Diet, Microbiome, and Human Health

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Chapter 4

Diet, Microbiome, and Human Health

Ashfaque Hossain, Saeed Akhter, and Yearul Kabir

Contents

4.1 Introduction

The influence of microorganisms residing in the intestine (gut microbiota) on human health and disease is an area of intense research as health and well-being is of primary concern to us. The gut microbiome (gut microbiota and its collective genomes) plays a key role in homeostasis in humans and a strong relationship exists between diet, microbiota, and our health (Nicholson et al. 2012, Martin et al. 2014). Dietary components and dietary metabolites have roles beyond basic nutrition, and the modulation of the gut microbiome composition by the alteration of food habits has potentialities in health improvement and even disease prevention (Holmes et al. 2012, Guzman et al. 2013). Understanding the complex interaction between diet and the composition and function of human gut microbiota is critical in advancing our knowledge in the formulation of ways of manipulation of microbiota to prevent various health conditions and to improve our health. A growing body of evidence suggests that reprogramming the gut microbiome or its function has beneficial effects on the host metabolism (de Vos and de Vos 2012, Goldsmith and Sartor 2014). The current knowledge of the complex and bidirectional interaction between the gut microbiome and dietary components in relation to human health and disease is reviewed in this chapter.

Virtually every surface of the human body is colonized by microorganisms. The human intestine is the habitat of many species of bacteria along with viruses, unicellular eukaryotes, and other organisms which have evolved and adapted to live, colonize, and grow there, forming a huge ecosystem, the gut microbiota (Bäckhed et al. 2005, Holmes et al. 2011). With an average length of 1.50 m and diameter of 6.4 cm, the human large intestine (colon) represents one of the largest interfaces where host–microbe interactions occur (Cummings and Macfarlane 1991). The intestine is home to an estimated 10^{14} microbial cells (Lepage et al. 2012). The microbes that we carry outnumber us 10:1 in terms of total human body cell (somatic and germ cells) counts. The combined number of genes in the microbiota genome is 150 times larger than the human genome (Neish 2009, Musso et al. 2010, Lepage et al. 2012). Taken together, the information reveals that the human gut microbiome is an ecosystem of the highest complexity. The vast majority of the microbial cells present in the human intestine are bacteria; the other members are viruses (5.8%; estimated 1200 viral genotypes are present; Breitbart et al. 2003), archaea (0.8%), and eukaryotes (0.5%) (Arumugam et al. 2011). Various factors influence the structure and function of gut microbiota. These include the availability of nutrients and antimicrobial compounds, temperature, pH, redox potential, degree of anaerobiosis, and presence of bacteriophages (Kinross et al. 2008). The metabolic activity of the human gut microbes is as robust as that of the liver and it has been suggested to function as an auxiliary, virtual organ (O'Hara and Shanahan 2006, DiBaise et al. 2012). A single layer of epithelial cells separates the gut microbiota from the internal milieu, and the structure and composition of the gut flora reflect the evolution and adaptation at both microbial and host levels promoting extensive, multiple levels of host–microbial interactions (McFall-Ngai et al. 2013). To maintain an intact and functional epithelial barrier is essential as the prevention of an unregulated uptake/translocation of the microbiome or its metabolites is required for the maintenance of host homeostasis (Guzman et al. 2013).

It is increasingly becoming clear that health and disease states can be explained at the individual level, at least in part, by the host–bacterial relationship. Directed manipulation of the

microbiome offers a promising avenue for therapeutic applications as studies have shown that the transfer of donor microbiota induces a variety of donor phenotypes into the recipients (Jia et al. 2008). In addition, such practice has also resulted in the accelerated recovery of sick recipients (Prakash et al. 2011, McFall-Ngai et al. 2013).

4.2 Gut Microbiota

Although it is generally accepted that the intestine of a newborn infant is sterile and is rapidly colonized by different microorganisms during and after birth, thus developing the gut microbiota (Mackie et al. 1999), recent evidence shows that colonization of the gut is initiated before birth following ingestion of microbe-containing amniotic fluid by the fetus (Mshvildadze and Neu 2010). The human placenta, although considered sterile, has recently been found to possess a unique microbiome. A population-based cohort of placental specimens collected under sterile conditions from 320 subjects following culture-independent metagenomic analysis showed that the placenta harbors a variety of microbes and the placental microbiome most closely resembles the oral microbiome (Aagaard et al. 2014).

How a child is born (natural delivery or caesarean section) and how that child is fed (breast feeding or bottle feeding) strongly influences the development of gut microbiota (Penders et al. 2006). Hygiene levels and medication are also important in determining the structure of the gut microbiota of infants. The gut microbiota is usually considered fully developed by the age of 4. Each person possesses a unique microbiota and it is stable over time in healthy adults(Vanhoutte et al. 2004, Vrieze et al. 2010). Pioneer bacteria involved in the initial colonization in newborn babies are important in determining the final composition of the microbiota in adults (Guarner and Malagelada 2003).

Metagenomic studies have brought to light the enormous richness and diversity of human gut microbiota compositions. Microorganisms colonize different parts of the gastrointestinal tract (GIT) and bacterial population density varies along the GIT (Guarner and Malagelada 2003, Tappenden and Deutsch 2007, Romano-Keeler et al. 2014). There is a qualitative and quantitative increase in complexity in the bacterial population from the stomach to the colon (Table 4.1). In addition, there is variation in the composition of the flora along the GIT in terms of surface adherent and luminal bacteria. Although the ratio of anaerobes to aerobes is lower at the mucosal surface than in the lumen, the anaerobes outnumber aerobes and facultative anaerobes by two to three orders of magnitude in the overall count (Sekirov et al. 2010).

4.2.1 Diversity of Microbes in Gut Microbiota

The GIT is one of the most complex ecosystems on earth; organisms from all the kingdoms of life such as bacteria, archaea (e.g., methanogens), and eukarea (fungi, helminths, and protozoa) as well as viruses are present in gut microbiota (Norman et al. 2014). Development of culture-independent methods such as 16S ribosomal RNA survey and direct sequencing vastly advanced our knowledge of gut microbiota. Such studies have shown that bacteria living in the human gut achieve the highest cell densities recorded for any ecosystem which is a complex community of the diverse array of bacterial species. Culture-independent metagenomic studies have also revealed that cultivable fecal bacteria represent a fraction of the total bacteria present in the GIT, with the proportion of undescribed species varying from 30% to 90% (Blaut and Clavel 2007, Lagier et al. 2012). In healthy adults, 80% of the identified fecal microbiota belong to four dominant phyla: the Gram-negative *Bacteroidetes* and *Proteobacteria* and the Gram-positive *Actinobacteria* and

Table 4.1 The Number and Type of Bacteria Present in Different Anatomic Sites of the Human Intestine

Anatomic Site	*pH*	*Number of Bacteria*	*Type of Bacteria*
Stomach	2.0	$1–10^2$	*Lactobacillus* *Streptococcus* *Helicobacter* *Peptostreptococcus*
Duodenum		$10^1–10^3$	
Jejunum	↑ 4.0–5.0 ↓	$10^3–10^4$	↑ *Streptococcus* *Lactobacillus*
Ileum		$10^7–10^9$	*Bacteroides* *Clostridium* *Streptococcus* *Actinomycinaea*
Colon		$10^{11}–10^{12}$	*Bacteroides* *Clostridium* *Bifidobacterium* *Enterobacteriaceae* *Akkermansia* *Prevotella* *Ruminococcus*

Source: Adapted from Guarner, F. and J. R. Malagelada. 2003. *The Lancet* 360: 512–519; Sekirov, I. et al. 2010. *Physiological Reviews* 90: 859–904.

Note: The pH and functions of the different parts are also listed.

Firmicutes. These include at least 17 families, corresponding to no less than 1250 different species of bacteria (Schuijt et al. 2013). A striking feature of the gut microflora is that the majority of gut bacteria (~65%) are Gram-positive bacteria, virtually all of which are obligate anaerobes; Gram-negative anaerobes account for another 20%–30% of the total gut bacterial population (Bäckhed et al. 2005). In a large-scale culture-independent study Frank et al. (2007) put a much higher number to the bacterial genus and species present in the human gut microbiome. According to this study the human gut microbiome consists of at least 1800 genera and 15,000–36,000 species of bacteria demonstrating that a staggering level of microbial diversity remains to be characterized within the human microbiome.

The firmicutes are with a low G + C content, bacteroidetes and actinobacteria are with a high G + C content (de Vos and de Vos 2012, Schuijt et al. 2013). Gut microbiota exists in a relatively stable condition within the host and takes part in wide ranging metabolic processes (Tremaroli and Bäckhed 2012) but there is substantial variation in the species composition between individuals (Diamant et al. 2011, Flint 2012). Microbiota of each individual has a conserved fraction (core microbiota) which is shared between individuals and which may be needed for correct functioning of the gut and a variable fraction (variable microbiota) (Booijink et al. 2010, Tremaroli and Bäckhed 2012). A core microbiota that comprises 50–100 bacterial species when the frequency of abundance at the phylotype level is not considered, and a core microbiome harboring more than 6000 functional gene groups is present in the majority of human guts surveyed (Zhu et al. 2010).

4.3 Diet Influences Gut Microbiota

The important factors which influence human health are genetics, environment, and diet. Food has a role beyond serving as nutrients. Dietary impacts on health are one of the oldest concepts in medicine (Goldsmith and Sartor 2014). The importance of food in health was acknowledged more than 2500 years ago by Hippocrates by his sayings "death sits in the bowels," "bad digestion is the root of all evil" and his "food as medicine" philosophy (Hawrelak and Myers 2004). The influence of the gut microbiome and its interaction with the host is pivotal to understand nutrition and metabolism (Sekirov et al. 2010, Chen et al. 2014). Diet can influence the composition of gut microbiota and gut microbiota has wide ranging health effects; both positive and negative (Laparra and Sanz 2010, Tremaroli and Bäckhed 2012, Scott et al. 2013). Several lines of evidence suggest that dietary factors might profoundly influence the structure and function of gut microbiota, rapidly and reproducibly (David et al. 2014). These include studies using the mouse model, human clinical studies, epidemiological studies, and metagenomic investigations in humans (Bäckhed et al. 2005, McFall-Ngai et al. 2013). Diet is one of the major determinants for the persistence of a particular bacterium in the gut because the diet provides nutrients not only for the host but also for the bacteria residing there (Blaut and Clavel 2007).

Dietary composition and caloric intake appear to swiftly regulate intestinal microbial composition and function. The relative proportion of the three main macronutrients (carbohydrates, proteins, and fats) influence gut transit time and pH, in addition to the composition of gut microbiota (Scott et al. 2013, Shen et al. 2014). The diet-induced alteration of gut microbiota may change the relative proportion of protective/beneficial bacteria making the host susceptible to disease and/or reducing its efficiency of food utilization (Walker et al. 2011).

Compelling evidence to support the notion that diet modulates the structure and function of gut microbiota came from the studies with resistin-like molecule knockout mice (which are resistant to diet-induced obesity). A high-fat diet resulted in a decrease in *Bacteroidetes* and an increase in *Firmicutes* and *Proteobacteria* in control mice (which became obese) and also in resistin-like molecule knockout mice (which did not become obese) (Hildebrandt et al. 2009). Also transplantation of microbiota from lean or obese humans to germ-free mice established the corresponding phenotypes. The structure of microbiota also shifted accordingly following high-fat or low-fat diets in these microbiologically humanized mice (Turnbaugh et al. 2008) clearly demonstrating that diet influences gut microbiota in a profound way and adiposity is transferrable by fecal transplantation which responds to dietary changes (Petrof and Khoruts, 2014).

Analyses of large metagenome datasets have indicated that the microbial composition of individuals can be described within a few distinctive enterotypes (classification of the human

gut microbiome according to the dominant microorganism present) which are independent of age, gender, and nationality and respond differently to diet and drugs (Arumugam et al. 2011). Enterotype *Bacteroides* (type-1) is associated with the consumption of a diet rich in protein and animal fat, while those who ate more fiber and carbohydrates and less animal fat and protein had *Prevotella* enterotype (type-2). Enterotype *Ruminococcus* (type-3) is not so distinct and is partly merged with the Bacteroides enterotype (Wu et al. 2011, Tremaroli and Bäckhed 2012). The enterotypes are stable and a long-term change in dietary habits are probably needed to induce a shift of one enterotype to another, as a 10-day dietary intervention failed to result in the alteration of the enterotype (Wu et al. 2011).

An interesting study by Filippo et al. (2010) showed how diet can impact the shaping of gut microbiota by comparing European children on a Western diet with rural African children who had a fiber-rich diet. Children from a rural African village in Burkina Faso showed a significant enrichment in *Bacteroidetes* with abundance of bacteria from the genus *Prevotella* and *Xylanibacter* and a depletion of *Firmicutes*. *Prevotella* and *Xylanibacter* encode enzymes enabling hydrolysis of cellulose and xylan. These African children indeed demonstrated a higher content of short-chain fatty acids (SCFAs) revealing that the gut microbiota in rural Africa may allow individuals to maximize energy intake from fibers while affording protection from inflammation and infection. In addition, these children showed depletion of *Firmicutes* which are usually abundant in people on a high-protein, high-fat diet (Filippo et al. 2010).

Studies with adult human volunteers have shown that changing the amount and type of carbohydrate consumed over periods of 4 weeks induced a significant change in the composition of the gut microbiota and its metabolic products (Brinkworth et al. 2009, Walker et al. 2011). Difference in gastrointestinal flora between breast-fed and formula milk-fed babies is another example of diet-induced changes in gut microbiota. Several studies have shown that although Bifidobacteria are the most prevalent bacteria in the GIT flora of both feeding groups, the amount is significantly higher in breast-fed than in formula-fed infants (Harmsen et al. 2000). Babies fed breast milk vs. formula milk display very large difference in inflammation and susceptibility to disease. Formula milk also causes a dramatic shift in gut flora from a simple flora dominated by *Bifidobacteria* to a complex adult gut flora; the number of *Escherichia coli* and Bacteroides was significantly higher in formula milk-fed than in breast-fed infants (Vandenplas et al. 2011).

Diet also influences the composition of gut microbiota in the elderly. Bacteroidetes are the dominant member of the gut microbiota and the relative abundance of various groups within the Firmicutes phylotype in the elderly differs from that of young adults (Claesson et al. 2012). Overall, the bacterial diversity of gut microbiota tends to decrease with age (Claesson et al. 2011). Diet not only influences the structure of gut microbiota, it may also induce change in the expression of bacterial cell surface constituents. Gut bacteria *Bacteroides thetaiotaomicron* exhibit changes in the capsular polysaccharide depending on the availability of nutrients (Bäckhed et al. 2005).

4.4 Influence of Gut Microbiota on Metabolism of Diet

A diverse population of bacterial species in the human gut performs important metabolic and immune functions that eventually delineate the nutritional and health status of the host (Selma et al. 2009, Martin et al. 2014). The association of gut microbiota with the host is based on molecular interactions that predominantly affect nutrition, immunity, and metabolism. These complex, site-specific, microbial communities contribute in vitamin synthesis, energy uptake, and the development of immunity in the host (Bik 2009). External environmental factors such as diet,

pathogens, or antibiotic treatment and genetic predepositions may disturb the microbial ecosystem leading to "dysbiosis" and impaired activity that can render a negative health effect (Hawrelak and Myers 2004).

The impact of gut microbiota on the nutritional and health status of the host is determined by the modulation of immune and metabolic functions. Enzymatic activities involving transformation of various dietary compounds is also attributed to the microbiome (Laparra and Sanz 2010). Intestinal microbiota is responsible for the fermentation of nondigestible dietary residue in addition to endogenous mucus of the epithelia (Roberfroid et al. 1995).

Several reports elucidate the role of certain specific bacterial species such as Bacteroidetes in the degradation of carbohydrates on account of their ability to possess a large numbers of genes encoding carbohydrate active enzymes and their ability to shift to the specific energy sources available in the gut. The genomic sequencing of gut symbiont *Bacteroides thetaiotaomicron* has shown to encode for 400 enzymes including transport, binding, and digestion of complex carbohydrates (Xu et al. 2003). Similarly, Firmicutes, Actinobacteria, and Verrucomicrobium phyla constitute a group of bacteria that would initiate the degradation of complex substrates including plant cell walls, starch particles, and mucin in the gut. Bacteroidetes and actinobacteria (Bacteroides enterotype) have been shown to associate with saturated fat and animal protein whereas carbohydrates and simple sugars (glucose and fructose) are linked with *Firmicutes* and *Proteobacteria* (Prevotella enterotype). Microbial diversity in the gut is greatly influenced by the type of nutrient as fat and protein are reported to decrease and carbohydrates increase the diversity of human gut microbiome (Delzenne et al. 2011a,b). The emerging concept of prebiotics and their impact on human health requires explorative studies to highlight the complex relationship between diet composition, gut microbiota, and metabolic outputs (Wu et al. 2011, Flint 2012).

Host metabolism is linked with the products of microbial metabolism through signaling mechanisms thereby directly affecting intestinal function, the liver, the brain, and adipose and muscle tissues leading to a rise in the level of obesity and the associated morbidities. Transformation of indigestible food components into molecules by gut microbiota, and providing energy to the host has been linked with obesity pathogenesis due to an excess energy intake (Bäckhed et al. 2007, Tremaroli and Bäckhed 2012).

Dietary bioactive compounds such as prebiotics, polyunsaturated fatty acids (PUFAs), and phytochemicals influence the composition of the gut microbiota and their ability to generate fermentation products. PUFAs including ω-3 and ω-6 fatty acids are associated with various aspects of immunity and metabolism (Laparra and Sanz 2010). The interactions between PUFAs and components of gut microbiota greatly alter their role in metabolism. A growing body of evidence shows that dietary factors can dramatically alter the gut microbiome in ways that contribute to metabolic disturbance and progression of obesity (Cani et al. 2009b). Dietary fat composition can both reshape the gut microbiota and alter host adipose tissue inflammatory/lipogenic profiles. In addition, there exists the interdependency of dietary fat source, commensal gut microbiota, and the inflammatory profile of mesenteric fat that can collectively affect the host metabolic state (Huang et al. 2013).

In a study published recently, a team of researchers found that in mice, just one of species of bacteria, *Akkermansia muciniphila* plays a major part in controlling obesity and metabolic disorders such as type 2 diabetes (Everard et al. 2013). It is the dominant bacterial species in the human gut, representing 3%–5% of the microbial community (Belzer and de Vos 2012). The abundance of this bacterium inversely correlates with body weight in rodents and humans. Interestingly, prebiotic feeding normalized *A. muciniphila* abundance, which correlated with an improved metabolic profile, reversed high-fat diet-induced metabolic disorders including fat-mass gain, metabolic

endotoxemia, adipose tissue inflammation, and insulin resistance (Karlsson et al. 2012). In addition, prebiotic feeding of *A. muciniphila* increased the intestinal levels of endocannabinoids that control inflammation, the gut barrier, and gut peptide secretion (Everard et al. 2013).

4.5 Gut Microbial Activity in Health and Diseases

The enormous gene pool of the microbiome provides various enzymes of diverse metabolic pathways which communicate with and affect the host in numerous ways, both beneficial and harmful, acting locally and systemically (Tables 4.2 and 4.3). The adaptable and renewable activity of the microbiome is normally a health asset but certain members of the microbiota can become a liability in genetically susceptible and immunocompromised individuals (Lepage et al. 2012, McDermott and Huffnagle 2014).

4.5.1 Gut Microbial Activity Beneficial to Human

The primary role of the gut bacteria beneficial to the host is the fermentation of non-digestible carbohydrates such as cellulose, hemicellulose, pectins, gum, and resistant starches resulting in generation of SCFAs. In addition to serving as an energy source, SCFAs stimulate differentiation and proliferation of epithelial cells (Slavin 2013, Sharma and Devi 2014). Interaction between the gut bacteria and the host is important in the differentiation and proliferation of IEC, a competent immune system and moderate inflammatory responses (Yu et al. 2012). Also, gut bacteria stimulate the intestinal endocrine cells to stimulate hormones, which in turn enter circulation and can modulate the host function (Sekirov et al. 2010). In addition, gut bacteria communicate directly with immune cells of the gut-associated lymphoid tissue (GALT) and terminal of visceral afferent nerves (Diaz-Heijtz et al. 2011). Gut microbiota also play nonimmune, protective roles by directly blocking intestinal pathogenic microbes to IEC and by enhancing mucosal integrity via epithelial cell stimulation. So the gut microbiota may influence many metabolic processes of the host. It is important that the state of homeostasis is maintained between human hosts and their gut microbiota as it dictates the health and disease states of the host depending upon the relative proportion of beneficial bacteria and harmful bacteria (Kaminogawa 2010). In Table 4.2 the major beneficial effects of gut bacteria are listed. For many of the health benefits offered by the gut bacteria, there are multiple mechanisms involved; only the most common mechanism is mentioned here due to limitations of space.

4.5.2 Dysbiosis Leads to Disease States

Human gut microbiota exists in a state of homeostasis with the host for the benefit of the host and the microbes under normal circumstances. The gut microbiota is a complex ecosystem whose diversity is enormous, and under specific conditions when there is a disruption in the state of homeostasis, it is able to overcome protective host responses and exert pathologic effects (Wallace et al. 2011); the intestinal microbiome is linked to a growing number of over 25 diseases and syndromes (de Vos and de Vos 2012). The intestinal mucosal immune system has developed specialized mechanisms for eliminating the pathogenic microbes while at the same time tolerating the beneficial gut microbiota (Schuijt et al. 2013). Altogether, in indirect or associative support in maintaining the state of host–microbe homeostasis, several mechanisms of mucosal immunity are involved. These include strongly developed innate defense mechanisms ensuring appropriate

Table 4.2 Health Benefits of Gut Microbiota

Health Benefit	*Mechanism/Main Finding*	*References*
Energy harvest	Harvest calorie from undigested ingredients of food.	Jumpertz et al. (2011)
Synthesis of B vitamins and vitamin K	Lactic acid bacteria and *Bifidobacteria* can de novo synthesize biotin, thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), cobalamine (B_{12}), folic acid, and vitamin K.	LeBlanc et al. (2013)
Absorption of minerals	SCFAs produced by gut bacteria enhance absorption of calcium, copper, iron, magnesium, and manganese.	Scholz-Ahrens et al. (2007)
Development of immune System	Gut immune maturation depends on colonization with a host-specific microbiota. Pathogens-associated molecular patterns (PAMP) of gut bacterial interact with TLRs and other pattern recognition receptors (PRP) of host cells to modulate development and stimulation both innate and adaptive arms of host immune system.	Chung et al. (2012), Ganal et al. (2012)
Neuronal networking	Interaction between the intestinal microbiota, the gut, and the central nervous system (CNS) is recognized as the microbiome–gut–brain axis. Dysbiosis leading dysregulation of microbiome–gut–brain axis leads to a variety of disease conditions.	Collins et al. (2012), De Vadder et al. (2014)
Angiogenesis	Gut microbiota-derived ligands induce proliferation, migration, tube formation and production of proangiogenic factors, from human intestinal microvascular endothelial cells (HIMECs); vessel sprouting and angiogenesis observed in the *ex vivo* and *in vivo* assays.	Schirbel et al. (2013)
Prevention of allergy	Alteration of gut microbiota resulted in elevated serum IgE concentrations, increased steady-state circulating basophil populations and exaggerated basophil-mediated Th2 cell responses and allergic inflammation. Altered microbiota leads to the induction of immune deviation in infancy. High counts of *Bacteroides* prevented clinical manifestation of atopy.	Hill et al. (2012)
Metabolism of xenobiotics	Gut microbiota modulates hepatic gene expression and function by altering its xenobiotic response to drugs without direct contact with the liver.	Björkholm et al. (2009)
Protection against infection	By outcompeting pathogen for attachment sites, by producing bacteriocins, and by limiting nutrients availability for the pathogens.	Guarner and Malagelada (2003), Canny and McCormick (2008)

Table 4.3 Dysiosis Leads to Disease States

Disease	*Mechanism/Main Findings*	*References*
Atherosclerosis	Metabolism by intestinal microbiota of dietary L-carnitine, a trimethylamine abundant in red meat produces trimethylamine N-oxide (TMAO) and accelerates atherosclerosis in murine model.	Koeth et al. (2013)
Type-2 diabetes	Metagenomic studies have shown that patients with type-2 diabetes gut microbial dysbiosis, show decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens.	Qin et al. (2012)
Metabolic endotoxemia	High fat diet-induced changes in gut microbiota leads to increased transit of LPS to systemic circulation which contribute to the development of metabolic endotoxemia and ultimately clinical signs of chronic diseases.	Chang and Li (2011)
Eczema	A diverse and adult-type microbiota in early childhood is associated with eczema and it may contribute to the perpetuation of eczema.	Nylund et al. (2013)
Irritable bowel syndrome	Overgrowth of aerobic bacteria in the small intestine using nitrate generated as a by-product of the inflammatory response; reduced number of *Bifidobacteria* in the gut leading to altered floral–mucosal interactions. The enteric nervous system, and brain–gut–brain axis are directly involved in the pathogenesis.	Kinross et al. (2008)
Altered gut permeability	Altered gut microbiota-induced glucagon like peptide-2 (GLP-2)-driven alteration of gut permeability.	Cani et al. (2009a)
Obesity	An obesity-associated gut microbiome with increased capacity for energy harvest.	Diamant (2011) Everard et al. (2013)
Allergy	Reduced biodiversity and altered gut flora composition early in life fails to confer maximum tolerogenic immunomodulatory effects.	Sjögren et al. (2009), Özdemir (2013) Trompette et al. (2014)
CRC	*Clostridium* and *Bacteroides* enhance the potency of DNA-damaging agents, for example, N-nitorso compounds and heterocyclic amines and increase the growth rate of colonic tumor; *Lactobacillus* and *Bifidobacteria* reduce tumorigenesis	Uccello et al. (2012)
Cancer (other types)	Microbiota changes observed. Microbial-induced inflammation contribute to cancer by stimulating production of cytokines that promote cell proliferation and/or inhibit apoptosis.	Bultman (2014)

Table 4.3 (*Continued*) Dysiosis Leads to Disease States

Disease	*Mechanism/Main Findings*	*References*
Autism	Abnormal energy metabolism by altered gut bacteria results in production of acyl-carnitine and altered production of propionic acid which results in alteration in mitochondrial function leading to neurodevelopmental disorder, autism.	Frye et al. (2013)
Asthma	Change in gut flora early in life leading to inappropriate immune tolerance contributes toward the development of asthma.	McLoughlin and Mills (2011)

function of the mucosal barrier, existence of unique types of lymphocytes and secretory immunoglobulin A (sIgA). Studies with germ-free animals have demonstrated that the gut microbiota plays a vital role in the development of an optimally functioning mucosal immune system (Mazmanian et al. 2005, Kostic et al. 2013).

Human gut microbiota converts L-carnitine into trimethylamine which can promote cardiovascular risk in humans (Zhu et al. 2014). Inadequate or excess stimulation of the immune system is a challenge to host microbe homeostasis and subsequent disease states. A number of cellular components [e.g., lipopolysaccharides (LPS), peptidoglycans, teichoic acids, flagella, superantigens, bacterial DNA, heat-shock protein] and the metabolic products of the gut microbiota are able to stimulate both the innate and adaptive components of the host immunity (Holmes et al. 2011, 2012). Chronic immune activation in response to signals from gut microbiota could pose the risk of chronic, low-grade inflammation, which studies have shown as a predisposing factor for a variety of multifactorial, multigenic complex diseases including obesity, diabetes, inflammatory bowel diseases, rheumatoid arthritis, cardiovascular disease, allergy, asthma, autism, colon cancer, and several infections, inflammatory, autoimmune, and neoplastic disease (Chang and Li 2011, Hormannsperger et al. 2012; Table 4.3). Numerous studies have shown that the modulation of structure and function of gut microbiota by probiotics, prebiotics, synbiotics, cobiotics, and immunobiotics offer a realistic therapeutic and preventive option for these diseases.

A recent study showed that gut bacteria enhances infectivity of certain viruses. The virus becomes covered with LPS molecules from natural gut bacteria, then virus–LPS conjugate interact with TLR4 (receptor of LPS molecules on mammalian cells) to make viral infection possible. Mouse mammary tumor virus (MMTV), poliovirus, and a reovirus which impairs bile duct function all have been shown to use the same LPS-dependent strategy. LPS–TLR4 interaction leads to stimulation of IL-10 production, which suppresses the body's antiviral reaction, further enhancing viral infection. The bacterial cell wall component peptidoglycan also promoted viral infectivity (Wilks and Golovkina, 2012).

4.6 Human Colon as a Fermenter

The main theme of the development of various functional foods such probiotics, prebiotis, cobiotics, synbiotics, and immunobiotics is the improvement of the colonic fermentation process which has been shown to exert profound health benefits on the human host (Blaut and Clavel 2007, Villena and Kitazawa 2014). The human colon is a highly active metabolic organ which acts a

fermenter where a variety of undigested food ingredients (mostly complex carbohydrates) are fermented for the benefit of both the microbes and the host (Figure 4.1; Valeur and Berstad 2010). With approximately 1.5 kg of bacteria in the colon (Xu and Gordon 2003), and metabolic activity comparable to the liver (Martin et al. 2009, DiBaise et al. 2012), fermentation in the gut thus plays an important role in the digestive physiology and energy metabolism of the host.

Because of the diversity and metabolic potential of the gut microbiota, gut fermentation is a complicated process resulting in a dynamic gut metabolom (total metabolites) where the end product produced by one organism serves as a growth substrate for the other (Vlieg et al. 2011).

The degradation of complex carbohydrates and plant polysaccharides as a source of energy for human and microbial cells is not completely accomplished by human enzymes and these nondigestible carbohydrates including xylans, resistant starch, cellulose, hemicellulose, pectins, gums, and inulin in addition to certain oligosaccharides that escape digestion are fermented by colonic microbiota resulting in the yield of energy and SCFA (acetate, propionate, and butyrate) (Martens et al. 2011, Pokusaeva et al. 2011). A considerable number of these polymers cannot be degraded by the host; however herbivores possess the ability to meet 70% of their energy requirement from microbial breakdown under the concept of mutualism. The gut microbiome is highly enriched with genes for carbohydrate metabolism encoding a large assortment of enzymes for carbohydrate metabolism (>115 families of glycosidic hydrolases and >21 families of polysaccharide lyase); in contrast the human genome has relatively few genes that encode carbohydrate-metabolizing

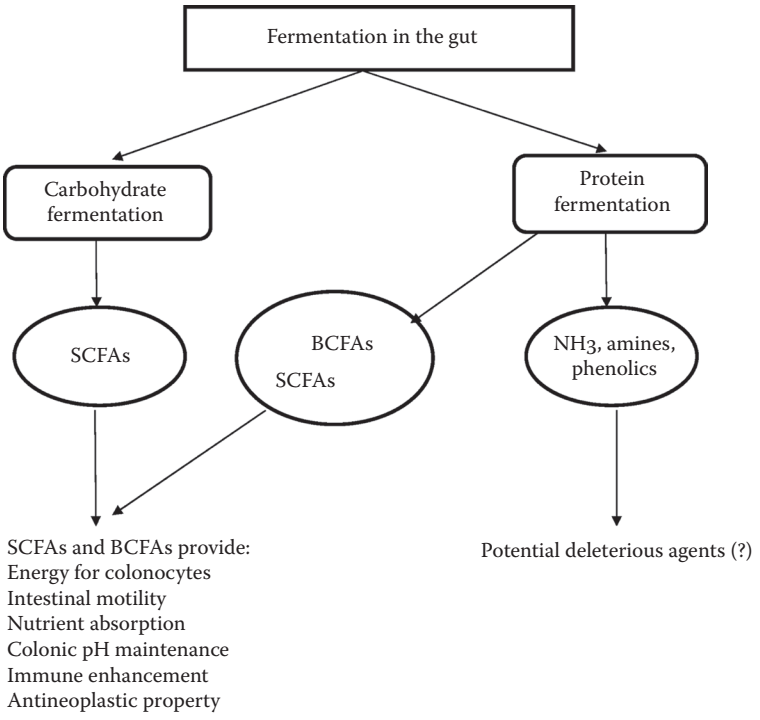

Figure 4.1 Fermentation of carbohydrates and proteins in the gut producing SCFAs, BCFAs, and other products. (From Cummings, J. H. et al. 1987. *Gut* 28: 1221–1227; Macfarlane, G. T. and S. Macfarlane. 2011. *Journal of Clinical Gastroenterology* 45: S120–S127.)

enzymes (Gill et al. 2006, Ley et al. 2006, Flint et al. 2008). During the fermentation process in the colon, the gut microbiota breaks down substances such as resistant starch and dietary fibers which are not completely hydrolyzed in the small intestine by the host enzymes resulting in production of a variety of postbiotics such as SCFAs and gases (e.g., hydrogen, methane, and carbon dioxide) (Topping and Clifton 2001).

A variety of factors influence the fermentation process; the most important factor is the type and amount of complex carbohydrates which pass through the small intestine undigested and reach the colon and its resident microbiota (Tremaroli and Bäckhed 2012, Besten et al. 2013). In addition, the type and amount of various SCFAs produced by fermentation is also influenced by pH, availability of inorganic terminal electron acceptors (e.g., sulfate and nitrate), and large intestine transit time (Macfarlane and Macfarlane 2011).

The microbial population colonizing different parts of the gut influences human health in several ways. Under normal physiological conditions, these microbial communities contribute nutrients and energy to the host through the fermentative process of nondigestible dietary components in the large intestine. Carbohydrates and proteins serve as the main fermentative substrates in the large intestine resulting in the production of a number of products including gases, hydrogen, carbon dioxide, SCFAs, branched chain fatty acids (BCFAs), lactic acid, ethanol, ammonia, amines, phenols, and indoles (Figure 4.1). The human microbiome is also responsible for the maintenance of a balance with the host's metabolism and immune system (MacFarlane and Cummings 1991, Flint et al. 2007, Flint 2012).

4.6.1 Short-Chain Fatty Acids

SCFAs are saturated organic fatty acids with 1–6 carbon atoms and are the principal anions which arise from bacterial fermentation of polysaccharide, oligosaccharide, proteins, peptide, and glycoprotein in the colon (Velázquez et al. 1997, Macfarlane and Macfarlane 2011). The main postbiotic fermentation products of resistant starch and dietary fibers are SCFAs; which includes acetate (C2), propionate (C3), and butyrate (C4)(Figure 4.2).

Various studies demonstrated that SCFA production is in order of acetate > propionate > butyrate in a molar ratio of approximately 3:1:1 mainly in the proximal and transverse colon (Cummings et al. 1987, Cummings and Myers 1991). SCFAs are produced in the range of 150–600 mmol/day (average of 400 mmol/day); more than 95% are immediately absorbed by the colon and the rest excreted in the feces. The SCFAs thus absorbed provide approximately 500 kcals of energy a day; which amounts to 5%–15% of the total caloric needs of an individual on a Western diet (Martin et al. 2014). Absorption of SCFAs by the colonic mucosa is an energy-independent process and the rate of absorption of different SCFAs are same, irrespective of their chain length. In addition to SCFAs, small amounts of isobutyrate, valerate, and isovalerate are also produced (Beyer-Sehlmeyer et al. 2003). The absorbed SCFAs are used as an energy source by the colonocytes (butyrate being the preferred substrate) and also by the other tissues including

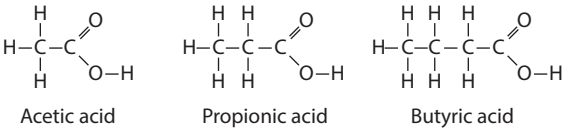

Figure 4.2 Short-chain fatty acids.

the liver and the muscles. In addition to serving as energy, SCFAs also have other important functions (Macfarlane and Macfarlane 2011).

4.6.2 SCFAs and Their Physiologic Effects

By facilitating the uptake of electrolytes and water, SCFAs reduce the osmotic effect of unabsorbed carbohydrate molecules and thus act as an antidiarrheal agent; an individual may suffer from diarrhea (antibiotic associated diarrhea) when the gut microbiota is disrupted resulting in impaired colonic fermentation (Binder 2010). Microbial fermentation products influence gastrointestinal motility and sensitivity and may play a role in the pathogenesis of irritable bowel syndrome. Research data indicate that inadequate β-oxidation of SCFAs as a pathogenic mechanism for ulcerative colitis (Bergman 1990, Cummings et al. 1996, Flint et al. 2007). Brighenti et al. (1995) demonstrated a significant role of acetate and propionate as modulators of glucose metabolism thus resulting in lower glycemic responses to oral glucose owing to the absorption of these SCFAs. Proliferation and differentiation of the IEC is modulated by SCFAs and thus contribute toward the creation of a protective barrier against pathogens (Hooper et al. 2012). SCFAs decrease colonic pH and increase colonic water absorption and thus maintain colonic health. Other health-promoting properties of SCFAs include laxation and vasodilation; SCFAs also play roles in gut motility and gut wound healing (Flint et al. 2007, Tan et al. 2014). SCFAs are incorporated as basic elements in a variety of biosynthetic processes such as lipogenesis, gluconeogenesis, and protein synthesis. SCFAs activate G protein-coupled receptors, GPR41 and GPR43 (Kim et al. 2013) leading to the activation of several intracellular pathways including transcriptional factors such as activating transcriptional factor-2 (ATF-2) and signal transduction mediators protein kinase C and mitogen-activated protein kinases (MAPKs) (Elamin et al. 2013, Kim et al. 2013).

An important method of regulation of gene expression is through acetylation (carried out by the enzyme histone acetyl transferase, HAT) and deacetylation (carried out by the enzyme histone deacetylase, HDAC) of the DNA-binding protein, histone. Acetylation leads to enhanced gene expression, while deacetylation reverses the process. By exerting a negative effect on HDAC activity (Davie 2003), SCFAs are reported to modulate the expression of a number of key regulatory proteins such as NFAT, NFκB, p53, and MyoD (Vinolo et al. 2011). Recent *in vivo* and *in vitro* studies suggest that SCFAs stimulate gut hormone secretion. Colonic enteroendocrine L cells express receptors for SCFAs (free fatty acid receptor, FFA2 and FFA3). The release of insulinotropic hormone, glucagon-like peptide-1 (GLP-1) and an anorectic hormone, peptide YY by colonic enteroendocrine L cells is mediated by SCFAs (Vinolo et al. 2011, Kaji et al. 2014). In an interesting study, Wichmann et al. (2013) showed that SCFAs, by modulating the secretion of the hormone GLP-1, increase intestinal transit time of food in case of energy insufficiency and thus allow for greater energy harvest and absorption in germ-free and antibiotics-treated mouse models.

Butyrate is produced in minimal amount in comparison to acetate and propionate, but is the most studied and is reported to have a more important role in colonic homeostasis and also in wide array of metabolic processes (Nordgaad 1998). Butyrate serves as the primary energy source for the colonocyte, supplying 70%–90% of its energy requirements. It promotes cell repair, proliferation, and differentiation (Canani et al. 2011). By stimulating the healthy growth of colonic cells, butyrate helps prevent colon cancer. Additional properties of butyrate which contribute to preventing colon cancer includes reduction of DNA damage, reduction of the exposure of the colonic mucosa to ammonia, inhibition of the conversion of primary to secondary bile acids (Kaji et al. 2014). Butyrate enhances villi development and promotes the gut barrier function and reduces epithelial permeability which is achieved by upregulation of mucin-associated genes (MUC1-4) in the intestinal

goblet cells. Butyrate also upregulates the expression of tight junction proteins such as zonulin and occludin, enhancing intestinal barrier function (Bordin et al. 2004, Gaudier et al. 2004).

Butyrate exerts a differential effect on healthy colonocytes and tumor cell lines. It activates the expression of genes involved in cell proliferation and differentiation in healthy colonocytes; whereas it activates genes which leads to apoptosis in tumor cells (Canani et al. 2011). Butyrate also possesses antimicrobial properties. It is reported to prevent colonization by *Salmonella enteritidis* in experimental animals. Mechanistic studies revealed that it upregulated host defense protein genes and also downregulated invasion genes in *Salmonella*, thereby reducing the ability of the bacteria to attach and invade host cells of the intestinal epithelium (Immerseel et al. 2006, Sunkara et al. 2012).

4.6.3 SCFAs as Modulators of the Immune System

Research in the field of immunology and gut microbiology has shown that SCFAs exert wide ranging effects on immune and inflammatory responses. SCFAs act as chemotactic factors for neutrophils and thus enhance the recruitment of circulating leukocytes to the inflammatory site. SCFAs modulate the production of these inflammatory mediators by neutrophils and other immune cells. Studies have shown that propionate and butyrate reduce the LPS-stimulated production of cytokine-induced neutrophil chemoattractant-2 (CINC-2), tumor necrosis factor (TNF)-α, and nitric oxide (NO) production by neutrophils (Maslowski and Mackay 2011, Vinolo et al. 2011), which contribute toward controlling the inflammatory process involved in the pathogenesis of various diseases including Crohn's disease (Segain et al. 2000).

As IEC are in direct contact with high concentrations of SCFAs, the effects of these fatty acids on these cells have been a topic of intense investigation. By changing the type or the amount of chemokines produced by intestinal cells, SCFAs may alter the recruitment of leukocytes and the pattern of inflammatory mediators produced in this tissue (Vinolo et al. 2011, Zeng et al. 2014). SCFAs are also involved in T cell differentiation. This is achieved by stimulation of production of prostaglandin E2 (PGE2), which by activating its receptor EP4 facilitates Th1 differentiation and Th17 expansion, two subsets of T helper involved in a variety of immune processes including inflammation (Smith et al. 2013).

Studies have identified various structural components and secreted products of gut microorganisms with direct roles in modulating the immune system. Flagellin from the probiotic strain *E. coli* Nissle is reported to induce β-defensin, an antimicrobial peptide implicated in the resistance of epithelial surfaces to microbial colonization (Schlee et al. 2007). A soluble protein p40, derived from probiotic bacteria *Lactobacillus rhamnosus* GG prevented cytokine-induced apoptosis in IEC through the activation of epidermal growth factor receptor (EGFR). Delivery of p40 to the colon prevented and treated colon epithelial cell injury and inflammation and ameliorated colitis in an EGFR-dependent manner (Yan et al. 2011). Butyrate exerts an anti-inflammatory effect by inhibition of NF-kB activation. Studies have shown that it downregulates production of TNF-α in human peripheral monocytes and in macrophage-like synoviocytes in rheumatoid arthritis patients by regulating mRNA degradation (Fukae et al. 2005).

4.6.4 Fermentation of Fat

The interaction of gut microbiota with dietary fat is more complex. Lipid metabolism by gastrointestinal microbes generates multiple fatty acid species that can affect host health. Metabolism of linoleic acid has been associated with several human colonic *Roseburia* species that form either vaccenic acid or a hydroxy-18:1 fatty acid. They may also act as precursors of conjugated linoleic

acid (CLA) *cis*-9, *trans*-11-18:2-, the health-promoting compound (Devillard et al. 2007). In a study with germ-free mice it has been found that representative gut bacteria *Lactobacillus plantarum* carry out the metabolism of PUFAs and generates oxo, hydroxyl, and conjugated fatty acids and partially saturated *trans*-fatty acids. These fatty acid intermediates, especially hydroxyl fatty acids, were detected in host organs. The evidence for the fact that the source of these hydroxy fatty acids is the bacterial metabolism of fatty acids came from the observation that the levels of hydroxy fatty acids were much higher in specific pathogen-free mice than in germ-free mice. These findings suggest that lipid metabolism by gastrointestinal microorganisms affect host lipid composition, which in turn may provide new ways for host health improvement by altering lipid metabolism related to the onset of metabolic syndrome (Kishino et al. 2013).

4.6.5 Fermentation of Protein

The dietary proteins which escape digestion in the small intestine, as well as proteins from mucous, enzymes, sloughed epithelial cells, dead host, and bacterial cells are fermented in the colon. Fermentation of proteins leads to the production of SFAs, BCFAs, and amines. In addition, protein fermentation also generates ammonia, phenols, indoles, and sulfurs (Figure 4.1; Macfarlane and Cummings 1991). In contrast to carbohydrate fermentation which takes place in the proximal and transverse colon, protein fermentation mainly occurs in the distal colon, when carbohydrates get depleted. It is generally accepted that protein fermentation is considered detrimental to the host's health; for example, colorectal cancer (CRC) and ulcerative colitis appear most often in the distal colon, which is the primary site of protein fermentation. Some of the proteolytic fermentation products, however, are used by gut microbiota as nitrogenous growth factors. Mucin contains a substantial amount of nitrogen in the form of amino sugars. Fermentation of these sugars releases ammonia which is absorbed from the colon by mucosal cells and contributes to the host's nitrogen balance (Windey et al. 2012).

4.6.6 Bacteria Involved in Gut Fermentation

Bacteroides are the most numerous and most versatile polysaccharide fermenting bacteria of the gut microbiota; other gut fermenters belong to the genera *Bifidobacterium, Ruminococcus, Lactobacillus,* and *Clostridium* (Guarner and Malagelada 2003). In addition to saccharolytic and proteolytic bacteria, methanogens and other bacteria that utilize intermediate fermentation products such as hydrogen, ethanol, lactate, and succinate are also present in large number (Guarner and Malagelada 2003, Nakamura et al. 2010). Gram-positive Firmicutes are the human colonic butyrate producers comprising the two most abundant groups related to *Eubacteriumrectale/Roseburia* spp. and *Faecalibacterium prausnitzii.* Mechanisms proposed in non-gut *Clostridium* spp. whereby butyrate synthesis leads to the energy generation through substrate-level phosphorylation and proton gradients has also been found to be true in majority of gut bacterial species involved in butyrate production (Louis and Flint 2009).

4.7 Concept of Probiotics, Prebiotics, Synbiotics, Cobiotics, and Immunobiotics: Mechanism of Action and Health Claims

Advances in knowledge about gut microbiota through recent studies with functional metagenomics has opened the possibilities of applying this knowledge for rational remodeling for human

benefit (Holmes et al. 2012, Guzman et al. 2013). Owing to the inherent plasticity of gut microbiota, the various physiologic features that can be targeted are relative susceptibilities to infections, metabolic syndromes, bioavailability of nutrients, development of innate and adaptive immunity, immune tolerance, development and functioning of the nervous system, and the intestinal barrier function (Delzenne et al. 2011a,b, Martin et al. 2014). Various gut microbiota modifying agents such as probiotics, prebiotics, synbiotics , cobiotics, and immunobiotics can be used to achieve a measurable benefit to the host.

Antibiotics can be used to eliminate or suppress undesirable bacteria from the human host, probiotics can introduce missing or suppressed beneficial bacteria in the gut microbiota, prebiotics can enhance the proliferation of beneficial microbes, synbiotics can synergistically enhance the potency of both probiotics and prebiotics to maximize sustainable changes in the human microbiome (Roberfroid 1998, Lourens-Hattingh and Viljoen 2001, Gibson et al. 2004, Takahashi et al. 2013). Cobiotics, on the other hand, can be beneficial both to the host and to gut microbiota in a targeted health effect, and immunobiotics can promote health through modulation of mucosal immune mechanisms (Greenway et al. 2013, Tomosada et al. 2013). Through strategic use of these gut microbiota modifying processes, either singly or in combination, remodeling of the gut microbiome to suit individual therapeutic needs can be considered (Preidis and Versalovic 2009, Goldsmith and Sartor 2014).

4.7.1 Probiotics

The main theme of probiotic action is its capacity to remodel gut microbiota, which results in subsequent health benefits. Lilley and Stillwell (1965) used this term for the first time describing it as a microbial substance that stimulated the growth of other microorganisms, followed by Sperty (1971) who narrated probiotics as tissue extracts that promoted microbial growth. Parker (1974) used the expression, probiotic as animal supplements containing organisms and substances that would contribute to create a balance of the intestinal flora. Similarly, Fuller (1989) defined probiotics as food supplements with live microorganisms to promote host health by balancing the intestinal flora. Subsequently, Fooks et al. (1999) stated the word probiotic to comprise of two Greek words meaning "for life." Despite a number of other definitions of the term probiotic, the currently prevailing and the most widely accepted one is that "probiotics are live microorganisms, administrated in certain quantities that confer health benefits to the host" (FAO/WHO 2001). Numerous lactic acid bacterial strains are considered as probiotics; however, all of them do not meet the required standard because of their sensitivity to the critical level of acidity and bile salts in the human GIT (Hekmat and Reid 2006). Probiotics are consumed by humans either as a live dietary supplement or as live microflora in fermented foods. The most well-known probiotic-containing food product is *yogurt* (Lourens-Hattingh and Viljoen 2001). Other fermented foods that contain probiotics are some juices and soy drinks, buttermilk, fermented and unfermented milk, some soft cheeses, *sauerkraut*, *miso, tempeh, kefir,* kimchi, pickles, and *kombucha* tea (Collins and Gibson 1999, Anuradha and Rajeshwari 2005, Shah, 2007, Soccol et al. 2010).

Another significant aspect of probiotics is strain specificity that may create a challenge in research on probiotics or probiotic-containing food products to establish the effectiveness of one strain relative to the other (Canani et al. 2007). Species of *Lactobacillus* and *Bifidobacterium* have been considered the most popular for use in a majority of probiotic products available in the market (FAO/WHO 2001). Rational yoghurts, frozen yogurts, and desserts are the reservoirs of *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* in many parts of the world (Senok 2009). Although these species have been associated with improved lactose digestion and immune

enhancement, all of these do not meet the criteria for a probiotic microorganism on account of their sensitivity to the conditions in the GIT where they do not survive in very high numbers. Moreover, other genera, such as *Escherichia* and *Enterococcus* are now marketed as probiotics; however, their safety as probiotics remains a concern for consumers (Eaton and Gasson 2001, Ishibashi and Yamazaki 2001, Senok et al. 2005).

Some of the fundamental properties of probiotic strains that would benefit human health include resistance to acid and bile, attachment to the human gut epithelial cells, colonization in the human intestine, and production of antimicrobial substances (Parvez et al. 2006). Additionally, probiotics would present a characteristic of not being pathogenic, toxic, mutagenic, or carcinogenic in the host organism and should be generally regarded as GRAS (generally recognized as safe) (Mattia and Merker 2008). Moreover, they must be viable during processing and storage, and should offer resistance to the physicochemical processing of the food and must be able to survive the digestion process. They must also possess the ability to adhere and colonize the gut mucosa and promote immunostimulation without an inflammatory effect (Saarela et al. 2000, Prado et al. 2008).

Abundant literature is available to elucidate the multiple health benefits of ingesting probiotic containing foods. Numerous studies explicated the role of probiotics as antimicrobial and antimutagenic (Lourens-Hattingh and Viljoen 2001), anticarcinogenic (Marteau et al. 2001), and antihypertensive (Liong et al. 2009) in humans. Probiotic bacteria not only promote the endogenous host defense mechanisms and the nonimmunologic gut defense (Salminen et al. 1998), but also generate increased humoral immune responses on ingestion thereby promoting the intestine's immunologic barrier (Kaila et al. 1992, Marschan et al. 2008).

Probiotics have demonstrated protection against allergic sensitization and allergic diseases as several studies reported an attenuating effect in allergic symptoms after probiotic treatment (Dotterud et al. 2010) suggesting these syndromes to be mediated by the induction of regulatory mechanisms, such as generation, proliferation, and activity of tolerogenic dendritic cells (DCs) and T cells (Rautava et al. 2006, Lyons et al. 2010). Speculation prevails whether probiotic bacteria imparted such an effect directly or whether it is yielded through probiotic-mediated stabilization of the intestinal microbiota (Bernardo et al. 2012). Probiotics have also been reported to positively impact mineral metabolism, especially bone stability (Arunachalam 1999), attenuation of symptoms of bowel disease, and Crohn's syndrome (Marteau et al. 2004).

More complex and increased beneficial effects of probiotics in humans entail stimulating nonspecific host resistance to microbial pathogens (Perdigon et al. 1986, 1988) and modulating the host's immune responses to deleterious antigens leading to downregulation of hypersensitivity reactions. Enhanced recovery from infection, and antimicrobial functions have been reported as some *Lactobacillus* strains exhibited suppression of pathogenic microorganisms including *Sal. enteritidis*, *E. coli*, *Shigella sonnei*, and *Serratia marcescens* (Drago et al. 1997). Granato et al. (2010) reported a series of physiological benefits of probiotic strains that include regulation of the intestinal flow, control of diarrhea, reduced cholesterol levels, improved lactose tolerance, better absorption of micronutrients, improved immunological system, better urogenital health, prevention of cancer, reduced catabolic products of the kidney and liver, and prevention of arteriosclerosis. The authors further emphasized the potential health outcomes of probiotic ingestion reporting reduced rate of onset of osteoporosis, better development, and improved bioavailability of nutrients. Other clinical studies explained the role of probiotics in improving the mucosal barrier function, increasing allergen-specific IgA levels, and more importantly affecting a range of other immune-modulatory properties (Marschan et al. 2008, West et al. 2009).

Probiotics have been used to treat diseases of the GIT with impressive success. Consumption of yoghurt (a ready source of probiotics) eliminated symptoms of lactose intolerance and improved

digestion in people who cannot efficiently absorb lactose (Guarner and Malagelada 2003). A recent study on 3758 children aged 1–5 years revealed that daily intake of a probiotic strain, *Lactobacillus casei* strain Shirota played an important role in the prevention of acute diarrhea in young children in a community setting during a 24-week period (Sur et al. 2011).

Saccharomyces boulardii has been shown to be a highly effective probiotic agent used in preventing gastroenteritis caused by *Shigella flexneri* in a murine model (Zbar et al. 2013). *E. coli* strain Nissle 1917 (EcN) is a less commonly used probiotic. In a study, this strain was found to be safe and well-tolerated and significantly reduced stool frequency in infants and toddlers suffering from acute diarrhea (Henker et al. 2007).

Impressive data are also available on the use of probiotics to boost immune response. Studies with various formulations of probiotics, either alone or in combination, were carried out to determine their effects on immune parameters, infectious outcomes, and inflammatory conditions in humans. These studies revealed that mucosal IgA production (especially in children), phagocytosis, and natural killer cell activity can be enhanced by some probiotic bacteria (Lomax and Clader 2009, Dong et al. 2010). Enteric bacteria induce local immune response in the gut in addition to systemic response. Probiotics have been used to enhance both humoral- and cell-mediated immune response against gastrointestinal pathogens. The specific IgA response was enhanced following probiotic administration in children infected with the rotavirus and in adults undergoing vaccination with an attenuated *Salmonella typhi* strain; enhanced phagocytic of circulating leukocytes was also noted (Majamaa et al. 1995, Schiffrin et al. 1995).

Application of probiotics offers great promise in the treatment of necrotizing enterocolitis (NEC) in premature babies. NEC, an extensive intestinal inflammatory disease of premature infants, is caused, in part, by an excessive inflammatory response to initial bacterial colonization due to the inappropriate expression of innate immune response genes. In a randomized placebo-controlled clinical trial, it was shown that probiotics (*Bifidobacterium infantis* and *Lactobacillus acidophilus)* significantly reduced the incidence of NEC. Probiotics were demonstrated to prevent NEC by modulating enterocyte genes that regulate innate immune-mediated inflammation (Ganguli et al. 2013). Bifidobacteria, the classic probiotic bacteria, exhibit beneficial effects through the modulation of host defense responses and protection against infectious diseases. The inhibitory effect of bifidobacteria can in part be attributed to the increased production of acetate which inhibits translocation of the Shiga-like toxin produced by *E. coli* O157:H7 from the gut lumen to the blood; indicating that acetate produced by protective bifidobacteria improves the intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection (Fukuda et al. 2011).

4.7.2 Prebiotics

A prebiotic is defined by the FAO as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota." Prebiotics are non-digestible food ingredients which upon ingestion function by promoting the growth and activity of certain specific colonic microbiota (Gibson and Roberfroid 1995, Roberfroid 2007). Primarily, these indigestible materials are nonviable food components benefitting the host through modulation of the microbiota (Gibson et al. 2004, Pineiro et al. 2008). There have been several criteria to define a prebiotic, however, a prebiotic would indicate resistance to gastric acidity, be fermentable by gut microbiota, and possess the ability to support the growth and/or activity of beneficial gut microflora (Schrezenmeir and de Vrese 2001, Roberfroid 2007).

The majority of prebiotics are dietary fibers such as oligosaccharides, although a variety of food ingredients can function as prebiotics. The proper functioning of prebiotics is related to

their metabolism by the probiotics (Gourbeyre et al. 2011). In the absence of dietary fibers in the colon, anaerobic bacteria derive energy from protein fermentation which leads to the generation of toxic and potentially carcinogenic compounds such as phenolic and ammoniac compounds (Kolida et al. 2002, Manning and Gibson 2004). Common prebiotic oligosaccharides are inulin (polymers composed mainly of fructose units, and typically with a terminal glucose), fructose oligosaccharides (FOS), galactooligosaccharides (GOS), sucrose oligosaccharide (SOC), *trans*-galacto-oligosaccharides (TOS), xylooligosaccharides (XOS), pyrodextrins, soy oligosaccharides, and isomaltose-oligosaccharides (Macfarlane et al. 2008). Different members of the gut microbiota have preferential prebiotic substrates; for example, growth of Bifidobacteria is more efficient on fructans in comparison to Clostridia and *Bacteroides* sp. (Prakash et al. 2011, Gourbeyre et al. 2011). GOS being derived from lactose usually consist of chains of galactose monomers (Scholtens et al. 2006), are versatile food ingredients, and possess prebiotic characteristics. However, utilization of GOS by bifidobacteria is still hard to analyze as no precise analytical methods for it exists. Selectivity in consuming several types of GOS by different bifidobacteria denotes targeting prebiotics to focus upon certain bifidobacterial species (Barboza et al. 2009).

4.7.2.1 Inulin and FOS

Inulin and FOS are the most widely consumed prebiotic materials by humans worldwide. These are fructans (polymers of fructose attached by β 1-2 linkage with a terminal glucose residue (Macfarlane et al. 2006). Inulins have a degree of polymerization (DP) of <200 (usually 2–60), whereas a FOS has a DP of <10. These are non-digestible carbohydrates and are transferred to colon and fermented almost quantitatively (ca. 100%; Roberfroid 2007). Inulin possess 25%–35% of food energy of starch and approximately 10% of sweetness of sugar/sucrose making it a versatile food ingredient in many processed foods (Roberfroid 1999). Numerous major dietary sources of prebiotics cover fructans such as inulin, oligofructose, and short-chain FOS. A "high performance" (HP) type of inulin is also available in the market. HP inulin has an average DP of 25 with the residual sugars as well as the oligomers removed. This product provides almost twice the fat mimetic characteristics of standard inulin with no sweetness contribution (Niness 1999). The energy obtained from the fermentation of inulin and FOS is mostly due to production of SCFA and lactate which contribute to 1.5 kcal/g of energy for both inulin and FOS. There is also increasing evidence from human and animal studies that such prebiotics can enhance satiety and decrease energy intake leading to improved control of body weight (Cani et al. 2009a).

Inulin, naturally occurring in onions in high concentration has been shown to possess fructose monomers in high number (10–60) while oligofructose derivatives, found in asparagus, wheat, and artichoke, exist in low number (3–7) of fructose monomers (Gibson et al. 1994). Improved conditions in bowel inflammation, reduction in the production of pro-inflammatory biomarkers on using long-chain inulin along with an increase in intestinal bifidobacteria and lactobacilli have been reported in the literature (Lindsay et al. 2006, Leenen and Dieleman 2007).

4.7.2.2 Health Benefits of Prebiotics

Prebiotics while in the GIT have been reported to deliver beneficial effects in various ways. Prebiotics influence intestinal transit time and normally determine bowel habits. Prebiotics stimulate the growth of a variety of intestinal microbiota including bifidobacteria. Studies have demonstrated the implication of prebiotics in reducing atherosclerosis, osteoporosis, obesity, type-2

diabetes, cancer, infections, and allergies risk in humans (Scholtens et al. 2006, Roberfroid 2007). Human milk oligosaccharides (HMO) are reported to have a range of biological activities beyond providing nutrition to the infant (Barile and Rastall 2013). In addition to the selective stimulation of beneficial bacteria, which is a common property of all prebiotics, certain prebiotics such as GOS can competitively block adhesion of pathogens to IEC. Evidence exists that fructans-type prebiotics such as inulin and FOS can reduce serum cholesterol levels and increase HDL/LDL ratio (Ooi and Liong 2010). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite reduction and glucose response after a meal (Cani et al. 2009b). In summary, prebiotic food ingredients are vital to support the growth and survival of probiotic organisms in the human intestine. So, beneficial probiotic bacteria need to be constantly introduced in the diet and supplied with proper fibrous diet (prebiotics) to sustain them in the gut so that a healthy microbe–host relationship is maintained.

4.7.3 Synbiotics

As probiotics and prebiotics offer health benefits to humans, it was hypothesized that by combining probiotics and prebiotics, it should be possible to achieve not only the combined effect, but also a synergistic effect. The term synbiotics was proposed by Roberfroid (1998) to describe such a combination of a probiotic and a prebiotic which was more potent than either of these ingredients. In addition to probiotics and prebiotics, studies are conducted to explore various combinations of synbiotics aiming at the modification of gut flora for health benefits. Synbiotics (*Bifidobacterium breve* Yakult and *Lb. casei* Shirota as probiotics, and GOS as a prebiotic) was used to treat D-lactate acidosis. It allowed the reduction in colonic absorption of D-lactate by both prevention of D-lactate-producing bacterial overgrowth and stimulation of intestinal motility, leading to the remission of D-lactate acidosis (Takahashi et al. 2013). Another recent study also showed the usefulness of synbiotics. Phenol and *p*-cresol, as metabolites of aromatic amino acids produced by gut bacteria, are regarded as bioactive toxins and serum biomarkers of a disturbed gut environment. A double-blind placebo-controlled trial on consumption of synbiotics (*Bif. breve* strain Yakult as probiotic and prebiotic GOS as prebiotic) demonstrated reduced serum total phenol levels and prevented skin dryness and disruption of keratinization in healthy adult women providing evidence of health benefits to the skin as well as the gut (Miyazaki et al. 2013). A synbiotic combination of inulin (prebiotic) and *Bifidobacterium longum* (probiotic) was found to be more potent in inhibiting azoxymethane (AOM)-induced aberrant crypt foci in rats in comparison to either inulin or the probiotic alone (Rowland et al. 1998).

4.7.4 Cobiotics

Cobiotics is a newly coined term to describe substances which are utilized by probiotics and also by the host. In contrast, prebiotics are only utilized by probiotics, but not by the host (Greenway et al. 2013). Certain enzymes react with food materials and release nutrients which are stimulatory to the probiotics. Enzymes protease and amylase when incorporated as a cobiotic combination, function as lactogenic factor (stimulate the growth of lactobacilli). Enzymes cellulose and hemicellulose, on the other hand function as a bifidogenic, that is, stimulate the growth of bifidobacteria. In a recent study, the effectiveness of cobiotics has been highlighted. A cobiotic consisting of prebiotic purified inulin, sugar-free blueberry pomace extract, and an oat preparation of purified beta-glucan was used to repair gastrointestinal dysbiosis and found to be highly effective in augmenting glucose control in a type-2 diabetic patient (Greenway et al. 2013).

4.7.5 Immunobiotics

The term "immunobiotics" was coined to identify bacteria that promote health through driving mucosal immune mechanisms, compared to those with strictly local effects (Podleski 2011, Tomosada et al. 2013). Immunobiotics are demonstrably beneficial for treating a variety of mucosal disorders, including inflammatory diseases. Immunobiotic microorganism *Lactobacillus jensenii* TL2937 has been found to interact with IEC and immune cells in experimental models through the modulation of Toll-like receptors (TLRs) to maintain a fine balance between tolerance and inflammation (Villena et al. 2013, Villena and Kitazawa 2014). Interestingly, immunobiotic bacteria has been found to stimulate the immune system at sites beyond the intestinal tract; consumption of *Lb. rhamnosus* CRL1505 (Lr1505) and *Lb. casei* CRL431 (Lc431) resulted in the stimulation of the immune system in the respiratory tract as demonstrated by increased activity of macrophages at those sites (Marranzino et al. 2012). Nasal administration *Lb. rhamnosus* strains have been reported to differentially modulate respiratory antiviral immune responses and induce protection against respiratory syncytial virus infection (Tomosada et al. 2013).

In an attempt to modulate virus-induced inflammation–coagulation interactions to treat acute respiratory virus infections, immunobiotic strain *Lb. rhamnosus* CRL1505 strain was used. The immunobiotic strain triggered activation of TLR-3 by modulating the production of proinflammatory and anti-inflammatory cytokines as well as the expression of tissue factor and thrombomodulin in the lung (Zelaya et al. 2014). The preventive treatment with the immunobiotic bacteria beneficially modulated the finely tuned balance between clearing respiratory viruses (respiratory syncytial virus and influenza virus) and controlling immune-coagulative responses in the lung, allowing normal lung function to be maintained in the face of a viral attack. These findings demonstrate that immunobiotic functional food offers novel preventive and therapeutic approaches to better control virus-inflammatory lung damage (Zelaya et al. 2014).

4.8 Microbiome Metabolites: Effects on Health

Abundant literature confirms the role of microbial metabolites of dietary components in disease prevention and disease risk (Qin et al. 2012, Goldsmith and Sartor 2014). The undigested nutrients including polysaccharides, lipids, and peptides that reach the large intestine unabsorbed positively impact the growth of gut microbiota (Bazzocco et al. 2008, Sekirov et al. 2010). Short SCFAs (acetate, propionate, and butyrate) that are formed as a result of microbial metabolism of dietary carbohydrates are directly linked to the proportion and composition of gut microbiota thereby affecting host health. Similarly, SCFAs are also produced by anaerobic microbial metabolism of peptides and proteins along with several toxic compounds such as ammonia, amines, phenols, thiols, and indols which are potentially harmful to human health (Cummings et al. 1996, Scott et al. 2013).

Phytochemicals, generally regarded as bioactive nonnutrient plant compounds, possess antioxidant, antiestrogenic, anti-inflammatory, immunomodulatory, and anticarcinogenic properties. The currently existing 25,000 phytochemicals exhibit either positive and deleterious effects on human health, for example, vegetables, the major source of nitrates in the human diet, may exert a damaging effect by interacting with several compounds forming nitrosamines, nitrosamides, and nitrosoguanidine that cause DNA damage. Contrarily, a plethora of literature confirms the positive role of dietary bioactive phytochemicals with potential benefit to human health. Nevertheless, components of the gut microbiota are associated with the fermentation, transformation, and bioavailability of these phytochemicals (Scalbert et al. 2002, Qin et al. 2012).

Laparra and Sanz (2010) elucidated the role of phytochemicals and their metabolic products as antimicrobial inhibiting pathogenic bacteria and stimulating the growth of beneficial bacteria in the gut. Moreover, phytochemicals and their derived products potentially affect colonic microbiota, as a part of them remains unabsorbed in the gut and this unabsorbed portion is subsequently metabolized in the liver. These metabolites are excreted through the bile in the form of glucuronides which eventually accumulate in the ileal and colorectal lumen (Bazzocco et al. 2008, Tzonuis et al. 2008).

The metabolism of dietary phenolic compounds results in different types of metabolites in the colon before absorption (Selma et al. 2009). Unabsorbed dietary phenolics and their metabolites possess the ability to act as antimicrobial or bacteriostatic agents. These metabolites inhibit the growth of selective pathogens and promote the growth of commensal bacteria, including some recognized probiotics (Lee et al. 2006, Laparra and Sanz 2010). The rapidly advancing field of medical sciences warrants recognition and understanding of how gut microbiota can beneficially interact with diet and modulate metabolism for improved long-term health status.

4.9 Perspectives

Research on the human microbiome, especially on the interplay between food and gut microbiota leading to health and disease conditions has become one of the most exciting fields in biology; generating fascinating insights into the relations of microbes and man as modulated by diet and the effects of the microbes on our health and well-being. The bidirectional interaction between host and microbe, which appears to influence the host at multiple levels, is crucial for our evolution, development, metabolism, immune function, and susceptibility to infectious and noncommunicable diseases. The influence of diet on gut microbiota is astounding; metagenomic analysis of gut microbial ecology is generating useful information regarding the functional contribution of gut microbiota to its host as modulated by diet and its relationship to health and disease states. It is anticipated that newer methodologies such as metaproteomics, the function-based approach relying on microbial protein expression; metabolomics, the functional analysis of complex microbial populations through analysis of their complete metabolite profiles; metatranscriptomics, which enables community-wide gene expression analysis of gut microbiota, will allow more thorough research leading to varied ways of harnessing the beneficial effects of the modulation of gut microbiota by diet as therapeutic, and even preventive options in future (Lozupone et al. 2012, David et al. 2014).

4.10 Conclusion

The human gut is a natural habitat for a large and dynamic bacterial community. The microbiome (genes and genomes of all the bacteria inhabiting the gut) of each person is distinct and variable but each individual possesses a shared core microbiome which is required for proper maintaining of cross species homeostasis between the gut bacteria and the host. The recent interest in the structure and function of the gut microbiome, its dynamic evolution throughout an individual's life in a host specific manner and how it is modified by diets, has resulted in a series of exciting findings. The advent of culture-independent techniques to study the microbiome has enabled scientists to decipher the dynamics of the complex bidirectional interactions between diet and the microbiome in relation to human health and diseases. Although details of the complex interactions between diet, the microbiome and host health is an emerging area of science, existing knowledge is being

used in leveraging the microbiome to develop dietary interventions to counterbalance dysbiosis and to increase overall well-being. However, it is essential to assess the efficacy of the pro/prebiotics to molecular detail and the long-term safety of probiotics, as the impact of prolonged perturbation of the microbiome is largely unknown. Collectively, the research findings reviewed here suggest that more integrative studies will provide all-encompassing knowledge of the complex, multi-level interactions between diet, the microbiome, and host health, which can be utilized to design microbiome-based biomarkers for those at risk of various infectious and metabolic diseases and formulate diet-driven microbiota alteration strategies to improve human health.

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