurs in 25% of all antibiotic-associated diarrhoea (Bartlett 2002). Another disease also called the antibiotic-associated diarrhoea is reported to coincide with the decline in the carbohydrate-fermenting butyrate-producing members of the phylum *Firmicutes* (Britton and Young 2012). Further, it has been shown that even short-term antibiotic treatment can bring about long-term changes in gut microbiota that is not necessarily reversible with the discontinuation of antibiotic treatment (Jakobsson

et al. 2010). The reduced microbial diversity would not only lead to invasion and proliferation of pathogenic flora due to lowered resistance (Chang et al. 2008; Britton and Young 2012) but is responsible for the progression of the disease (Freter 1955).

Over the past decade, increased morbidity and mortality, as well as relapse of *C. difficile* infection, have become more common (Khanna et al. 2012) due to the emergence of strain 027 of *C. difficile* (Karas et al. 2010; Marsh et al. 2012). Antibiotic resistance, sporulation ability and toxin production are suggested to be the potential contributors to virulence of historical ribotypes and *C. difficile 027* (Warny et al. 2005; Drudy et al. 2007; Merrigan et al. 2010; Lanis et al. 2010, 2012, 2013). TcdA and TcdB are two large clostridial toxins produced by *C. difficile* responsible for major virulence causing extensive tissue damage in human disease (Taylor et al. 1981; Libby et al. 1982; Lyerly et al. 1986). Among the two toxins, TcdB is the critical virulence factor (Lyras et al. 2009), antigenically variable and more lethal and causes more extensive brain haemorrhage (Lanis et al. 2013).

Due to ever-increasing severity of CDI, many studies have been initiated to unravel the details of the disease progression that perhaps would aid in designing effective treatment. In humans, bile acids are secreted in the small intestine in response to consumption of food so as to facilitate absorption of fats and fat-soluble vitamins and nutrients (Britton and Young 2012). Cholate and chenodeoxycholate are the primary bile acids that are conjugated to either of the two amino acids—glycine and taurine (Ridlon et al. 2006). Deoxycholate, a secondary bile acid produced by the action of 7-dehydroxylase on cholate, was reported to be a potent *C. difficile* spore germinant but highly toxic to its vegetative cells. Further, bile acid (taurocholate) and amino acid (glycine) were shown to enhance *C. difficile* spore germination by 1000-fold (Sorg and Sonenshein 2008). Antibiotic treatment perhaps reduced members of microbiota that were involved in the conversion of cholate to deoxycholate, thus resulting in increased levels of cholates and their derivatives. This in turn facilitates the germination of spores and growth and propagation of vegetative cells of *C. difficile* (Britton and Young 2012). However, chenodeoxycholate is shown to inhibit spore germination (Sorg and Sonenshein 2008), and hence non-metabolizable derivates of chenodeoxycholate could serve as therapeutics (Sorg and Sonenshein 2010). Competitive exclusion of toxigenic *C. difficile* by non-toxigenic *C. difficile* (Sambol et al. 2002), direct antagonism by intestinal microbiota such as *Bacillus thuringiensis* that secretes thuricin CD bacteriocin with narrow-spectrum activity against *C. difficile* spores (Rea et al. 2010) and faecal transplantation (Khoruts et al. 2010) are suggested as potential curative measures.

8.6 Autism Spectrum Disorders (ASD) and Gut Microbiome

The gut microbes are now reported to make neuroactive compounds, including neurotransmitters and metabolites that act on brain via the vagus nerve that connects the brain and the digestive tract (Schmidt 2015). Disruptions of the healthy

microbiome are suggested to result in anxiety, depression and even autism. Autism spectrum disorders (ASD) are complex neurobiological disorders characterized by stereotyped behavioural patterns leading to visible impairment in social interactions and communications (Johnson and Myers 2007). Both genetic and environmental factors play an important role in ASD aetiology. Genetically, ASD is linked with autosomal recessive inheritance, X-linked inheritance and sporadic chromosomal anomalies. Among the environmental factors, gut microbes have the potential to interact with central nervous system (Collins and Bercik 2009). Autistic children's gut had reduced bacterial richness compared to neurotypical children. Altered gut microbiota was not due to demographics or special diets but due to antibiotic treatment that is suggested to aggravate ASD-related behavioural symptoms (Kang et al. 2013). High levels of gram-negative bacteria *Bacteroides vulgatus* and *Desulfovibrio* have been reported in autistic children (Finegold et al. 2010). Lipopolysaccharides (LPS) present in the cell walls of many pathogenic gram-negative bacteria are suggested to damage many tissues including the brain (Minami et al. 2007) leading to increased permeability of the blood–brain barrier, thus facilitating the accumulation of high levels of mercury in the cerebrum that may aggravate ASD symptoms (Adams et al. 2008). Glutathione—an important antioxidant responsible for heavy metal detoxification in the brain—has been shown to be reduced in rats exposed to LPS (Zhu et al. 2007). Depletion of glutathione could also be caused by p-cresol—formation of which is catalysed by a glycyl radical enzyme (p-hydroxyphenylacetate decarboxylase) from *C. difficile*, a gram-positive bacteria (Selmer and Andrei 2001). As discussed above *C. difficile* is known to play a crucial role in development of gastrointestinal illness (GI). Thus the presence of autistic symptoms and their correlated GI severity seems to be linked to reduced richness and diversity of gut microflora that in turn might alter the physiological functionality and microbial GI robustness due to decrease in microbial redundancy in ASD children (Kang et al. 2013). Although a statistically significant correlation between autistic symptoms and abundances of unclassified *Veillonellaceae, Prevotella* and *Coprococcus* genera is established, severity of GI symptoms is not a significant predictor of these microbial changes among autistic children (Kang et al. 2013). ASD children are reported to have a strong preference for starches, snack and processed foods while rejecting most fruits, vegetables and proteins (Field et al. 2003; Sharp et al. 2013). Although the aetiological factors contributing to feeding problems in ASD patients remain elusive (Mulle et al. 2013), neurobehaviourally influenced aetiology of higher rates of constipation and encopresis is reported in ASD (Ibrahim et al. 2009). The major function of gut microbiome of healthy individuals is to help in breaking down complex plant polysaccharides and other dietary matter. The altered gut microbiome of the ASD patients reported is unable to assist in the breakdown of the plant polysaccharides thus causing GI distress (Mulle et al. 2013). Hence, interventions aimed at restoring the microbial balance in the gut of ASD individuals might improve behaviours (Mulle et al. 2013).

8.7 Gut Microbiome, Obesity and Diabetes

The relation between obesity and gut microbiota was known as early as three decades ago, and the gut microbiota is shown to shift in response to host adiposity and nutrient intake (Musso et al. 2011). Several studies have suggested the involvement of gut microbiota in host metabolism, energy utilization and storage (Musso et al. 2011) leading to the development of fat mass and fat storage (Backhed et al. 2004; Everard and Cani 2013). *Bacteroides intestinalis*, *Bacteroides fragilis* and *Escherichia coli* are suggested to be involved in generation of secondary bile acids in the colon (Fukiya et al. 2009), and bile acids are known to exert metabolic regulatory functions in addition to favouring dietary lipid absorption (Keitel et al. 2008; Lefebvre et al. 2009). The development of obesity was found to be associated with the enrichment of *Firmicutes*—specifically *Mollicutes*—at the expense of *Bacteroidetes* in mice fed with high-fat/high-sugar diet compared to those fed with low-fat/high-polysaccharide diet (Turnbaugh et al. 2008). The microbiome of the obese mice showed enrichment in genes coding for enzymes that enable the extraction of energy from otherwise indigestible alimentary polysaccharides suggesting increased energy extraction capacity of the gut flora of obese individuals (Turnbaugh et al. 2006; Musso et al. 2011). Further, gut microbiota is shown to play a major role in the onset of insulin resistance and type 2 diabetes (Bäckhed et al. 2004, 2007; Cani et al. 2007a; Shen et al. 2013) triggering low-grade inflammation—a common feature characterizing obesity and several other metabolic disorders (Everard and Cani 2013). Microbiota-derived lipopolysaccharides (LPS) are reported to be the key molecule involved in early development of inflammation and metabolic diseases (Cani et al. 2007b). Animal model studies have established that obesity is transmissible along with gut microbiota (Musso et al. 2011) as transplantation of microbiota from obese mice to germ-free wild-type recipient mice resulted in increased adiposity compared to those that received microbiota from conventionally raised lean wild-type littermates (Turnbaugh et al. 2006).

Diet also plays an important role in changing the microbial diversity of gut microbiome. High-fat diet when given to both obese and lean genotypes was found to be associated with a decrease in *Bacteroidetes* and an increase in both *Firmicutes* and *Proteobacteria* (Hildebrandt et al. 2009; Turnbaugh et al. 2009). On the other hand, germ-free mice were found to be resistant to diet-induced obesity caused by consumption of a high-fat or high-sugar 'Western' diet (Backhed et al. 2007). A study by Ley et al. (2005) clearly demonstrated that both—genetic obese and diet-induced obese—had increased abundance of *Firmicutes* in their gut microbiome.

While type 2 diabetes is a metabolic disorder caused due to obesity-linked insulin resistance, type 1 diabetes (T1D) is a T-cell-mediated autoimmune disease due to slow and progressive destruction of insulin-producing β cells (Zipris 2008). Both genetic and environmental factors are known to contribute to autoimmunity disorders. Altered gut microbiota, impaired intestinal mucosal barrier and mucosal immunity are reported to contribute to T1D pathogenesis (Musso et al. 2011). Although specific details of how the gut microbiota regulates the T1D are unknown, T1D-resistant MyD88 KO mice were shown to harbour a lower *Firmicutes*/*Bacteroidetes*

ratio with an increased proportion of *Lactobacilli*, *Rikenellae* and *Porphyromonadaeae* (Wen et al. 2008). The dynamic link between gut microbiota, adiposity and diabetes indicates that manipulation of gut microbial communities by dietary interventions (e.g. probiotics or prebiotics) and translocation could be an approach to treat obesity and improve metabolic health (Flint et al. 2014).

8.8 Gut Microbiome and Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) involves chronic and recurring immune responses with relapsing and remitting inflammations in gastrointestinal tract. Aetiology is multifarious including genetic, microbial and environmental factors contributing to disease development (Cho and Blaser 2012). IBD primarily includes two subtypes, namely, ulcerative colitis (UC) and Crohn's disease (CD). UC remains confined to the colon and rectum, while CD can affect different areas of GI tract including the mouth. These are characterized as autoimmune diseases with the identification of pathways involving NOD2, ROS, CARD9 and Th17 cells in genetically susceptible hosts (Cho and Blaser 2012). Genetic predisposition is in itself not sufficient for the onset and progression of inflammation. Microbial dysbiosis plays a key role in the onset and progression of IBD, indicating the complex interplay between the gut microbiome and genetic susceptibility to IBD (Knights et al. 2013).

Microbial dysbiosis refers to the shift in relative abundances of dominant taxa and decrease in overall diversity of gut community (Sokol and Seksik 2010). It remains unclear whether this dysbiosis is the cause of or the response to the disease; nevertheless stable and healthy gut commensal bacteria are necessary to suppress the pathogenic infection (Kamada et al. 2012). Broadly, IBD is associated with reduced gut diversity, an increase in proportion of *Gammaproteobacteria* and reduced number of *Firmicutes* (Sokol and Seksik 2010). A significant decrease in abundance of two genera *Roseburia* and *Phascolarctobacterium* is associated with both UC and CD subjects (Morgan et al. 2012). In gut, species of the genus *Roseburia* are associated with production of butyrate and utilization of acetate (Duncan et al. 2002), whereas species of the genus *Phascolarctobacterium* are associated with production of propionate in coculture with *Paraprevotella* (Watanabe et al. 2012). Therefore, apart from changes in composition, functional imbalance has also been witnessed in IBD subjects including upregulation of sulphur metabolism pathways and downregulation of butanoate and propanoate metabolism. Few microbial clades are differentially abundant in CD and UC patients; proportion of *Faecalibacterium* of *Ruminococcaceae* family (acetate producers) is reduced, and members of the family *Enterobacteriaceae* show significant increase in abundance in CD (Kang et al. 2010), whereas a significant reduction in members of *Leuconostocaceae* is seen in UC (Morgan et al. 2012).

Epidemiological studies on concordance rates for IBD in German monozygotic twins (16% for UC and about 35% for CD) suggest stronger genetic influence in CD as compared to UC and also indicate the role of environmental factors in the development of chronic inflammation (Spehlmann et al. 2008).

Dietary intake is also correlated with incidence of IBD. Diet with high amounts of total fats, PUFAs, omega-6 fatty acids and meat was associated with an increased risk of CD and UC, whereas high fibre and fruit intake were related to decreased risk for CD. High vegetable intake was linked with decreased risk for UC (Hou et al. 2011). Recently, blow-out of 'Western diet', rich in protein but low in fruits and vegetable, is also being considered as a reason for increasing IBD incidence.

Hence, there exists an interaction network between genetics, host gut microbiome and diet providing feedback to host immune responses. For instance weakened immune response to commensal bacteria in gut can result from mutations in *NOD*2 and *GPR*35 and, as a result, cause imbalance in taxonomic structure of gut microbiota which can subsequently lead to metabolic dysbiosis. Altered metabolic capabilities of gut microbiome may further lead to diminished antibacterial activity through different pathways and consequent taxonomic and metabolic imbalance (Knights et al. 2013). Recently, even the alterations in gut virome have been observed in IBD patients (Ray 2015).

Based on studies done so far, treatments used for IBD are accompanied with potential risks and side effects. However, use of probiotics and prebiotics with clinical course is being tested for its cure of which using *Faecalibacterium* as a probiotic is a promising strategy in counterbalancing the gut commensal bacteria composition in CD patients (Sokol et al. 2008). Symbiosis factors from microbes can also be employed in therapeutics for inflammation, for example, PSA (polysaccharide A) produced by *B. fragilis* is reported to suppress the production of interleukin-17 (pro-inflammatory) from intestinal immune cells (Mazmanian et al. 2008). Apart from these, researchers are trying faecal bacteriotherapy (FBT) in which faeces from healthy donor are transplanted into the gut as a treatment of UC, though it has not yet approved regulatory authorities.

8.9 Microbiome and Gastric Cancers

It is evident from the preceding discussion that the gut microbiota has significant influence on inflammation of the gut particularly the distal large intestine (Louis et al. 2014). The chronic inflammation of the gastrointestinal tract progresses to inflammatory bowel disease (IBD), and IBD patients are reported to show an increased incidence of colorectal cancer (CRC) also known as colitis-associated cancer (CAC) (Jess et al. 2005; Danese et al. 2011). More than 95% of CRC cases show an association with dietary lifestyle and more recently gut microbiota, while less than 5% are hereditary (Rustgi 2007; Watson and Collins 2010; Irrazábal et al. 2014). CRC is ranked third among the most common causes of cancer-related deaths in the world (AICR 2007; Jemal et al. 2011; Irrazábal et al. 2014). Several pathogenic bacteria have been implicated in promoting CRC via pro-inflammatory interactions with host cells (Sears and Garrett 2014; Zackular 2014; Zackular et al. 2014; Louis et al. 2014). Relative abundance of *Ruminococcaceae*, *Clostridium*, *Pseudomonas* and *Porphyromonadaceae* was higher, while the relative abundances

of *Bacteroides*, *Lachnospiraceae*, *Clostridiales* and *Clostridium* were found to be less in patients with adenomas (Zackular et al. 2014). Further, patients with carcinomas had higher relative abundances of *Fusobacterium*, *Porphyromonas*, *Lachnospiraceae* and *Enterobacteriaceae* and lower abundances of *Bacteroides*, *Lachnospiraceae* and *Clostridiales* (Zackular et al. 2014). Furthermore, *Helicobacter pylorus* has been identified as the primary cause of gastric cancer (Tu et al. 2008). However, it is now becoming increasingly clear that collective activities of the metabolic products of the microbiota greatly influence the predisposition to and protection against CRC (Gill and Rowland 2002; Schwabe and Jobin 2013). Nitrosation of amines produced by fermentation of proteins in the large intestine by *Bacteroides* and *Firmicutes* leads to formation of N-nitroso compounds that have the potential to promote cancer (Rowland 2000; Louis et al. 2014) as indicated by the positive correlation between dietary intake of NOCs and CRC in European populations (Loh et al. 2011). Nitroreductases and nitrate reductases encoded by *Proteobacteria* are suggested to be facilitating nitrosation (Louis et al. 2014). Ammonia—a product of protein fermentation—is reported to be potentially carcinogenic at low concentrations (Windey et al. 2012). Although polyamines are essential for maintenance of structural integrity of membranes and nucleic acids, higher levels of polyamines are associated with several diseases including cancer (Louis et al. 2014) and certain gut bacteria including enterotoxigenic *B. fragilis* that upregulate polyamine production (Pegg 2013). Further, pathogens such as *Shigella flexneri*, *Streptococcus pneumoniae*, *Salmonella enterica* and *H. pylori* are known to exploit polyamines to increase their virulence (Di Martino et al. 2013). Colonocyte barrier breakdown by toxic sulphide produced as hydrogen sulphide in the gut by sulphate-reducing bacteria related to *Disulfovibrio* spp. could be another causative agent of CRC as indicated by higher stool sulphide levels in CRC patients, although increased levels of *Disulfovibrio* spp. have not been reported (Carbonero et al. 2012). However, several bacterial pathogens such as *B. fragilis*, *E. coli* NC101 strain, *Fusobacterium* spp. and *Campylobacter* spp. seem to be directly and specifically involved in promoting CRC (Sears 2009; Arthur et al. 2012; Kostic et al. 2013). Further, there is a complex interplay between diet, bile acid and gut microbiota (Louis et al. 2014). Higher-fat intake is positively correlated with secondary bile acids (Ou et al. 2013), and secondary bile acid deoxycholic acid is reported to promote liver cancer (Yoshimoto et al. 2013). Furthermore, higher levels of bile acids are reported from faecal samples of CRC patients (Ou et al. 2012).

Both animal and human studies suggest that dietary supplementation with non-digestible carbohydrates can reduce protein fermentation in the large intestine, leading to decrease in the genotoxicity of faecal water (Windey et al. 2012), thus reducing the incidence of IBD as well as CRC.

Conclusions

We owe our very persistence in nature to the plethora of the microbiota that has colonized our various organ systems. Particularly, the gut microbiota provides important benefits in terms of primary breakdown of the food ingested, immune development as well as mental wellbeing. However, the full import of the role of

human microbiome on the health as well as disease has just begun to emerge with the advent of culture independent research technologies. Although specific microbes have been implicated in causing and/or promoting specific diseases, it is now becoming clear that it is the overall community structure of microbiota that is the 'Lakshman Rekha' that separates health and disease, and diet seems to play a very crucial role in altering the community structure of the gut microbiota. The way to a man's heart is certainly through his stomach but via the microbiota.

Acknowledgements P.H., U.S., V.G., N.N. and N.K.M. gratefully acknowledge Council of Scientific and Industrial Research (CSIR) and University Grant Commission (UGC) for providing research scholarships. We also gratefully acknowledge Department of Biotechnology (DBT), Government of India (under project BT/PR3301/BCE/08/875/2011) for providing infrastructural support.

Competing Interests No competing interests to be disclosed.

References

Adams JB, Romdalvik J, Levine KE, Hu LW. Mercury in first-cut baby hair of children with autism versus typically-developing children. Toxicol Environ Chem. 2008;90:739–53.

Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science. 2012;338:120–3.

Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanism underlying the resistance to diet-induced obesity in germ free mice. Proc Natl Acad Sci USA. 2007;104:979–84.

Bäckhed F, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA. 2004;101:15718–23.

Bartlett JG. Antibiotic-associated diarrhea. N Engl J Med. 2002;346:334–9.

Berg RD. The indigenous gastrointestinal microflora. Trends Microbiol. 1996;4:430–5.

Bianconi E, et al. An estimation of the number of cells in the human body. Ann Hum Biol. 2013;40:463–71.

Britton RA, Young VB. Interaction between the intestinal microbiota and host in Clostridium difficile colonization resistance. Trends Microbiol. 2012;20:313–9.

Buss SN, et al. Gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol. 2015;53:915–25.

Cani PD, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007a;56:1761–72.

Cani PD, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia. 2007b;50:2374–83.

Carbonero F, Benefiel AC, Gaskins HR. Contributions of the microbial hydrogen economy to colonic homeostasis. Nat Rev Gastroenterol Hepatol. 2012;9:504–18.

Chang JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, Young VB. Decreased diversity of the fecal microbiome in recurrent Clostridium difficile-associated diarrhea. J Infect Dis. 2008;197:435–8.

Chassaing B, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature. 2015;519:92–6.

Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet. 2012;13:260–70.

Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology. 2009;136:2003–14.

Danese S, Malesci A, Vetrano S. Colitis-associated cancer: the dark side of inflammatory bowel disease. Gut. 2011;60:1609–10.
Di Martino ML, et al. Polyamines: emerging players in bacteria–host interactions. Int J Med Microbiol. 2013;303:484–91.
Dominguez-Bello MG, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA. 2010;107:11971–5.
Drudy D, Kyne L, O'Mahony R, Fanning S. gyrA mutations in fluoroquinolone-resistant Clostridium difficile PCR-027. Emerg Infect Dis. 2007;13:504–5.
Duncan SH, Hold GL, Barcenilla A, Stewart CS, Flint HJ. *Roseburia intestinalis* sp. nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. Int J Syst Evol Microbiol. 2002;52:1615–20.
Everard A, Cani PD. Diabetes, obesity and gut microbiota. Best Pract Res Clin Gastroenterol. 2013;27:73–83.
Field D, Garland M, Williams K. Correlates of specific childhood feeding problems. J Paediatr Child Health. 2003;39:299–304.
Finegold SM, et al. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe. 2010;16:444–53.
Flint HJ, Duncan SH, Louis P. Gut microbiome and obesity in treatment of the obese patient. 2014:73–82.
Freter R. The fatal enteric cholera infection in the guinea pig achieved by inhibition of normal enteric flora. J Infect Dis. 1955;97:57–65.
Fukiya S, et al. Conversion of cholic acid and chenodeoxycholic acid into their 7-oxo derivatives by Bacteroides intestinalis AM-1 isolated from human feces. FEMS Microbiol Lett. 2009;293:263–70.
Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. Genes Nutr. 2011;6:209–40.
Gill CIR, Rowland IR. Diet and cancer: assessing the risk. Br J Nutr. 2002;88:s73–87.
Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology. 2009;137:1716–24.
Hoffmann C, et al. Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. PLoS One. 2013;8:e66019.
Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol. 2011;106:563–73.
Human Microbiome Project Consortium. A framework for human microbiome research. Nature. 2012;486:215–21.
Ibrahim SH, Voigt RG, Katusic SK, Weaver AL, Barbaresi WJ. Incidence of gastrointestinal symptoms in children with autism: a population-based study. PLoS One. 2009;124:680–6.
Irrazábal T, Belcheva A, Girardin SE, Martin A, Philpott DJ. The multifaceted role of the intestinal microbiota in colon cancer. Mol Cell. 2014;54:309–20.
Jakobsson HE, et al. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One. 2010;5:e9836.
Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
Jess T, Gamborg M, Matzen P, Munkholm P, Sørensen TI. Increased risk of intestinal cancer in Crohn's disease: a meta-analysis of population-based cohort studies. Am J Gastroenterol. 2005;100:2724–9.
Johnson CP, Myers SM. Identification and evaluation of children with autism spectrum disorders. Pediatrics. 2007;120:1183–215.
Kamada N, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science. 2012;336:1325–9.
Kang S, et al. Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. Inflamm Bowel Dis. 2010;16:2034–42.
Kang DW, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One. 2013;8:e68322.

Karas JA, Enoch DA, Aliyu SH. A review of mortality due to Clostridium difficile infection. J Infect. 2010;61:1–8.
Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome, and immune system: envisioning the future. Nature. 2011;474:327–36.
Keitel V, Kubitz R, Häussinger D. Endocrine and paracrine role of bile acids. World J Gastroenterol. 2008;14:5620–9.
Khanna S, Tosh PK. A clinician's primer on the role of the microbiome in human health and disease. Mayo Clin Proc. 2014;89:107–14.
Khanna S, et al. The epidemiology of community-acquired Clostridium difficile infection: a population-based study. Am J Gastroenterol. 2012;107:89–95.
Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. J Clin Gastroenterol. 2010;44:354–60.
Knights D, Lassen KG, Xavier RJ. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. Gut. 2013;62:1505–10.
Kostic AD, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013;14:207–15.
Lanis JM, Barua S, Ballard JD. Variations in TcdB activity and the hypervirulence of emerging strains of Clostridium difficile. PLoS Pathog. 2010;6:e1001061.
Lanis JM, Heinlen LD, James JA, Ballard JD. Clostridium difficile 027/BI/NAP1 encodes a hypertoxic and antigenically variable form of TcdB. PLoS Pathog. 2013;9:e1003523.
Lanis JM, Hightower LD, Shen A, Ballard JD. TcdB from hypervirulent Clostridium difficile exhibits increased efficiency of autoprocessing. Mol Microbiol. 2012;84:66–76.
Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. 2009;89:147–91.
Ley RE, Backed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci USA. 2005;102:11070–5.
Li K, Bihan M, Yooseph S, Methé BA. Analyses of the microbial diversity across the human microbiome. PLoS One. 2012;7:e32118.
Libby JM, Jortner BS, Wilkins TD. Effects of the two toxins of Clostridium difficile in antibiotic-associated cecitis in hamsters. Infect Immun. 1982;36:822–9.
Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)—Norfolk Study. Am J Clin Nutr. 2011;93:1053–61.
Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol. 2014;12:661–72.
Lyerly DM, Phelps CJ, Toth J, Wilkins TD. Characterization of toxins A and B of Clostridium difficile with monoclonal antibodies. Infect Immun. 1986;54:70–6.
Lyras D, et al. Toxin B is essential for virulence of Clostridium difficile. Nature. 2009;458:1176–9.
Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr. 1999;69:1035s–45s.
Marsh JW, Shutt KA, Brooks MM, Pasculle AW, Muto CA, Harrison LH. Perirectal swab surveillance for Clostridium difficile by use of selective broth preamplification and real-time PCR detection of tcdB. J Clin Microbiol. 2012;50:4078–82.
Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453:620–5.
Merrigan M, et al. Human hypervirulent Clostridium difficile strains exhibit increased sporulation as well as robust toxin production. J Bacteriol. 2010;192:4904–11.
Mikelsaar M, Zilmer M. Lactobacillus fermentum ME-3—an antimicrobial and antioxidative probiotic. Microb Ecol Health Dis. 2009;21:1–27.
Minami T, Oda K, Gima N, Yamazaki H. Effects of lipopolysaccharide and chelator on mercury content in the cerebrum of thimerosal-administered mice. Environ Toxicol Pharmacol. 2007;24:316–20.

Morgan XC, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012;13:R79.
Mulle JG, Sharp WG, Cubells JF. The gut microbiome: a new frontier in autism research. Curr Psychiatry Rep. 2013;15:337–49.
Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med. 2011;62:361–80.
Ou J, DeLany JP, Zhang M, Sharma S, O'Keefe SJ. Association between low colonic short-chain fatty acids and high bile acids in high colon cancer risk populations. Nutr Cancer. 2012;64:34–40.
Ou J, et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am J Clin Nutr. 2013;98:111–20.
Pegg AE. Toxicity of polyamines and their metabolic products. Chem Res Toxicol. 2013;26:1782–800.
Pertea M, Salzberg SL. Between a chicken and a grape: estimating the number of human genes. Genome Biol. 2010;11:206–12.
Peterson J, et al. The NIH human microbiome project. Genome Res. 2009;19:2317–23.
Ravel J, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA. 2011;108:4680–7.
Ray K. IBD: gut microbiota in IBD goes viral. Nat Rev Gastroenterol Hepatol. 2015;12(3):122.
Rea MC, et al. Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against Clostridium difficile. Proc Natl Acad Sci USA. 2010;107:9352–7.
Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res. 2006;47:241–59.
Rowland RHIR. Metabolic activities of the gut microflora in relation to cancer. Microb Ecol Health Dis. 2000;12:179–85.
Rustgi AK. The genetics of hereditary colon cancer. Genes Dev. 2007;21:2525–38.
Sambol SP, Merrigan MM, Tang JK, Johnson S, Gerding DN. Colonization for the prevention of Clostridium difficile disease in hamsters. J Infect Dis. 2002;186:1781–9.
Schmidt C. Mental health: thinking from the gut. Nature. 2015;518:S12–5.
Schnorr SL. The diverse microbiome of the hunter-gatherer. Nature. 2015;518:S14–5.
Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer. 2013;13:800–12.
Sears CL. A dynamic partnership: celebrating our gut flora. Anaerobe. 2005;11:247–51.
Sears CL. Enterotoxigenic Bacteroides fragilis: a rogue among symbiotes. Clin Microbiol Rev. 2009;22:349–69.
Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. Cell Host Microbe. 2014;15:317–28.
Selmer T, Andrei PI. p-Hydroxyphenylacetate decarboxylase from Clostridium difficile. A novel glycyl radical enzyme catalysing the formation of p-cresol. Eur J Biochem. 2001;268:1363–72.
Sharp WG, Berry RC, McCracken C, Nuhu NN, Marvel E, Saulnier CA, Klin A, Jones W, Jaquess DL. Feeding problems and nutrient intake in children with autism spectrum disorders: a meta-analysis and comprehensive review of the literature. J Autism Dev Disord. 2013;43:2159–73.
Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. Mol Asp Med. 2013;34:39–58.
Sokol H, Seksik P. The intestinal microbiota in inflammatory bowel diseases: time to connect with the host. Curr Opin Gastroenterol. 2010;26:327–31.
Sokol H, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA. 2008;105:16731–6.
Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for Clostridium difficile spores. J Bacteriol. 2008;190:2505–12.
Sorg JA, Sonenshein AL. Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid. J Bacteriol. 2010;192:4983–90.
Spehlmann ME, et al. Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study. Inflamm Bowel Dis. 2008;14:968–76.

Taylor NS, Thorne GM, Bartlett JG. Comparison of two toxins produced by Clostridium difficile. Infect Immun. 1981;34:1036–43.
Todar K. The normal bacterial flora of humans. Todar's Online textbook of Bacteriology, 2012. http://www.textbookofbacteriology.net/normalflora_3.html
Tu S, et al. Overexpression of interleukin-1β induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. Cancer Cell. 2008;14:408–19.
Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe. 2008;3:213–23.
Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007;449:804–10.
Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.
Turnbaugh PJ, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480–4.
Warny M, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet. 2005;366:1079–84.
Watanabe Y, Nagai F, Morotomi M. Characterization of *Phascolarctobacterium succinatutens* sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. Appl Environ Microbiol. 2012;78:511–8.
Watson AJ, Collins PD. Colon cancer: a civilization disorder. Dig Dis. 2010;29:222–8.
Wen L, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008;455:1109–13.
Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. Mol Nutr Food Res. 2012;56:184–96.
World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR, 2007.
Yoshimoto S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499:97–101.
Zackular J. Characterizing the role of the gut microbiome in colorectal cancer. Doctoral dissertation, University of Michigan; 2014.
Zackular JP, Rogers MA, Ruffin MT, Schloss PD. The human gut microbiome as a screening tool for colorectal cancer. Cancer Prev Res. 2014;7:1112–21.
Zar JH. Measures of variability and dispersion, in Biostatistical analysis 5th Ed. (Editor: Lynch D) Pearson Publishing Company, USA 2010, 33–46.
Zhu Y, Carvey PM, Ling Z. Altered glutathione homeostasis in animals prenatally exposed to lipopolysaccharide. Neurochem Int. 2007;50:671–80.
Zipris D. Innate immunity and its role in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes. 2008;15:326–31.

Dr. Rup Lal Dr. Rup Lal is an eminent professor of the University of Delhi, Delhi. His primary research interests include microbial diversity at pesticide-polluted sites, genetic and biochemistry of hexachlorocyclohexane (HCH) degradation, and development of HCH bioremediation technology. Prof. Rup Lal is also interested in the evolution of lin genes and genetic manipulation of rifamycin producer *Amycolatopsis mediterranei*. His group has already sequenced genomes of *Amycolatopsis mediterranei* S699, *Thermus* sp. RL, *Acinetobacter* sp. HA, and *Sphingobium indicum* B90A.

Dr. Mallikarjun Shakarad Dr. Mallikarjun Shakarad is a professor at the Department of Zoology, University of Delhi. His area of expertise is in the evolution of life histories in insect systems and the influence of microbiota in altering the health span and life histories in higher animal systems.