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Preface

Colorectal cancer is the third leading cause in both incidence and mortality amongst men and women. The American Cancer Society estimated that 136,830 people are affected by it and 50,314 died in 2014. Developing countries are also showing increased incidences of colorectal cancer. Genetic and environmental factors contribute to the disease, of which major contribution is due to the latter. While 10–15 % of cancers are due to familial and hereditary factors, diet is associated with 50–80 % cases. Animal, epidemiological, case–control and cohort studies have indicated that various nutrients and components of food aid in the development or prevention of colorectal cancer. Excess consumption of red and processed meat, roasted coffee, etc. have shown a consistent increase in the incidence of colorectal cancer, indicating that compounds formed in food containing free amino acids and sugars interact at elevated temperatures to form mutagens or carcinogens, namely, heterocyclic amines and polycyclic aromatic hydrocarbons. These mutagens can cause alterations and damage to DNA, causing colorectal cancer. The usual remedy for colorectal cancer includes surgeries and invasive chemotherapy or radiation regimen; however, the cancer grows undetected and recurs after treatment. Several lifestyle and dietary factors could prevent this ailment.

This book comprises of seven chapters. The introductory chapter describes the gut system and its microbiome. Four chapters describe the dietary habits (probiotics, synbiotics and bioactive carbohydrate, namely, prebiotics and dietary fibres) that could modify and reduce the risk of developing colorectal cancer. Probiotics, prebiotics and synbiotics have developed into a major research focus area due to their specific health benefits in human. The applications of these include functional foods, health supplements and nutraceuticals. Emphasis is also laid on the dietary fibres that have shown significant health benefits against colorectal cancer. Consumer interest in the relationship between diet and health has improved the dietary habits presently. This has also paved the way for functional food and nutraceuticals that supply the nutrients for beneficial normal reactions in the body. The chapters discuss the concept, definition and status of the "biotics" in the current market as well as their different physiological and molecular mechanisms of action.

Short-chain fatty acid, which is involved in CRC considerably and forms the molecular pathway in preventing colorectal carcinogenesis, is discussed in Chap. 6. Short-chain fatty acid is formed in the colon as a result of the microbial fermentation of undigested bioactive carbohydrates including prebiotics (oligosaccharides, inulin, lactulose, lactitol) and dietary fibres by

Bifidobacterium and *Lactobacillus*. Acetate, propionate and butyrate are major SCFA products. Butyrate, specifically, is involved in the inhibition of histone deacetylase, resulting in histone hyperacetylation and growth inhibition in the colonic epithelial cells. Apart from these, it is also involved in minimizing inflammation, thereby exhibiting immunomodulatory effects.

Several benefits of probiotics, prebiotics, synbiotics and dietary fibre are novel and have not been fully explored. Similarly, the mechanism of action of these also has not been understood with reference to colorectal cancer and other diseases. This book will be of practical and scientific use to academicians, research scholars, students, health professionals, nutritionists, etc. and could support the cause of preventing CRC by adopting smart food habits.

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Abbreviations

3-MA	3-Methyladenine
7,8-DiMeIQx	2-Amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline
AAD	Antibiotic-associated diarrhoea
AAR	Annual incidence rates
ACA	Anticancerous activity
ACF	Aberrant crypt foci
AGJ	Artificial gastric juice
AIJ	Artificial intestinal juice
Akt	Protein kinase-B
AMA	Antimicrobial activity
AMP	Antimicrobial peptides
AOM	Azoxymethane
ATP	Adenosine triphosphate
AXOS	Arabinoxylo-oligosaccharides
AαC	2-Amino-9H-pyrido[2,3-b]indole
B(α)P	Benzo(α)pyrene
CAGR	Compound annual growth rate
cAMP	Cyclic adenosine monophosphate
CD	Crohn's disease
CDK	Cyclic dependent kinase
CFTR	Cystic fibrosis transmembrane conductance
cfu/g	Colony-forming unit per gram
CH_4	Methane
CHOS	Chito-oligosaccharides
CIN	Chromosomal instability
Cl^-	Chloride ion
CLA	Conjugated linoleic acid
CMC	Carboxymethyl cellulose
CO_2	Carbon dioxide
CRC	Colorectal cancer
CS	Caesarean section
CSC	Cancerous stem cell
DC_R	Dentritic cells
DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline
DNA	Deoxyribonucleic acid
EcN	*E. coli* strain Nissle 1917
EGFR	Epidermal growth factor receptor

EPA	Environmental Protection Agency
ERK	Extracellular signal-regulated kinase
FAO	Food and Agriculture Organization
FOS	Fructo-oligosaccharide
FP	*Faecalibacterium prausnitzii*
FP7	Framework Programme for Research
Fru	Fructose
Gal	Galactose
GIA	Global Industry Analysts
GIT	Gastrointestinal tract
GLP1	Glucagon-like peptide 1
GLP2	Glucagon-like peptide 2
Glu	Glucose
Glu-P-1	2-Amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole
Glu-P-2	2-Aminodipyrido[1,2-a:3′,2′-d]imidazole
GOS	Galacto-oligosaccharide
GPCRs	G-protein-coupled receptors
GRAS	Generally recommended as safe
GTO	Gentio-oligosaccharides
H^+	Hydrogen ion
H_2	Hydrogen
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
HCA	Heterocyclic amines
HCO_3^-	Carbonate ion
HDAC	Histone deacetylase
HepG2	Human hepatoma cell lines
HTS	High-throughput sequencing technologies
IARC	International Agency for Research on Cancer
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IDL	Intermediate-density lipoprotein
IL	Interleukin
IMO	Isomalto-oligosaccharide
IQ	2-Amino-3-methylimidazo[4,5-f]quinoline
ISCs	Intestinal stem cells
kD	Kilodalton
kJ	Kilojoule
LAB	Lactic acid bacteria
LcS	*L. casei* strain, Shirota
LDL	Low-density lipoprotein
MAPK	Mitogen-activated protein kinase
MeAαC	2-Amino-3-methyl-9H-pyrido[2,3-b]indole
MeIQ	2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline
MeIQx	2-Amino-3,8-dimethylimidazo [4,5-f]quinoxaline
MLN	Mesenteric lymph nodes
MMPs	Metalloproteinases
MNNG	N-Methyl-N′-nitro-N-nitrosoguanidine

MOS	Mannan-oligosaccharides
MSI	Microsatellite instability
Na^+	Sodium ion
NF-κB	Nuclear factor-κB
NH_3	Ammonia
NH_4^+	Ammonium ion
NIH	National Institutes of Health
NK	Natural killer
NOC	N-Nitroso compounds
PAH	Polycyclic aromatic hydrocarbons
PARP	Poly (ADP-ribose) polymerase
PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
PI	Prebiotic index
PI3K	Phosphatidylinositol 3-kinase
POS	Pectin-oligosaccharides
PPAR-γ	Peroxisome proliferator-activated receptor-gamma
RD	Resistant dextrin
ROS	Reactive oxygen species
RS	Resistant starch
SCFA	Short-chain fatty acids
SO_4	Sulphate
SOS	Soy-oligosaccharide
TGOS	Trans-galacto-oligosaccharide
TIM	Tissue inhibitor matrix metalloproteinase
TNF	Tumour necrosis factor
TOS	Trans-oligosaccharide
Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]-indole
Trp-P-2	3-Amino-1-methyl-5H-pyrido[4,3-b]indole
UC	Ulcerative colitis
USD	United States Dollars
USNFIA	United States National Food Ingredient Association
UTI	Urinary tract infection
VLDL	Very-low-density lipoprotein
WHO	World Health Organization
XOS	Xylo-oligosaccharide

Contents

Introduction: Gastroinstestinal System and Colorectal Cancer

1

Abstract

The gastrointestinal (GI) system is an important part of the human body involved in digestion. Animals and humans, live in harmony with microbes and establish a symbiotic relationship with them. The human microbiota contains as many as 10^{14} bacterial cells, which is ten-fold higher than the number of human cells present in our body. With the development of high-throughput sequencing technique, it is possible to study the gut microbiota and its role in health and disease. It performs a number of essential protective, structural and metabolic functions for host health, including food processing, digestion of complex host-indigestible polysaccharides, pathogen displacement, and synthesis of vitamins. The gut microbiota may exert beneficial functions beyond the gut such as controlling obesity, gastrointestinal disorders, inflammatory bowel disease, and irritable bowel syndrome. Also, the altered microbiota has been linked to neuropsychological disorders (depression). The gut microbiota has also been implicated in the development of colorectal cancer (CRC). CRC is one of the major health problems in the world, and the risk factors associated with it are genetic as well as environmental. It evolves through a stepwise accumulation of genetic and epigenetic alterations, leading to the transformation of normal colonic mucosa into invasive cancer. In this chapter, the role of the gut microbiota in health and disease, CRC and its types, prevalence and mechanism are briefly discussed.

Keywords

Gut microbiota • CRC • Intestinal stem cells • Colon stem cell • Crypt • Metagenomics

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1.1 Introduction

Human system needs energy for proper metabolic activities, development and growth, which comes from the breakdown of food. Food as such cannot be absorbed into the blood until they are broken down into small soluble chemicals. This chemical digestion works with the help of enzymes. Once the digestion is completed, the small molecules pass through the wall of the stomach and small intestine by either active or passive diffusion. The digestive system of the body is termed as gastrointestinal system.

1.1.1 Gastrointestinal Tract

The gastrointestinal (GI) system is an important part of the human body, is involved in the digestion of food, and it includes all the parts of the body from mouth to anus. It is uniquely constructed to perform its specialized function of turning food into the energy needed for the body and packaging the residue for waste disposal. The different components and functions of the GI system are briefly discussed below (Table 1.1) (US Department of Health and Human Services 2010).

Table 1.1 Overview of the functions of gastrointestinal organs

Organ	Major function
Mouth and other associated	Ingestion: food is voluntarily placed into oral cavity
	Propulsion: voluntary phase of deglutition (swallowing) initiated by tongue propels food into the pharynx
	Mechanical breakdown: mastication (chewing) by teeth and mixing movement by tongue
	Chemical digestion: salivary amylase in saliva, produced by salivary gland, begins chemical breakdown of starch
Pharynx	Propulsion: peristaltic waves move food bolus to stomach, thus accomplishing involuntary phase of deglutition
Stomach	Mechanical digestion and propulsion: peristaltic waves mix food with gastric juice and propel it into the duodenum
	Chemical digestion: digestion of protein begun by pepsin
	Absorption: absorbs a few fat soluble substances (aspirin, alcohol, drugs)
Small intestine and associated accessory organs	Mechanical digestion and propulsion: segmentation by smooth muscle of the small intestine continually mixes content with digestive juices and moves the food along tract and through ileocecal valve at a slow rate, allowing sufficient time for digestion and absorption
Large intestine	Chemical digestion: some remaining food residues are digested by gut microbiota
	Absorption: absorbs most remaining water, electrolytes and metabolites produced by bacteria
	Propulsion: propels feces towards rectum by peristalsis, haustral churning, and mass movement
	Defecation: reflex triggered by rectal distension; eliminates feces from body

Mouth It is at the beginning of the digestive tract and is the first place where digestion starts with intake and first bite. Food is broken down into pieces and further digested by salivary enzymes and enters into the esophagus.

Esophagus It is present in the throat near the trachea and delivers food to the stomach upon swallowing.

Stomach The stomach is a main storage tank that produces a mixture of acid, mucus, and digestive enzymes to further rupture the ingested food and then release them into the small intestine.

Small Intestine It is a 22.8-foot long tube and is made up of three segments – the duodenum, jejunum, and ileum. Peristalsis also works in this organ, namely moving the food through and mixing it with digestive secretions from the pancreas and liver. The main function of duodenum is to further break down the digested food. The jejunum and ileum are responsible for absorption of

nutrients into the bloodstream. In the small intestine, the contents start out as semisolid and is then converted into a liquid form after passing through the organ with the help of water, bile, enzymes, and mucous. After absorption of nutrients, the leftover food residue liquid moves on to the large intestine or colon.

Pancreas Its main function is to secrete digestive enzymes into the duodenum that is required for further digestion. It also produces insulin, a chief hormone for metabolizing sugars, which it secretes directly into the bloodstream.

Liver It is the body's chemical "factory" and is within the digestive system. It helps to process the nutrients absorbed from the small intestine. It takes the raw materials absorbed by the intestine and helps in the synthesis of various chemicals required for the whole system. The liver also detoxifies potentially harmful chemicals.

Gallbladder It is a small organ where bile is stored and concentrated before it is released into the small intestine. There it helps to absorb and digest fats.

Colon (Large Intestine) It is a six-foot long muscular tube that connects the small intestine to the rectum. It is made up of the cecum, the ascending (right) colon, the transverse (across) colon, the descending (left) colon, and the sigmoidal colon, which connects to the rectum. The main function of the colon is to process the waste obtained from the small intestine so that emptying of the bowels is easy and convenient.

Rectum It is an 8-inch chamber that connects the colon to the anus, and it serves as a temporary storehouse for feces. The expansion of the rectal walls causes the stretch receptors within the walls to stimulate the urge to defecate.

Anus It is the opening where the gastrointestinal tract ends and exits the body. It is present below the rectum and is the last portion of the colon. The function of the anus is to transmit feces from the rectum to the external environment.

1.1.2 Gut Microbiota as an Organ

Microbiota is the total microorganisms (including bacteria, archaea, eukaryotes, and viruses) that live in and/or on a human or a specific part such as the gastrointestinal tract, oronasopharyngeal cavity, skin, urogenital tract (Reid et al. 2011; Sommer and Bäckhed 2013). The human microbiota contains as many as 10^{14} bacterial cells, which is tenfold higher than the number of human cells present in our body (Sekirov et al. 2010). This area of research has gained lots of interest in the last 10 years and improved our knowledge and understanding about the resident species and their functions. In the last 5 years (since 2010), more than 4,500 publications are available on PubMed against 635 publications in 2005–2009. The recent research on gut microbiome has brought forth its significance in health and the input of high-throughput sequencing technologies (HTS). Here, the NIH-funded Human Microbiome Project and MetaHIT project financed by the European Commission under the 7th FP program are worth mentioning.

The mucosal surface of the human gastrointestinal (GI) tract is about 200–300 m^2 and is colonized by 10^{13}–10^{14} bacteria with >500 different species and subspecies (Hao and Lee 2004). The gastrointestinal microflora is divided into two types:

1. Autochthonous flora (indigenous flora), which colonize particular habitats, i.e., physical spaces in the GI tract;
2. Allochthonous flora (transient flora), which cannot colonize particular habitats except under abnormal conditions (Hao and Lee 2004).

Most pathogens are allochthonous microorganisms with few exceptions where autochthonous may turn as opportunistic pathogens when the gut microbiota is disturbed.

The structure and composition of the gut flora reflect the natural selection at both the microbial and host levels, which promotes mutual cooperation within and functional stability of this complex ecosystem (O'Hara and Shanahan 2006; Ley

Table 1.2 Variations in microbial numbers and composition across the length of the gastrointestinal tract

Organ	Population density	Bacterial diversity	Function and pH
Stomach	10^1–10^2	*Lactobacillus*	HCl secretion
		Streptococcus	Macromolecule
		Helicobacter	Digestion
		Peptostreptococcus	pH 2
Duodenum	10^1–10^3	*Streptococcus*	Main digestion, absorption of monosacchrides, amino acid, fatty acid, and water pH 4–5
		Lactobacillus	
Jejunum	10^3–10^4	*Bacteroides*	
		Clostridium	
		Streptococcus	
		Actionmychaea	
Ileum	10^7–10^9		
Colon	10^{11}–10^{12}	*Bacteroides*	Bile acids, vitamin B12, and water absorption pH 7
		Clostridium	
		Bifidobacterium	
		Enterobacteriaceace	
		Eubacteria	

et al. 2006; Rawls et al. 2006). Composition of the microbes in different parts of the gut is heterogeneous and is influenced by different factors such as acid, bile, and pancreatic secretions, which hinder the colonization in the stomach and proximal small intestine (Ridlon et al. 2014; Endesfelder et al. 2014). However, bacterial density is higher in the distal small intestine and increases in the large intestine to 10^{11}–10^{12} bacteria per gram of colonic content (Table 1.2) (Whitman et al. 1998; Ley et al. 2006). Moreover, surface-adherent and luminal microbial populations are different (Carroll et al. 2011; Ringel et al. 2015). For example, *Bacteroides*, *Bifidobacterium*, *Streptococcus*, members of Enterobacteriacea, *Enterococcus*, *Clostridium*, *Lactobacillus*, and *Ruminococcus* are predominant in feces, whereas only *Clostridium*, *Lactobacillus*, and *Enterococcus* are identified in the mucus layer and epithelial crypts of the small intestine (Swidsinski et al. 2005.

Koren et al. (2012) studied the dynamics of the gut microbiota throughout the course of pregnancy. During the first trimester, the gut microbiota is similar in many aspects to that of a healthy nonpregnant male and female controls, but by the third trimester, the structure and the composition of the community resembles a disease-associated dysbiosis that differs among women. The fetal gut is thought to be sterile and colonization begins immediately after birth, and it undergoes dramatic changes during the development. However, some evidence suggests the presence of bacteria in the intrauterine environment (Prince et al. 2014). The bacteria found in the meconium might have translocated from the mother's gut via the blood stream and may influence the microbiota of the infant before birth (Madan et al. 2012). Recently, microbiome is also discovered in preterm and full-term placentas with a unique bacterial community consisting of low number of organisms and large number of species (Aagaard et al. 2014). These are mostly nonpathogenic commensals belonging to the phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria and very much resemble the microbiome of the oral cavity (Aagaard et al. 2014). Experimental evidence suggests that a growing embryo and fetus does not (always) develop in a sterile world but may encounter the presence of bacteria, at least in the placenta, which needs further study to elucidate the role of this placenta.

After birth of the baby, development of microbiota is influenced by genetic, epigenetic, and environmental factors such as delivery mode,

use of antibiotics, and breastfeeding (Bermon et al. 2015). The delivery mode at childbirth has an impact on early microbiota composition and could subsequently influence on long-lasting metabolic effects and postnatal maturation of immune system development (Dominguez-Bello et al. 2010; Biasucci et al. 2010). Vaginally delivered children display a microbiota that shares characteristics with the vaginal origin (Huurre et al. 2008), whereas the GI tract of babies delivered by cesarean section is mainly colonized by environmental microflora (Biasucci et al. 2008). The reduced microbial stimulation during infancy would result in slower postnatal maturation of the immune system and development of an optimal balance between TH1 and TH2-like immunity (Björkstén et al. 2001; Björkstén 2006). Infants born by cesarean section (CS) show the presence of lower numbers of bifidobacteria and *Bacteroides* when compared to the vaginal-born ones. This suggests that the microbiota derives, at least in part, from the mother during the delivery and modulates the early colonization of microbiota and immune development (Min and Rhee 2015).

There are significant differences in the microbiota of breast-fed infants when compared to the formula-fed infants (Azad et al. 2013; Guaraldi and Salvatori 2012). Breast milk contains beneficial bacteria like bifidobacteria and oligosaccharides, which serves as synergistic synbiotics and helps in the colonization of these bacteria in such infants. The difference in the gut microbiome of a formula-fed baby when compared to the former may underpin the health risks associated with formula feeding. Hence, the colonization process of the infant gut microbiome is chaotic and associated with life events, such as illnesses, dietary changes, and antibiotic treatment, suggesting that differences in the colonization patterns of multiple babies would most likely reflect differences in their daily lives (Koenig et al. 2011).

A balanced gut microbiota is vital for the development of an appropriate innate and adaptive immune response (Walker 2013; Bermon et al. 2015). The disruption of the normal colonization process could lead to alterations in the important symbiotic relationship, which is necessary for immune homeostasis. Initial colonization by microbes in infants is very important to balance immune homeostasis (Walker 2013). For example, initial colonization of infants is hampered by various factors such as birth by cesarean section or receiving excessive perinatal antibiotics leading to aberrant mucosal immune function (Fig. 1.1). As a result, later in childhood, they are susceptible to immunity-related diseases like asthma and autoimmune diseases (e.g., celiac disease). The gut microbiota performs a number of essential protective, structural, and metabolic functions for host health, including food processing, digestion of complex host-indigestible polysaccharides, pathogen displacement, and synthesis of vitamins (Table 1.3) (Grenham et al. 2011; Trompette et al. 2014; Neish 2014; Clarke et al. 2014). The gut microbiota produces numerous chemicals of a hormonal nature that are released into the bloodstream, and they act at distal sites and are implicated in many aspects of physiological functions of the gastrointestinal tract, including the regulation of secretion, absorption and digestion, and gut motility (Clarke et al. 2014) (Table 1.4).

Unlike other endocrine systems or organs, which secrete a single or at most a small number of humoral agents, the gut microbiota has the potential to produce hundreds of products (Clarke et al. 2014). The gut microbiota is the most complex and biochemically heterogeneous than any other endocrine organ in the human being. This complexity is attributed to the biochemical and metabolic activity of the microbial cells. They contribute approximately a weight of 1–2 kg in an average adult (Clarke et al. 2014).

1.1.3 Gut Microbiota and Health

With recent advancements in research, there is enough evidence towards a possible coevolution of the host and its indigenous microbiota (Sekirov et al. 2010). This coevolution process helps them to establish a symbiotic relationship with the host (Prakash et al. 2011). The gut microbiota contributes to human health by performing important functions like providing protection, structural

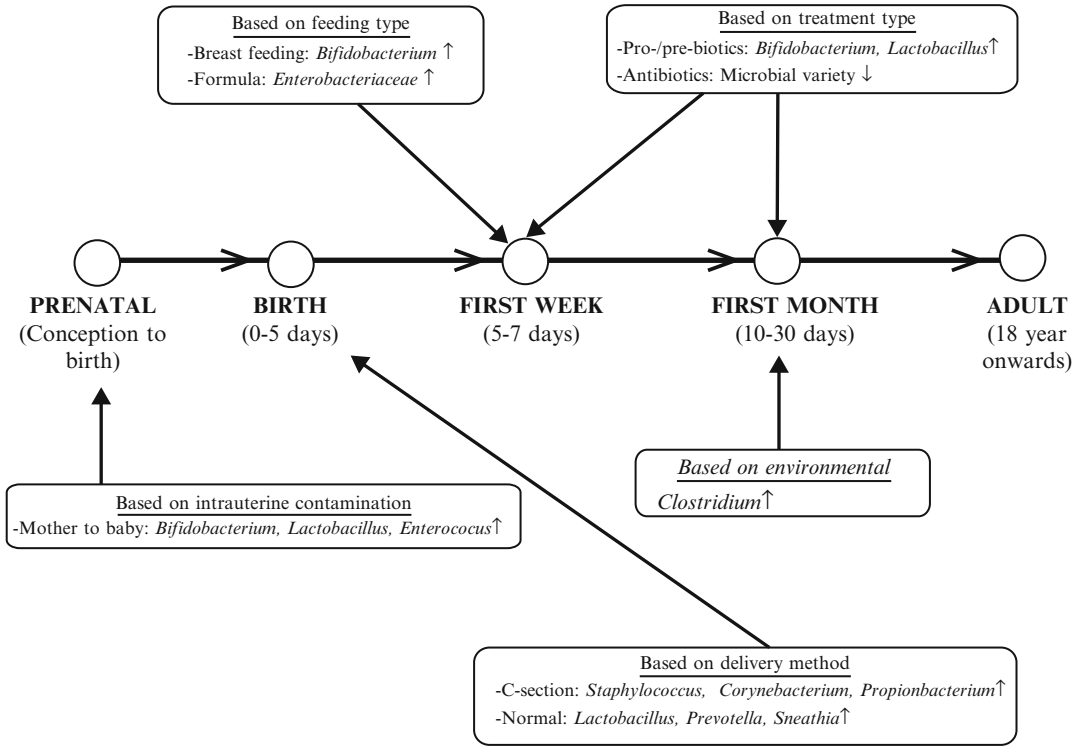

Fig. 1.1 Factors influencing the development of the intestinal microflora in the infant gut

Table 1.3 Function of commensal gut microbiota

Protective functions	Structural functions	Metabolic functions
Pathogen displacement	Barrier fortification	Control IEC differentiation and proliferation
Nutrient competition	Induction of IgA	
Receptor competition	Apical tightening of tight junction	Metabolize dietary carcinogens
Production of antimicrobial factors, e.g., bacteriocins, lactic acid, short-chain fatty acids	Immune system development	Synthesize vitamins, e.g., biotin, folate
		Ferment nondigestible dietary residues and endogenous epithelial-derived mucus
		Ion absorption
		Salvage of energy

and functional stability and various metabolic functions (Prakash et al. 2011; Sekirov et al. 2010).

Protective Effect The gut microbiota mainly protects its host by serving as a physical barrier to incoming pathogens by competitive exclusion, such as occupation of attachment sites, consumption of nutrient sources, and production of antimicrobial substances. It also stimulates the host to produce various antimicrobial compounds. To colonize and proliferate in the intestine, microbes develop a mechanism to outcompete each other. Commensal bacteria produce antimicrobial components, including bacteriocins and proteinaceous toxins that specifically inhibit members of the same or similar bacterial species (Hammami et al. 2013). Antimicrobial peptides (AMPs) are low molecular weight proteins produced in the mammalian GIT, that act by disrupting the sur-

Table 1.4 Peptide hormones of the gastrointestinal tract

Hormone	Produced by	Function
Gastrin	Gastric antrum Duodenum	Gastrin stimulates gastric juice production and smooth muscle contraction in the stomach and small and large intestines and controls the pyloric sphincter
Glucose-dependent insulinotropic peptide (GIP)	Mucosa	Inhibits gastric acid secretion
Insulin	Pancreatic β cells	Anabolic hormones
Motilin	Mucosa	Also known as the "housekeeper of the gut"; it helps in improving gastrointestinal motility or peristalsis
Pancreatic polypeptide	Pancreas	Self-regulates endocrine and exocrine pancreatic secretion activities and affects hepatic glycogen levels and gastrointestinal secretions
Neurotensin	Mucosa	Delays gastric emptying
Cholecystokinin (CKK)	Mucosa	Stimulates the digestion of fat and protein
Peptide tyrosine tyrosine (PYY)	Mucosa	Decreases gastric acid secretion
Neurotensin	Mucosa	Delays gastric emptying
Secretin	Mucosa	Promotes the secretion of digestive juice from pancreas with high amounts of bicarbonates and also stimulates stomach to produce pepsin (the protein digestive enzyme) and liver to produce bile
Glucagon	Pancreatic α cells	Stimulates release of insulin and pancreatic somatostatin
Cholecstokinin	Mucosa	Gall bladder and pancreatic secretion
Somatostatin	Gastric antrum, pancreatic δ cells	Endocrine/paracrine; inhibits secretion of gastrin, secretin, GIP, motilin, pancreatic polypeptide, pancreatic secretion, glucagon, insulin
Histamin	Mast cells close to parietal cells	Attaches to H2 receptor and sensitizes cell to stimuli such as gastrin
Calcitonin	Esophageal tumor	Not normally produced in the GIT

face structures of both commensal and pathogenic bacteria (Sekirov et al. 2010). Examples of these include defensins, cathelicidins, C-type lectins, etc.

Certain commensal bacterium produces short-chain fatty acids (SCFA), which can alter the local pH to inhibit the growth of certain intestinal pathogens. *Bifidobacterium* blocks colonization of pathogenic *E. coli* through acidification of its environment (Fukuda et al. 2011). The SCFA, butyrate, downregulates the expression of several virulence genes, for example, those encoding the Type 3 secretion system (T3SS) proteins in *Salmonella enteria* serovar Enteritidis and Typhimurium (Gantois et al. 2006). The indigenous microbiota is also essential for the development of the immune system. Experiments with germ-free mice show underdeveloped lymphatic systems, with fewer Peyer's patches and isolated lymphoid follicles, fewer intestinal dendritic cells supporting the role of bacterial signals in B cell development (Prakash et al. 2011). The development of T-cell receptor, for example regulatory T, T helper type 1 and 2 cells, and T helper 17 cells, is dependent on signals from intestinal bacteria. Capsular polysaccharide A, produced by *Bacteroides fragilis*, *a* commensal bacteria, beneficially influences the immune system (Mazmanian et al. 2008). *Lactobacillus rhamnosus* JB-1 has potent immunoregulatory

effects. It changes the nerve-dependent colonic migrating motor complexes (MMCs), enteric nerve function, and behavior (Al-Nedawi et al. 2015). Short-chain fatty acids, such as butyrate, may also exert potent immunomodulatory effects by suppressing the nuclear factor-kB activation and/or by acting on G-coupled receptors. These effects are also demonstrated with acetate (Prakash et al. 2011).

Metabolic Function The combined biochemical capacity of the microbiota mediates diverse beneficial roles, including vitamin synthesis, bile salt metabolism, and xenobiotic degradation. It also provides biochemical pathways required for the fermentation of nondigestible substrates and endogenous mucus. Through fermentation, bacterial growth is stimulated, producing short-chain fatty acids (SCFA) and gases. The role of SCFA is discussed in more detail in Chap. 6.

Structural and Histological Function The GIT of a new born is structurally and functionally immature, and its postnatal development is influenced by a number of factors, including exposure to a developing gut microbial community (Walker 2013). Various animals' models such as germ-free model and antibiotic-treated animal model provided insights on the role of the gut microbiota to provide structural stability to GIT. Butyrate produced by the gut microbiota helps to secrete various factors such as inducing the secretion of mucins, trefoil factors, and antimicrobial peptides. In addition to involvement in cell and tissue development, some bacterial communities may strengthen the barrier at the level of the tight junctions and development of villi, crypts and development of the villus microvasculature (Prakash et al. 2011). Gut microbes are vital for keeping the human gut healthy. More importantly, their functions are multifactorial and they exert beneficial functions beyond the gut. Experiments with the germ-free model showed that a number of extraintestinal processes and organ systems are dysregulated, highlighting the contribution of the indigenous gut microbes for their development and maintenance (Sekirov et al. 2010). Recent reports indicate that altered microbiota has been linked to metabolic and neuropsychological disorders (depression and autism spectrum disorder). They help in controlling obesity and gastrointestinal disorders, including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (Cammarota et al. 2015; Zhou and Foster 2015).

The gut microbiota is also implicated in the development of CRC (Candela et al. 2011; Compare and Nardone 2011; Hamer et al. 2012), by causing dysbiosis leading to IBD, which is considered to be a risk factor for this cancer (Man et al. 2011). The complex interaction within and between microbiota and the host may contribute to colon carcinogenesis through the induction of chronic inflammation and the promotion of immune evasion (Turner et al. 2013). Analysis of colon tumor and normal tissue shows increased level of *S. bovis*, enterotoxigenic *Bacteroides fragilis*, and *Escherichia coli* at these sites (Ellmerich et al. 2000; Gold et al. 2004). Fragilysin producing *Bacteroides* spp. is associated with relapses of IBD and the induction of colon tumors in Apc/Min mice (Hajishengallis et al. 2012).

1.1.4 Colorectal Cancer

Colorectal cancer is the cancer of the colon or rectum (large intestine) due to the abnormal growth of cells that invade or spread to other parts of the body. Colorectal cancer evolves through a stepwise accumulation of genetic and epigenetic alterations, leading to the transformation of normal colonic mucosa into invasive cancer (Arends 2013). CRC could be seen in three different patterns based on its origin and is classed as inherited, familial, and sporadic (Roper and Hung 2013). Inherited and familial CRC accounts for 10 and 25 %, respectively, and derive, at least in part, from germ line mutations, whereas sporadic CRC derives from somatic mutation and accounts for approximately 70 % of CRC. The development of CRC is attributed to the loss of genomic stability leading to the acquisition of multiple mutations (Ewing et al. 2014; Munteanu and Mastalier 2014). The types of genomic instability include (1) chromosomal

instability (CIN), (2) microsatellite instability (MSI), (3) aberrant DNA methylation, and (4) DNA repair defects. The genome-wide analysis of gene mutations shows the presence of somatic mutations in several hundred genes in CRC and an average of 80 mutations in any single CRC. This observation highlights the heterogeneity of the disease (Casey et al. 2013). The most frequent and characteristic genetic changes in colorectal carcinogenesis includes alterations in APC, KRAS, SMAD4, TP53, and the mismatch repair (MMR) genes, MLH1 and MSH2.

The environmental risk factors that contribute to the development of CRC includes (1) lifestyle factors (e.g., nutrition, tobacco use, physical activity), (2) naturally occurring exposures (e.g., ultraviolet light, radon gas, infectious agents), (3) medical treatments (e.g., radiation and medicines, including chemotherapy, hormone drugs, drugs that suppress the immune system), (4) workplace and household exposures, and (5) pollution (www.cancer.org; Roper and Hung 2013; Hagland and Søreide 2015). A high body mass index (BMI) is a clear risk factor for CRC, which could be reduced by physical activity.

1.1.4.1 Statistics

The most commonly diagnosed cancers worldwide are those of the lung (13 %), breast (11 %), and CRC (9.7 %) (Ferlay et al. 2013). Cancer-related deaths are due to lung (19.4 %), liver (9.1 %), and stomach (8.8 %). CRC is the third most common cancer in the world, and approximately 95 % of it is adenocarcinomas. Other types of cancer includes mucinous carcinomas and adenosquamous carcinomas. According to a recent report of the American Cancer Society, there are approximately 93,090 cases of colon cancer and 39,610 cases of rectal cancer to be diagnosed in US in the year 2015 (American Cancer Society 2015). According to the same report, between 2007 and 2011, CRC incidence and mortality rate dropped by 4.3 % and 2.5 % per year, respectively, among adults above the age of 50 years. However, in adults younger than 50 years, CRC incidence rates have increased by 1.8 % per year. Moreover, the incidence is higher in men than in women. According to another report, there are 1,360,602 (9.7 %) new cases and 693,933 (8.5 %) deaths due to CRC worldwide (Dušek et al. 2014).

CRC is the second most common cancer in Europe with 447,136 (13 %) new cases every year and 214,866 (12.2 %) deaths. Variation of CRC incidence among Asian countries are very wide and is found higher in all developed Asian countries when compared to the south Asian countries (Mohandas 2011). The incidence of colorectal cancer has been increasing rapidly in recent decades, and mortality has also increased except in Japan and Singapore (Hyodo et al. 2010). The burden of CRC has risen rapidly in some economically developed Asian countries like Japan, South Korea, and Singapore. According to the National Cancer Registry Programme conducted by ICMR in India, the annual incidence rates (AARs) for colon cancer and rectal cancer in men are 4.4 and 4.1 per 100,000, respectively (NCRP 2013). The AAR for colon cancer in women is 3.9 per 100,000. Colon cancer ranks 8th for both the genders, while rectal cancer ranks 9th among men. It is predicted that 2.4 million cases of colorectal cancer will be diagnosed annually worldwide by 2035.

1.1.4.2 Role of Mutagens/Carcinogens

A mutagen is a substance that induces mutation in cells or organisms, and a carcinogen is a substance that is responsible for causing cancer by initiating unregulated growth in cells or tissues of multicellular animals. Study shows that 157 of 175 known carcinogens (approximately 90 %) are also mutagens (Griffiths et al. 2000). The somatic mutation theory of cancer holds that these agents cause cancer by inducing the mutation of somatic cells. Carcinogens may have different levels of cancer-causing potential, and the risk associated with the development of cancer is multifactorial and depends on how the subjects are exposed to a carcinogen, the length and intensity of the exposure, and the person's genetic makeup (NCI, USA). According to the International Agency for Research on Cancer (IARC), carcinogens can be classified into the following groups based on the cancer-causing

potential of a substance: (1) Group A: carcinogenic to humans, (2) Group B: likely to be carcinogenic to humans, (3) Group C: suggestive evidence of carcinogenic potential, (4) Group D: inadequate information to assess carcinogenic potential, and (5) Group E: not likely to be carcinogenic to humans. Examples of food carcinogens include heterocyclic amines (HCA), polycyclic aromatic hydrocarbons (PAH), N-nitroso compounds (NOC), mycotoxins (aflatoxins) and acrylamide. Preclinical and clinical studies have indicated that they are responsible for CRC, breast cancer, and prostate cancer (Raman et al. 2013.

Heterocyclic Amines (HCA) They are procarcinogens formed during the reaction between creatine or creatinine (found in muscle meats), amino acids, and sugars at high temperatures (Sugimura et al. 2004). HCA are classified into two groups, based on reaction with 2 mM of nitrite. Group I HCA includes 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]-indole (Trp-P-1); 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2); 2-amino-9*H*-pyrido[2,3-*b*]indole (Aαc); 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC); 2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*]imidazole (Glu-P-1); and 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2). They lose their mutagenicity through conversion of the amino to hydroxyl groups. However, no such changes are observed with Group II HCA. These include 2-amino-3-methylimidazo[4,5-f]quinolone (IQ); 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ); 2-amino-3,4-dimethylimidazo[4,5-f]quinolone (MeIQx); 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx); and 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx) (Sugimura et al. 2004). Some HCAs can produce tumors in the colon, mammary glands, and prostate leading to the development of cancer (Sugimura et al. 2004; Goldman and Shields 2003).

Polycyclic Aromatic Hydrocarbons (PAH) Benzo(α)pyrene (BαP) is one of the best studied among more than 100 types of PAHs. B(α)P is isolated from coal tar in 1930 and shows tumor-inducing capacity in mouse skin (Rubin 2001). Human beings are exposed to PAHs from dietary sources and cigarette smoke. PAHs have been found particularly in charcoal-broiled, grilled, and smoked meats (Cross and Sinha 2004). However, the influence of PAH from the diet to CRC risk remains unclear because of the difficulties associated with quantification of individual intake.

N-Nitroso Compounds (NOC) These are among the most powerful chemical carcinogens, and so even small amounts in the human body could be hazardous (Cross and Sinha 2004). They are strong alkylating agents capable of causing mutation and subsequently leading to cancer. They are present in the diet containing a variety of cured meats and fish products (Goldman and Shields 2003). N-nitrosamines are formed on simultaneous ingestion of nitrite or nitrogen oxides and a nitrosatable substrate such as a secondary amine. Dietary N-nitrosamines have been linked to esophageal and other gastrointestinal cancers (Loh et al. 2011; Fritschi et al. 2015).

Mycotoxins They are toxic for animal and human health and are produced by molds as secondary metabolites (Bennett and Klich 2003). The toxic effect of mycotoxinsis referred to as mycotoxicosis, and its severity depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual, and possible synergistic effects of other chemicals to which the individual is exposed to (Peraica et al. 1999). Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds especially responsible for liver carcinoma (Goldman and Shields 2003). Aflatoxins are produced by two major *Aspergillus* species. *A. flavus*, produces only B aflatoxins, and *A. parasiticus* produces both B and G aflatoxins. Aflatoxins M1 and M2 are oxidative metabolic products of aflatoxins B1 and B2 (Peraica et al. 1999). Aflatoxins, except aflatoxin M1, are classified as Group 1 carcinogens.

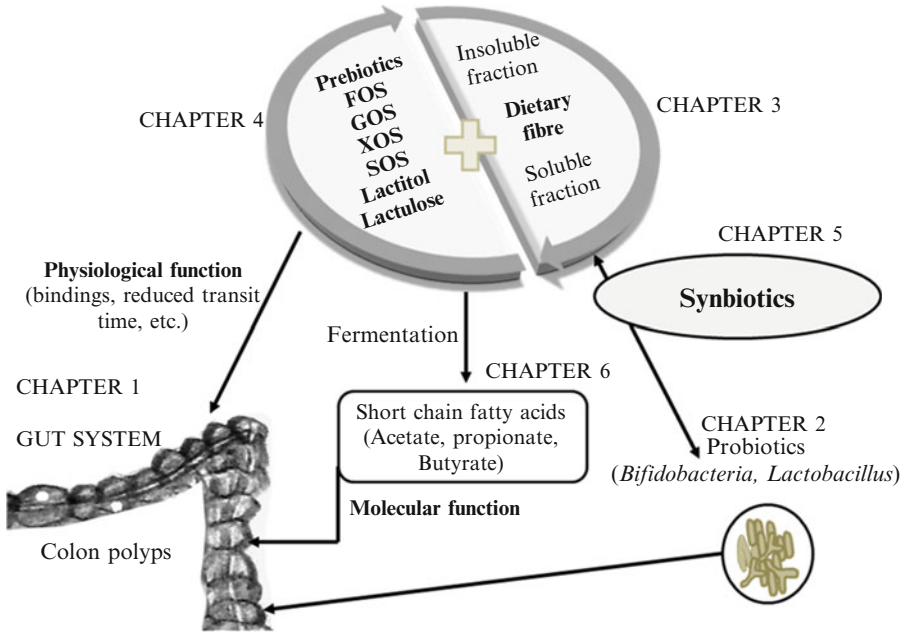

Fig. 1.2 Schematic diagram summarizing the various topics discussed in different chapters related to colorectal carcinogenesis and strategies to prevent its development

1.1.4.3 Origin of Colorectal Cancer

Intestinal stem cells (ISCs) are undifferentiated, multipotent, and self-renewable, and they are involved in tissue homeostasis and repair. They are located at the base of mucosal invaginations called crypts (Vaiopoulos et al. 2012). Each crypt comprises three main terminally differentiated cell lineages (enterocytes, goblet cells, and endocrine cells) that reside in the top third of the crypt, and possibly Paneth-like cells (Boman and Huang 2008; Medema and Vermeulen 2011). These cells are in continuous replacement process, as they constantly shed into the lumen when they become senescent (Papailiou et al. 2011). This process is regulated by stem cells and is under microenvironmental influence (Medema and Vermeulen 2011). The Wnt/β-catenin, Hedgehog (Hh), Notch, the phosphatidylinositol 3-kinase (PI3K)/Akt, and the bone morphogenetic proteins (BMPs) signaling pathways play a key role for the self-renewal of ISC. It is hypothesized that pathways important for control of the normal stem cell may be equally important for loss of control of the cancer stem cell (Davies et al. 2011). However, colon cancer stem cells (CSC) also contain a population of cells with a self-renewal capacity, which often give rise to more differentiated progeny, and are capable of driving or initiating tumor growth with highly tumorigenic and chemoresistant properties. Potential markers of colon CSCs have been proposed, including CD133, CD166, integrin β1, and signal transducers CD24, CD44, ALDH1, and LGR5.

As microenvironment plays an important role in cancer initiation, it is necessary to define and characterize regional niches within the intestine. Stem cells reside at the base of colon crypts and are likely to target the cancer-initiating mutations (Turner et al. 2013). In one study, microbial populations are identified in an intestinal-crypt-specific unique environment, adjacent to stem cells (Pédron et al. 2012). Hence, a detailed investigation of intestinal microbes relevant to colon stem cells is needed to avert cancer. To further understand the role of the gut microbiota in the initiation of CRC, it is important to identify and characterize those bacteria and profile the metabolic products generated by the microbiota and how diet influences this metabolite production (Turner et al. 2013).

1.1.4.4 Strategies to Decipher Gut Microbiota

Around a decade back, bacterial ecosystem analysis is based on classical cultivation method. With the rapid development of advanced molecular technologies such as PCR-denaturing gradient gel electrophoresis, it has been shown that the gut microbial ecosystem is far more complex than previously thought (Wang et al. 2015). Metagenomics and several next-generation sequencing technologies help us to better understand the complexity associated with gut microbiome. 16S rDNA sequence analysis and metagenomics are two effective DNA-sequencing approaches used to study uncultivated gut microbial communities. Further development of functional metagenomics and metatranscriptomics, metaproteomics and metabolomics complements the understanding of the human gut microbiome.

The subsequent chapters in the book describe how probiotics, synbiotics, and bioactive carbohydrates, namely prebiotics and dietary fibers, could modify and reduce the risk of developing CRC (Fig. 1.2). The benefits of functional foods have not been fully explored, and their mechanism of action has not been understood, with reference to diseases, including CRC.

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Probiotics and Colorectal Cancer

2

Abstract

Probiotics are living microbes that when taken in an adequate amount confers a health benefit to the host. Predominant probiotic microorganisms include *Lactobacillus* spp, *Bifidobacterium* spp., and *Enterococcus* spp. The yeast, *Saccharomyces boulardii*, and other bacterial species like *Bacillus* and *Clostridium butyricum* have been studied extensively. Several mechanisms have been proposed for probiosis, mainly attributed to their abilities to strengthen the intestinal barrier, to modulate the host immune system, and to produce antimicrobial compounds. The main therapeutic and health benefits of probiotics include prevention of diarrhea, hypercholesterolemia, and inflammatory bowel disease and improvement in lactose utilization and mineral absorption, anti-*Helicobacter pylori* activity, and anticancerous activity (ACA). The ACA of probiotic is mainly accredited to the (1) inactivation of cancerogenic compounds, (2) lowering of intestinal pH, (3) modulation and enhancement of the host's innate immunity through the secretion of anti-inflammatory molecules, (4) alteration of the intestinal microflora, (5) antiproliferative effects via regulation of apoptosis and cell differentiation, and (6) inhibition of tyrosine kinase signaling pathways. *Faecalibacterium prausnitzii* anti-inflammatory commensal could be used as a potential probiotic against colitis and CRC.

Keywords

Antimicrobial activity • Bifidobacteria • Lactobacilli • Anticancer activity • *Faecalibacterium prausnitzii* • Gut microbiota • Postbiotics • Probiotics

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2.1 Probiotics and Colorectal Cancer

2.1.1 History, Definition, and Statistics

2.1.1.1 History

The concept of probiotics was introduced more than 100 years back by Elie Metchnikoff, a Russian scientist who received a Noble prize for his work on phagocytosis (Metchnikoff 1907). He postulated that lactic acid bacteria (LAB) could offer health benefits capable of promoting longevity. He developed a diet with milk fermented with a bacterium, which he called as "Bulgarian bacillus." In 1906, Henry Tissier from Pasture Institute isolated bifidobacteria from breast-fed infants and reported the role of this Y-shaped organism in modulating and inhibiting pathogens in infants. In Japan, in early 1930s, Dr. Shirota, a physician, isolated *L. acidophilus* Shirota (subsequently named *L. casei* Shirota) from the intestine to develop fermented milk (Oozeer et al. 2006). A brief summary of the history of probiotic is listed in Table 2.1.

Table 2.1 Timeline of probiotics history

Year	Contribution	Reference
1908	Postulated that LAB offered health benefits capable of promoting longevity	Elie Metchnikoff
1899	First to isolate a *Bifidobacterium*	Henry Tissier
1917	Isolated a nonpathogenic strain of *Escherichia coli* from the feces of a First World War soldier who did not develop enterocolitis during a severe outbreak of shigellosis	Alfred Nissle
1965	First coined the word "probiotics" and defined as microbially derived factors that stimulate the growth of other organisms	Lilly and Stillwell
1974	Defined probiotics as "organisms and substances which contribute to intestinal microbial balance"	Parker
1989	Emphasized the requirement of viability for probiotics and redefined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"	Roy Fuller
2001	"live microorganisms which when administered in adequate amounts confer a health benefit on the host"	FAO/WHO
2014	"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"	International Scientific Association for Probiotics and Prebiotics (ISAPP)

2.1.1.2 Definitions

The term probiotic, Greek meaning "for life," was first coined by Lilly and Stillwell in 1965. In 1974, Parker defined the microorganisms and substances that contribute to the intestinal microbial balance as probiotics. More than 6,000 research papers have been published in the area of probiotics in the last decade, indicating the importance given to it by the scientific community. An Expert Committee defined the term "Probiotic," popularized by R. Fuller in 1989, as "Living microorganisms, which upon ingestion in certain numbers exert healthy benefits beyond inherent general nutrition." Ever since the 1990s, probiotics research has become an emerging field, which could be quantified in terms of exponential increase in publications. According to the United States National Food Ingredient Association (USNFIA), probiotics include bacteria, yeast, fungi and these are sources of direct-fed microbial of live naturally occurring microorganisms (Miles and Bootwalla 1991). In 2001, an Expert Consultation of international scientists working on behalf of the Food and Agriculture Organization of the United Nations (FAO) and the WHO redefined probiotics as live microorganisms that when administered in adequate amounts confer a health benefit upon the

host (FAO/WHO 2001). An expert panel was convened in October 2013 by the International Scientific Association for Probiotics and Prebiotics (ISAPP) to discuss the field of probiotics (Hill et al. 2014).

Most probiotic microorganisms include *Lactobacillus* spp. (de Vrese and Schrezmeir 2008), *Bifidobacterium* spp. (Turroni et al. 2014), and *Escherichia coli* Nissle (EcN) (Franz et al. 2011). The yeast *Saccharomyces boulardii* (Dinleyici et al. 2014) and bacterial species like *Bacillus* (Cutting 2011) and *Clostridium butyricum* (Takahashi et al. 2004) have been studied extensively.

2.1.2 Probiotics as Functional Food

Functional foods are designed to provide a specific health benefit, performance, and/or well-being extending beyond the provision of simple nutrients. Most widely known functional foods are carotenoids, dietary fibers, fatty acids, flavonoids, isothiocyanates, minerals, phenolic acids, plant stanols/sterols, prebiotics and probiotics, etc. (Hasler 2002). The majority of widely used probiotics includes species of lactobacilli, bifidobacteria, nonpathogenic *Escherichia coli*, bacilli, and yeasts, including *Saccharomyces boulardii*. The scientific and clinical evidence in support of the therapeutic potential of probiotic bacteria in human health has been increasing steadily (Balakrishnan and Floch 2012).

2.1.2.1 Lactobacilli

Lactobacilli are Gram-positive, nonspore-forming, nonmotile, catalase-negative, and rod-shaped fermentative bacteria with low G+C content belonging to the phylum Firmicutes (Herbel et al. 2013). They have complex nutritional requirements and are strictly fermentative, aerotolerant or anaerobic, aciduric or acidophilic (de Vrese and Schrezmeir 2008). Lactobacilli are predominant member of the LAB, encompass more than 25 species, and include a diverse range of organisms. They use lactose, glucose, and other sugar as carbon source and produce lactate via homofermentative metabolism. However, some lactobacilli are heterofermentative and produce alcohol in addition to lactic acid from sugars. These acid-producing bacteria can grow well at low pH environments and inhibit growth of other organisms. They are found in a variety of habitats, including human and animal mucosal membranes, on plants or material of plant origin, sewage, and fermented milk products.

2.1.3 Bifidobacteria

They are nonmotile, nonsporulating, pleomorphic rod-shaped fermentative bacteria with high G+C content belonging to the phylum Actinobacteria. Most strains are strictly anaerobic. Bifidobacteria were first isolated from the feces of a breast-fed infant and then named *Bacillus bifidus* (Tissier 1900). Due to the close resemblance to properties of lactobacilli, they are classified as members of the genus *Lactobacillus* during a large part of the 20th century. In the recent past, they have been reclassified and recognized as a different genus. They are found in human milk and first to be colonized in the gut of a baby (Arboleya et al. 2011). They are a major part of the normal human gut microbiota throughout life. They appear in the stools of a baby a few days after birth and increase in number thereafter. The number of bifidobacteria in the colon of adults is 10^{10}–10^{11} cfu/g, but this number decreases with age.

2.1.3.1 Enterococcus

Enterococci are Gram-positive, nonspore-forming, catalase-negative, oxidase-negative, facultative anaerobic cocci that occur singly, in pairs, or in chains and also belong to the group of LAB of the phylum Firmicutes (Foulquié Moreno et al. 2006). They play an important role in the ripening of cheeses, probably through proteolysis, lipolysis, and citrate breakdown, hence contributing to taste and flavor. They are also present in other fermented foods, such as sausages and olives. Furthermore, the production of bacteriocins by enterococci is well documented. However, their role in these products has not been fully elucidated. Commercial enterococcal probiotics

include *E. faecium* SF68® (NCIMB 10415, produced by Cerbios-Pharma SA, Barbengo, Switzerland) and *E. faecalis* Symbioflor 1 (SymbioPharm, Herborn, Germany). Both strains are produced in the form of a pharmaceutical preparation. However, safety of enterococci is a concern, as they are known to be opportunistic pathogens and are a prevalent cause of nosocomial infections that cause bacteremia, endocarditis, and other infections. Several virulence factors have been mentioned for this species, and the number of vancomycin-resistant enterococci is increasing with time (Franz et al. 2011).

2.1.3.2 *E. coli* Nissle 1917 (EcN)

It is a nonpathogenic strain of the Enterobacteriaceae family that has probiotic properties. It was isolated from a healthy soldier during a *Shigella* outbreak in 1917 with a hypothesis that commensal strain could protect from presumably infectious gastroenteritis (Nissle 1959). Since then, EcN has had a long history of use in clinical settings to treat gastrointestinal disorders. However, the molecular mechanism of its beneficial activity is not well understood (Schultz 2008).

2.1.3.3 *Faecalibacterium prausnitzii*

Faecalibacterium prausnitzii (FP)is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients (Sokol et al. 2008). Its anti-inflammatory effects are tested on cellular and TNBS colitis models and are partly due to the secreted metabolites that are able to block NF-κB activation and IL-8 production (Qiu et al. 2013; Martín et al. 2014). These results suggest that counterbalancing dysbiosis using FP as a probiotic is a promising strategy in CD treatment. They also produce butyrate, which helps in keeping colonic cells healthy, and so reduce the risk of disease such as CRC and IBD.

2.1.4 Selection Criteria for Probiotic Organism

To select potential and safe probiotic strains, FAO/WHO in 2002 set guidelines for their evaluation in food (FAO/WHO 2002). The functional requirements of probiotics should be established by using in vitro methods, and the results of these studies should be reflected in controlled human studies. Probiotic strains should (1) preferably be of human origin for human use; (2) be robust in terms of acid and bile tolerance; (3) adhere to the epithelial surface of humans and persist in the GI tract; (4) survive action of toxic metabolites, primarily phenols produced during the digestion process, as well as antibiotics and phage; grow in anaerobic and storage conditions of the food carrier; (5) resist against degradation by digestive enzymes present in the intestine (e.g., lysozymes); and (6) must be safe for use.

2.1.5 Probiotics as Health Promoter

Postbiotics are nonviable bacterial products or metabolic by-products from probiotic microorganisms that have biologic activity in the host and are an important attribute of probiosis (Patel and Denning 2013; Cicenia et al. 2014). Several mechanisms have been proposed for probiosis which is mainly attributed to their abilities to fortify the intestinal barrier, to modulate the host immune system, and to produce antimicrobial molecules (Dobson et al. 2012). In vitro and in vivo studies indicate that probiotics provide several beneficial effects to host (Fig. 2.1) (Uccello et al. 2012; Raman et al. 2013; Baboota et al. 2013; Liévin-Le Moal and Servin 2014). Each of these benefits will be discussed in the subsequent sections.

2.1.5.1 Antimicrobial Activity (AMA)

Probiotic must survive and at least transiently colonize and provide protection against pathogens in the intestinal tract of the host (Šušković et al. 2010). The role of AMA of probiotics against human and food spoilage organism is crucial, well documented, and critically reviewed elsewhere (Liévin-Le Moal and Servin 2014). The ability of lactobacilli to prevent and treat the urinary tract infection (UTI) has long been observed and is now supported by some clinical evidence (Reid et al. 2009). Several mechanisms

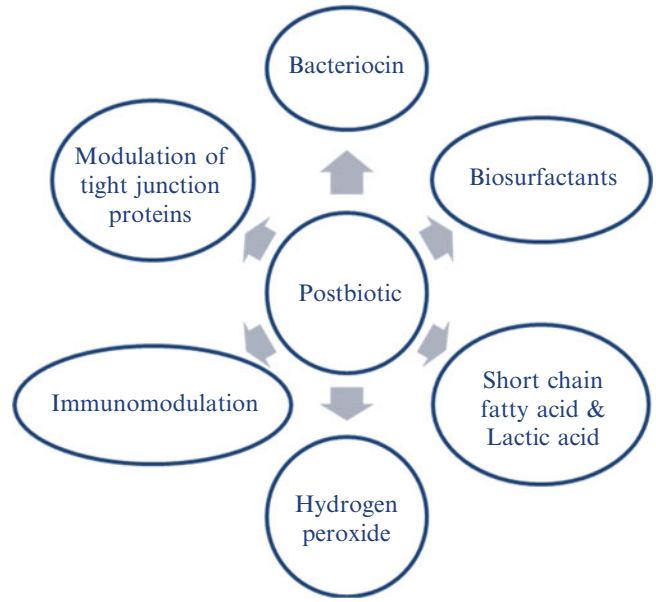

Fig. 2.1 Postbiotics produced by probiotic strains

have been postulated for AMA of postbiotics, which include presence of antiadhesion factors, production of hydrogen peroxide and bacteriocins, and modulation of immune or signaling systems (Fig. 2.1) (Reid et al. 2011; Liévin-Le Moal and Servin 2014; Cicenia et al. 2014).

2.1.5.2 Production of Acids

Probiotic produces short-chain fatty acids and lactic acids as end products, which provide an acidic environment unfavorable for the growth of many pathogenic and food spoilage microorganisms. Acids are generally thought to exert their AMA by interfering with the maintenance of cell membrane potential, inhibiting an active transport, reducing intracellular pH, and inhibiting a variety of metabolic functions (Lorca and de Valdez 2009; Šušković et al. 2010). A detailed discussion of these is carried out in chapter 6.

2.1.5.3 Hydrogen Peroxides

AMA of hydrogen peroxide is ascribed to its strong oxidizing effect on the bacterial cell and destroy basic molecular structure of the cellular proteins. Hydrogen peroxide could protect against urogenital infections, especially in the case of bacterial vaginosis. It is more effective as a sporicidal than as a bactericide. H_2O_2-producing lactobacilli might also exert control over vaginal cancer through specific interactions of reactive oxygen species, such as superoxide anion, hydroxyl radicals, and hypochlorous acid (Bauer 2001). Lactobacilli exhibit self-protection against toxic activity of H_2O_2 through the production of Fe^{3+}-activated extracellular peroxidase (Martín and Suárez 2010).

2.1.5.4 Production of Bacteriocin

Bacteriocins are ribosomally synthesized antimicrobial compounds that are produced by all major lineage of bacteria and archaea, including many members of the LAB, and are a heterogeneous group of peptides with respect to their size, structure, mode of action, antimicrobial potency, immunity mechanism, and target cell receptors (Dobson et al. 2012). Bacteriocins are divided into four main classes (Fig. 2.2). Three classes of bacteriocins in lactobacilli have been defined by Klaenhammer (1993). Later, a fourth class of "complex bacteriocins" was added to the list. Bacteriocins as therapeutics could be viable alternatives to antibiotics because of their (1) potency based on in vitro and in vivo assays, (2) low toxicity, (3) the availability of both broad- and narrow-spectrum peptides, (4) the possibility

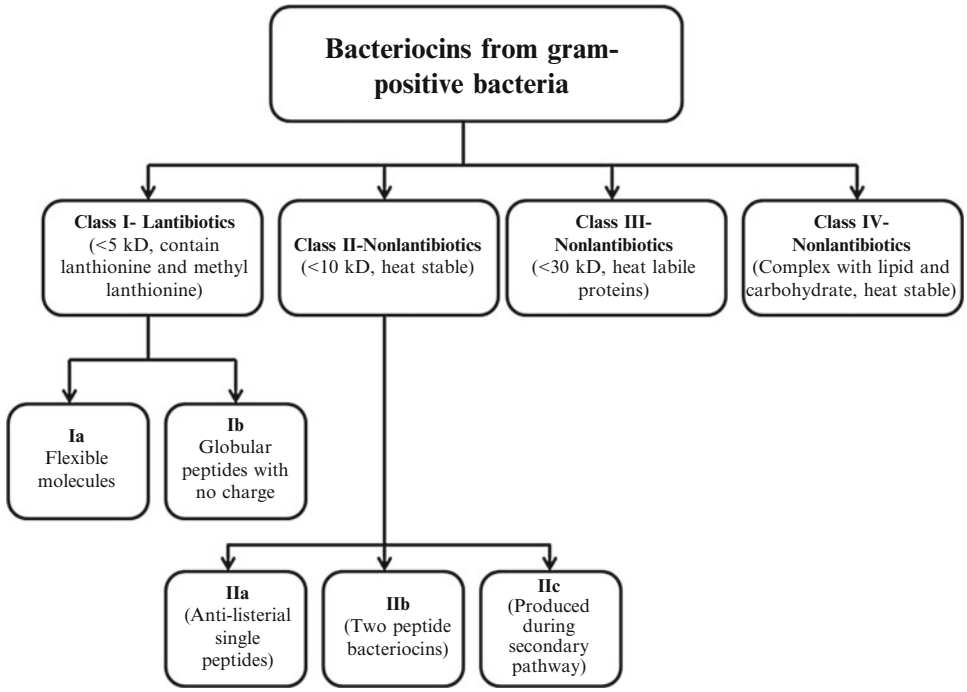

Fig. 2.2 Classification of bacteriocins from Gram-positive bacteria

of in situ production by probiotics, and (5) the fact that they can be bioengineered (Cotter et al. 2013). Corr et al. (2007) showed that the bacteriocin Abp118 produced by *Lactobacillus salivarius* UCC 118 was essential for protecting the host against infection by invasive *Listeria monocytogenes*. Results suggest that secretory antimicrobial proteins other than lactic acid and hydrogen peroxide produced by LAB exhibit a broad range of activity against Gram-positive and Gram-negative organisms (Liévin-Le Moal and Servin 2014). However, the overall AMA of probiotics is multifactorial and is due to the synergistic action of lactic acid and proteinaceous substances (Servin 2004)

2.1.5.5 Biosurfactant

Biosurfactants are "surface-active" compounds that are synthesized by microorganisms and may have a role in the restoration and maintenance of microbial homeostasis (Reid et al. 2011). Biosurfactants produced by probiotics can serve as antiadhesive and antimicrobial agents and a defense weapon against other colonizing strains in the urogenital and gastrointestinal tracts (Gudiña et al. 2010). However, detailed and extensive studies are needed to characterize them produced from probiotic strains.

2.1.5.6 Modulation of Tight Junction Proteins

Probiotics are capable of modulating the permeability of epithelial barriers, changing the inflammatory potential of epithelial cells, or directly modulating the activity of immune cells (Ulluwishewa et al. 2011; Caricilli et al. 2014).

2.1.5.7 Immunomodulation

The gut microbiota protects the host by priming the immunological defense mechanism (Weng and Walker 2013). The effects of few selected LAB on the immune system are listed in Table 2.2. The probiotic strains exhibit potentially useful properties, including anti-inflammatory effects, improvement of mucosal barrier homeostasis, beneficial effects on intestinal microbiota, and a reduction of visceral hypersensitivity (Barbara et al. 2012).

Table 2.2 Immodulatory activity exerted by selected strains of probiotics

Effect	Strains	Reference
Enhancement and maintenance of tight junction formation	*B. infantis*	Ewaschuk et al. (2008)
	B. bifidum	Khailova et al. (2009)
	Probiotic mixture VSL#3	Mennigen et al. (2009)
	E. coli Nissle 1917	Ukena et al. (2007)
	L. plantarum WCFS	Karczewski et al. (2010)
Production of secretory IgA	*L. acidophilus*	Torii et al. (2007)
	L. casei	de Moreno de Leblanc et al. (2011)
Transcription of NF-κB	*L. acidophilus*	Chen et al. (2013)
	L. acidophilus, *L. casei*, and *L. rhamnosus*	van Baarlen et al. (2011)
Induction of proinflammatory cytokines	*Lactobacillus* strains	Maassen et al. (2000)
	L. acidophilus strain L-92	Torii et al. (2007)
	L. rhamnosus GR-1	Li et al. (2014)
Induction of anti-inflammatory cytokines	*L. reuteri* and *L. casei*	Smits et al. (2005)
	Streptococcus salivarius	Kaci et al. (2014)
Regulatory cells (Treg)	*B. infantis* 35624	O'Mahony et al. (2008)
Bacterial DNA induce TLR9 signaling → anti-inflammatory effect in murine colitis	Probiotic mixture VSL#3	Lammers et al. (2003)
Elevation of serum level of IL-10	Probiotic mixture VSL#3	Hart et al. (2004)
Induction of maturation of dendritic cells (DC)	*L. reuteri* and *L. casei*	Smits et al. (2005)
Enhancement of immunoreactivity of spleen cells and phagocytes and serum antibody response to orally and systemically administered antigens	*L. casei* and *L. bulgaricus*	Perdigon et al. (1986)
	L. casei or *L. acidophilus*	Perdigón et al. (1988)
Activation of the gene for human beta-defensin 2 in intestinal mucosa		Ganz (2003)

2.1.6 Probiotics and Gut Microbiota

The gut microbiota is a highly specialized organ containing host-specific assemblages of microbes whereby metabolic activity directly impacts human health and disease (Bocci 1992; O'Hara and Shanahan 2006). They live in harmony with the gut of the host and help to maintain the integrity of the gastrointestinal tract (GIT) and other organs, namely, lungs, heart, brain, and liver (Kau et al. 2011). Dysbiosis, perturbations in the composition of gut microbiota, may disrupt the host-microbes interaction and eventually may contribute to disease susceptibility. Probiotics could positively manipulate the composition and the function of the gut microbiome, mainly by executing any of the mechanisms of probiosis discussed earlier (Hemarajata and Versalovic 2013). They also upregulate the integrity of the intestinal barrier, which may result in the maintenance of immune tolerance, modulate the intestinal immunity, decrease the translocation of bacteria across the intestinal mucosa, and reduce the disease phenotypes such as GIT infections IBS and IBD (Caricilli et al. 2014). Different tools such as culture-dependent methods, metagenomic sequencing, functional metagenomics and metatranscriptomics, metaproteomics and metabolomics are used to study the effect of probiotics on the composition, diversity, and functioning of the gut microbiota (Wang et al. 2015).

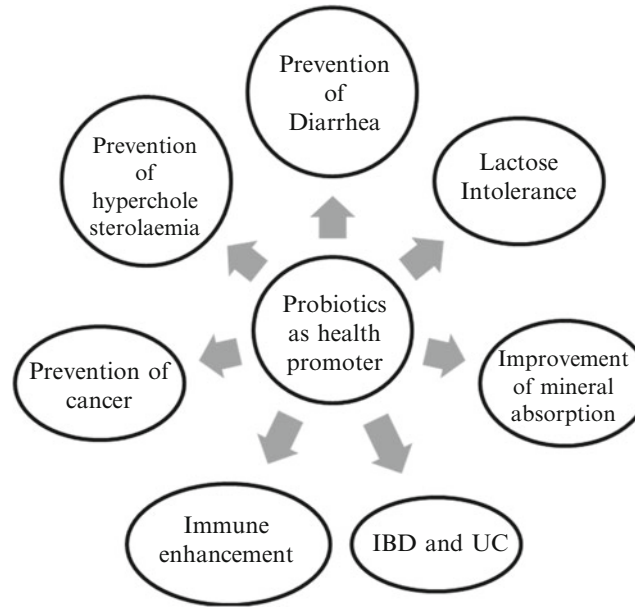

Fig. 2.3 Probiotics as health promoter. Proposed benefits of consuming probiotic microorganisms

2.1.7 Probiotics and Health

The main therapeutic and health benefits of probiotics are summarized in the Fig. 2.3.

2.1.7.1 Prevention of Diarrhea

Diarrhea (Greek διαρροια = flowing through) means the increased liquidity or decreased consistency of stools. It is usually associated with an increased frequency of stools and an increased fecal weight. WHO defines diarrhea as three or more watery stools on two or more consecutive days (de Vrese and Marteau 2007). Although probiotics have a major influence on the gastrointestinal flora composition and exhaustive research being done on the prevention and treatment of acute gastroenteritis, antibiotic-associated diarrhea (AAD), traveler's diarrhea in infants, the reports on their clear benefits on pediatric disorders remain unclear (Vandenplas et al. 2013). However, data support the use of probiotics for the adjunct treatment of acute viral gastroenteritis and for the prevention of gastrointestinal diseases (Weichert et al. 2012).

A growing number of research publications indicate that some probiotic strains may help to maintain the health in old people, suggesting both health and cost-saving benefits in offering fermented dairy products. These benefits include establishment of a balanced intestinal microflora and resistance and/or prevention of diarrhea (Malaguarnera et al. 2012).

2.1.7.2 Prevention of Hypercholesterolemia

Hypercholesterolemia is one of the major risk factors for cardiovascular disease, which is the leading cause of death in many countries, including India. Even a 1 % reduction in the serum cholesterol reduces the risk of CHD by 2–3 % (Manson et al. 1992). The development of new dietary supplements to reduce serum cholesterol has gained plenty of attention. Reduction of cholesterol by probiotics has been demonstrated in humans, mice, and pigs (Kawase et al. 2000; Haberer et al. 2003; Sadrzadeh-Yeganeh et al. 2010). Cholesterol-lowering activities of probiotics are mainly attributed to the assimilation of cholesterol by growing cells, binding of cholesterol to the cellular surface, incorporation of cholesterol into the cellular membrane, deconjugation of bile via bile salt hydrolase, coprecipitation of cholesterol with deconjugated bile, binding of bile by fiber,, and production of short-chain fatty acids by oligosaccharides (Ooi and Liong 2010; Bordoni et al. 2013). However, more clinical evidence is lacking to strengthen this hypothesis.

2.1.7.3 Improvement in Lactose Utilization

Lactose intolerance is due to the insufficient activity of lactase in the human gut that causes various degrees of abdominal discomfort. Probiotics, including lactobacilli and bifidobacteria, produce β-D-galactosidase, which auto digests lactose and improves lactose tolerance (de Vrese and Schrezenmeir 2008).

2.1.7.4 Anti-*Helicobacter Pylori* Activity

Helicobacterpylori infects over 50 % of the population worldwide, and in developing countries 80 % of the middle-aged adults may be infected (De Falco et al. 2015). It is associated with gastritis, gastric and duodenal ulcers, and gastric malignancies. Its eradication would eliminate a major worldwide cause of cancer death (Graham 2015). Currently, no vaccine is available for *H. pylori* and antibiotic resistance is increasing. So the need of the hour is to identify alternative treatment strategies. Results obtained from in vitro and preclinical studies of using probiotics for *H. pylori* reveal the feasibility of this approach (Felley and Michetti 2003; Ruggiero 2014). Several clinical trials indicate that administration of probiotics can reduce the side effects of *H. pylori* eradication treatment, increase tolerability, and often increase the overall efficacy.

2.1.8 Improvement of Mineral Absorption

Probiotics show a positive influence on the bone metabolism and bone mass density and is mainly attributed to the increased mineral solubility due to the production of short-chain fatty acids, production of phytase enzyme by the bacteria to overcome the effect of mineral depressed by phytate, reduction of intestinal inflammation followed by increase in the bone mass density and hydrolysis of glycoside bond in the food in the intestines by gut microbiota (Parvaneh et al. 2014). However, detailed studies are needed to validate the results from animal model.

2.1.8.1 Inflammatory Bowel Disease (IBD)

IBD is an idiopathic chronic condition of the GIT characterized by intermittent periods of inflammation and remission (Nanau and Neuman 2012). It is divided into two subtypes: ulcerative colitis (UC) and Crohn's disease (CD). UC and CD differ based on the localization in the intestine and features of the inflammation. CD is often described as a prototype of T-helper 1-type disease. It may affect a portion of the intestine in a segmental fashion and present a transmural inflammation, extending along the entire intestinal wall. UC (T-helper 2-mediated condition) is a diffuse and a continuous inflammation and is confined to the mucosa of the colon. Microscopic lesions are present only in the mucosa layer for UC, while it affects the full thickness of the intestinal wall in CD (Nanau and Neuman 2012). The etiopathogenesis of IBD may be multifactorial, as is the pathophysiology. It is attributed to the alterations in the gastrointestinal motility, visceral hypersensitivity, intestinal microbiota, loss of epithelial cell barrier function, overexpression of proinflammatory mediators in different effector T lymphocyte subsets (Th1 and Th17, Th2), deficient protective and regulatory signals and/or abnormal antigen presentation, dysfunction of the brain-gut axis or certain psychosocial factors (Dai et al. 2013). Probiotics offer an alternative by altering the intestinal flora by (1) competitive exclusion, whereby probiotics compete with the microbial pathogens for a limited number of receptors present on the surface of the epithelium; (2) immunomodulation and/or stimulation of an immune response of gut-associated lymphoid and epithelial cells; (3) antimicrobial activity and suppression of pathogen growth; (4) enhancement of barrier function; and (5) induction of T cell apoptosis in the mucosal immune compartment (Sheil et al. 2007).

2.1.8.2 Anticancerous Activity

CRC arises by a well-defined series of sequences caused by mutations, activations, and deletions of oncogenes and tumor suppressor genes leading to histological changes (the adenoma-carcinoma sequence) (Commane et al. 2005). It is likely that

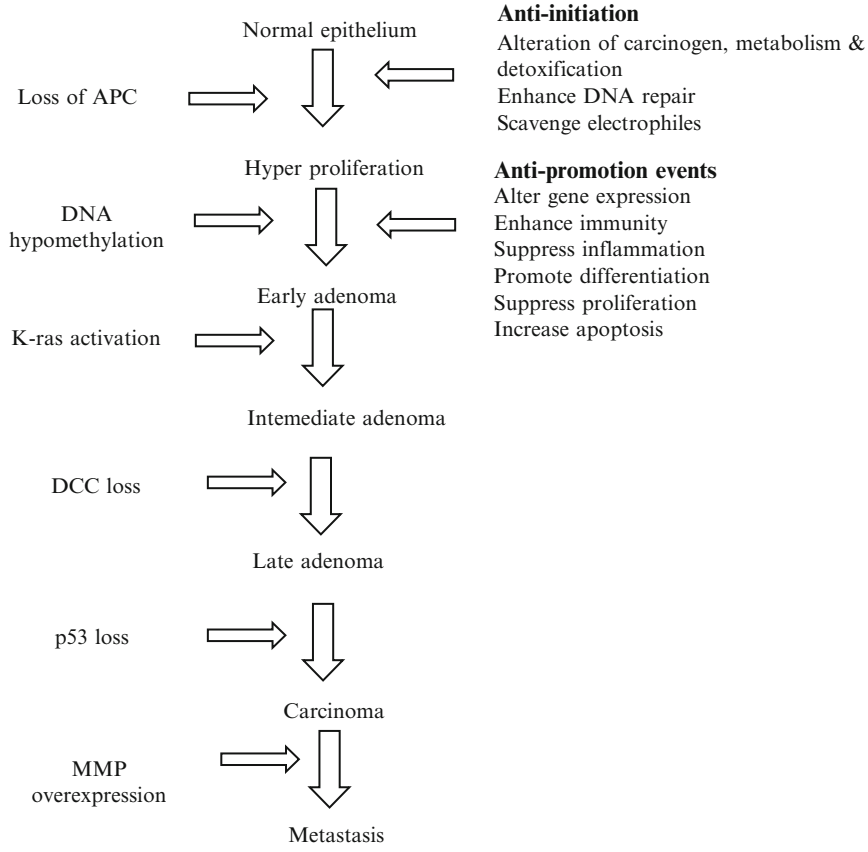

Fig. 2.4 Sequential steps involved in the development of CRC. Probiotics could act on various steps as shown in the *boxes* (Commane et al. 2005)

different probiotic strains may exert effects at different stages of carcinogenesis (Fig. 2.4) (Table 2.3).

The ACA of probiotic is mainly accredited to (1) inactivation of cancerogenic compounds, (2) lowering of intestinal pH, (3) immunomodulation, (4) alteration of the intestinal microflora, (5) regulation of apoptosis and cell differentiation, and (6) inhibition of tyrosine kinase signaling pathways (Uccello et al. 2012; Raman et al. 2013). These have been discussed in detail in section 2.5.

Antimutagenesis is a phenomenon in which mutation process is inhibited by the natural or synthetic compounds leading to a decrease in the levels of spontaneous and induced mutations (Bhattacharya 2011). Antimutagens are divided into two subgroups: (1) dysmutagens, which affect the extracellular modification of the mutagens (thereby preventing DNA damage), and (2) bioantimutagens, which decrease the mutation rate by virtue of their involvement in the cellular processes.

It is well known that probiotic strains bind and reduce the activity of the mutagens. Binding of mutagen is dependent on different factors such as presence of peptidoglycans, polysaccharides and secretory glycoproteins in the strains; the growth phase of the organism; and mutagen type (Raman et al. 2013).

2.1.9 Mechanism of Action of Probiotics on Colorectal Cancer

This section focuses on the role of probiotics in preventing colon cancer. Mechanism of ACA of probiotics is ascribed to the following:

Table 2.3 Anticancerous activity of probiotics by modulating immune response

Model	Effect	Strains	Reference
Methylcholanthracene-induced tumor in mice	Host innate immunity by stimulating the spleenic NK cell activity	*L. casei* strain, Shirota (LcS)	Takagi et al. (2001)
Mouse model	Improves the immune system	Intrapleural injection of an inactivated strain of LcS	Yasutake et al. (1999)
Mouse model	Potent immunomodulatory effects by upregulating or potentiating the generation of Tregs by tolerogenic DCs in mesenteric lymph nodes (MLN)	Probiotics mixture, designated IRT5	Kwon et al. (2010)
Mouse model	Downregulated expression of CXCR4 mRNA and MHC class I, as well as increasing apoptosis in tumor tissue	*Lactobacillus acidophilus*	Chen et al. (2012)
Mouse model	Activation of dendritic cells and IFN-γ production	*Enterococcus faecalis* CECT7121	Molina et al. (2015)

1. Inactivation of carcinogenic compounds
2. Alteration of the intestinal microflora
3. Lowering of intestinal pH
4. Immunomodulation
5. Regulation of apoptosis and cell differentiation
6. Inhibition of tyrosine kinase signaling pathways

2.1.10 Inactivation of Mutagenic and Carcinogenic Compounds

Potential probiotic strains bind the mutagen through the cell surface and peptidoglycans (sugar and protein moieties) and exert antimutagenic activity and ACA (Vorobjeva and Abilev 2002; Raman et al. 2013) (Table 2.4). Probiotic bacteria, along with dietary ingredients, help in detoxification and biotransformation of procarcinogens and carcinogens into less toxic metabolites, thus preventing tumor formation (Pool-Zobel et al. 2005). Biotransformation of mutagens/carcinogens occurs in the gut with the help of phase I and phase II type of enzymes. These regulate the toxic, mutagenic, and neoplastic effects of environmental carcinogens. Phase I enzymes cause bioactivation, and phase II enzymes cause the inactivation of mutagens/carcinogens (Hammons and Lyn-Cook 2004). *Lactobacillus* strains could help in reducing the load of mutagens by exhibiting antigenotoxicity and activity (Table 2.4).

The probiotic human strain *L. rhamnosus* 231 (Lr 231) binds to MNNG and MeIQx, leading to their biotransformation and subsequent detoxification (Ambalam et al. 2011). Administration of viable Lr 231 protects rats from MNNG-induced colon inflammation (Gosai et al. 2011).

2.1.10.1 Alteration of the Intestinal Microflora

The GIT, particularly the colon, is denselypopulated with bacteria containing commensal and pathogenic bacteria. Inflammation in GIT results in the enrichment of certain bacterial groups with procarcinogenic traits, including *Fusobacterium* spp., *Streptococcus gallolyticus* subsp. *gallolyticus* (formerly known as *Streptococcus bovis* biotype 1), enterotoxic *B. fragilis* and adherent-invasive *E. coli* (Louis et al. 2014). A diet rich in animal fat and red meat stimulates the growth of secondary bile salt-producing and sulfate-reducing bacteria, respectively. Gut bacteria are important contributors to bile acid metabolism and thus may play a role in the biology linking of bile acids to CRC (Sears and Garrett 2014). Commensal microbes may act by inhibiting fecal putrefactive bacteria, such as coliforms, mainly

Table 2.4 Consolidated table on antimutagenic activity of probiotics

Bacteria	Mechanism involved	Mutagen used	Reference
Streptococcus cremoris Z-25, *L. acidophilus* IFO13951, and *B. bifidum* IF014252	Cellular fraction and cell wall	Heterocyclic amines	Zhang and Ohta (1991)
L. helveticus	Release of peptides in the fermented milk	4-nitroquinoline-*N*'-oxide	Matar et al. (1997)
Four strains of *L. gasseri* and *B. longum*	Binding was dependent on the mutagen, pH, bacterial strain, and the complex cell wall structure	Trp-P-1, Trp-P-2, Glu-P-1, IQ, and MeIQ	Sreekumar and Hosono (1998a, b)
Bacillus subtilis-fermented soybean product – Natto	Dependent on the bacterial strain, mutagen, pH, incubation time, presence of metal ions, sodium chloride and alcohol, enzymes, and acetylation	HCA Trp-P-1 and IQ	Rajendran and Ohta (2001)
L. rhamnosus GG	Cell wall carbohydrate components' hydrophobic and electrostatic interactions	Alatoxin B_1 (AFB_1)	Haskard et al. (2000)
Lactobacilli and bifidobacteria were found reversible	Bacterial cell wall or components	AFB_1	Peltonen et al. (2001)
L. plantarum KLAB21 (Kimchi, a Korean fermented food)	Three secretory glycoproteins (16, 11, and 14 kD)	*N*-methyl-*N*'-nitro-N-nitrosoguanidine (MNNG), NQO, 4-nitro-*O*-*phenylenediamine* (NPD) and AFB_1	Rhee and Park (2001a)
Soymilk fermented with *S. thermophilus*, *L. acidophilus*, *B. infantis*, *B. longum*	Antimutagenic molecules during bacterial fermentation of milk	3,2-dimethyl-4-amino-biphenyl (DMABP)	Hsieh and Chou (2006)
L. plantarum KLAB21	Culture-free supernatants	MNNG, NQO, 4-nitro-*O*-*phenylenediamine* (NPD), and AFB_1	Rhee and Park (2001b)
Eight LAB species	Whole cell	AαC, PhIP, IQ, MeIQx, DiMeIQx	Stidl et al. (2008)
Three *B. longum*	Fermented skim milk	Trp-P-1 and Trp-P-2	Sreekumar and Hosono (1998a, b)
B. longum PS+	Polysaccharides	Trp-P-1	Sreekumar and Hosono (1998a, b)
Lactobacilli and bifidobacteria	Butyrate production	HCA	Lankaputhra and Shah (1998)
L. plantarum 301102	Exopolysaccharides (EPS)	Trp-P-1	Tsuda et al. (2008)
LAB and *B. adolescentis* ATCC 15703	Growth-dependent extracellular bioactive compounds	B (α) P and sodium azide	Chalova et al. (2008)
Lactobacillus strains	Biotransformation of mutagens/carcinogens	4-NQO and MNNG	Cenci et al. (2002); Caldini et al. (2005)
L. casei DN 114001	Metabolism of mutagens	IQ, PhIP and MeIQx	Nowak and Libudzisz (2009)
L. rhamnosus 231	Binding and biotransformation	MNNG, NQo, MeIQx	Ambalam et al. (2011)
L. rhamnosus IMC 501	Binding and biotransformation	4-NQO	Verdenelli et al. (2010)

by adhering to enterocytes and producing SCFA. The enteric flora modification in interleukin-10 knockout mice by probiotic *L. salivarius* UCC118 results in a reduced prevalence of colon cancer (O'Mahony et al. 2001). Thus, probiotics may counteract CRC development also through a mechanism of competition with pathogenic intestinal microbiota. *Faecalibacterium prausnitzii* (FP) is an anti-inflammatory commensal that may be useful as probiotic to reduce colitis and CRC.

Probiotic strains, namely bifidobacteria and lactobacilli, downregulate the expression of xenobiotic-metabolizing enzymes that mediate carcinogenesis through various enzymes, such as β-glucuronidase, azoreductase, and nitroreductase, and decrease the risk of tumor development (Wollowski et al. 2001). SCFAs produced as metabolites of probiotic metabolism could inhibit the generation of carcinogenic products from procarcinogens by lowering the enzyme levels (Roy et al. 2006).

2.1.10.2 Lowering of Intestinal pH

SCFA and lactic acid produced as metabolite by probiotic bacteria decreases the load of pathobionts and help in maintaining homeostasis by lowering the intestinal pH (Roy et al. 2006). They also help in lowering the solubility of bile acids and ammonia absorption and increase mineral absorption (Cook and Sellin 1998). Butyrate is the preferred energy source of colonocytes and has been implicated in the control of the machinery regulating apoptosis and cellular differentiation (Canani et al. 2011; Donohoe et al. 2011).

2.1.10.3 Immunomodulation

Recent studies have shown that an initial colonization by a sufficiently diverse microbiota is essential for proper development and regulation of the innate and adaptive immune system (van Baarlen et al. 2013). Altered gut microbiota may lead to dysbiosis and loss of metabolic and immune homeostasis. Probiotics can be used to manipulate gut microbiota and maintain immune homeostasis. Dendritic cells (DCs) and natural killer (NK) cells are important during the early defense against cancer (Fernandez et al. 1999). Probiotics may regulate myeloid DC maturation, polarizing the subsequent T-cell activity toward Th1, Th2, or even T-reg responses (Stagg et al. 2004). This desmutagenic potential of probiotics has been recently addressed, and it is now evident that probiotic strains that induced IL-12 help in the maturation of human monocyte-derived DCs, blood DCs, mouse spleenic cells, and lymph node DCs, which in turn helps in the activation of NK cells to produce IFN-γ (Rizzello et al. 2011). Hu et al. (2015) reported that *L. plantarum* can enhance the anti-tumour immune response and delay tumour formation. Animal studies show probiotics has the potential to prevent CRC by modulating the host immune system.

2.1.10.4 Regulation of Apoptosis and Cell Differentiation

Apoptosis is a form of genetically programmed cell death, which plays a key role in the regulation of cell numbers (Zhong et al. 2014). The decreased ability to trigger apoptosis leads to alteration in the regulation of cell proliferation and, subsequently, an important pathogenic event associated with many cancers. However, regulating the cell survival and death at molecular level in the apoptotic process could provide an avenue for chemoprevention and could hold therapeutic potential. Probiotic could play an important role in the regulation of cell apoptosis via intrinsic and extrinsic pathways and facilitate the prevention of CRC (Uccello et al. 2012). There are studies supporting the evidence that probiotics may exert anti-CRC activity through apoptosis-mediated signaling pathways (Table 2.5).

2.1.10.5 Inhibition of Tyrosine Kinase Signaling Pathway

Tyrosine kinase signaling pathway plays a central role in controlling cellular differentiation and proliferation and is tightly controlled and regulated. Deregulation in this pathway leads to the development of cancer. Since these effects are initiated by the receptor, tyrosine kinase activation, they are the key targets for inhibitors (Arora and Scholar 2005). p40, a *Lactobacillus rhamnosus* GG (LGG)-derived soluble protein, ameliorates intestinal injury and colitis, reduces apoptosis, and preserves barrier function by transactivation of the EGF receptor (EGFR) in intestinal epithelial cells (Yan et al. 2013).

Table 2.5 Antiproliferative effects of probiotics via regulation of apoptosis and cell differentiation

	Bacterial strain	Mechanism	Reference
In vivo model			
Rat Model	Synbiotic combination of resistant starch and *Bifidobacterium lactis*	Proapoptotic action due to the carcinogen, AOM	Le Leu et al. (2005)
			Le Leu et al. (2010)
Mice model	*Lactobacillus acidophilus*	Severity of the colorectal carcinogenesis was minimized and induced apoptosis	Chen et al. (2012)
In vitro model			
Human cancer cell lines (AGS, HeLa, MCF-7 and HT-29)	*Enterococcus faecalis*	Induced apoptosis via secretory protein	Nami et al. (2014)
Caco-2 cells	*E. coli* Nissle 1917 commensal *E. coli* K12	Lower inflammasome activation and secretory metabolites	Becker et al. (2014)
	Lactobacillus reuteri	Proliferation due to COX-2, cyclin D1 and cell survival due to Bcl-2, Bcl-xL	Iyer et al. (2008)
		Enhance MAPK activities	
Colo320 and SW480 intestinal epithelial cells	VSL#3	Suppress the COX-2 expression	Otte et al. (2009)
HT-29 colon cancer cells	Exopolysaccharides of *L. acidophilus* and *L. rhamnosus*	Autophagic cell death activated due to induction of Beclin-1, GRP78, Bcl-2, and Bak	Kim et al. (2010)
Colorectal carcinoma cell line LS513	*L. acidophilus* and *L. casei*	Enhanced the apoptosis-induction capacity of 5-fluorouracil	Baldwin et al. (2010)
HT-29 cells	Milks fermented by *Lactobacillus helveticus*, Bifidobacterium, *L. acidophilus* or a mix of *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*	10–50 % decrease cell confluency	Baricault et al. (1995)
Human multidrug-resistant (MDR) myeloid leukemia (HL60/AR) cells	*Lactobacillus kefiri* P-IF	Induction of apoptosis was associated with activation of caspase 3, decreased expression of Bcl-2, and decreased polarization of mitochondrial membrane potential	Ghoneum and Gimzewski (2014)
HT29, AGS, MCF-7, and HeLa, as well as a normal human cell line	*Lactobacillus plantarum* 15HN and *Lactococcus lactis* sub sp. *Lactis* 44Lac	Apoptosis is the main cytotoxic mechanism for secreted metabolites	Haghshenas et al. (2014)
CaCo-2 cells	*L. acidophilus* and *L. casei*	Reduced cell proliferation and increased cell apoptosis	Soltan et al. (2015)

Probiotic *Bacillus polyfermenticus* suppresses colon cancer cell growth in vitro and in vivo. The ACA is partially attributed to the downregulation of receptors ErbB2 and ErbB3 required for tumor development (Ma et al. 2010).

Saccharomyces boulardii regulates the intestinal mucosal inflammatory response by downregulating MAPK signaling pathways that are located downstream of many growth factor receptors, including the epidermal growth factor receptor (EGFR) (Uccello et al. 2012). *S. boulardii* prevents cancer cell colony formation, reduces EGF-mediated cell proliferation, and increases apoptosis in Apc^{Min} mice with intestinal tumor (Uccello et al. 2012). The probiotic *S. boulardii* generates in vivo mitogen and metabolic signals that are transduced into intestinal mucosal cells, downstream from the apical membrane to the nuclei, using recruitment substrates and serine, threonine, or tyrosine kinases (Buts and De Keyser 2010). Thus, probiotics can act as modulator of singling pathways associated with growth and cancer initiation, thereby may also serve as a novel therapeutic or prophylactic role in CRC. However, detailed studies are needed to further understand the molecular mechanism involved in prophylactics.

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Bioactive Carbohydrate: Dietary Fibers and Colorectal Cancer

3

Abstract

The health benefits of dietary fibers, namely, the insoluble and soluble fractions, against colorectal cancer are focused here. The role of food and occupational-originated environmental carcinogens or mutagens (heterocyclic amines, HCA, and polycyclic aromatic hydrocarbon, PAH) and the effects of dietary fibers against colorectal cancer (CRC) have recently gained ample interest. Epidemiological and clinical studies show that increasing the consumption of dietary fiber decreases the incidences of CRC. They act differently from drugs, and their effects could be categorized majorly into physiological and cellular or molecular mechanisms. The benefits accruing through the former include increase in intestinal viscosity, detoxification by binding the promutagens and mutagens, dilution and fecal bulking, pH maintenance, growth of favorable bacteria, formation of short-chain fatty acids (in particular, butyrate), and reduced transit time. The major pathway in cellular mechanism includes butyrate formation, which modulates the gene expression and assists in the elimination of damaged DNA. Immunomodulation by phase I and phase II enzymes, antitumor activity by induction of TNF-α and NK-cells, and antiproliferative activity are other cellular mechanisms that induce apoptosis and death of cells containing damaged DNA. Role of various dietary fibers and their fractions against CRC could prove the way for reducing the incidence of the carcinogenesis.

Keywords

Dietary fiber • Colorectal cancer • Short-chain fatty acids • Immunomodulation • Heterocyclic amines • Polycyclic aromatic hydrocarbons • Butyrate • Proliferation • Anticancer • Diet

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3.1 History, Definition, and Statistics

The benefits of consuming fibers studied using coprolites have been reported approximately 10,000 years back. Though the high amounts of fiber was consumed in ancient times, its consumption decreased over the centuries as it was considered as a bothersome intestinal waste until the scientific community became aware of its benefits and once again started showing interests. Hippocrates in 430 BC compared the laxative effects of coarse and refined wheat and emphasized the importance of whole grain flour in maintaining healthy bowel system. In the 9th century AD, Hakim, a Persian physician, also put forth similar views. By 1920s, McCance and Laurence (1929) developed the concept of undigestible carbohydrates, which was the stepping stone for the modern concept of dietary fibers as nondigestible plant substances. Even World War II saw minimum death from major ailments such as heart diseases due to the use of unrefined flour (www.world-foodhistory.com). In 1930, Kellogg, McCarrison, and Walker suggested the health benefits of wheat bran, bread, fruits, and beans, and in 1941, dietary fiber was reported to influence the secretory activities of the alimentary tract (Duckworth and Godden 1941).

Hipsley (1953) was the first to coin the phrase "dietary fibre," to define nondigestible components, which constitute the plant cell wall. These included cellulose, hemicelluloses, lignin, and other plant components, which resist enzymatic hydrolysis in human gut. Between 1972 and 1976, a group of scientists adopted Hipsley's term to develop the "dietary fibre hypotheses" (Hipsley 1953; Burkitt et al. 1972; Painter 1975). The hypotheses postulated an inverse relationship between the consumption of dietary fiber and the incidences of colon cancer and heart diseases. The dietary fiber definition was further broadened in 1976, which included all the undigestible polysaccharides and storage saccharides (gums, modified celluloses, mucilages, oligosaccharides, and pectin) (Trowell et al. 1976).

With the growing interest and knowledge about the benefits of dietary fiber, numerous researchers embarked on developing analytical methods to quantify these dietary fibers (Southgate 1978; Furda et al. 1979; Baker et al. 1979; Schweizer and Wursch 1979; Furda 1981; Baker and Dorris 1981; Heckman and Lane 1981; Asp et al. 1983). The focus was primarily on removing the digestible portions from the indigestible portion in the food, using enzymes. The success of the techniques, however, was limited and depended on the commercially available enzymes. By 1981, the general consensus on the methodology for the quantification of dietary fiber, as defined by Trowell et al. (1976) was achieved in the spring workshop of the Association of Official Analytical Chemists (AOAC) in Ottawa, Canada (Prosky et al. 1985).

The enzymatic-gravimetric methodology was modified due to numerous controversial views, and a rough method was obtained (Prosky et al. 1985), which was adopted by AOAC as the first Official Method of Analysis for total dietary fiber (AOAC Official Method 985.29, Total Dietary Fibre in Foods-Enzymatic-Gravimetric Method) (AOAC 1995). Same year, AACC adopted the same method as AACC Approved Method 32–05 (Prosky et al. 1985; AACC 1995). These methods extend rapidly and were widely accepted by the research communities. Because of its widespread acceptance and use, AOAC 985.29/AACC 32–05 became the de facto operating definition for dietary fiber and was accepted in Ottawa workshop (Prosky et al. 1985). Due to nutritional differences in dietary fiber fractions, this definition was modified as Official Method 991.42, Insoluble Dietary Fibre in Food and Food Products-Enzymatic-Gravimetric Method, Phosphate Buffer (AOAC 1995). Through several collaborative studies, Lee, Mongeau, Li, Theander, and others developed and validated official methods for total, insoluble, and soluble dietary fiber that was jointly approved by AOAC/AACC (AOAC 1995; AACC 1995). Hence, the dietary fiber quantification definitions till date could be summarized as following:

1. AOAC 992.16: Enzymatic-Gravimetric Method for Total Dietary Fibre
2. AOAC 993.21: Nonenzymatic-Gravimetric Method for Total Dietary Fibre in Food and Food Products with <2 % Starch

3. AOAC 994.13: Gas Chromatographic-Colorimetric Gravimetric Method (Uppsala Method) for Total Dietary Fibre (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin)

Still, several researchers acknowledged the latest definition as insufficient in quantifying certain unique components of dietary fibers, which led to further modification, and following AACC prepared the analytical reference standards and values for total, insoluble, and soluble dietary fiber (Caldwell and Nelsen 1999). Concise accounts of various definitions for dietary fiber are shown in Table 3.1.

According to the CODEX Alimentarius, in 2009, the dietary fiber was defined as the "edible carbohydrate polymers with three or more mono-

Table 3.1 Various attempts to define dietary fiber

Year	Definitions	Scientists/Organization
1953	The term "dietary fiber" was coined	Hipsley
1972–1976	This describes the remnants of plant cell wall that are resistant to hydrolysis by human alimentary enzymes	Trowell and coworkers
1976	Broadened the definition to include all digestion-resistant polysaccharides, including gums, modified celluloses, mucilages, oligosaccharides, pectins, cellulose, hemicellulose, lignin and linked waxes, cutin, and suberin	Trowell and coworkers
1976–1981	Developed methods to quantify the components that were included in the definition	Asp, Schweizer, Furda, Theander, Baker, and Southgate
1979	Initial steps for developing an international consensus on definition and methodology for dietary fiber	Prosky
1981	Consensus on dietary fiber definition and analytical approach	AOAC Spring Workshop in Ottawa, Ontario, Canada
1981–1985	Validation of the consensus methodology in multinational collaborative studies	Prosky, Asp, Furda, Schweizer, DeVries, and Harland
1985	Enzymatic-gravimetric method adopted to quantify total dietary fiber. This method that is equivalent to AACC Approved Method 32–05 becomes a working definition for dietary fiber	AOAC Official Method of Analysis 985.29
1985–1988	Collaborative studies on developing methods for fractionation of insoluble and soluble dietary fiber	
1991	Enzymatic-gravimetric method equivalent to AACC Approved Method 32–07 for the fractionation of insoluble dietary fiber was first adopted	AOAC Official Method of Analysis 991.42
1988–1994	Development, validation, and procedures for official or approved method status for the various collaborative approaches that fit the definition of dietary fiber	Lee, Mongeau, Li, Theander, and coworkers
1992	Reassurance on the consensus on physiological dietary fiber definition	Ist Survey-International
1993	Affirms consensus on physiological dietary fiber definition and complete components	IInd Survey-International
1995	Reaffirmation on the consensus on physiological dietary fiber definition and its components	AOAC International Workshop on Definition of Complex Carbohydrates and Dietary Fiber

(continued)

Table 3.1 (continued)

Year	Definitions	Scientists/Organization
1999	"Dietary fiber consists of the remnants of edible plant cells, polysaccharides, lignin, and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans." It identifies a macroconstituent, including cellulose, hemicellulose, lignin, gums, modified celluloses, mucilages, oligosaccharides and pectins, and associated minor substances, such as waxes, cutin, and suberin	AOAC 985.29/AACC 3205, AOAC 991.43/ AACC32-07, and equivalent methods
2009	Edible carbohydrate polymers with three or more monomeric units that are neither digested nor absorbed in the human small intestine are naturally occurring in the food that has been obtained from food raw material by physical, enzymatic, or chemical means and has beneficial physiological effects, and edible synthetic carbohydrate polymers, which have a beneficial physiological effect	CODEX Alimentarius

meric units that are neither digested nor absorbed in the human small intestine, are naturally occurring in the food, that has been obtained from food raw material by physical, enzymatic, or chemical means and has beneficial physiological effects, and edible synthetic carbohydrate polymers, which have a beneficial physiological effect" (Jones 2014).

Defining dietary fiber is challenging and controversial, as it is not a single chemical entity but a group of related compounds with more than one physiological health benefits. Currently, dietary fibers find application in various markets and are available in pure form and/or as additives. Dietary fibers are recommended in regular diet as they improve gastrointestinal health and associated bowel syndromes. TechNavio (a London-based global research firm) anticipate a significant growth for the dietary fiber market in North America and is expected to reach $ 3.76 billion by 2018 (CAGR). The firm attributes the steady growth of this market (in the USA) to its health beneficial factors against obesity, constipation, and chronic illnesses such as heart disease, cancers, and gastrointestinal diseases. Some examples of various types of dietary fiber supplements in the market are shown in Table 3.2.

3.2 Types and Components of Dietary Fiber

Dietary fibers are primarily plant material comprising of complex, nonstarch carbohydrates, lignin, and analogous polysaccharides. They pass through the small intestine, reach intact to the colon where they are available for fermentation by the resident bacteria resulting in new metabolites, thereby modulating the nutrient absorption/metabolism and preventing several diseased conditions. They contribute no calories to the diet, but the metabolites released by the colonic bacteria during fermentation are used to meet their energy requirements. However, ruminants acquire their energy from these fibers since these bacteria in the rumen hydrolyze the fibers to molecules that could be absorbed and metabolized by the host (Turner and Lupton 2011).

Dietary fibers, based on their source, are broadly classified into two types, namely, terrestrial (cereals, fruits, vegetables, and fungi) and marine dietary fibers (marine algae). They can also be divided into many different broad categories, nonstructural or simple polysaccharides and structural polysaccharides (Fig. 3.1). Based on their chemical, physical, and functional proper-

Table 3.2 Nonprescription fiber product in market

Brand Name (Active ingredient)								
Benefibre Products (wheat dextrin)	Citrucel Products (methylcellulose)	FibreChoice (inulin)	Fibrecon Caplets (calcium polycabophil)	Hydrocil (psyllium)	Metamucil Clear and Natural (inulin)	Konsyl Products (psyllium hydrophilic mucilloid)	Metamucil (psyllium husk)	Unifibre (powdered cellulose)
Benefibre Fibre Ultra Caplets	Citrucel Powder (sugar free available)	FibreChoice Chewable Tablets				Konsyl Original	Metamucil Original	
Benefibre Plus Heart Health Caplets	Citrucel Soft Chews	FibreChoice Sugar-free Tablets				Konsyl Easy Mix	Metamucil Fibre Powder Drink Mixes	
Benefibre Chewable Tablets	Citrucel Caplets	FibreChoice Weight Management Tablets (chromium and picolinic acid)				Konsyl Fibre Easy to Swallow Capsules	Metamucil Fibre Wafers	
Benefibre Powder	Citrucel Fibre Shakes	FibreChoice Plus Calcium (500 mg and 200 IU Vitamin D)					Metamucil Fibre Heart and Digestive Capsules	
Benefibre for Children	Citrucel Fibre Smoothie	FibreChoice Plus Antioxidants					Metamucil Strong Bones (300 mg calcium carbonate)	
Benefibre Plus Calcium (300 mg)							Metamucil Fibre Singles	
Benefibre Stick Packet								

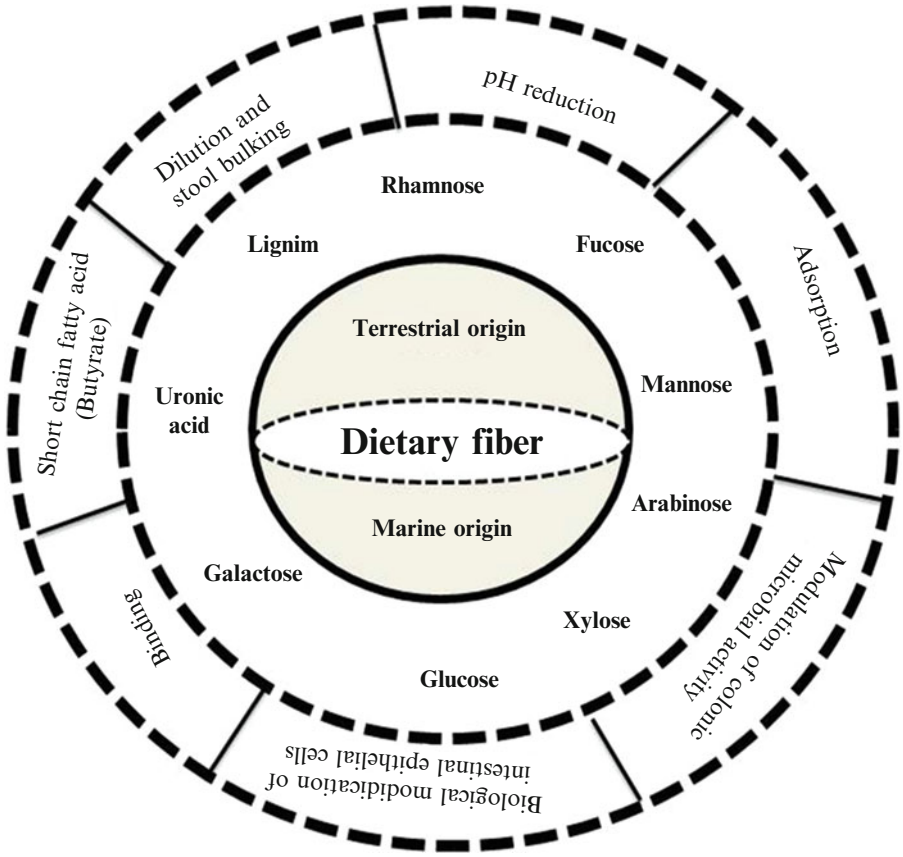

Fig. 3.1 Classification of dietary fibers and their health benefit

ties, the latter can further be grouped into soluble and insoluble polysaccharides. These fractions include neutral sugars, Klason lignin, arabinoxylan, inulin, pectin, bran, cellulose, β-glucan, and resistant starch.

Terrestrial plant sources, including cereal, vegetable, and fruit fibers have been widely explored for their assistance against colorectal cancer. Oats have high amounts of soluble dietary fiber (Sjödin et al. 1985; Qu et al. 2005). Marine dietary fibers are those that originate from marine algae, and their importance towards medical applications has come into focus, recently. The terrestrial and marine dietary fibers vary in their composition. Marine dietary fibers mostly contain predominantly a single type of glucan moiety such as galactose or fucose and other smaller moieties such as glucose, xylose, or arabinose. Presence of sulfated polysaccharides is a distinct character of red and brown algae (Kim et al. 2010). Brown algal sulfated polysaccharide, fucoidan (brown algal polysaccharide), has recently shown to exhibit anticarcinogenic and antimutagenic properties in vitro (Kim et al. 2010). The binding of mutagens and carcinogens to marine dietary fibers is a novel area (Raman and Doble 2014, 2015).

Very low levels of fiber intake lead to an increase in constipation, which is the most notable response of the body to the diet with lesser fiber content. The mechanism of action of dietary fibers on metabolic health is not well understood. However, it is speculated that it changes the intestinal viscosity, nutrient absorption and rate of passage, and production of short-chain fatty acids and gut hormones (Lattimer and Haub 2010). A generous dietary fiber intake has been reported to impart several health benefits, includ-

ing reduced cancer threat, coronary heart diseases, strokes, hypertension, diabetes, obesity, and gastrointestinal disorders, including gastroesophageal reflux disease, duodenal ulcer, diverticulitis, constipation, and hemorrhoids (Qu et al. 2005). Dietary fibers are reported to have anticarcinogenic, antimutagenic, antitumorogenic, antiproliferative, antigenotoxic, anti-inflammatory, and antioxidant activity. They also improve serum lipid concentration, control blood glucose in diabetes, promote bowel regularity, assist in weight loss and immunomodulation, and lower blood pressure. Increased intake of soluble fiber improves glycemia and insulin sensitivity in nondiabetic and diabetic individuals.

3.2.1 Dietary Recommendations

Dietary Reference Intake value for the dietary fibers has been suggested based on the decreased risk of coronary heart disease (Turner and Lupton 2011). The different levels of dietary fibers that are recommended for men and women for different age groups are shown in Fig. 3.2. Females have a lower recommended value than males of the same age group. There are no dietary intake recommendations for infants. No tolerable upper intake level for dietary fibers has been suggested. However, very high levels could induce and/or reduce the absorption and mineral bioavailability of calcium, iron, magnesium, and zinc.

The relationship between the dietary fiber and minerals is a controversial subject (Ismail-Beigi et al. 1977; Hara et al. 1996; Greger 1999; El-Zoghbi and Sitohy 2001). Dietary-fiber-associated substances such as phytates and polyphenols impair the absorption of the minerals (Ca, Zn, Mg, Mn, Cu and Fe) (Coudray et al. 2003; Prescha et al. 2014). Soluble fibers, including pentosans, pectin gums, and mucilage increase the bioavailability of minerals (Chawla and Patil 2010). Physicians recommend the consumption of fiber-rich foods to reduce the occurrence of obesity, cardiovascular diseases, type-2 diabetes, and cancers.

3.3 Dietary Fiber and Gut Microbiota

Epidemiological and experimental studies have suggested that dietary fibers play an important role in preventing colon cancer. Dietary fibers reduce the contact time of carcinogens within the intestinal lumen and promote the growth of commensals in the gut would positively alter the gut metabolism in a various ways. Gastrointestinal tract is inhabited by trillions of bacterial species. The bacterial gut population shifts to a healthier composition in the presence of fermentable dietary fiber, which forms the substrate for the bacterial fermentation (Zhu et al. 2011; Van Der Kamp et al. 2010). By modulating the gut micro-

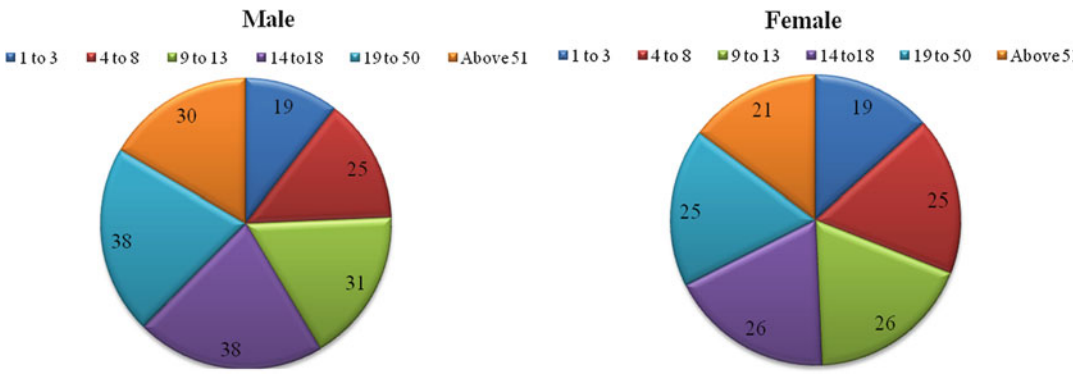

Fig. 3.2 Recommended intake levels for dietary fiber (g/day) (Turner and Lupton 2011). Intake of dietary fibers in female during pregnancy and lactation are increased to 28 and 29 g/day, respectively

biota landscape, dietary fibers reduce the risk of type-2 diabetes mellitus, obesity, cardiovascular diseases, and colon cancer and improve immunity (Table 3.3). Dietary fibers when undergoing bacterial fermentation are beneficial; however, certain dietary fibers are subjected to concurrent anaerobic proteolytic fermentation and cause detrimental effects, resulting in a yin-yang effect (Davis and Milner 2009).

Bacterial fermentation is principally controlled by the amount, type, and the physiochemcial characteristics of the fiber accessible to bacteria in the colon (Lupton 2004). De Filippo et al. (2010) conducted a study to understand the impact of diet in shaping the gut microbiota in the children of Europe and rural Africa and observed that the latter had enriched with Bacteroidetes and depleted with Firmicutes in comparison to the former. Fiber from oat bran, pectin, and guar are highly fermentable than cellulose and wheat bran (McBurney and Thompson 1990; Lupton 2004). The type of dietary fiber also influences the gut microbial composition. For instance, inulin (a polymer of fructose monomers found in onions, garlic, and asparagus) stimulates the growth of bifidobacteria but hampers the growth of potential pathogenic bacteria such as *E. coli*, *Salmonella*, and *Listeria* (Gibson et al. 1995; Bosscher et al. 2009). Human colon stimulator, xylo-oligosaccharides, decreases the butyrate-producing bacteria *Faecalibacterium prausnitzii*, while total butyrate concentration is high in the distal vessel (Christophersen et al. 2013). Xylo-oligosaccharides also affect the levels of sulfate-reducing bacteria, *Bacteroides fragilis*, accentuating that dietary carbohydrates modify gut microbiota and their ability to change the physiological properties of the colonic environment (Christophersen et al. 2013). Diet rich in nonstarch polysaccharides and resistant starch has intense effect on the type of fecal bacteria, including *Ruminococcus bromii*, which contribute to starch degradation and short-chain fatty acid (SCFA) production (Abell et al. 2008).

Table 3.3 Soluble and insoluble dietary fiber and its health benefits and the mechanism of action

Dietary fiber	Medical benefit	Mechanism of action
Soluble fiber	Crohn's disease	Enhancement of short-chain fatty acid production, and mainly acetate
	Celiac disease	Normalization of intestinal microbiota
	Colitis	Effects on epithelial permeability
	Colon cancer	Trophic effects on enterocytes, short-chain fatty acid production
	Metabolic syndrome	Anti-inflammatory effects
	Arthritis	Enhancement of immune response
	Cardiovascular diseases	Reductions of blood pressure and reduction of LDL serum concentrations
Insoluble fiber	Type-2 diabetes	Increase insulin sensitivity, loss of weight, improved energy density
	Colon cancer	Increased bulking effect, laxation, and gut transit time
	Cardiovascular diseases	Reduction of blood pressure and reduction of LDL serum concentrations

The large colon is inhabited predominantly by saccharolytic metabolic bacteria, although amino-acid-fermenting bacteria and syntrophic species are also present. Hence, dietary fiber and carbohydrate availability determines the composition and metabolic activities of the gut microbiota and several physiologic properties of the microbiota, which are attributed due to the fermentation of dietary fiber and production of SCFA (Cummings and Macfarlane 1991). Low dietary fiber intake and low SCFA production are reported in colon-cancer-risk subjects when compared to healthy individuals. These are also accompanied by distinct fecal microbial profiles between the two groups (Chen et al. 2013). Authors also observed that *Clostridium*, *Roseburia*, and *Eubacterium* spp. are signifi-

cantly less prevalent, while *Enterococcus* and *Streptococcus* spp. are more prevalent in the colon-cancer-risk group (Chen et al. 2013). Low pH resulting from the dietary fiber fermentation increases the biosynthetic requirements for nitrogen-containing precursors and inhibits the toxin accumulation in the colon (Smith and Macfarlane 1996).

Dietary fiber consumption has significant benefits on health. Many of the benefits could be attributed to the fermentation of dietary fiber in the colon and the production of SCFA, specifically butyrate. SCFA production and its role in preventing colorectal cancer are discussed in detail in Chap. 6.

3.4 Dietary Fiber and Health

Experimental and epidemiological studies have emphasized the anticarcinogenic effects of dietary fibers against colon cancer. The general benefits of dietary fiber are shown in Table 3.3.

3.4.1 Anticancer and Antitumor

Epidemiological and clinical studies demonstrate that intake of dietary fibers is inversely related to CRC (Lattimer and Haub 2010).

Trametes versicolor glucans (fungal glucans) have demonstrated superior anticancer effects in humans. Fungal polysaccharide has shown to increase the survival of advanced stage gastric, colon, and colorectal cancer patients (Mitomi et al. 1992), with one study showing improved immune parameters (including blood NK-cell activity, leukocyte cytotoxicity, proportion of helper cells and lymphocyte suppressor cells) (Sun and Zhou 2014). In several animal models, a wide range of polysaccharides have shown antitumorogenic effects. Aqueous and acid glucan extracts from *A. subrufescens* have demonstrated anticancer activities in animal models (Kobayashi et al. 2005; Murakawa et al. 2007). Anticancer effects have been reported following the intake of polysaccharide in various forms from different fungal sources, including *G. lucidum* (Lu et al. 2002), *G. frondosa* (Hishida et al. 1988; Kurashige et al. 1997; Kubo and Nanba 1997), *Hordeum vulgare* (Cheung et al. 2002; Hong et al. 2004; Modak et al. 2005), *Laminaria angustata* (Teas et al. 1984), *Lentinula edodes* (Nanba et al. 1987), *Pleurotus ostreatus* (Kurashige et al. 1997), *Saccharomyces cerevisiae* (Hong et al. 2004), *Sclerotinia sclerotiorum* (Suzuki et al. 1991). Glucomannan from *L. edodes* also enhanced the survival rate of animals injected with cancer cells (Fujii et al. 1978). Apple and citrus pectins have also been accounted to exert anticancer effects, including reduced tumor incidence (Watanabe et al. 1979; Ohkami et al. 1995; Pienta et al. 1995; Hayashi et al. 2000; Nangia-Makker et al. 2002).

Anticarcinogenic/antimutagenic activities of dietary fibers are attributed to their ability to produce SCFA. Dietary fibers may act against CRC by binding mutagens and carcinogens/procarcinogens and diluting them through fecal bulking, SCFA formation, and reducing the pH by supporting the colonic bacterial fermentation (Perrin et al. 2001). Anticarcinogenic effects have been observed in the case of prebiotics as well. Protective effect of dietary fibers against dimethylhydrazine, HCA, and PAH are reported in vitro and in vivo (Santarelli et al. 2008). Consumption of cereals, pulses, fiber-rich fruits and vegetables, and marine algae has been reported to reduce the prevalence of colorectal adenomas and CRC (Santarelli et al. 2008). It is observed that a high intake of dietary fibers, particularly, cereal fibers and whole grains, reduce significantly the risk of CRC (Aune et al. 2013). The mode of action of dietary fibers (water insoluble and water soluble fractions) on CRC risk can be summarized into physiological and cellular/molecular mechanisms (Fig. 3.3).

Dietary fiber intake has been claimed to influence the physiological mechanism. It binds to procarcinogens, and carcinogens enhance fecal bulking or reduce the system pH. The foremost physiological effects of dietary fibers include aiding in gastric emptying, reducing the intestinal transit time, and helping in the production of ceco-colonic microbial flora by fermentation (Sjödin et al. 1985; Nishiyama et al. 1992; Lewis

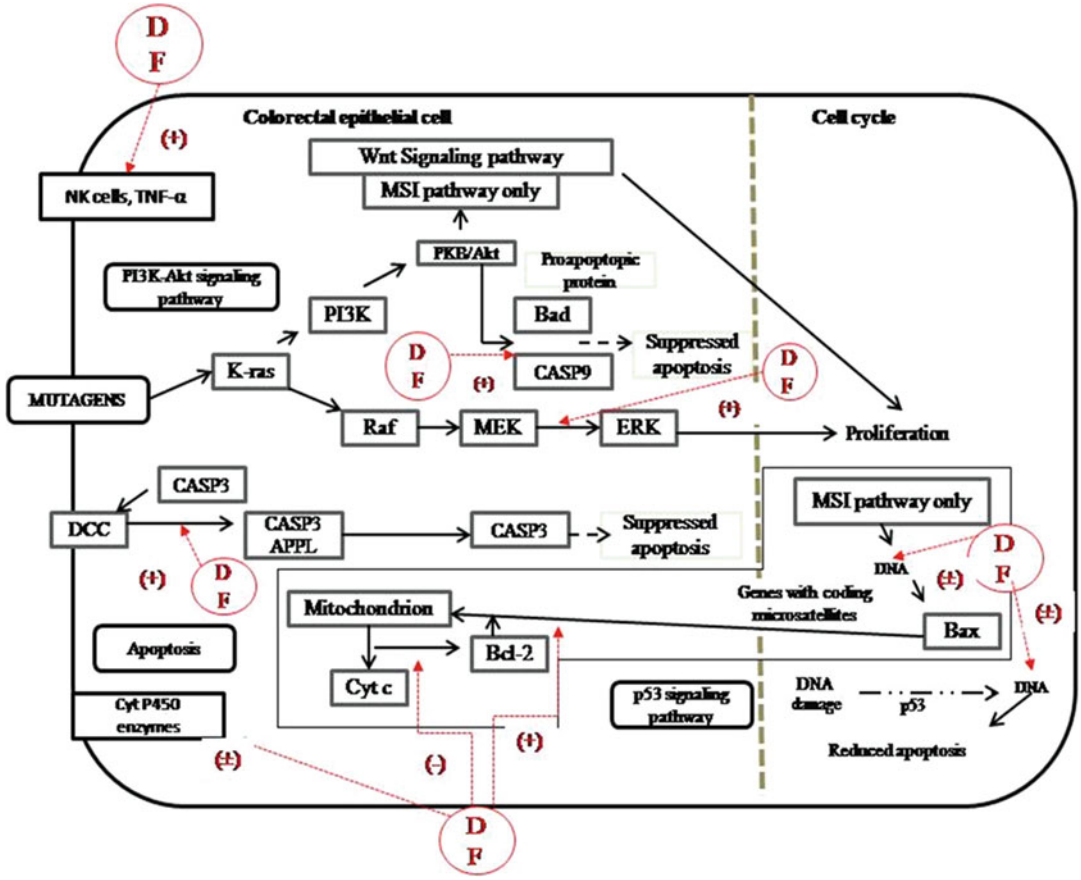

Fig. 3.3 Pathway showing the mechanism of action of dietary fibers in inducing apoptosis and preventing colon carcinogenesis

and Heaton 1997). Processing techniques modify the amount of soluble dietary fibers found in cereals, fruits, and vegetables, which are fermented by a wide variety of anaerobic bacteria resulting in an increase in the bacterial biomass, an increase in the fecal mass, a change in intracolonic pH, and production of short-chain fatty acids and various gases as metabolic end products (Lattimer and Haub 2010; Aune et al. 2011). On the other hand, the insoluble fibers predominantly serve as bulking agents, which result in shorter transit time through the intestine and increased fecal mass. The short-chain fatty acids resulting from the colonic fermentation of dietary fiber are largely absorbed via the portal blood and reach both the liver and the peripheral tissues (Perrin et al. 2001; Comalada et al. 2006).

Fucoidan, the marine polysaccharide from brown algae, induces apoptosis in human colon cancer cells, mediated via the death-receptor-mediated and mitochondria-mediated apoptotic pathways. At cellular level, dietary fiber, specifically fucoidan, increases the levels of cleavage at caspase −3, −7, −8, and −9 (death-receptor pathways) and aids in dietary fiber-mediated apoptosis (Yuan et al. 2006; Kim et al. 2010). It also influences PI3K-Akt signaling pathway, which in turn controls the Wnt signaling pathway and cell proliferation. Downregulation of ERK pathway by dietary fibers, specifically by fucoidan, also induces apoptosis. These are also accompanied by changes in Bcl-2 and Bax and p38 kinase (Kim et al. 2010). Fucoidan is also observed to induce PARP cleavage. An important function of

PARP is to facilitate the repair of the single-strand DNA nicks. Thus, presence of cleaved-PARP is a valuable marker for apoptosis. In p53 signaling pathway, an alteration in the Bcl-2 family protein contributes to mitochondrial membrane permeability, resulting in activation of caspase-9 through Bid-cleavage and mitochondria-mediated pathway. Death-receptor- and mitochondria-mediated pathways are two distinct yet interconnected pathways that trigger caspase activation. These two are the cellular mechanisms involved in the colorectal cancer prevention by the dietary fibers (Fig. 3.3) (Yuan et al. 2006; Kim et al. 2010).

3.4.2 Anti-inflammatory

Inflammation is an immediate host response of the body to injuries of the tissue due to microbial infection or other noxious stimuli. Uncontrolled inflammation and inadequate control of it may evoke chronic conditions that could be associated with cancer (Kundu and Surh 2012). SCFA could be associated with the reduction in inflammatory condition.

3.4.2.1 Leukocyte Recruitment and SCFA

The process of inflammation begins with the recruitment of leukocyte that migrates from the bloodstream to the inflamed tissue through a multistep process involving expression and activation of adhesion molecules and chemokines (Luster et al. 2005). SCFA aids in the leukocyte recruitment (Maslowski et al. 2009; Vinolo et al. 2009). Evidence indicates the directional migration of neutrophils induced by SCFA, which depend on the activation of GPR43, a G protein-coupled receptor (Maslowski et al. 2009; Vinolo et al. 2011). The receptors GPR41 and GPR109A that are related to GPR43 are activated by SCFA (Tazoe et al. 2009), thus indicating the role of SCFA in the migration of neutrophils to the inflammatory sites (Vinolo et al. 2011).

Apart from neutrophil migration, SCFA modulates the expression and secretion of cell adhesion molecules and chemokines, which play a vital role in leukocyte recruitment (Zapolska-Downar and Naruszewicz 2009; Vinolo et al. 2009). Cell adhesion molecules, including selectins, integrins, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 are required for adhesion and transendothelial movement of leukocytes (Böhmig et al. 1997). SCFA reduces the adherence of monocytes and lymphocytes to human umbilical vein endothelial cells, which is associated with the reduction of NF-κB and PPARγ activities and expression of adhesion molecules (intercellular adhesion molecule-1, ICAM-1, and vascular cell adhesion molecule-1, VCAM-1) (Böhmig et al. 1997; Zapolska-Downar and Naruszewicz 2009). Leukocyte recruitment is altered by SCFA as it modulates the amount and type of adhesion molecules and chemokines, which in turn reduces the chronic gastrointestinal (GI) tract inflammatory response.

3.4.2.2 Proinflammatory Mediators and SCFA

Cytokines and proinflammatory mediators play a key role in both the extrinsic and intrinsic pathways of inflammation-associated carcinogenesis. Macrophages are the major source of inflammatory mediators, (Kundu and Surh 2012) and on activation they produce considerable amounts of tumor necrosis factor alpha (TNF-α), IL-1β, IFN-γ and IL-6, chemokines, and nitric oxide (NO) (Vinolo et al. 2011; Kundu and Surh 2012). Butyrate reduces the lipopolysaccharide (LPS)- and cytokine-stimulated production of proinflammatory mediators, including TNF-α, IL-6, IFN-γ, and NO, and increases the release of the anti-inflammatory cytokine IL-10 (Vinolo et al. 2011; Zimmerman et al. 2012).

Histone deacetylases (HDAC) and histone acetyltransferases modulate the degree of protein acetylation and gene expression and, thus, affect the expression of proinflammatory mediators (Waldecker et al. 2008; Vinolo et al. 2011; Zimmerman et al. 2012). Butyrate also augments the acetylation of nonhistone proteins such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), tumor suppressor gene in medulloblastoma (MyoD), and tumor protein p53(Vinolo et al. 2011).

3.4.2.3 Gastrointestinal Barriers and Gut Microbiome

The gut microbiota maintains an intact GI barrier, and its disruption can cause an inflammatory process (Zhu et al. 2011). The primary barrier is the interaction between the microbiota and the gut epithelial cell layer, which is an active process, in which certain inflammatory mediators are produced. This involves ligands of toll-like receptors (TLRs) such as LPS and flagellin that activate TLR-4 and −5 to modulate different aspects of host metabolism and immune response (Vijay-Kumar et al. 2010). The secondary barrier is formed by epithelial cell secretion of mucus, which protects the intestinal epithelium from the intestinal microbiota, including invasive microbes (Salzman 2011). The mucus layer is composed of mucin proteins produced by goblet cells (Zhu et al. 2011). In the small intestine, the Paneth cells directly sense invasive enteric bacteria through TLR activation and then release the antimicrobial peptides (Paolillo et al. 2009). Therefore, mucus not only forms a physical barrier and provides a nutrition source for the microbiota but also contains protective mediators, including secreted antimicrobial peptides and immunoglobulin (IgA) (Salzman 2011; Delzenne et al. 2011). Consequently, the mucosal immune system and the homeostasis of the gut microbiota are mutually dependent on each other and their equilibrium maintains a stable intestinal environment.

3.4.3 Immunomodulation

A large collection of literature suggests the immunomodulatory effects of dietary polysaccharides, and they have a significant impact on the gut microbes and microbial ecology (Flint et al. 2007; Macfarlane et al. 2008) and, therefore, influence the host nutrition, immune modulation, resistance to pathogens, intestinal epithelial development and activity, and energy metabolism (Segain et al. 2000; Ishizuka et al. 2004; Mazmanian and Kasper 2006; Jacobs et al. 2009; Garrett et al. 2010). Immune-stimulating effects of oral polysaccharides have been observed in human subjects.

Arabinogalactans, galactomannans, laminarin, glucomannans, and mixed polysaccharide products (Ambrotose® products) have been shown to be metabolized by human colonic bacteria. Orally ingested fucoidans, glucans, and mannans (or their fragments) have been detected in numerous tissues and organs throughout the body (Sakurai et al. 1996; Irhimeh et al. 2005). Arabinogalactans are shown to augment lymphocyte proliferation and the number of CD8+ (transmembrane protein) lymphocytes (Nantz et al. 2001) so as to amplify the IgG subtype response to pneumococcal vaccination (Udani et al. 2009). *Panax quinquefolius* (North American ginseng) furanose extract is revealed to reduce the frequency of acute respiratory illness and symptom duration (McElhaney et al. 2006). Fucoidan from *Undaria pinnatifida* (wakame) is also found to stimulate as well as suppress the immune effects in humans; increase stromal-derived factor-1, IFN-g, CD34+ cells, and CXCR4-expression in CD34+ cells; and decrease the blood leukocytes and lymphocytes levels (Irhimeh et al. 2007).

Immune-stimulating effects are also reported from dietary fiber from fungal sources, including glucan products of *Agaricus subrufescens* (Takimoto et al. 2004; Chan et al. 2007; Yuminamochi et al. 2007), *Lentinula edodes* (shiitake) (Hanaue et al. 1988; Vetvicka et al. 2008), *Saccharomyces cerevisiae* (Tsukada et al. 2003), *Laminaria digitata* (Rice et al. 2005), *Sclerotium rolfsii* (Rice et al. 2004), *Sclerotinia sclerotiorum* (Suzuki et al. 1991), and *Phellinus linteus* (Lim et al. 2004; Oh et al. 2006). Furanose extract from *P. quinquefolius* and pectins from *Buplerum falcatum* and *Malus* (apple) spp. have also been reported to enhance immune function in healthy young animals (Sakurai et al. 1999; Lim et al. 2003; Biondo et al. 2008). Galactomannan (guar gum) from *Cyamopsis tetragonolobus* or highly methoxylated pectin are observed to stimulate immune effects and antibody production in older animals (Yamada et al. 2003). Similar reports on the effectiveness of oral

polysaccharide against infection and immune modulation have been demonstrated in vivo (Murphy et al. 2008). Similar examples include HSV-1 injection and feeding of *Avena* (oat) spp. soluble glucans (Murphy et al. 2008), feeding of *E. vermiformis* and *Avena* spp. particulate glucans (Yun et al. 2003), *E. coli* injections and feeding laminarin (Neyrinck et al. 2007), HSV injection and feeding with fucoidans (Hayash et al. 2008), *Staphylococcus aureus* or *Candida albicans* injections and feeding of *S. cerevisiae* glucans (scleroglucan) (Rice et al. 2004), and injection of fecal solution and feeding of the aqueous extract of *A. subrufescens* (*A. blazei* Murrill) (Bernardshaw et al. 2006). In human subjects with seasonal allergic rhinitis, *S. cerevisiae* β(1 → 3) and (1 → 6) glucan decreased the interleukin (IL-4, IL-5) and percentage of eosinophils and increased the IL-12 in nasal fluid (Kirmaz et al. 2005), while a placebo-controlled study of patients with recurring aphthous stomatitis (canker sores) on consumption of β (1 → 3) and (1 → 6) glucans found to have an increased lymphocyte proliferation and reduced ulcer severity scores (Koray et al. 2009).

In vivo benefits of dietary fibers against inflammatory bowel diseases have been reported in many cases. They include fucoidan from *Cladosiphon okamuranus* Tokida (Matsumoto et al. 2004), galactomannans from *Cyamopsis tetragonolobus* (Naito et al. 2006), pectin from *Malus* spp. (Lim et al. 2003), and mixed polysaccharide supplements (Koetzner et al. 2010). Animals tested with ovalbumin established anti-inflammatory effects when treated with aqueous extracts of *A. subrufescens* (Chan et al. 2007) and *Ganoderma tsugae* (Lin et al. 2006) and pectin of *Pyrus pyrifolia* (Lee et al. 2004) that contained soluble fibers. Heteroglycans from *Lycium barbarum*, *Lentinus lepidus*, and *A. subrufescens* have confirmed immune-stimulating effects in vivo in cancer models (Ito et al. 1997; Gan et al. 2004). Glucans from *T. versicolor* have also shown decreased tumor growth and vascular density in cancer-induced models (Ho et al. 2004).

3.5 Mechanism of Action of Dietary Fiber and Its Fractions on Colorectal Cancer

3.5.1 Physiological Mechanism

Various mechanism has been demonstrated by which dietary fiber acts against CRC, which includes binding carcinogens/procarcinogens and mutagens, fecal bulking, reduced transit time, dilution, and fermentation leading to the formation of favorable metabolites, SCFA.

3.5.1.1 Binding Procarcinogens and Carcinogens to the Dietary Fibers

Structural polysaccharides fasten strongly to 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), which could be due to the presence of high amounts of Klason lignin, pectin, and uronic acid (Sjödin et al. 1985; Nishiyama et al. 1992). Such binding is suggested to involve both reversible adsorption and chemical interaction of functional groups of mutagens or carcinogens to dietary fiber. Binding is significantly affected by the pH (pH 6.8) of the system. The percentage of Trp-Ps taken up by carboxymethyl cellulose (CMC) and agar in artificial gastric juice (AGJ) at a pH of 1.2 is 52–56 % and 58–78 %, respectively. It increases in artificial intestinal juice (AIJ) at a pH of 6.8 to 97–98 % and 87–89 %, respectively. The percentage of IQ and MeIQ adsorbed by CMC is 21–27 % in AGJ and 100 % in AIJ. Fungal dietary fiber such as *Agaricus blazei* β-glucan is also reported to have antimutagenic effects against human lymphocyte, human hepatoma cell lines (HepG2), which is possibly due to its protective action against the DNA damage in the cells (Angeli et al. 2006). The mutagenic activity of β-glucan against 3-amino-1-methyl-5H-pyrido[4,3-b] indole (Trp-P-2) and ROS-induced DNA damage show dose-dependent protective effect within the

range between 20 and 80 μg/ml. The protection of DNA in the cells is also accredited to the ability of glucans to bind to BαP and scavenge the free radicals produced during the metabolism. Marine dietary fibers, namely, alginic acid and calcium derivatives of alginate are also reported to adsorb mutagens, and their activity is directly correlated to uronic acid content (Sjödin et al. 1985; Nishiyama et al. 1992).

3.5.1.2 Laxation, Fecal Bulking, and Dilution of Procarcinogens and Carcinogens in the Gastrointestinal System

Dietary fibers exhibit general hydrocolloid property, namely, water-holding capacity by the fiber moieties. This assists in laxative effect in the large intestine where they undergo fermentation, enhance the microbial growth, increase the bacterial mass, stimulate peristalsis, and induce fecal bulking. Rapidly fermented polysaccharides, including pectin, exhibit high activity because of their large hydrophobic surface area. Fecal bulking aids in dilution of intraluminal contents and, thereby reduces the contact time of mutagen and carcinogen to the intestinal lining, thus aiding in cancer prevention (Lewis and Heaton 1997). These characteristic features of dietary fiber are similar to prebiotics, including inulin, fructo-oligosaccharide and galacto-oligosacchrides.

3.5.1.3 Bacterial Fermentation in the Gastrointestinal System

Bacterial fermentation process produces SCFA, which maintains gut health and intestinal morphology and function. Details of the role of SCFA in preventing colon cancer are discussed in Chap. 6.

Commonly formed SCFA are acetic, propionic, and butyric acids at a molar ratio of 60:20:20. These help in maintaining the homeostasis and are associated with enhanced sodium absorption and bicarbonate excretion. Butyrate also acts as an energy source for colonocytes and is associated with the lowering of luminal pH to 5.0–5.6 (Rechkemmer et al. 1988; Oltmer and Engelhardt 1994) and alteration of the colonic microflora. SCFA lessens and acts on the advanced stages of cancer development, and its mechanism is detailed in the later section (Comalada et al. 2006). The strong fastening property, fecal bulking (100 %), and dilution effect of dietary fibers, together with useful by-products formed in human colon during fermentation, significantly aid in the physiological detoxification of carcinogens and mutagens.

3.5.2 Cellular Mechanism

Cellular mechanism has been classed into antitumor activity, formation of SCFA, and immunomodulation of xenobiotic enzymes that assist in reducing the rate of CRC (Fig. 3.3).

3.5.2.1 Antitumor Activity of Dietary Fibers

Lignans of wheat bran exhibit antitumor activity against human colon cancer cell lines (SW480) in dose- and time-dependent manner (Qu et al. 2005). *Marginisporum crassissimum*, *Sargassum vulgare*, and *Fucus vesiculosus* exhibit antitumor activity in several cell lines (mouse melanoma cell lines, B16-BL6; human colon carcinoma cell lines, HT-29, HCT-15, HCT 116) and in vivo (transplanted with Sarcoma 180 cells and H22) (Hyun et al. 2009). λ-carrageenan exhibits antitumor activity possibly due to the induction of natural killer cells (NK) and proliferation of lymphocyte and tumor necrosis factor-α (TNF-α) (Yuan et al. 2006). The administration of 5-flurouracil (5-Fu) and λ-carrageenan helps in restoring the immune function in chemo-treated mice.

Treating HCT-15 cells with fucoidan from sulfated seaweed polysaccharide leads to apoptotic events, including DNA fragmentation, chromatin condensation, and increase in the population of sub-G1 hypodiploid cells (Hyun et al. 2009). Fucoidan reduces the expression of Bcl-2 and increases that of Bax in a time-dependent manner. It also increases the activity of caspase-9, −8, −7, and −3 and aids in the cleavage of poly (ADP-ribose) polymerase (PARP). Apoptosis is also accompanied by strong activation of extracellular signal-regulated kinase (ERK) and p38 kinase

and inactivation of phosphatidylinositol 3-kinase (PI3K)/Akt in a time-dependent manner (Hyun et al. 2009; Kim et al. 2010). Similar anticarcinogenic effects of low molecular weight κ-carrageenan (27 and 67 kD) were observed against HCT-116 (Raman and Doble 2015). Maghemite nanoparticles of ι-carageenan are also reported to show anticarcinogenesis against HCT-116 emphasizing the collective preventive action of carrageenan (red marine algal polysaccharide) on colon cancer cells (Raman et al. 2015).

3.5.2.2 Formation of SCFA Due to the Fermentative Hydrolysis of Dietary Fibers in the Gastroinstestinal System

Soluble dietary fibers, including arabinoxylans, inulin, β-glucan, pectin, etc. undergo fermentation in the small intestine and are converted to acetic, propionic, and butyric acids. The role of butyrate varies between normal and cancerous cells, and this phenomenon is termed as "butyrate paradox." Details of the role of SCFA against colon cancer are described in Chap. 6. Butyrate inhibits colonic tumor cells and promotes healthy colonic epithelial cells, but the signaling pathway is still unclear. It is reported that the chemopreventive benefits of butyrate depends on its amount, time of exposure with respect to the tumorogenic process, and the type of fat in the diet (Lupton 2004). Although cell culture studies support protection (Lupton 2004), most animal studies do not. Butyrate, a fermented by-product of dietary fiber, maintains gene expression and modifies epigenetic hyperacetylation of histones and nonhistone proteins and consequently acts as a histone deacetylase inhibitor and regulates the expression of critical cell cycle (Lin et al. 2012), alteration of DNA methylation that results in enhanced accessibility of transcription factors to nucleosomal DNA (Lin et al. 2012). It also induces cell differentiation, suppresses proliferation, and enhances apoptosis to eliminate DNA-damaged cells that might otherwise progress to malignancy, thus reduces the risk of CRC (Le Leu et al. 2010) (Fig. 3.4).

Lactate, also improves gut health and gut-associated immune defense and increases the surface area for absorption. Propionate and acetate induce apoptosis in human colorectal carcinoma cell lines through the loss of mitochondrial trans-

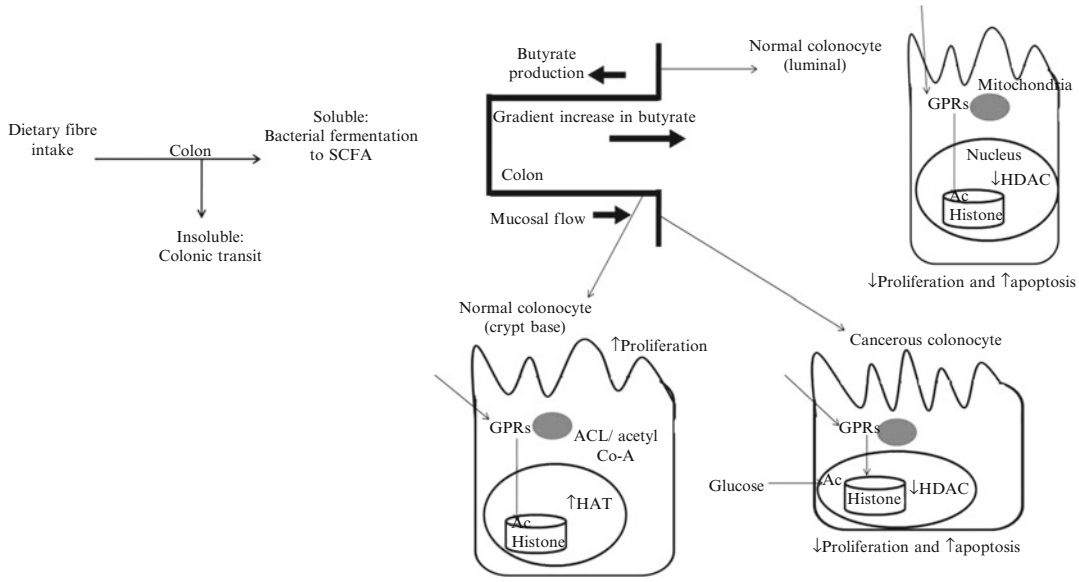

Fig. 3.4 Proposed model for the function of dietary fibers in preventing colon carcinogenesis by forming short-chain fatty acids (Bultman 2014)

membrane potential, generation of ROS, caspase-3-processing, and nuclear chromatin condensation (Cousin et al. 2012).

3.5.2.3 Immunomodulatory Activities of Dietary Fibers

Xenobiotic metabolizing enzymes that contribute in carcinogen activation and its metabolism fall into two categories, namely, phase I and phase II enzymes. The former include cytochrome-b5, cytochrome-b5 reductase, cytochrome P450, and cytochrome P450 reductase. The latter include glutathione S-transferase, uridine diphospho-glucuronyl transferase, and DT-diaphorase, which reduce the activation of procarcinogens to reactive carcinogenic intermediates and their elimination from the body. HCA and PAH are metabolically activated by cytochrome p4501A1, 1A2, and 1B1 to their intermediates and ultimately to active carcinogens (Shimada and Fujii-Kuriyama 2005). Studies with Caco-2 cells indicate that SCFA induces glutathione transferase π (Beyer-Sehlmeyer et al. 2003), thus assisting in modulating the xenobiotic metabolizing enzymes. Subsequently, dietary fibers rich in soluble polysaccharides assist in altering the metabolic pathway and the activity of the enzymes involved and induces apoptosis.

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Bioactive Carbohydrate: Prebiotics and Colorectal Cancer

4

Abstract

Prebiotics are defined as the indigestible food components that are selectively fermented by the intestinal microbes and promote changes in the gut environment, gut microbial community structure, and their metabolism. Prebiotics have been reported to avert colorectal cancer development by altering the composition and activity of the gut microflora. Administration of prebiotics has been reported to reduce colon cancer and its biomarkers. Oligosaccharides such as inulin, fructo-oligosaccharide, galacto-oligosaccharide, lactulose, soy-oligosaccharide, xylo-oligosaccharide, lactitol, resistant starch, etc., are some of the commercially available prebiotics. Short-chain fructo-oligosaccharides thwart colon carcinogenesis possibly by modulating the colonic ecosystem. Galacto-oligosaccharides, lactulose, and fructo-oligosaccharides are observed to increase the intestinal concentration of lactate, stool frequency, and weight; and decrease fecal concentration of secondary bile acids, fecal pH, and nitroreductase and β-glucuronidase activities, thus preventing colon carcinogenesis. Lactulose is reported to reduce the proliferation and occurrence of adenoma. Resistant starch prevents cancer by modulating gene expression and DNA methylation. Prebiotics undergo fermentation by gut microbes leading to the formation of short-chain fatty acids and enhance the immunity of the host. Butyrate has been observed to have direct effects in preventing colon cancer. Prebiotics are observed to modulate the colonic gut environment and aid in the growth and development of *Bifidobacteria* spp.

Keywords

Prebiotics • Short-chain fatty acids • Oligosaccharides • Inulin • Fructo-oligosaccharides • Galacto-oligosaccharides • Lactulose • Bifidogenic effects • Colon • Gut microbes

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4.1 History, Definition, and Statistics

Prebiotics was initially introduced by Gibson and Roberfroid (1995) by switching "pro" for "pre," which means "before" or "for" and defined it as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon." These descriptions overlap with the definition of dietary fiber with the exception in selectivity for certain species of saccharides. For instance, bifidobacteria showed increased growth specificity when ingested with fructo-oligosaccharides and inulin (Gibson and Roberfroid 1995; Gibson et al. 1995), transgalactosylated oligosaccharides (Tanaka et al. 1983; Ito et al. 1993; Rowland and Tanaka 1993), and soybean oligosaccharides (Hayakawa et al. 1990).

Gibson and Roberfroid (1999) also observed that these prebiotics stimulated the growth of favorable and beneficial bifidobacteria and lactobacilli (Gibson and Roberfroid 1999). Apparently, prebiotics must endure the digestive process, the gut pH, and the enzymatic hydrolysis before it reaches the colon and preferably persist throughout the large intestine so that its benefits are evident distally (Gibson et al. 2004). In the colon, prebiotics act as the dietary bulking agent or as the available substrate for resident colonic bacteria, so it decreases the pH and increases the production of short-chain fatty acids (SCFA) that may reduce the number of pathogenic microorganisms (Morisse et al. 1993).

Prebiotics or oligosaccharides are normally found in plants and are relatively short-chain carbohydrates. They occur widely in nature. They are also detected in relatively smaller quantities, as free sugars or glycoconjugates in human milk and the colostrum of various animals (Bucke and Rastall 1990). They are maintained as sugar reserves in seeds and tubers and are utilized as growth commences. However, the effects of prebiotics or these oligosaccharides on the gastrointestinal physiology have been highlighted in recent years with renewed significance to human health (Van Loo et al. 1999). In the United States, the adult use of probiotics and/or prebiotics was four times higher in 2012 than that in 2007. Oligosaccharides of low molecular weight have been the subject of recent interest. They form the most accessible source of carbon for colonic bacteria, which on ingestion reach the ileocaecal region in a relatively unmodified form (Oku et al. 1984; Nilsson et al. 1988). Certain oligosaccharides (Table 4.1) have been reported to have an ability to support the growth of gut microorganisms whose metabolism has positive physiological consequences (Fuller and Gibson 1998; Gibson and Roberfroid 1999).

Today, the industry is keen on developing a range of oligosaccharides that could be incorporated into the food to improve their "prebiotic effect." This is especially true in the Western countries where their conventional daily diet has relatively small quantities of oligosaccharides (ca. 2–5 g per day) (Macfarlane and Cummings 1991). Fructo-oligosaccharides are well researched prebiotics that occur naturally in the diet. A detailed description of the prebiotic oligosaccharides is given elsewhere in this chapter.

Certain prebiotics aid in the growth and metabolic activities of bifidobacteria and lactobacilli (Rowland and Tanaka 1993; Fuller and Gibson 1998; Bouhnik et al. 1999), the beneficial microorganisms that exert therapeutic and prophylactic influence on the health of infants and adults (Salminen et al. 1998). A beneficial effect of *Bifidobacterium* spp. in breast-fed babies against childhood diseases includes the prevention of colonization of transient pathogens, which were reported earlier (Fuller 1991, 1992). Similarly, reduced levels of bifidobacteria may partly explain the augmented vulnerability of elderly people to certain disorders and reduction in their immunity (Rowland and Tanaka 1993; Gibson and Roberfroid 1995; Buddington et al. 1996). The indigenous gut flora may be compromised due to the reduction in the number of *Bifidobacterial* spp. This may reduce capacity to fight pathogens. Suitable prebiotics could increase the number of bifidobacteria or lactobacilli leading to an improved microbial balance in the colon and colonization resistance. Although this hypothesis is still a controversy, static batch culture fermentation

Table 4.1 Major types of prebiotics and benefits

Components	Source	Microflora stimulated	Potential benefits
Saccharides			
Oligosaccharides	Onion, garlic, chicory root, burdock, asparagus, Jerusalem artichoke, soybean, wheat bran, milk, honey, leek, banana, rye, barley, salsify (Mussatto and Mancilha 2007)	*Bifidobacterium* species	Increase in bifidobacterium
			Suppression of putrefactive bacteria
			Prevention of constipation and diarrhea
Fructo-oligosaccharides (inulin, oligofructose)	Same as above	*Bifidobacterium* species	Augmentation of bifidobacteria and reduction of pH
		Lactobacillus acidophilus	Improvement of gastrointestinal health
		Lactobacillus casei	Improvement of calcium absorption
		Lactobacillus plantarum	
Fructans	Ash-free white powder from tubers of Jerusalem artichoke	*Bifidobacterium* species	Increase in bifidobacteria
Stachyose and raffinose	Soybean extract	*Bifidobacterium* species	Growth factor
Peptides			
Casein macropeptide	Bovine milk	*Bifidobacterium* species	Increase in bifidobacteria
Human kappa casein and derived glycomacropeptide	Human milk: chymotrypsin and pepsin hydrolysate	*Bifidobacterium bifidum*	Increase in bifidobacteria
Polyols			
Lactitol	Synthetic sugar alcohol of lactose	*Bifidobacterium* species	Increase in bifidobacteria
Lactulose	Synthetic derivative of lactose	*Bifidobacterium* species	Increase in bifidobacteria

Chart adapted from the International Food Information Council Foundation: Media Guide on Food Safety and Nutrition: 2004–2006. *Examples are not an all-inclusive list

with human fecal bacteria has supported this view. The study indicated that fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), isomalto-oligosaccharides (IMO), and lactulose alter the gut microflora, increasing the level of bifidobacteria and/or lactobacilli and reducing the levels of harmful bacteria, including clostridia and bacteroides (Rycroft et al. 2001). In vitro study with a simulation model for fermentation in the colon implicated that inulin also aids in increasing the levels of lactobacilli but to a lesser extent of bifidobacteria levels, in the proximal regions (McBain and Macfarlane 2001). These studies indicated that GOS increased both bifidobacteria and lactobacilli in the proximal and transverse regions of the colon model. Similar results were observed in rodents (Apajalahti et al. 1998). When inulin was fed along with the diet, the caecal bacteria increased, as did the production of SCFA, causing a reduction in the pH (Apajalahti et al. 1998). Molecular sequencing indicated an increase in the population of bifidobacteria and a decrease in the pathogenic bacteria, namely, clostridia and desulfovibrios. An increase in the lactic acid bacteria and a decrease in the enterobacteria are observed in the gut of rats containing human flora and fed with resistant starch (Silvi et al. 1999). Modulations of the flora are reported in human volunteers when they are fed with prebiotics. GOS and FOS are reported to augment the

fecal bifidobacteria in a dose-dependent manner (Ito et al. 1990; Bouhnik et al. 1999). The rate of increase depended on the initial levels of the bacteria. Individuals with the lowest starting populations of bifidobacteria showed maximum increase (Rycroft et al. 2001). Molecular-based techniques have confirmed the beneficial effects of prebiotics, namely, FOS and lactulose, in human trials (Tuohy et al. 2001, 2002). The FOS have also been seen to be a highly effective prebiotic when incorporated in a biscuit and consumed at a rate of 8 g per day (Tuohy et al. 2001). Other prebiotics, namely, inulin, trans-galacto-oligosaccharide, and lactulose, are found in wide usage in Japan.

Prebiotics find application in various day-to-day food products for humans and animals, which include food and beverages, dietary supplements, and animal feed. These form a major market with tremendous potential (Table 4.2). Prebiotic demand for food and beverage applications is anticipated to attain $ 4.5 billion USD in 2018 (Transparency Market Research 2015). Dietary supplements, a recent trend, have picked up pace in the past few years, and they find applications in the food as nutritional supplements, infant formulae, and specialty nutrients. According to Global Industry Analysts (GIA), the prebiotic sales are approximately $ 1 billion USD in 2011 (Spinner 2013). The US market for prebiotics is projected to reach $ 225.31 million USD by the year 2015. The European market is motivated by the development of prebiotic enrichment into new food such as, meat and snack products, and the US market is continuing to meet the demand for fructans, which form the largest product segment in the US prebiotic market. Market analysis shows that the prebiotic-rich infant formulae are projected to grow at a compound annual growth rate (CAGR) of 11.3 % between 2012 and 2018 (Transparency Market Research 2015). The most recent application for prebiotics has been in the animal feed sector and as pet food, and it has become a highly lucrative market. Prebiotic demand for animal feed applications is expected to cross 70,000 tons by 2018 (Transparency Market Research 2015).

Table 4.2 Major prebiotic applications in various food and the prebiotic markets

Major prebiotic applications	Major markets
Natural food (whole grains, onions, bananas, garlic, honey, leeks, artichokes)	Europe[1]
Beverages	Asia-Pacific[2]
Dairy products	North America[3]
Cereals	Rest of the world[4]
Baked food	
Fermented meat products	
Dry foods	
Supplements	
Dietary supplements	
Food supplements	
Nutritional supplements	
Specialty nutrients	
Infant formula	
Animal feed	

The criteria for the classification of food ingredient as prebiotics are listed below (Roberfroid 2007):

1. It should resist digestion at gastric pH.
2. It should not be hydrolyzed by the gut enzymes.
3. It should not be absorbed in the upper gastrointestinal tract.
4. It is fermented by gut microbes.
5. It should induce selective stimulation of growth and activity of gut microbes that are associated with health and well-being (Gibson and Roberfroid 1995).

Prebiotics, the nondigestible food substances, are accountable for the growth of the favorable bacterial species in the gut, thus benefiting the host. The popularity of the prebiotics is rapidly rising due to its wide application as a functional food, which includes prebiotic-enriched dairy products, health drinks, nutrition bars, breakfast cereals, beverages, bakery products, meat products, mineral supplements, weight loss products, green foods, infant food, and pet food (Table 4.3). This could be due to the improved consumer awareness towards health and nutrition. Europe has been the largest consumer of prebiotic-rich

Table 4.3 Food rich in prebiotics and the weight of prebiotics in percentage

Food containing prebiotics	Weight (%)
Raw chicory root	64.6
Raw Jerusalem artichoke	31.5
Raw dandelion greens	24.3
Raw garlic	17.5
Raw leek	11.7
Raw onion	8.6
Cooked onion	5
Raw asparagus	5
Raw wheat bran	5
Raw banana	1

food, owing to increased awareness of its beneficial health effects. The demand for prebiotics in Europe is predicted to reach $ 1.9 billion USD in 2018. The Asia-Pacific is the second major consumer and is expected to be a potential market in the coming years. Asian demand for prebiotic ingredients is expected to grow at a CAGR of over 11 % from 2012 to 2018 (Transparency Market Research 2015). Abbott Laboratories; BENEO-Orafti SA; Bright Food (Group) Corporation Limited; Cargill Incorporated; Cosucra Groupe Warcoing SA; Kraft Foods Group, Inc.; FrieslandCampina Domo; Jarrow Formulas, Inc.; Parmalat S.P.A.; Roquette Freres; Royal Cosun; and Yakult Honsha Co. Ltd. are some of the leading companies known to manufacture prebiotic ingredients and prebiotic-rich food (Transparency Market Research 2015).

To assess the ability of prebiotic in selectively stimulating *Bifidobacterium* and *Lactobacillus*, *PrebioticIndex* (PI) was introduced, which can be calculated using the following formula:

$$\mathrm{PI} = (\mathrm{Bif}/\mathrm{Total}) - (\mathrm{Bac}/\mathrm{Total}) + (\mathrm{Lac}/\mathrm{Total}) - (\mathrm{Clos}/\mathrm{Total}),$$

where

Bif	–	*Bifidobacterium*
Bac	–	*Bacteroides*
Lac	–	*Lactobacillus*
Clos	–	*Clostridium*
Total	–	Total bacteria

4.2 Types of Prebiotics

Prebiotics, a group of nutritional compounds, have the ability to promote the growth of specific beneficial (probiotic) gut bacteria and suppress putrefactive and pathogenic bacteria. Table 4.4 and Fig. 4.1 show the major types of prebiotics and their structures, respectively. Prebiotics undergo fermentation in the colon and aid in the proliferation of beneficial gut microbes (Fig. 4.2). The effect of prebiotic oligosaccharides could be broadly grouped into two (Conway 2001):

(a) Effect on the microbes:
- *Bifidobacterium* count increases
- *Lactobacillus* count increases based on the type of prebiotic
- Clostridia and enterobacteria decreases with the type of the prebiotics
- Reduced shedding of *S. typhimurium*

(b) Effect on the host health and physiology:
- Reduction in blood lipids
- Increase in mineral absorption
- Reduced risk of cancer development

4.2.1 Inulin

Inulin is found in around 36,000 plants, including herbs – chicory root, burdock root, and dandelion root; fruits – apples, bananas; sweet vegetables – onions, garlic, asparagus, leeks, and Jerusalem artichokes; and raw apple cider vinegar. Inulin-type prebiotics contain fructans of inulin type. Fructans are naturally occurring plant oligo- and polysaccharides with one or more fructosyl-fructose linkages forming the majority of glycosidic bonds. Inulin-type fructan has β-(2→1)-fructosyl-fructose glycosidic bond that gives it unique structural and physiological properties. They tolerate the enzymatic hydrolysis by human salivary and small intestinal digestive enzymes. Inulin-type prebiotics include fructo-oligosaccharides (FOS), oligofructose, and inulin. These terms have been interchanged in both the scientific literature and in food applications. Commercially available inulin-type prebiotics could be extracted from chicory root or synthesized from fundamental

Table 4.4 Summarized table showing various prebiotics, their chemical structure, and their degree of polymerization (Cummings and Stephen 2007)

Prebiotic	Chemical structure	Degree of polymerization
Inulin	β(2 → 1)-Fructans	2–65
Fructo-oligosaccharides	β(2 → 1)-Fructans	2–9
Galacto-oligosaccharides	Galactose oligomers and some glucose/lactose/galactose units	2–5
Soy-oligosaccharides	Mixture of raffinose and stachyose	3–4
Xylo-oligosaccharides	β(1 → 4)-linked xylose	2–4
Isomalto-oligosaccharides	α(1 → 4)-glucose and branched α(1 → 6)-glucose	2–8
Resistant starch/dextrin	Mixture of glucose oligomers	Various

molecule sucrose. Depending on the source and processing conditions, inulin-type prebiotics could have different chemical compositions. Hence, some inulin-type prebiotics are relatively high in free sugars (fructose, glucose, and sucrose), while others have no free sugar. Mixtures consisting wholly of inulin-type oligosaccharides, polysaccharides, or both could be observed based on the processing conditions.

Inulin, oligofructose, and FOS resist enzymatic digestion in the upper gastrointestinal tract and reach the colon virtually intact and undergo bacterial fermentation. Inulin-type prebiotics are truly bifidogenic and stimulate the growth of *Bifidobacteria* spp. However, their effects on other gut organisms are less clear. A minimal dose of inulin-type prebiotic is needed to induce a bifidogenic effect. However, depending on the composition there could be variations in terms of total number of bifidobacteria and individual *Bifidobacteria* spp.

4.2.2 Fructo-oligosaccharides (FOS)

Fructo-oligosaccharides (FOS), the oligosaccharides occurring naturally in plants (onion, chicory, garlic, asparagus, banana, artichoke, etc.), are polymers of fructose linearly arranged and linked by β(2 → 1) bonds. The number of fructose units range from 2 to 60 and often end in a glucose unit (Fig. 4.1). FOS are not hydrolyzed by small intestinal glycosidases and reach the cecum structurally unchanged, where they are metabolized by the intestinal microflora to form SCFA, L-lactate, CO_2, hydrogen, and other metabolites (Kolida et al. 2002; Sabater-Molina et al. 2009). This low molecular weight saccharide has low sweetness intensity, is calorie free, is noncarcinogenic, and is considered as a soluble dietary fiber. Additionally, FOS show prebiotic effect, improve mineral absorption, and decrease the levels of serum cholesterol, triacylglycerols, and phospholipids (Kolida et al. 2002; Sabater-Molina et al. 2009). FOS, similar to inulin, are considered as a prebiotic ingredient and are added to food supplements and infant formulae to encourage the growth of non-pathogenic gut microflora (Kolida et al. 2002; Sabater-Molina et al. 2009). A subgroup of inulin and a prebiotic is regularly added to dairy foods and baked goods. It improves the taste and stimulates the growth of the beneficial bacteria, bifidobacteria. Consumption of FOS increases fecal bolus and the frequency of depositions. A dose of 4–15 g/day given to healthy subjects was reported to reduce constipation in adults and newborns during their first month (Sabater-Molina et al. 2009). It also reduces traveler's diarrhea and combats several other diseases (Cherbut et al. 2003).

4.2.3 Galacto-ligosaccharides (GOS)

GOS or trans-oligosaccharide (TOS) and trans-galacto-oligosaccharide (TGOS), a collective term for the group of carbohydrates containing oligo-galactose and some lactose and glucose, are commercially produced from lactose by enzymatic hydrolysis using β-galactosidase (Macfarlane et al. 2006).

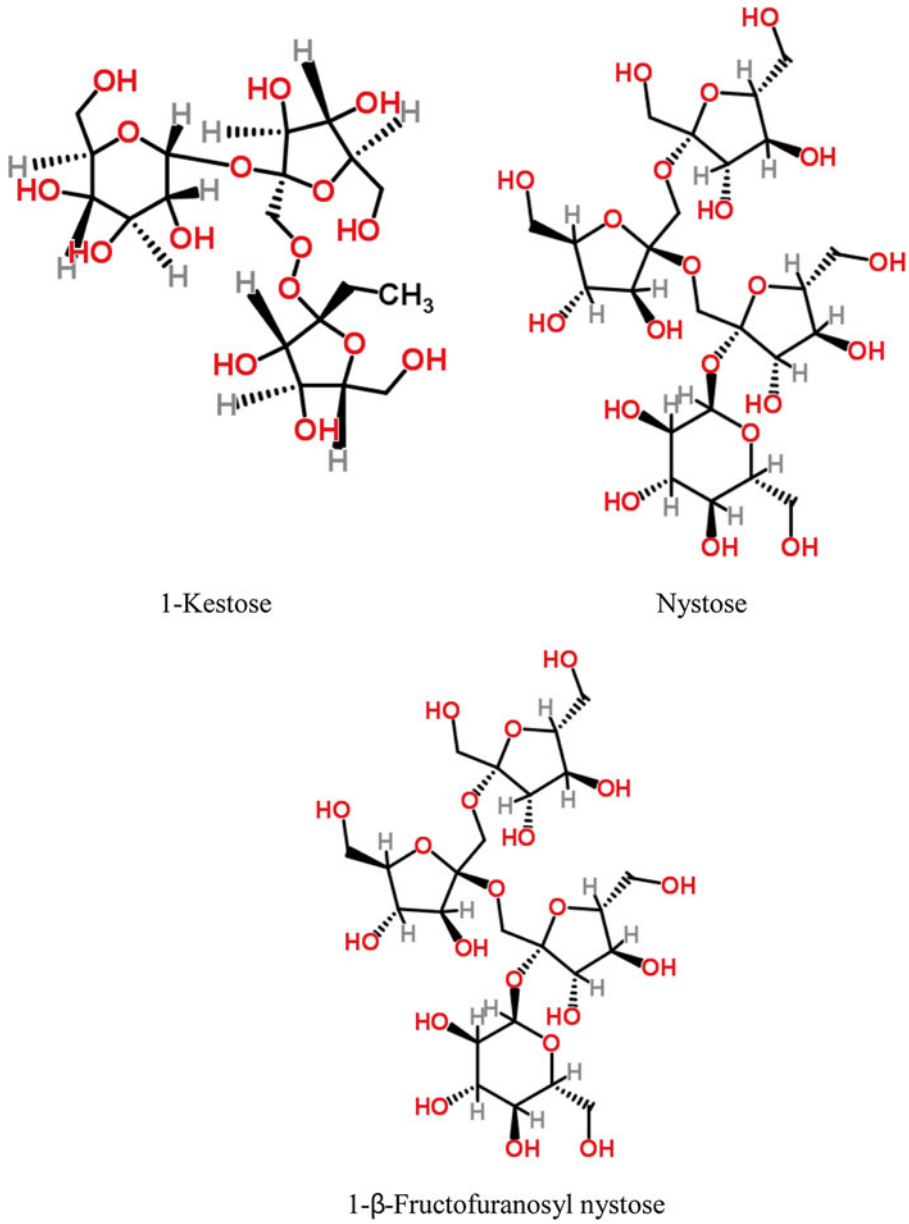

Fig. 4.1 Some short-chain fructo-oligosaccharides

Oligosaccharides are the third largest component in the human milk, and high levels are found in the colostrums (24 % of total colostrums carbohydrates) (Bode 2006). Galacto-oligosaccharides are naturally found in human and bovine milk (Alander et al. 2001). Milk contains high amounts of neutral oligosaccharides than acidic oligosaccharides. The principal sugar components of oligosaccharides are sialic acid, *N*-acetylglucosamine, L-fucose, D-glucose, and D-galactose, which result in a complex of over 130 different oligosaccharides. Human milk contains large amounts of galactose with a backbone of lactose (β-D-galactopyranosyl-(1 → 4)-α-d-glucopyranose) and external galactoses leading to the formation of galactosy-lactose; 1 → 3,

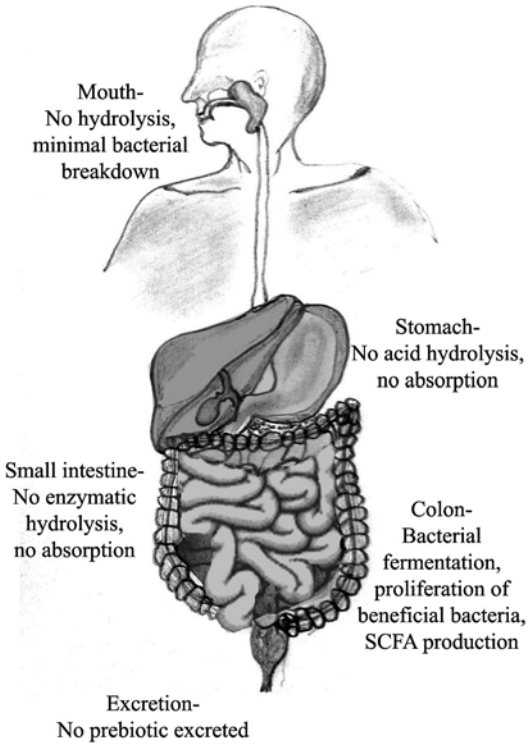

Fig. 4.2 Behavior of prebiotics in human digestive system

1→4, and 1→6 galactosyl-lactose; and 1→6 galactosyl-lactose. These oligosaccharides contribute mainly in protecting human infants from gastrointestinal pathogenic bacteria (Fanaro et al. 2005). The total concentration of lactose in human milk has been estimated to be approximately 1 g/l (Boehm et al. 2005).

GOS and FOS show laxative effects that are attributed to their ability to act as soluble fibers. These pass undigested into the colon and undergo bacterial fermentation (Delzenne 2003). It is suggested that constipation could cause reduction in the number of bifidobacteria and treatment with GOS could correct this, consequently, benefitting bowel function (Hamilton-Miller 2004). Studies on human infants (Ben et al. 2004; Fanaro et al. 2005) and adults (Ito et al. 1990, 1993; Bouhnik et al. 1997) have emphasized the effect of GOS on bifidobacteria and the mixture of GOS and FOS on colonic flora. Studies have emphasized the laxative effects of GOS in the elderly (Teuri et al. 1998) and in adults (Ito et al. 1990; Deguchi et al. 1997; Teuri et al. 1998; Alles et al. 1999). Further clinical trials have also emphasized the beneficial bowel functions of a mixture of GOS and FOS in infants (Fanaro et al. 2005).

Bacterial fermentation of GOS increases biomass, leading to an increase in fecal bulk. Undigested oligosaccharides and fermentation byproducts may cause an osmotic effect in the gut, thus increasing the water content of feces, which in turn stimulates peristalsis in the colon (Cummings et al. 2001). Furthermore, GOS alter the bowel function through influencing colonic environment. In vitro studies indicate a lowering of colonic pH because of an increase in short-chain fatty acids with bacterial fermentation (Bouhnik et al. 1997; Blaut 2002). These stimulate the growth of lactobacilli and bifidobacteria and repress the growth of pathogens (Blaut 2002). SCFA may also have an effect on the intestinal motility; however, the clinical relevance and mechanisms of action remain unclear (Cherbut et al. 2003).

The microbial fermentation of GOS may enhance the production of gases in the colon (Cummings et al. 2001) and may cause gastrointestinal symptoms, including flatulence. Studies indicate that a maximum of 12 g of GOS daily are well tolerated by the body (Teuri et al. 1998; Niittynen et al. 2007).

GOS have been commercialized by Yakult Honsha and Nissin Sugar from Japan, Corn Products Intl. from the United States, and Borculo Domo Ingredients and Clasado from Europe (Torres et al. 2010). Recently, new products have come into the market, which are mixtures containing oligosaccharides of different degree of polymerization (CUP-oligo, Oligomate 55, TOS-100, Vivinal-GOS, Bimuno, Purimune, and Promovita-GOS). Food-grade GOS are transparent syrups or white powders.

4.2.4 Lactulose (Lactosucrose)

Lactulose, a disaccharide, comprises galactose and fructose (4-*O*-β-D-galactopyranosyl-D-fructose) that results from heat processing of milk (Tratnik 1998) or alkaline isomerization of lactose (Mussatto and Mancilha 2007).

It stimulates the growth and activity of *Bifidobacterium* species so it has been known as bifidus factor (Mizota et al. 1987; Matijevic et al. 2009). It also stimulates some species of *Lactobacillus*, *Clostridium*, and *Peptostreptococcus* in the large intestine in humans (Kneifel et al. 2000). Mizota et al. (2002) observed that fecal microflora varied with lactulose intake. Bifidobacteria increased from 22.4 to 50.5 % during intake and then decreased to 26.2 % after the treatment. Lactulose, as a prebiotic, has been observed to act as an energy source for bacteria and improve the quality of fermented skim milk in cocultures of the yogurt and probiotic cultures (*L. acidophilus*, *L. rhamnosus*, and *B. lactis* in combination with *S. thermophilus*) (Oliveira et al. 2011). It also acts as an osmotic active laxative characterized by shorter transit time and accelerated bowel movement. Lactulose is degraded by gut microbes into SCFA, which reduces the pH of the colon, raises the osmotic pressure, and increases the stool volume (Mizota et al. 2002). The amount of acetic acid rises from 30 to 35 μg/g in the feces during treatment, thus causing the fall in pH from 6.5 to 6.3 (Mizota et al. 2002s). Lactulose aids in the growth of the gut bacteria and enhances the biomass by utilizing ammonia and nitrogen. Acidification of gut inhibits ammonia production and reduces the residence time and passage of free ammonia from intestine to blood, thus reducing the blood ammonia levels (Mizota et al. 2002). In addition to ammonia, its administration also minimizes the concentration of putrefactive substance in the intestine, which includes skatol, indole, *p*-cresol, and phenol. Other physiological functions of lactulose include (Bouhnik et al. 2004; Seki et al. 2007) the following:

- Improvement of blood glucose responses
- Inhibition of secondary bile acid formation, thus preventing carcinogenesis (Challa et al. 1997)
- Activation of immune responses
- Treatment of salmonellosis
- Enhancement of mineral absorption such as calcium and magnesium

Physiological effects of this nonabsorbable carbohydrate also involve increased SCFA production (Jenkins et al. 1999; Gibson and Rastall 2006) that affects mucosal and systemic immunity in the host (Hooper et al. 2002). Hydrolyzed products, which include lipopolysaccharides, peptidoglycans, and lipoteichoic acids, exhibit immunomodulatory properties.

4.2.5 Lactitol

It is an emerging prebiotic, and it exists as lactitol monohydrate. Lactitol monohydrate (β-galactosidosorbitol) is highly soluble in water, with mild sweet taste (Riggio et al. 1990). It is a disaccharide alcohol, not absorbed in the human small intestine, and it reaches the colon as a potential substrate for the microbial fermentation (Kontula et al. 1999; Probert et al. 2004). Intake of high amounts of these sugar alcohols may have adverse consequences on the host system, including diarrhea.

4.2.6 Fructans (Polydextrose)

Polydextroseexpresses prebiotic effects by decreasing fecal pH, increasing residual concentration of SCFA and the number of bifidobacteria in feces (Jie et al. 2000; Probert et al. 2004). The nature of the glycosidic bonds in polydextrose enables it to undergo only partial fermentation by gut microbes and oppose enzymatic attack (Figdor and Rennhart 1981; Achour et al. 1994). It is a water-soluble, randomly bonded, condensation polymer of glucose with small amounts of sorbitol and citric acid. Polydextrose is not sweet and can be used as a low-calorie bulking agent in a wide range of food, including baked goods, confectionery, dairy products, and functional beverages.

4.2.7 Soy-Oligosaccharides (SOS)

Though soy is commonly consumed, the prebiotic potential of its oligosaccharides (raffinose and stachyose) has been less explored. Soybean seed is known as a rich source of galacto-

oligosaccharides, such as raffinose and stachyose. Raffinose, a trisaccharide, contains galactose connected to glucose unit of sucrose. Stachyose is a tetrasaccharide comprising of galactose linked to the terminal galactose unit of raffinose (Kim et al. 2003). These are linked by α(1 → 6) linkages. Noteworthy amounts of sucrose are observed in soy-oligosaccharides, together with the lower amounts of fructose, rhamnose, and arabinose. Seeds of legumes, lentils, peas, beans, chickpeas, and mustard are rich in raffinose (Johansen et al. 1996; Sánchez-Mata et al. 1998).

Humans do not possess α-galactosidases, essential for hydrolyzing the α(1 → 6) linkages in the oligosaccharides. Hence, these prebiotics are not digested when consumed. Intact oligosaccharides reach colon to undergo preferential fermentation by beneficial bifidogenic microorganisms, which contain the enzyme (Liu and Xu 2008). Fermentation results in production of gases (CO_2, H_2, CH_4, etc.) and SCFA that are remarkable for their prebiotic activity and associated health benefits (Gibson and Roberfroid 1995). High dietary levels of soy-oligosaccharides result in harmful effects on the intestine. The presence of soy-oligosaccharides in piglets' diet reduces the nutrient digestibility and growth performance and increases the incidence of diarrhea, since the endogenous enzymes for their hydrolysis are not present in their gut. The prebiotic activity of soy-oligosaccharide is dose dependent. Normally, in pigs, 40–50 % of oligosaccharides and fructans are digested in the small intestine; and are completely digested in the large intestine, resulting in an increase in the number of lactobacilli and *Bifidobacterium* and a decrease in clostridia and enterobacteria (Nemcova et al. 1999).

Fermentation of SOS by lactobacilli has been reported in the SHIME with the inoculums of fecal bacteria inoculums. In vitro studies indicate that SOS has similar effects as other galactooligosaccharides on gut bacteria. Growth of pure beneficial microbes has been observed with purified individual compounds or mixture of oligosaccharides.

4.2.8 Xylo-oligosaccharides (XOS)

Xylo-oligosaccharideshave immense prebiotic benefit and are included into many food products. XOS are found in bamboo shoots, fruits, vegetables, milk, corn, wheat, and honey (Vazquez et al. 2000). It is produced on an industrial scale from lignocellulosic plant material, which is rich in xylan, by chemical and enzymatic methods. The latter is preferred in the food industry as it lacks undesirable side reactions and by-products. The fermentation of XOS results in decrease in pH and the formation of SCFA, which serves as fuel and regulate cellular processes.

XOS are mixtures of oligosaccharides formed from xylose residues that are linked through β(1 → 4) linkages (Aachary and Prapulla 2008). Based on the xylose residues (2–10) involved in the XOS, it could be classified into xylobiose, xylotriose, and so on. Xylobiose with degree of polymerization of 2 is considered as XOS for food applications (Vazquez et al. 2000). XOS also have α-D-glucopyranosyl uronic acid or its 4-*O*-methyl derivative. Acetyl groups or arabinofuranosyl residues as side groups are also present, and these variations lead to diverse biological properties. Their biological properties include antioxidant activity (contributed by linked phenolic components), antiallergy, blood and skin-related effects, antiinfection, anti-inflammatory property, immunomodulatory activity, and antihyperlipidemic effects (Izumi et al. 2004).

4.2.9 Isomalto-oligosaccharides (IMO)

Isomalto-oligosaccharide is a nondigestible low-calorie health sweetener, which maintains the growth of colonic beneficial bacteria in the colon, thus acting as a prebiotic. It is composed of glucose monomers linked by α(1 → 6) glycosidic linkages. IMO has a mixture of isomaltose (Glu α1 → 6 Glu), isomaltotriose (Glu α1 → 6 Glu α1 → 6 Glu), and panose (Glu α1 → 6 Glu α1 → 4 Glu).

IMO can be easily assimilated by colonic bacteria and selectively increase the amount of *Bifidobacterium* approximately by 2–4 times. Panose, isomaltose, isomaltotriose, and isomalto-900 selectively aid in the proliferation of the bifidobacteria. Human trials indicate that colonic microflora flourish in the presence of IMO. The minimum effective dose of IMO is 8–10 g/day and Isomalto-900 is 13–15 g/day (Kohmoto et al. 1991). The hydrolysis of IMO in human jejunum by the enzyme isomaltase makes them available as substrate for colonic microbiota.

IMO naturally occurs in honey, sugarcane juice, and products derived thereof, such as treacle or food-grade molasses (Lina et al. 2002). It is generally used as a low-calorie sweetener mixed with a variety of other foods and beverages for the purpose of sweetening. It can be used to replace maltose syrup. Commercial Isomalto-900 is produced by incubating α-amylase, pullulanase, and α-glycosidase with cornstarch (Kohmoto et al. 1988).

4.2.10 Resistant Starch (RS) and Dextrin (RD)

Starch that escapes human digestive system and is transported to the lower gut for bacterial fermentation is designated as resistant starch. RS stimulates increased fecal bulk and low colonic pH, controls blood sugar level, improves bowel health, and reduces cardiovascular risk (Slavin et al. 2009). It is found in grains, cereals, tubers (especially potatoes), legumes, seeds, and some nuts (Goldring 2004; Fuentes-Zaragoza et al. 2011). RS is a linear molecule of a-1,4-D-glucan and has a relatively low MW (120 kD) (Fuentes-Zaragoza et al. 2011).

Five different types of resistant starch have been reported, and they are as follows:

- Type 1: physically amylase inaccessible – type1-RS1 includes the starch in plant cells with undestroyed cell walls. It is nondigestible by human gut enzymes and, together with plant tissue fragments, passes to the large bowel to undergo fermentation (Onyango et al. 2006). It is heat stable, which enables its use in conventional foods.
- Type 2: native high amylase – type2-RS2 is composed of native starch granules containing uncooked starch or poorly gelatinized starch.
- Type 3: retrograded type – type3-RS3 includes the substances precipitated from pap or starch gel (1.5 % amylose, 10 % amylopectin) during retrogradation. During the storage of the gel, starch helix undergoes aggregation forming thermally stable crystal structures, which is resistant to amylolytic enzyme (Lafiandra et al. 2014). Its cooking stability enables its usage in conventional foods (Champ et al. 2003). Storey et al. (2007) classified "retrograded resistant maltodextrins" as type3-RS.
- Type 4: chemically modified – type4-RS4 includes chemically or physically modified starch or by a combination of these two processes. New functional groups are added into the starch during chemical modifications that prevent digestion. In physical method, during high-temperature processing or in the presence of catalyst, dextrinization occurs, releasing free glucose that binds randomly to the chain, resulting in resistant starch (Brown 1996; Onyango et al. 2006).
- Type 5: fatty acid complex type – type5-RS5 is formed from high AM-starches (amylomalto-starches), which involve high gelatinization temperature and are susceptible to retrogradation. Frohberg and Quanz (2008) defined RS5 as water-insoluble linear poly α-1,4-glucan that is not susceptible to degradation by alpha-amylases. They also established that these aid in SCFA formation, particularly butyrate, in the colon.

RS is safe for consumption and is a natural component of some foods. Similar to soluble fiber, RS if consumed regularly (5–6 g/day) is proved to be beneficial in lowering blood sugar levels (Behall et al. 2006). RS has a low calorific value (8 kJ/g) compared to fully digestible starch (15 kJ/g) (Rochfort and Panozzo 2007). It could be fermented by human gut microbes and alter the composition of the microbial species and their metabolic activities, and formation SCFA,

which prevent the growth of cancerous tumors (Fuentes-Zaragoza et al. 2011). RS consumption has been reported to reduce postprandial glycemic and insulinemic responses, thereby assisting in the management of diabetes and associated cholesterol and triglycerides levels. Other benefits include lowering of colonic pH, increased excretion frequency, fecal bulking, prevention of constipation and hemorrhoids, and decreased production of carcinogens and ammonia levels. Others also reported that RS aids in the absorption of calcium, magnesium, zinc, iron, and copper in rats (Lopez et al. 2001). However, in humans, RS aids in the absorption of only calcium (Fuentes-Zaragoza et al. 2011).

FOS and RS may act synergistically (Rodríguez-Cabezas et al. 2010; Fuentes-Zaragoza et al. 2011), increasing lactobacilli and bifidobacteria in caecum and colon. FOS are more active in the first part of the large bowel, while RS reaches the distal part of the colon. Administration of FOS or raw potato starches stimulates different bacterial populations and metabolites in the caecum, proximal, and distal ends of the colon, and feces (Fuentes-Zaragoza et al. 2011). Compared to RS, FOS double the pool of fecal fermentation products, such as lactate, while the situation is just the opposite in the distal region. These observations confirm that each prebiotic demonstrates unique properties, which should be considered before their application for colon diseases. Furthermore, complementary kinetics of different prebiotics and their benefits should be considered in promoting health (Younes et al. 2001; Fuentes-Zaragoza et al. 2011). Younes et al. (2001) examined the synergistic effect of inulin and RS in male Wistar rats and observed that these prebiotics significantly increase the mineral absorption in the intestine, without altering their plasma levels.

Resistant dextrin is defined as short-chain glucose polymers, with no sweet taste and which show resistance to hydrolysis by human digestive enzymes (Ohkuma and Wakabayashi 2001). Most probably, dextrinization undergoes the mixed mechanism.

Nutriose, a commercial resistant dextrin, is a non-viscous soluble fiber made from starch using a highly controlled dextrinization process and is totally soluble in cold water without inducing viscosity (Lefranc-Millot 2008). It is produced from wheat (Nutriose FB®06) or maize starch. It has wide applications in food and pharmaceutical industries and is incorporated in fiber-enriched drinks, enteral nutrition, granulation binders, low-calorie food, and sugar-free confectionery (Serpelloni 2011). About 15 % of this is absorbed in the small intestine and 75 % is fermented in the large intestine, while the leftovers are excreted in the feces (Pasman et al. 2006). It decreases the number of *Clostridium perfringens* in human feces, reduces fecal pH, and increases SCFA production (Pasman et al. 2006). Also, it exhibits a progressive and significant impact on short-term satiety, which is time and dosage correlated (Guerin-Deremaux et al. 2011). It aids in a beneficial shift in the bacterial microbiome to butyrogenic genera such as *Peptostreptococcus*, *Fusobacterium*, and *Bifidobacterium* (Guerin-Deremaux et al. 2011).

Fibersol-2 is a well-known soluble, nondigestible, starch-derived resistant maltodextrin and produced from cornstarch by pyrolysis and subsequent enzymatic treatment forming random 1, 2- and 1, 3- α or β linkages (Ohkuma and Wakabayashi 2001). It is readily dispersible in water with no flavor and odor and is highly compatible with dry drink mix applications. It shows stability to acid and heat and could be used in retorted products (juices, sauces, puddings, fluid milks). It also shows superior freeze-thaw stability. Studies indicate that Fibersol-2 could effectively reduce the blood glucose and insulin, blood triglycerides, and serum cholesterol. It increases the fecal bulk and reduces transit time, thus maintaining colon health and reducing the incidences of colon cancer (Ohkuma and Wakabayashi 2001).

Arabinogalacto-oligosaccharides, arabinoxylo-oligosaccharides, arabino-oligosaccharides, galacturonan-oligosaccharides, rhamnogalacturonan-oligosaccharides, and pectic-oligosaccharides are some of the newly introduced prebiotics that exhibit fermentative action in pure intestinal bacteria, including *Bifidobacterium*, *Lactobacillus*, *Bacteroides* sp., *Clostridium* sp., *Escherichia coli*, and *Klebsiella* sp. (Oosterveld et al. 2002). Other oligosaccharides, which exhibit prebiotic activity,

include chitosan-oligosaccharide, chitin-oligosaccharide, gluco-oligosaccharides, and oligosaccharides from melibiose, mannan-oligosaccharides, oligodextrans, and gentio-oligosaccharides (White et al. 2002). Some probiotic bacteria have been reported to produce by themselves polysaccharides (but not oligosaccharide) with prebiotic effects for their growth (Dal Bello et al. 2001).

4.3 Prebiotics and Gut Microbiota

The gastrointestinal tract of the human embryo is virtually sterile and is colonized by microbes at birth (Fuller 1991, 1992). The microbiome that develops depends on the method of delivery of the infant (Salminen et al. 1998) and hygiene precautions associated with parturition (child birth) (Zetterstrom et al. 1994). In addition to characteristic vaginal flora, lactobacilli, yeast, streptococci, staphylococci and *Escherichia coli*, the neonate may come in contact with fecal microbes and skin bacteria during birth (Fuller 1991; Goldin and Gorbach 1992). Further, inoculation from the general environment and other external contacts may also play a key role in determining gut microbiome (Gronlund et al. 1999). During acquisition period, some bacteria briefly colonize the gut while others become indigenous. Thus, the neonatal gut faces a rapid microbial succession in the first few days to months of development by luminal redox potential (Eh) but more often by the feeding regime that follows its birth (Zetterstrom et al. 1994). Initial colonizers utilize the available oxygen, within 48 h, and allow succession of obligate anaerobes belonging to bifidobacteria, bacteroides, and clostridia groups. Hence, feeding method significantly influence the gut environment. Historically, breast-fed infants are thought to have relatively higher proportions of bifidobacteria (Fuller 1991, 1992). Breast feeding and bifidobacteria in the gut could be linked to the lower risks of gastrointestinal, respiratory and urinary tract infections (Kunz and Rudloff 1993). However, as the nature of commercial diet has altered and enriched in recent years, the naturally flourished bifidobacterial predominance in breast-fed infants is less significant. Moreover, these also display the positive capability of prebiotic-enriched diet in influencing the gut microbial composition and the health.

Studies have indicated that metabolic equilibrium is maintained by gut flora and an altered gut microflora could be associated with several diseased conditions, including cancer, cardiovascular diseases, obesity, type-2 diabetes, antibiotic-associated diarrhea, hypercholesterolemia, and others that are very commonly prevalent in the 21st century.

Prebiotics are unique in their functionalities, which selectively stimulate the growth of beneficial gut bacteria and contribute to the health of the host. It is reported that changes in the composition of gut microbiota occur not only at phyla but also at species levels; for instance, a lower bifidobacteria count could be associated with overweight and obesity. Overweight mothers give birth to neonates with a decreased number of bifidobacteria suggesting that obesogenic microbiota is an "inheritable" trait.

Bifidobacteria serve as a perfect model for the concept of role of prebiotics in gut management. Bifidobacteria population (and other microorganisms in the Group Firmicutes) is slightly lower in individuals with obesity (Ito et al. 1990). Similar observation is reported in the case of type-2 diabetes mellitus patients in comparison to nondiabetic patients (Mazmanian et al. 2008). These conclusions recommend that bifidobacteria play an important element in the development of obesity and related comorbidities. Feeding prebiotics like dietary fructans or inulin-type fructans to mice indicates that the oligosaccharide is used as energy substrate by bacteria, which assist in its growth and reduce the levels of lipopolysaccharide, glucose tolerance, and fat mass (Ghanim et al. 2009; Larsen et al. 2010; Basu et al. 2011; Lathrop et al. 2011). Further, prebiotic approach also prevents the overexpression of several host genes related to adiposity and inflammation. These dietary fructans on feeding in rodents increase the amount of endocrine L cells in jejunum and colon and promote the production and release of the active forms of GLP1 and GLP2 in the portal vein. GLP1 is reported to participate in the prebiotic-motivated appetite loss, loss of fat mass, and hepatic insulin resistance (Cani

et al. 2006), while GLP2 adds to the reduced permeability of the intestinal wall and endotoxemia associated with obesity (Cani et al. 2009; Delzenne et al. 2011).

4.4 Prebiotics and Health

Prebiotics have been utilized in the food industry for their functional and health promoting properties. These are used in food and beverage industries for their individual unique and inherent properties such as increase in emulsification, gel formation, low sweetness, low glycemic index, and modulation of viscosity. Common prebiotics that are used in improving gut health and act as supplements to food are dietary fructans, inulin, galacto-oligosaccharides, fructo-oligosaccharide, lactulose, lactitol, and resistant starch (Lin et al. 2005; Gibson et al. 2010).

Fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are "Generally Regarded as Safe" (GRAS). Others include xylo-oligosaccharides (XOS), isomalto-oligosaccharides(IMOS),gluco-oligosaccharides, pectin oligosaccharides (POS), mannan-oligosacharides (MOS), gentio-oligosaccharides (GTO), chito-oligosaccharides (CHOS), soybean oligosaccharides (SOS), and polydextrose, which are not commercially available in high purity (Gibson et al. 2004; Rizzello et al. 2011).

The possible health benefits of prebiotics could be broadly summarized as follows:

- Prevention of cancer and tumor growth
- Alleviation of lactose intolerance
- Lowering of serum cholesterol
- Enhancement of the immune system
- Prevention of vaginal infections
- Alleviation of allergic conditions

4.4.1 Prebiotics and Cancer

Femia et al. (2002) reported that the protective effect of probiotics on azoxymethane-induced carcinogenesis in vivo is lower in comparison to the effects of prebiotics (oligofructose-inulin), which could be due to the lower proliferation in the colon at the promotion stage. Similar beneficial effects of these prebiotics are observed against 1,2-dimethylhydrazine-induced colon cancer (Hughes and Rowland 2001; Pool-Zobel 2005). An addition of 5–15 % inulin or oligofructose in feed is reported to lower the incidence of breast tumor in rats and mice and lung metastasis. High-fat diets combined with inulin and *Lactobacillus acidophilus* treatment significantly reduced formation of aberrant crypt foci (ACF) in rats (Bolognani et al. 2001).

Consumption of fermented dairy products has been reported to lower the prevalence of colon cancer. Furthermore, preliminary in vivo studies have suggested that prebiotics could act as antimutagens (Taper et al. 1997). RS suppresses the formation of colonic aberrant crypt in a dose-dependent manner and may retard the growth and development of neoplastic lesions in the colon. This suggests the usefulness of RS as a preventive agent for individuals with high risk of developing colon cancer (Liu and Xu 2008).

Prebiotics may directly or indirectly affect carcinogenesis, colonization of bacteria and their proliferation, and inflammation. Fermentation of prebiotics leads to SCFA formation, which affects colonic mucosa. Treatment of human colon carcinoma cell lines, L97 and HT29, with the supernatant fractions of inulin fermentation inhibited their growth and induced apoptosis (Munjal et al. 2009). Butyrate produced during fermentation inhibits the growth of budding premalignant and malignant cells. Preclinical studies have also supported the view that butyrate might be a chemopreventive agent and may aid in preventing carcinogenesis (Scheppach and Weiler 2004) or act as guardian against colon cancer by promoting cell differentiation (Kim et al. 1982).

4.4.2 Prebiotics and Lactose Intolerance

Lactose intolerance is one of the worst problems affecting the world population. Lactose acts as an osmotic and non-digestible carbohydrate.

These subjects have low amounts of lactase (β-galactosidase) in the intestinal mucosal cells, which help to digest lactose to glucose and galactose. It worsens with age. Intake of milk could be avoided to avert this issue, but it will limit the availability of dietary calcium (Hamilton-Miller 2004).

Fermented dairy products (yogurt and milk containing *L. acidophilus*) have been observed to be easily digested by them. This could be due to the β-galactosidase activity of the bacteria that hydrolyzes lactose (Hamilton-Miller 2004). *L delbrueckii* var *bulgaricus* and *S salivarius* var *thermophilus* are also used for fermenting milk. These organisms pass through the stomach and are hydrolyzed by the pancreatic secretions (Hamilton-Miller 2004). The role of probiotics in improving lactose digestion and intolerance has been reported by many scientists (Mustapha et al. 1997).

The effects of prebiotics on lactose intolerance are uncertain. However, National Institute of Health (NIH) (2010) recommends that milk and milk products should not be avoided (even by lactose-intolerant individuals) as they have potential beneficial impact on the gut microbiota. Few strategies suggested to overcome lactose intolerance are to recondition the gut with smaller servings of milk and increase it over a few weeks, consume hard cheese as it has low lactose content and consume lactose-free milk and lactose tablets.

4.4.3 Prebiotics and Cholesterol

The World Health Organisation (WHO 2009) predicted that by 2030, cardiovascular diseases will be the leading cause of mortality, affecting about 23.6 million people around the world. Hypercholesterolemia contributes to about 45 % of heart attacks in Western Europe and 35 % in Central and Eastern Europe from 1999 to 2003. Thus, risk of heart attack is three times high in patients with hypercholesterolemia.

Prebiotics aid in the growth of beneficial gut bacteria and enhance SCFA production in the gut, which has direct effects on cardiovascular diseases and lipid profile (Wolever et al. 2002).

Yogurt containing live cultures of *L acidophilus* (Anderson and Gilliland 1999) and yogurt fermented with *L acidophilus* with added fructooligosaccharides (Schaafsma et al. 1998) are able to reduce the serum cholesterol; a reduction of 4.4 % is observed with the latter. Similarly, inulin is also reported to lower cholesterol (Davidson et al. 1998).

In a randomized, double-blind, and crossover study using hypercholesterolemic subjects to assess the prebiotic effect of inulin on blood cholesterol level, it is observed that a daily intake of 20 g inulin significantly reduced the serum triglycerides (Causey et al. 2000). Similar conclusions are reached in another study with 10 g of inulin (Letexier et al. 2003). In addition, the levels of plasma triacylglycerol are also low (Letexier et al. 2003). Another randomized, double-blind, placebo-controlled, and parallel design model also indicated that prebiotics affect the lipid profiles (Brighenti et al. 1999). A daily consumption of 50 g of rice-based ready-to-eat cereal (inulin-18 %) reduced plasma total cholesterol and triacylglycerols by 7.9 and 21.2 %, respectively. Others also observed that the purified diet containing 10 % long-chained fructan significantly reduced the blood cholesterol (29.7 %), LDL (25.9 %), IDL (39.4 %), and VLDL (37.3 %) (Mortensen et al. 2002). Other indigestible and fermentable compounds such as germinated barley, oligodextrans, lactose, hemicellulose-rich substrates, resistant starch and its derivatives, etc., have also been reported to exert hypocholesterolemic effects (Gibson et al. 2004). Cholesterol-lowering effect of resistant starch is also reported by others (Fernandez et al. 2000). They also observed that prebiotic significantly reduced plasma cholesterol (27.4 %) and LDL (28.0 %). In another randomized, placebo-controlled and parallel-designed study, it is seen that feeding male Wistar rats with the starch from Chinese yam (*Dioscorea opposita* cv. Anguo) significantly lowered plasma total cholesterol, LDL-cholesterol, and triglyceride (Wang et al. 2008). In another study, it is reported that daily feeding of 25 g/kg of β-cyclodextrin to male Wistar rats significantly reduced the plasma cholesterol and triacylglycerols (Favier et al. 1995).

4.4.4 Prebiotics and Osteoporosis

Several studies have noted that prebiotics (inulin, oligofructose, galacto-oligosaccharides, resistant starches, or lactulose) effectively stimulate calcium absorption in vivo (Scholz-Ahrens and Schrezenmeir 2002; Scholz-Ahrens et al. 2007). These studies are carried out in diseased models or altered physiological status such as gastrectomized, ovariectomized, cecectomized, and magnesium-, calcium-, or iron-deficient rats. It is observed that prebiotic effects of calcium absorption occur at large intestine level (Baba et al. 1996). Brommage et al. (1993) reported that calcium absorption in cecectomized rats could be enhanced by stimulating them with lactulose. Griessen et al. (1989) reported that in healthy adult volunteers, the calcium absorption from milk (21.4 %) and lactose-free milk (26.8 %) is similar. Lactose increased the calcium absorption in β-galactosidase-deficient subjects too. Increase in calcium absorption is observed with increased concentration of lactulose in postmenopausal women (van den Heuvel et al. 2000). Inulin, fructo-, and galacto-oligosaccharides are also seen to increase calcium absorption in adolescents (van den Heuvel et al. 2000; Cashman 2003).

Further, the effect of prebiotics on calcium absorption showed a dose-dependent effect and it depended on the amount of calcium in the diet and prebiotics in the feed. Galacto-oligosaccharides stimulated the calcium absorption in intact rats when they are fed with a diet containing 5 g of calcium/kg than with 0.5 g of calcium/kg (Chonan and Watanuki 1996). Similar prebiotic effect of oligofructose on metabolic calcium balance in ovariectomized rats is reported by others (Scholz-Ahrens et al. 2007). The effect is significant when they are fed with 10 g of calcium/kg of diet than recommended level (5 g of calcium/kg of diet). Linear increases in the intestinal calcium absorption in rats are observed when they are fed with different concentrations of lactulose (Brommage et al. 1993).

Oligofructose, transgalacto-oligosaccharides, lactulose, and resistant starch also stimulate the intestinal magnesium absorption in rat models (Scholz-Ahrens and Schrezenmeir 2002; Scholz-Ahrens et al. 2007) and may contribute to the bone mineral density.

The possible mechanisms by which prebiotics enhance calcium absorption in intestine could be as follows:

- Prebiotic hydrolysis by the gut enzymes leads to SCFA production. This reduces the luminal pH and increases the solubility of calcium in the large intestine.
- SCFA also aid in transcellular calcium absorption by increasing the exchange of cellular H^+ for luminal Ca^{2+}.
- Butyrate is involved in cell growth and hence increases absorptive surface area and increases mucosal calbindin D_{9K} levels in the large intestine.
- Increase in the metabolites, namely, polyamines that lead to cell growth, increase in the absorptive surface area, and so stimulate the gene expression of calbindin D_{9K}.

The possible mechanism of mineral absorption in reducing the risk of osteoporosis could be speculated as the augmentation of bone mineral density and assisting in bone regeneration and strength. Prebiotic-mineral absorption induces increased solubility of minerals due to acidic pH (SCFA), incidence of SCFA-salt conjugates, augmented absorption surface, increased expression of calcium-binding proteins, degradation of phytic acid-mineral complex that liberates associated minerals, release of bone-modulating factors, such as phytoestrogens, from food and stabilization of intestinal microflora and intestinal mucus (Scholz-Ahrens and Schrezenmeir 2002; Coudray et al. 2003; Scholz-Ahrens et al. 2007).

4.4.5 Cancer

Extensive researches have been carried out and are still ongoing to understand the effects of different prebiotics on colon cancer. Non-digestible oligosaccharides selectively utilized by probiotics in the colon help in reducing the risk of colon

cancer. Hsu et al. (2004) reported that both XOS and FOS markedly reduced the number of aberrant crypt foci in the colon of DMH-treated male Sprague-Dawley rats. Studies indicate that GOS, FOS, and lactulose boost the intestinal lactate concentration and SCFA, stool frequency, and fecal weight. They also decrease the secondary bile acids, colon pH, and nitroreductase and β-glucuronidase activities, signifying the apparent role of prebiotics in CRC (Bruno-Barcena and Azcarate-Peril 2015) The results suggest that dietary supplementation of prebiotics may have beneficial impact on gastrointestinal health and prevent colon cancer. The mechanism of action of prebiotics in preventing colon cancer is detailed in the following sections.

4.4.6 Anti-inflammatory

Prebiotic consumption imparts antitumorigenic effects through improvement of the immune response. Immunostimulating effects and anti-inflammatory activities are reported for the prebiotics arabino-(glucurono) xylans and 4-*O*-methylglucuronoxylan and highly branched heteroxylan. Partially *O*-acetylated XOS and deacetylated XOS show mitogenic activity and development of the mitogen-induced proliferation of rat thymocytes, representing its immunostimulatory potential (Nabarlatz et al. 2005). XOS exhibit dose-dependent direct mitogenic and comitogenic activities, which is similar with immunogenic water-soluble arabinoglucuronoxylan (Ebringerova et al. 1998). Feeding modified arabinoxylan to C57BL/6 and C3H mice improves the activity of NK cells and their binding to tumor cells indicating the induction of host immunity (Ghoneum and Abedi 2004).

Prebiotics may show indirect immunogenic effects by manipulating the intestinal microflora and altering the immune parameters such as NK cell activity, secretion of IL-10 and interferon, and lymphocyte proliferation (Janardhana et al. 2009). GOS is reported to decrease the incidence of colitis in Smad3-deficient mice by altering the function and trafficking of NK- cells (Gopalakrishnan et al. 2012). Prebiotics also modulate the immune parameters in gut-associated lymphoid tissue (GALT), secondary lymphoid tissue, and peripheral circulation (Brisbin et al. 2008). Influence of prebiotics on the GALT and modulation of human immune system and colon cancer are research areas that need in-depth study. Although prebiotics are shown to have immunomodulatory effect, more studies are necessary to establish the mechanistic role of prebiotic-induced immunomodulation against cancer.

4.5 Mechanism of Action of Prebiotics against Colorectal Cancer

The mechanism of action of anticarcinogenic effects of prebiotics could be classified as summarized below (Table 4.5). A schematic diagram showing the potential effects of prebiotics in preventing cancer is given in Fig. 4.3.

4.5.1 Stimulation of Beneficial Gut Microbes

Prebiotics alter gut environment and encourage lactobacilli and bifidobacteria to bind and eliminate toxins from the system. Intake of long-chain inulin-type fructans in rodents increases the bifidogenic effects, lowers pH and modulates immunity, and reduces the azoxymethane (AOM)-induced colonic pre-neoplastic aberrant crypt foci (ACF) and colon tumors (Verghese et al. 2002). Inhibitory effects of short FOS and inulin are also reported in ACF-induced rat models (Rowland et al. 1998). Both XOS and FOS are seen to inhibit colonic ACF in dimethylhydrazine (DMH)-treated rats by lowering cecal pH and serum triglyceride. Gain in total cecal weight, increase in bifidobacterial population, and reduction in ACF in the colon are evident (Hsu et al. 2004). GOS are also observed to considerably hamper the development of DMH- or AOM-induced colorectal tumors (Bouhnik et al. 1997). Inulin, FOS, and GOS undergo fermentation in the large intestine, stimulate microbial growth,

Table 4.5 Prebiotics and their mechanism of action in various disease conditions

Prebiotic	Medical benefits	Mechanism of action	References
Inulin	Crohn's disease	Enhancement of immune response	Hijová et al. (2013)
	Colitis	Effect on innate immunity	Macfarlane et al. (2008)
			Ramirez-Farias et al. (2009)
	Obesity	Modification of microbiome and increase in bifidobacteria	Hopping et al. (2009)
	Diabetes type 2		Costabile et al. (2010)
	Colon cancer		Ramnani et al. (2010)
	Constipation		
Fructo-oligosaccharide	Crohn's disease	Increase in bifidobacteria	Scholtens et al. (2006)
	Colitis	Decrease in colon pH	Benjamin et al. (2011)
	Obesity	Reduction in lipid accumulation	Aller et al. (2011)
	Constipation	Secretion of anti-inflammatory substances	Cummings et al. (2001)
	Traveler's diarrhea	Local induction of reactive oxygen species	Arslanoglu et al. (2008)
	Colon cancer		
			Boutron-Ruault et al., (2005)
Galacto-oligosaccharide	Crohn's disease	Improvement of growth performance and immune responses	Saavedra and Tschernia (2002)
			Macfarlane et al. (2008)
	Colitis	Diminishment of intestinal bacterial overgrowth	Drakoularakou et al. (2010)
	Obesity		

and increase bacterial mass, fecal bulking, and peristalsis (Den Hond et al. 2000; Niittynen et al. 2007). Bulking reduces the transit time and contact time to luminal lining, thus preventing cancer.

4.5.2 SCFA and Lactic Acid

Bacterial fermentation of prebiotics in the colon aids in the formation of SCFA, which assists in maintaining gut health and intestinal morphology and function (Scheppach 1994; Roy et al. 2006). Commonly formed SCFA are acetic, propionic, and butyric acids. Butyrate serves as fuel for colonocytes, lowers colon pH, and serves as histone deacetylase inhibitor, altering DNA methylation resulting in improved access of transcription factors to nucleosomal DNA (Hammers et al. 2008). Lactate has been reported to improve gut health and elevate gut-associated immune defense mechanism (Scott et al. 2013).

Butyrate reduces colonic cell proliferation and induces differentiation in colonic epithelial cells. Sodium butyrate was reported as a powerful inhibitor of growth and inducer of differentiation and apoptosis, thereby possessing a beneficial effect against colon cancer development. Fermentation by gut bacteria on a high amylose starch diet produced butyrate, which increased the detoxification of electrophilic products associated with oxidative stress (Lishaut et al. 1999).

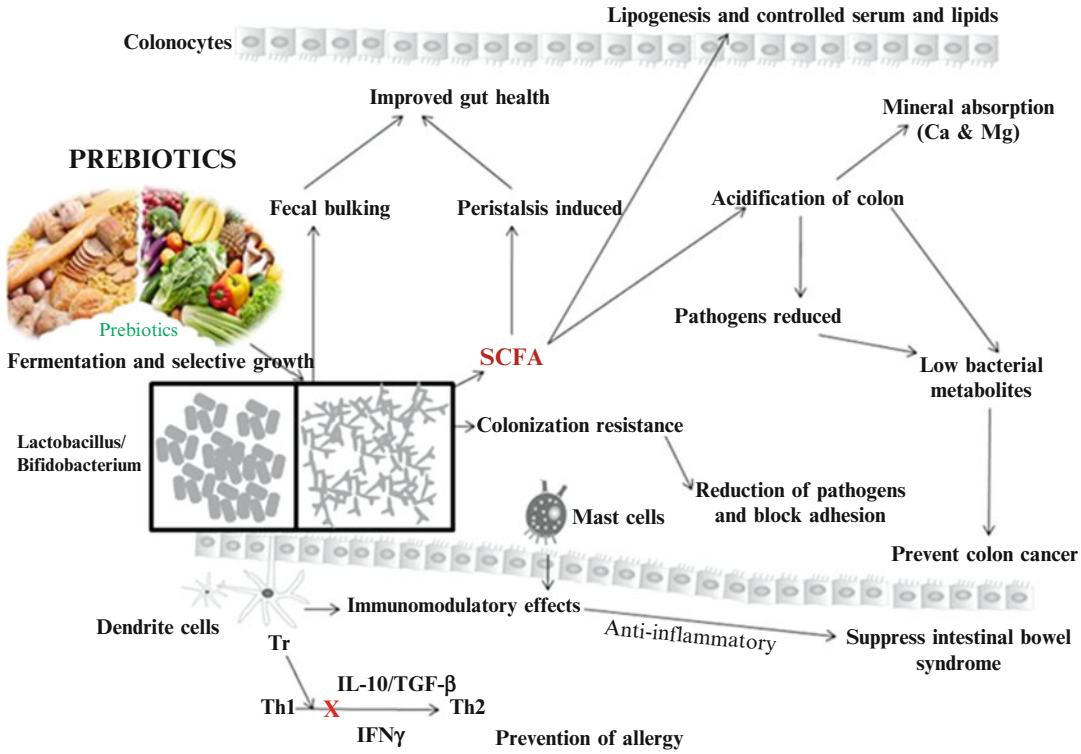

Fig. 4.3 Potential health benefits and mechanism of prebiotic action (Aachary 2009)

The ability of prebiotic resistant starch type-3 Novelose 330 to reduce the incidence of colon carcinogenesis *via* induction apoptosis in rats was established, and the effect was attributed to the increased production of butyrate (Bauer-Marinovic et al. 2006). The role of individual SCFA in preventing colon carcinogenesis has been discussed in detail elsewhere in the book.

4.5.3 Absorption of Micronutrients by Colon Cells

Increased mineral absorption (calcium, magnesium, selenium), vitamin D, and antioxidants due to the intake of short- and long-chain fructans aid in preventing cancer risk and maintaining the normal bowel structure (Scholz-Ahrens and Schrezenmei 2002). Inulin, oligofructose, FOS, GOS, SOS, RS, lactitol, and others have beneficial effect on mineral absorption and metabolism (Scholz-Ahrens et al. 2007).

4.5.4 Prebiotics Modulate Xenobiotic Metabolizing Enzymes

Xenobiotic metabolizing enzymes consist of phase I and phase II enzymes, and they are considered as indices of colon cancer.

Phase I enzymes consist of cytochrome b5, cytochrome b5 reductase, cytochrome P450, cytochrome P450 reductase, cytochrome P450 2E1.

Phase II enzymes consists of glutathione S-transferase (GST), uridine diphospho-glucuronyltransferase, and DT-diaphorase.

Both phase I and phase II enzymes assist in the inactivation of procarcinogens and their elimination from the system (Johnson et al. 2012). RS is reported to induce glutathione transferase π in rat colon (Wollowski et al. 2001). SCFA also induce glutathione transferase π as observed in Caco-2 cell lines (Johnson et al. 2012). Intake of prebiotics alone or in combination (horse chestnut/flaxseed) minimizes the β-glucuronidase

activity and improves the β-galactosidase and β-glucosidase activity highlighting the anticarcinogenic behavior of prebiotics (Hijová et al. 2012). Reduced bacterial β-glucuronidase activity and low toxic fecal ammonia are reported in corn hemicelluloses-fed healthy human subjects (Sugawara et al. 1990). Similarly, AXOS also stimulates an increase in the uptake and assimilation of nitrogen and excretion of ammonia through feces in humans (Cloetens et al. 2010). The protective effect of fructans and other prebiotics on AOM-induced carcinogenesis could be due to the downregulation of gene expression of inducible NO synthetase and cyclooxygenase-2 (Femia et al. 2002). The role of xenobiotics in preventing cancer is a new area of research that might catch attention in future.

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Synbiotics and Colorectal Cancer

5

Abstract

Synbiotics are defined as the combination of appropriate probiotics and prebiotics, where the latter form the substrate for the growth and development of selective indigenous or introduce beneficial bacteria in the colon. The prebiotics commonly in use are inulin, fructo-oligosaccharide, galacto-oligosaccharide, lactose, etc., and the probiotics investigated include *Bifidobacterium* and *Lactobacillus* spp. Prebiotics form the food for the growth of probiotics. The mechanism of action of synbiotics is a collective effort of pro- and prebiotics. Administration of synbiotics could prevent the initiation or early stage of cancer and also treat the existing tumors. Synbiotic interventions bring about significant alterations in the composition of the colonic microbiome leading to the altered metabolic activity of the organ. It reduces the exposure of cytotoxic agents, including mutagens and carcinogens, to the intestinal lining; decreases cell proliferation in the colonic tissue; and develops mucosal structure. It is reported that administration of synbiotics results in noteworthy changes in the fecal flora, with an increase in *Bifidobacterium* and *Lactobacillus* and a reduction in the harmful pathogens such as *Clostridium perfringens*. These also inhibit the fecal water to cause necrosis in the colonic cells and improve epithelial barrier functions. Synbiotics are also reported to show immunomodulatory effects.

Keywords

Synbiotics • Prebiotics • Probiotics • Gastrointestinal tract • Short-chain fatty acids • Microbial • Dietary supplement • Anticancer • Pathogen • Colorectal cancer

M. Raman et al., *Probiotics and Bioactive Carbohydrates in Colon Cancer Management*, DOI 10.1007/978-81-322-2586-7_5

5.1 History, Definition, and Statistics

The significance of synbiotics, as a nutritional supplement, in preventing non-communicable and communicable diseases rose recently. It is a novel area of research in functional foods and nutraceuticals that is being explored and is emerging for its several hidden health benefits.

The concept of synbiotics is not well developed and demands validation and further research. Roberfroid (1998) introduced the concept of prebiotic in 1995, and it has become very popular since then. However, the concept of synbiotic has, till now, not been widely applied and is a new notion in the development of functional foods or nutraceuticals. Synbiotic ("Syn" means "together" and "bios" means "life") is defined as a supplement that contains both a prebiotic and a probiotic that work together to improve the "friendly flora" of the human intestine and is of commercial value. Two concepts were projected by Kolida and Gibson (2011), and these are complementary and synergistic.

1. Complementary concept: a single or a combination of probiotics and prebiotics that are independently chosen to stimulate the beneficial gut microbial population. Prebiotics promote indirectly the growth and activity of the probiotics.
2. Synergistic concept: host beneficial probiotics and the prebiotics to specially enhance the survival, growth, and activity of these probiotic strains(s) are selected. However, the prebiotic may also increase the levels of beneficial resident gastrointestinal microbiota.

The prime reason behind the use of synbiotics is that the human gastrointestinal system (GI) is populated with a large number and variety of bacteria that have specific nutrient needs (bifidobacteria), and hence by selecting specific food or food ingredients, it is probable to augment the numbers of these target bacteria. When probiotics and prebiotics are administered concurrently, a synergistic activity is observed, which indicates the selective function of the prebiotic compound in supporting the growth of probiotic compound (Gibson and Roberfroid 1995; Fooks and Gibson 2002). This synergistic action not only stimulates and amplifies the survival of desired probiotics but also promotes the autochthonous-specific strain of intestinal tract (Fotiadis et al. 2008).

General health benefits attributed to these probiotics include prevention of tumors *in vivo* (Russell et al. 2013); management of allergy caused by food, including atopic eczema (Isolauri et al. 1999) and diarrhea in children (Isolauri et al. 1991); delay in aging (Duncan and Flint 2013); and aid in adjusting the blood lipid levels (Omar et al. 2013). To exert these health benefits, probiotics must be able to survive the alteration while passing through the stomach and colonize the colon (Webb 2011).

So Farnworth (2001) added a new point to the existing definition of synbiotics, which says: "Synbiotics encourage the growth of the probiotic organism by providing the specific substrate to the probiotic organism for its fermentation." Hence, the synbiotic concept was introduced as "the mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare" (Gibson and Roberfroid 1995) (Fig. 5.1).

An ideal synergistic synbiotic supplement would contain a suitable single or multistrain probiotic and a mixture of prebiotics. Prebiotics selectively favor the ingested probiotics and produce additive or synergistic effect (Kolida and Gibson 2011; Kondepudi et al. 2012; Raman et al. 2013). It also favors the growth of the endogenous beneficial bacteria and reduces the number of cancer-promoting bacteria or pathogenic bacteria.

Currently available and known synbiotic supplements in the market include a combination of bifidobacteria and fructo-oligosaccharides (FOS), *Lactobacillus* GG and inulins, and bifido-

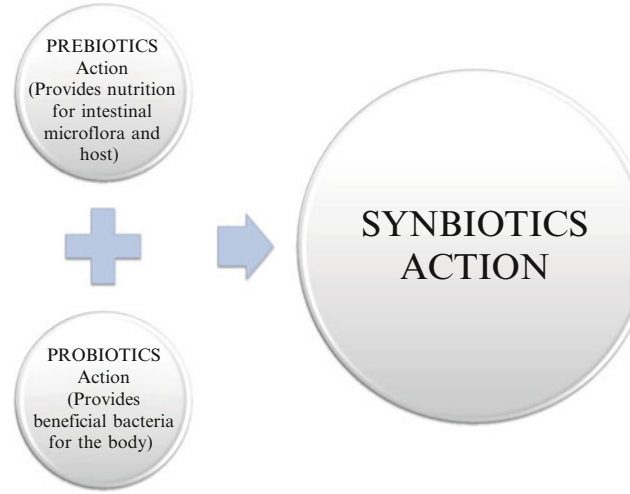


Fig. 5.1 Concept of synbiotics

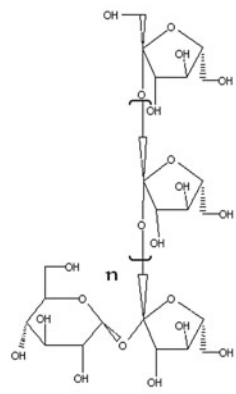
Table 5.1 Examples of common synbiotics

Synbiotics
Lactobacilli + lactitol
Lactobacilli + inulin
Lactobacilli + fructo-oligosaccharide or inulin
Lactobacillus rhamnosus GG + inulin
Bifidobacteria + fructo-oligosaccharide
Bifidobacteria + galacto-oligosaccharide
Bifidobacteria and lactobacilli + fructo-oligosaccharide or inulin

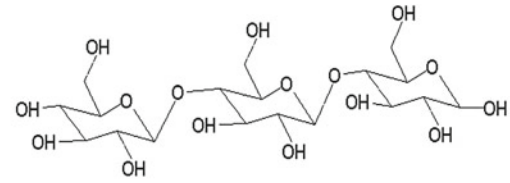
bacteria and lactobacilli and FOS or inulins (Table 5.1). FOS is a potential prebiotic as they are fermented by selective beneficial species of the gut (Gibson and Roberfroid 1995; Havenaar 2011; DuPont and DuPont 2011). They are also used as a source of natural sweetener and syrups for persons suffering from digestive problems (Charalampopoulos and Rastall 2012). Oral treatment with yacon syrup rich in FOS (50 %) markedly accelerates colonic transit time in healthy individuals and increases defecation frequency and satiety sensation (Geyer et al. 2008; Genta et al. 2009), indicating the importance of prebiotics in enhancing the growth of beneficial gut microbes (Fig. 5.2). Most of the synbiotics are marketed as yogurt. Synbiotic-rich yogurt, smoothies, etc. are few commercially available synbiotics marketed today.

5.2 Synbiotics and Gut Health

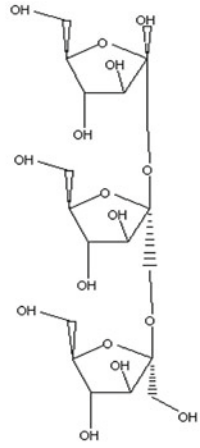
The gut, though the largest organ of the human system, is an important target for various kinds of stresses caused by factors including sepsis, trauma, burn, shock, bleeding, and infection (Shimizu et al. 2013). Foreign substances including food particles and antigens of the microbial origin that promote infectious and multiple organ dysfunction syndrome attack the gut; hence, gut microflora has a crucial role in health and diseases. Imbalanced gut bacteria, deteriorated intestinal epithelium, immune system, and commensal bacteria trigger development as well as aggravate the infectious complications including colon cancer and others (Shimizu et al. 2013). Studies have indicated that the altered gut microbiota (dysbiosis) is associated with several diseases that are predominantly prevalent in the 21st century. Under conditions of critical illness, it is difficult to maintain the normal gut flora. These ensue not only due to the various stresses but also due to the invasive treatment methodologies employed. Shimizu et al. (2006) reported the disturbed gut flora is critical in stressed and ill patients and that the gut balance could be reestablished using synbiotics. They reported that patients with severe systemic inflammatory response syndrome (SIRS) had 100–10,000 times



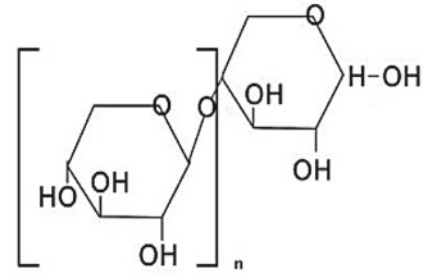
Inulin



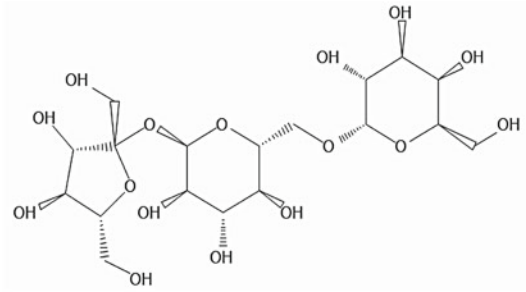
Galactooligosaccharide



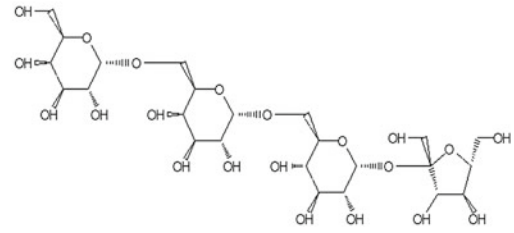
Fructo-oligosaccharide



Xylo-oligosaccharide



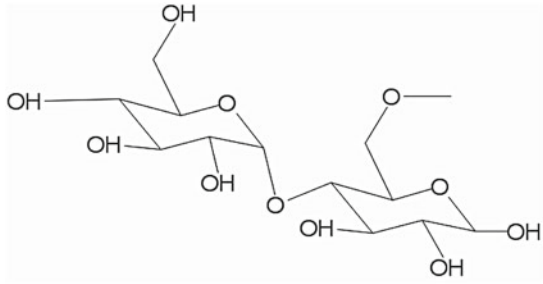
Raffinose



Stachyose

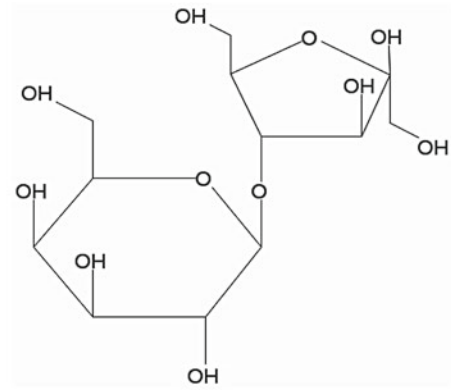
Soy-oligosaccharide

Fig. 5.2 Different types of prebiotics commercially used as an ingredient in synbiotics

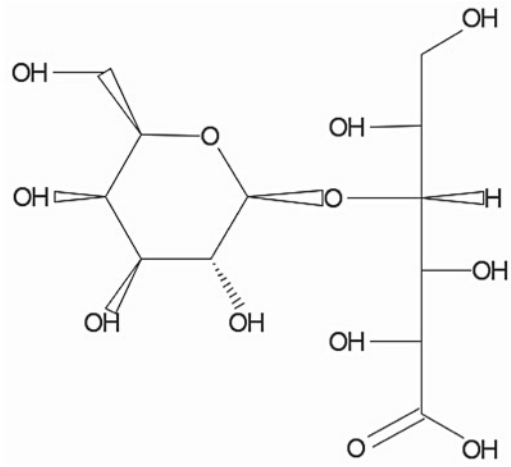


$2 < n < 20$

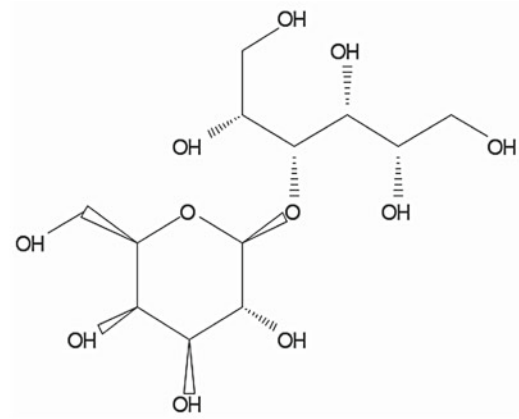
Maltodextrin



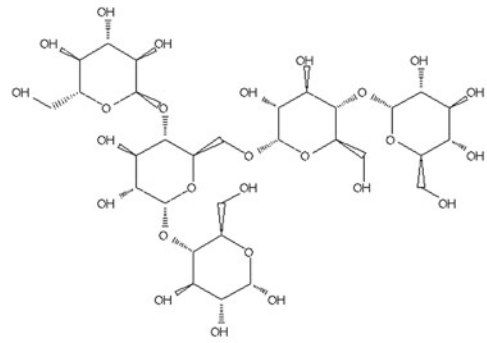
Lactulose



Lactobionic acid



Lactitol



Resistant starch

Fig. 5.2 (continued)

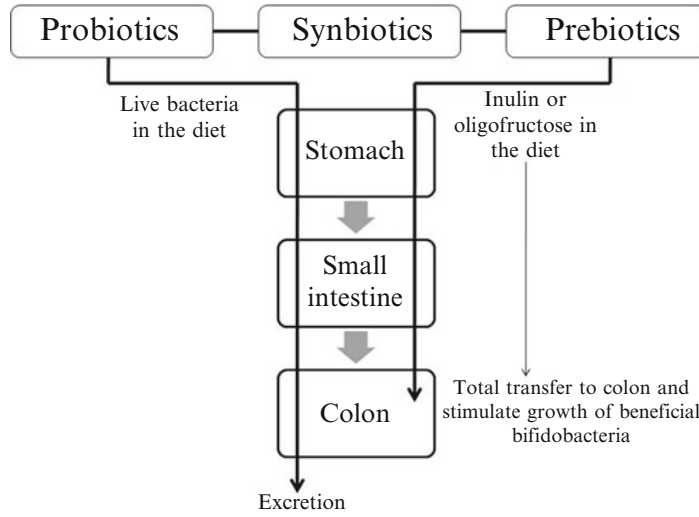


Fig. 5.3 Function of synbiotics in the human gut system

fewer total anaerobes, including *Bifidobacterium* and *Lactobacillus*, and 100 times more pathogenic *Staphylococcus* bacteria when compared to normal healthy human. In such cases, total organic acids, including acetic and butyric acids, were decreased considerably while the latter completely disappeared. The alteration in the normal gut flora could ultimately lead to mortality. Clinical studies indicated that an improved gut flora is observed with the administration of synbiotics (Shimizu et al. 2011). Also, the ratio of Firmicutes to Bacteroidetes (F/B) determines the gut health (Finegold et al. 2014).

Combination of prebiotics with probiotics such as FOS and *Bifidobacterium* or lactitol and *Lactobacillus in vitro* and *in vivo* has been found to stimulate the survival and activity of the microorganism (Chakraborti 2011). These synbiotic combinations improve the survival, implantation, and growth of newly added probiotic strains. Synbiotics comprising of *Bifidobacterium breve* strain Yakult and *Lactobacillus casei* strain Shirota as probiotics and galacto-oligosaccharides as prebiotics produced considerably greater levels of the beneficial *Bifidobacterium* and *Lactobacillus* and short-chain fatty acids (SCFA) and lower the incidence of infectious complications than the control with no synbiotics (Shimizu et al. 2009). The beneficial effects of these synbiotics were attributed to the increased levels of the two beneficial bacteria and increased production of SCFA in the gut. The mechanisms of action of SCFA are detailed in Chap. 6.

The probiotics in the sybiotics face several challenges in the host, including competition from other indigenous pathogenic or benign microorganisms, metabolic interactions between these indigenous microbiota, pH in the upper gastrointestinal tract, and the lack of anatomical and substrate heterogeneity (to mimic the human proximal and distal large intestines) (Gibson and Wang 1994) (Fig. 5.3). Another obstacle to probiotic survival is the high concentrations of bile acids (1–10 mM) in the colon (Begley et al. 2005). These constraints are overcome, and the flourishing of the probiotics takes place due to the utilization of the prebiotics present in the synbiotics. Su et al. (2007) demonstrated a positive effect of prebiotics on the survival and retention of specific probiotic inocula *in vitro* and *in vivo*.

The prebiotics include fructo-oligosaccharides (FOS), soybean oligosaccharide (SOS), or inulin, and the probiotics include *Lactobacillus acidophilus*, *Bifidobacterium lactis*, or *Lactobacillus casei* (Table 5.1). They observed that prebiotics SOS- or FOS-containing diet stimulated the growth of *L. acidophilus* and maintained it at its highest level. Soybean extract enhances the growth of bifidobacteria, while SOS moderately enhances the growth of these possibly due to the reduced percentage of polymerized sugars, namely, raffinose and stachyose. FOS and inulin

dietary treatment exerted similar effects on the gut *B. lactis*. The prebiotics, namely SOS, FOS, and inulin, enhanced the survival and retention time of *L. Casei* (Fig. 5.2). β-glucan hydrolysate and concentrate, and fiber gum as prebiotics are not comparable to SOS and FOS. Similar results are reported in randomized, double-blinded, crossover study comprising of healthy children. Administration of milk-based fruit juice containing probiotic, *Lactobacillus* GG with galactooligosaccharide (GOS), appreciably amplifies the amount of allochtonous *Lactobacillus rhamnosus* and autochthonous bifidobacteria, and lactobacilli in the feces of treated children. GOS is soluble dietary fibers that pass through the colon undigested, where it is hydrolyzed and fermented by gut microbes. Further, the monomeric units of GOS contain galactose and lactose, which are naturally found in human milk.

Xylo-oligosaccharide with *Bifidobacterium animalis* subsp. *lactis* as synbiotic (8 g XOS + 10^9 cfu Bi-07/d) when administered to healthy adults (25–65 years) for 21 days led to healthy bowel habits and improved composition of the gut microbiota, blood lipid concentration, and immune function. Nevertheless, in this double-blind, placebo-controlled, randomized, factorial, crossover study with the synbiotics, the symptoms such as bloating, abdominal pain, or flatulence are not controlled. XOS supplementation as a synbiotic had benefits improving the gut microbiota and markers of immune function and obesity (Finegold et al. 2014). Rycroft et al. (2001) evaluated the fermentative properties of some prebiotics *in vitro* and established that XOS and lactulose produced the highest increase in bifidobacteria, while FOS encouraged the growth of lactobacilli. Guerra-Ordaz et al. (2014) administered a combination of the prebiotic oligosaccharide lactulose and the probiotic strain of *Lactobacillus plantarum* as synbiotic against enterotoxigenic *Escherichia coli* (ETEC) and observed a reduced incidence of diarrhea. Inclusions of the probiotic were reported to increase the number of *L. plantarum* bacteria in the ileum and colon and the total lactobacilli in the colon.

Human milk oligosaccharides (diverse and complex than animal milks) received lot of interest in recent years due to their ability to augment the growth of bifidobacteria and cell adhesion as well as aid in the growth of lactic acid bacteria, which might be a potential probiotic (Kunz et al. 2000; Martín et al. 2003; Collado et al. 2009). Hence, human milk is considered as a synbiotic food that can help in the growth of beneficial bacterial in the gut of breast-fed infant (Martín et al. 2003). Some studies have also recommended that human milk oligosaccharides may encourage the establishment of autochthonous microbiota in children (Heyman and Ménard 2002).

Even though synbiotics are increasingly being used for various health treatments, the potential benefits of these to gut are limited and are the cumulative effects of pro- and prebiotics (Figs. 5.4 and 5.5). Hence, new combination of synbiotics are being experimented and developed. They exist as capsules and sachets. Pro-wel is a commercially available synbiotic (Patel and Patel 2010). Usually, the probiotic intake ranges from 1 to 10 billion cfu a few times a week, with varying prebiotic dose.

5.2.1 Anticancer

Colorectal cancer (CRC), though widely distributed all around the world, has the highest rate of mortality and occurrence in developed countries and is gradually increasing in developing countries, including India; in South America; and in Africa. This conspicuous geographic gap is largely due to varying food habits such as low content of insoluble and soluble vegetable dietary fiber, fondness for red meat, surplus consumption of refined carbohydrates, and low amounts of protective micronutrients, in addition to obesity and physical idleness (Crawford and Kumar 2003). These types of food could generate or enhance the potentiality of the carcinogens or mutagens, thereby producing DNA damage in the crypt cells leading to mutation of genes, including adenomatous polyposis coli (APC),

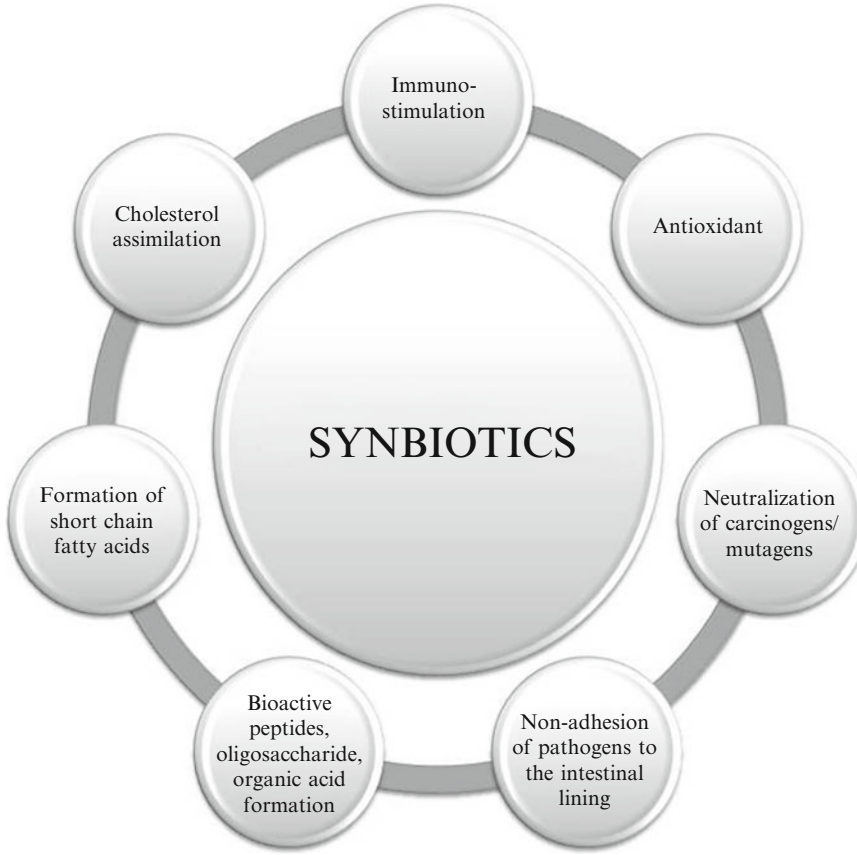


Fig. 5.4 Proposed function of synbiotics in the colon

Kirsten-ras (K-ras), and p53 (protein 53 kD) (Brady et al. 2000; Fotiadis et al. 2008; Sidhu et al. 2010). Several researchers demonstrated the detoxifying and antimutagenic properties of probiotics and prebiotics and concluded that these have beneficial protective effects on CRC (Wollowski et al. 2001; Fotiadis et al. 2008; Gupta and Garg 2009; Patel and Patel 2010). Extensive work is still pursued to demonstrate the potential preventive effects of these dietary interventions in combination on CRC (Liong 2008). Several studies indicate that modification of the gut microflora through these dietary modifications might interfere with the process of carcinogenesis, thus minimizing the risk of CRC. Epidemiological and experimental studies in humans and *in vivo* studies have indicated that pro- and prebiotics can minimize the risk of carcinogenesis by modulating the bacterial enzyme activities, antigenotoxic effects, and precancerous lesions (Burns and Rowland 2000).

Synbiotic combination of *Bifidobacteria* and oligofructose (FOS) has been established to hinder colon carcinogenesis in rats when compared to individual administration (Liong 2008). Administration of inulin and *B. longum* (6×10^9 cfu/day) jointly to azoxymethane (AOM)-treated Sprague Dawley rats significantly (26 %) reduced the total aberrant crypt foci (ACF) than when they were ingested alone (Rowland et al. 1998). Consumption of *B. lactis* and resistant starch was also able to augment the apoptotic response to AOM in rats, which was probably due to the fact that the resistant starch acted as the substrate for the optimal activity of the probiotic species. Combination of oligofructose-enriched inulin, *L. rhamnose*, and *B. lactis* prevents the AOM-induced suppression of NK-cells (natural killer

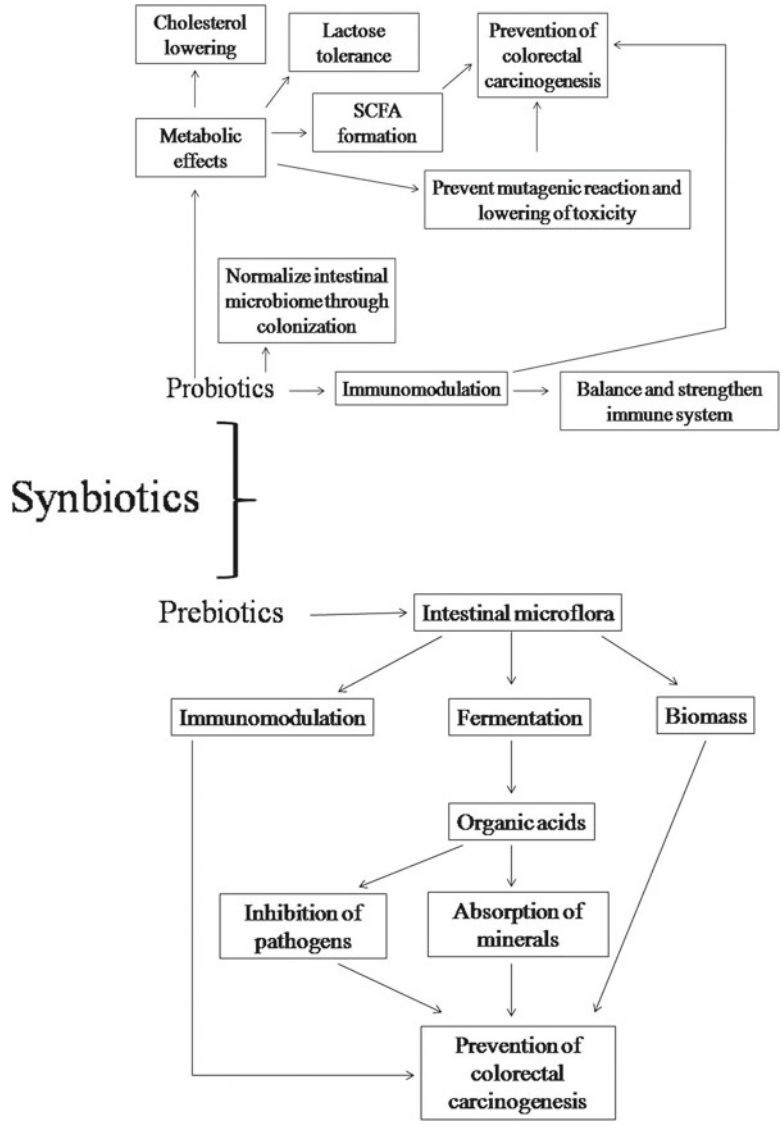


Fig. 5.5 An overview of synbiotics

cell) activity in Peyer's patches, while administration of individual components were not effective (Roller et al. 2004). These studies indicate that synbiotics have a critical role in CRC treatment (Fotiadis et al. 2008). However, prebiotics when administered alone have shown contradictory results on carcinogen-induced ACF, which might partly be due to the differences in the type of carcinogen/mutagen and the treatment regimes. Inulin (10 % in diet) had no significant effects on total ACF in the colon or their growth in F344 rats (Rao et al. 1998). Nevertheless, a significant decrease in ACF/cm^2 of colon was observed. Combination of *B. longum* and lactulose produced a 48 % inhibition of colonic ACF, which was considerably better than either of these when administered alone (Challa et al. 1997; Rowland et al. 1998). Scientists also observed that *B. longum* with inulin showed a synbiotic action with a reduction in the total ACF (74 %) in rats than when *B. longum* (29 %) or inulin (21 %) was administered individually. Also, large ACF reduced considerably (59 %) in the former, while the individual treatment had no effect. Liong (2008) reported fewer tumors in cancer-induced rats when they were fed with

cereal bran. He also suggested that synbiotics are more beneficial than the administration of either probiotic or prebiotic alone.

Dietary synbiotics, containing *L. rhamnosus* GG and *Bif. lactis* Bb12 as probiotics and oligofructose-enriched inulin as prebiotic, reduced the cancer risk factors in polypectomized and colon cancer patients in a 12-week randomized, double-blind, and placebo-controlled trial (Rafter et al. 2007). Administration of these also altered the composition of the fecal bacteria, namely, increase in the population of protective (beneficial) bacteria and reduction in the numbers of cancer-promoting (harmful) bacteria (Liong 2008; Daniel 2010). They also established that certain CRC intermediate biomarkers were modified due to synbiotic intervention as well as colorectal proliferation and the fecal water to induce necrosis in colonic cells decreased. Additionally, the function of epithelial barrier in the colon in polypectomized patients was also improved. Exposure to genotoxins was also reduced at the end of the intervention period. Liong (2008) postulated that the synbiotics modified the composition of the colonic bacterial ecosystem, which subsequently altered metabolic activity of the colon and prevented the amplified secretion of interleukin-2 (IL-2) by peripheral blood mononuclear cells in the polypectomized patients and interferon in the cancer patients (Rafter et al. 2007; Liong 2008). *B. longum* with raftiline HP (a derivative of inulin) beneficially altered the caecal physiology and bacterial metabolic activity and thereby, reduced the tumor risk and the incidence of an AOM-induced colonic lesions *in vivo* (Rowland et al. 1998).

5.2.2 Antiinflammation and Immunomodulation

Mice fed with a high-fat diet had an increased gut permeability and metabolic endotoxaemia (Safavi et al. 2013), which led to the leakage into the body of the Gram-negative intestinal microbiota, thus leading to the onset and progression of inflammation and metabolic diseases (Cani et al. 2009, 2012). Probiotics such as *Lactobacillus rhamnosus* GG and *Lactobacillus casei* DN-114-001 protect and improve the epithelial barrier and effectively suppress the Gram-negative bacteria into the gastrointestinal channel (Parassol et al. 2005; Strowski and Wiedenmann 2009; Frazier et al. 2011). A few probiotic strains, including *L. plantarum* 299, can mitigate bacterial translocation (Klarin et al. 2008). In a placebo-controlled trial, modification of the enteric flora in IL-10 knockout mice by probiotic *Lactobacilli* was associated with reduced prevalence of colon cancer and mucosal inflammatory activity (O'Mahony et al. 2005).

Synbiotics aid in producing higher amounts of SCFA than probiotics and subsequently protect the onset of CRC (Le Leu et al. 2010; Borowicki et al. 2011). One possible explanation for this is the interaction of the immunomodulating properties of the probiotic bacteria with butyrate, which is produced during prebiotic fermentation resulting in an upregulation of apoptosis (Le Leu et al. 2010). In addition to SCFA, probiotics are also involved in the production of conjugated linoleic acids (CLA), which are shown to exert numerous health benefits, including antiinflammatory and anticarcinogenic effects (Kelley et al. 2007).

CLA is a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds and is a potential prophylactic intervention for multiple inflammatory diseases, including obesity, hyperinsulinemia, hypertension, immune response, and apoptosis (Bassaganya-Riera et al. 2002, 2004; Bassaganya-Riera and Hontecillas 2010). *In vivo* studies indicate that CLA reduce the incidence of colonic tumors (Liew et al. 1995; Kim and Park 2003). Conjugated linoleic acid exerts a protective effect on inflammatory bowel disease and could be a promising chemopreventive agent against CRC *in vivo*. Dietary CLA ameliorate CRC activity, decrease colitis, and prevent adenocarcinoma formation in part through a PPAR-γ dependent mechanism (Evans et al. 2010). It also decreases the macrophage percentage in the mesenteric lymph nodes. Ewaschuk et al. (2006) demonstrated *in vitro* and *in vivo* that a mixture of the probiotic strains containing *Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*,

Bifidobacterium breve, *B. infantis*, *B. longum*, and *Streptococcus thermophilus* are able to produce CLA from linoleic acid, induce the upregulation of PPAR-γ (a nuclear hormone receptor expressed by monocytes, macrophages, T-cells, dendritic cells, and gastrointestinal epithelial cells in the gut mucosa), reduce colonic tumor cell viability, and thus induce apoptosis (Tontonoz et al. 1998; Clark et al. 2000). Short-term synbiotic treatment of ulcerative colitis, a relapsing inflammatory disease of the colon resulted in a significant improvement of the clinical appearance of the disease due to the changes in the mucosal microbiota (Furrie et al. 2005).

5.3 Mechanism of Action

Unlike probiotics, the possible anticarcinogenic activity of synbiotics is not tacit. As prebiotic oligosaccharides are fermented by the probiotic bacteria and other bacteria residing in the colon, butyrate and other SCFA are formed leading to the butyrate-mediated anticarcinogenic effects (Fig. 5.5) (Chakraborti 2011).

Mechanism of action of synbiotics could be normally summarized as follows:

Synergistic action
Prebiotics form substrate for the specific beneficial allochtonous and autochthonous bacteria to survive and flourish
Modification of the colonic microbiome, leading to altered metabolic activity in the colon

The prebiotics in the synbiotics might elevate the levels of minerals, calcium and magnesium, in the colon and may enhance the growth of bifidobacteria and lactobacilli in the large intestine. Moreover, *in vitro* and animal studies have shown that these bacteria bind and inactivate carcinogen and hence, have the ability to directly inhibit the growth of tumor and prevent the bacterial conversion of the precarcinogens into carcinogens (Chakraborti 2011; http://170.107.206.70/drug_info/nmdrugprofiles/nutsupdrugs/syn_0327.shtml). The bacterial fermentation of the dietary components in the gut system could be associated with the production of cancer-preventive agents and, hence, the minimization of the risk of colorectal cancer. The bacterial fermentation by-products are mainly SCFA, specifically acetate, propionate and butyrate, and gases. Gases are eliminated, and SCFA, together with nutrients and growth signals, play a vital role in the prevention of CRC. Also, secondary bile acid is reduced (Mai et al. 2004). Femia et al. (2002) observed that synbiotic combination of fructans and *B. lactis* (Bb12) or *L. rhamnosus* GG reduced the AOM-induced colorectal adenomas and carcinogenesis by increasing SCFA production. This also lessens the proliferative activity and the expression levels of GST placental enzyme pi-type, inducible NO-synthase, cyclooxygenase-2 enzymes, which are involved in the colorectal carcinogenesis.

The details of the promising mechanism of probiotics and prebiotics are discussed in Chaps. 2 and 4, respectively. The mechanism of action of SCFA in reducing cancer is discussed in Chap. 6.

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Short-Chain Fatty Acids

6

Abstract

Short-chain fatty acids are formed in the colon as a result of microbial fermentation (*Bifidobacterium* and *Lactobacillus*) of undigested bioactive carbohydrates, including prebiotics and dietary fiber, and their significant role in orchestrating colon carcinogenesis is of current interest among researchers. Acetate, propionate, and butyrate are the major by-products of SCFA formation. Propionate and butyrate are extensively involved in the cell differentiation, growth arrest, and apoptosis of cancer cells. The anticancer effects of acetate, propionate, and butyrate (molar ratio of approximately 3:1:1) is supported immensely by epidemiological, *in vitro* and *in vivo*, studies. The role of lactate has also been considered by several researchers. Diet and dietary habits alter the composition of the gut microflora. An inverse relationship between dietary fiber intake and the incidence of human colon cancer has been observed. Prebiotics and dietary fibers curtail the risk of colon cancer through diverse mechanisms. The interplay between propionate and butyrate, and gastrointestinal epithelial cells (colonocytes) in reducing colon carcinogenesis is a current topic of interest for many researchers. About 70–90 % butyrate is metabolized by the colonocytes. SCFA also modulate colonic and intracellular pH, cell volume, ion transport and regulate cell proliferation, differentiation, and gene expression. Propionate inhibits cell growth and activates apoptosis in colorectal carcinoma cells. Butyrate inhibits histone deacetylase resulting in histone hyperacetylation and growth inhibition in the colonic epithelial cells. This link between histone hyperacetylation, hyperacetylation-induced transcriptional regulation and growth inhibition has been considered as the foremost factors in preventing colon cancer. However, "butyrate paradox," another concept, has not been confirmed till date. The function of SCFA in the suppression of inflammation and immunomodulation is discussed.

Keywords

Anticancer • Apoptosis • Butyrate • Colon acetate • Fermentation • Inflammation • Metabolism • Propionate • Short-chain fatty acid

M. Raman et al., *Probiotics and Bioactive Carbohydrates in Colon Cancer Management*, DOI 10.1007/978-81-322-2586-7_6

6.1 Introduction

Interest in short-chain fatty acids (SCFA) has been rekindled in the last few years because of their beneficial effects on colonic and systemic health. Previously, they were considered as major etiological factors in carbohydrate-induced diarrhea (Binder 2010). However, studies conducted by physiologists on cattle indicated that they derive most of their energy from cellulose and SCFA in the rumen, where they are easily formed and rapidly absorbed (Topping and Clifton 2001). This has led to the general conviction that SCFA could be beneficial to human gut health. Studies on native East Africans indicated that they consumed diet rich in unrefined cereals and were at lower risk of colorectal cancer, diverticular disease, and constipation than the others (Topping and Clifton 2001), thus once again strengthening the impact of SCFA on health.

Prebiotics and dietary fibers, principally comprising of oligosaccharides and non-starchy soluble polysaccharides of low molecular weight, undergo varying degrees of breakdown during transit through human gut (the microbiome resembles an obligate herbivores) by a complex bacterial ecosystem, forming products that primarily comprise SCFA and gases. Carbohydrates are classified into two basic groups based on their digestibility in the gut (Lattimer and Haub 2010). The first group is simple carbohydrates (starch and simple sugars), which are enzymatically hydrolyzed and are absorbed in the small intestine, while the second group is complex carbohydrates or dietary fibers, including cellulose, lignin, and pectin, which are resistant to digestion in the small intestine and undergo bacterial fermentation in the colon (Lattimer and Haub 2010) (Fig. 6.1). About 95–99 % SCFA are produced from resistant starch and dietary fibers by the bacterial fermentation (*Bifidobacterium*, *Ruminococcus bromii*) and are absorbed and transported via portal vein to the liver. The unabsorbed fractions are distributed to other organs of the body and tissues for metabolism (Topping and Clifton 2001). *Faecalibacterium prausnitzii*, *Clostridium leptum* (or clostridial cluster IV) cluster and *Eubacterium rectale/Roseburia* spp., belonging to the *Clostridium coccoides* (or clostridial cluster XIVa) cluster of firmicute bacteria, are the two most important groups of butyrate producers (Louis and Flint 2009).

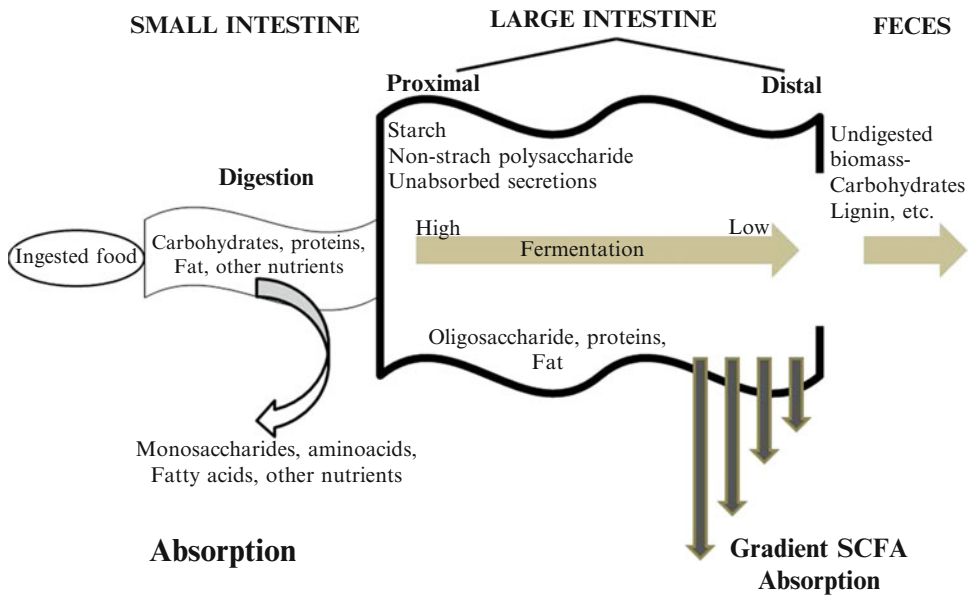

Fig. 6.1 An overview between intake of food its digestion and fermentation in the gastrointestinal tract. Prebiotics and dietary fiber form substrate for probiotics and undergo fermentation to produce short-chain fatty acids

Production of SCFA happens predominately in the proximal colon and is transported to distal regions by the fecal stream (Fig. 6.1). This is the site of greatest organic disease. Hence, delivery of SCFA, specifically butyrate, to this section is significant. Peripheral venous SCFA are high due to comparatively low visceral extraction and higher absorption rate during circulation. Conversely, SCFA concentration in human peripheral venous blood, except for acetate, is normally low, reflecting lower SCFA production rate and greater visceral extraction in humans. SCFA contribute to

- the normal large bowel function and
- prevent pathology through their metabolism by colonocytes in the lumen, colonic musculature and vasculature.

The function of SCFA, including their ion transport property, nutrient (energy supplies) for the colonic epithelium, modulator of intracellular pH and cell volume, and other functions such as regulators of proliferation, differentiation, and gene expression, are of keen interest for researchers today (Fig. 6.2). The increased scope of SCFA and their benefits in human health have achieved plenty of attention in the last three decades. There is mounting evidence that SCFA play a noteworthy role in maintaining gut health and in the prevention and management of several noncommunicable diseases, including cancer.

Soluble sugars comprising of monosaccharides and oligosaccharides undergo 100 % fermentation in the colon. This is followed by pectin/psyllium (90–95 %), bran (70 %), cellulose (20 %), and lignin (0–5 %), with their varying fermentation rates (Roberfroid 2005). Dietary fiber, resistant starch, and complex carbohydrates contribute largely to a high rate of SCFA production. Protein, mucus, sloughed cells, and gastrointestinal secretions may also contribute to SCFA production. Fermentation of proteins and amino acids by proteolytic bacteria yields branched SCFA, CO_2, CH_4, H_2, phenols, and amines (Roberfroid 2005). The primary effect of SCFA on colonic function is the result of their uptake and metabolism by colonocytes. Nonstarch polysaccharides, which resist digestion by intrinsic human intestinal digestive enzymes, do not account for SCFA production and are termed as "carbohydrate gap." It is shown that consumption of greater resistant starch is associated with reduced risk of tumors, which may be due to

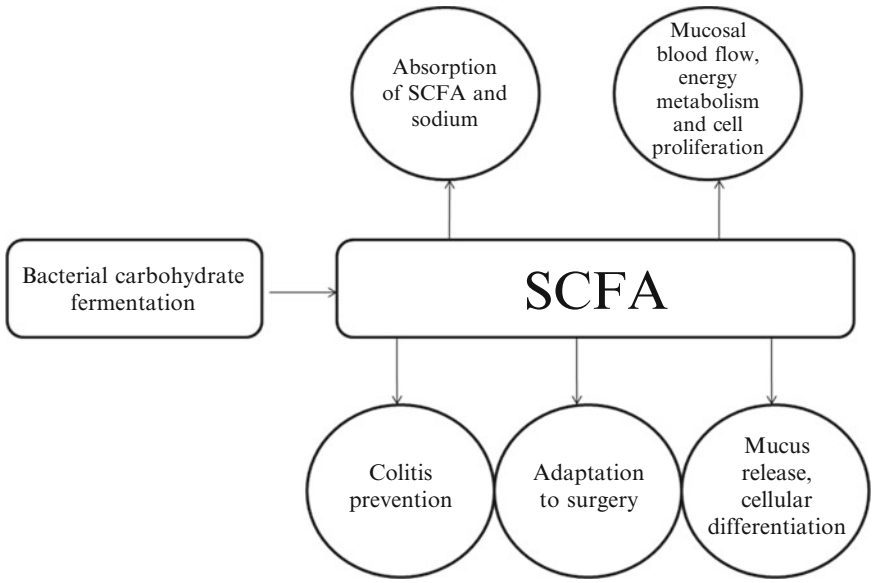

Fig. 6.2 Short-chain fatty acids and their health benefits

SCFA and colonocyte proliferation. However, in epidemiological and animal studies, this was negatively correlated with colorectal cancer risk. This inconsistency may be due to the particular features of the digestive system of the rodents (coprophagy). *In vitro* and *in vivo* studies indicate that butyrate produced by resistant starch fermentation may be promoting apoptosis in tumor cells. It has been shown that resistant starch promotes colon maintenance by alleviating infectious diarrhea and promoting colonic mineral absorption. High amylase maize starch, a kind of resistant starch, has been included in prebiotics as it promotes the survival of lactic acid bacteria (LAB).

6.2 Production of Short-Chain Fatty Acids

Basic fermentative action in the human colon includes hydrolysis of polysaccharides, oligosaccharides, and disaccharides to their monomeric sugars that upon fermentation results in increased biomass (Savage 1986; Topping and Clifton 2001). Bacterial cell-associated and secreted hydrolases assist in carbohydrate hydrolysis, and this leads to metabolizable energy for microbial growth and maintenance, and also metabolic end products, including SCFA (Fig. 6.3). The production of SCFA is determined by several factors, including the number and type of microflora present in the colon (Roberfroid 2005), substrate source (Cook and Sellin 1998), and gut transit time. A large variety of microfloral population inhabit the human colon (10^{10}–10^{11} cfu/g wet weight) (Hill 1995). SCFA production and overall stoichiometric reaction have been summarized for hexose as follows (Cummings 1997; Topping and Clifton 2001). The significance of fermentation by intestinal bacteria is broad.

$$59\ C_6H_{12}O_6 + 38\ H_2O \rightarrow 60\ CH_3COOH\ \text{(acetate)} + 22\ CH_3CH_2COOH\ \text{(propionate)} + 18\ CH_3CH_2\ CH_2\ COOH\ \text{(butyrate)} + 96\ CO_2 + 268\ H^+ + \text{heat} + \text{additional bacteria.}$$

More than 50 genera and over 400 species of bacteria have been identified in human feces. Bacterial numbers, fermentation, and proliferation are elevated in the proximal end of the colon where the carbohydrate availability is the maximum. Therefore, the principal site of colonic fermentation is the cecum and proximal colon, whereas the distal colon is deprived of carbohydrate and water and, hence, does not participate in colonic fermentation (except polydextrose, the second generation of prebiotics). Species such as *Bifidobacterium* and *Lactobacillus* and their carbohydrate substrates have been associated with an improved health, resulting in the emergence of the idea of pro- and prebiotics. The former involves the delivery of specific bacteria to the colon and the latter includes the administration of

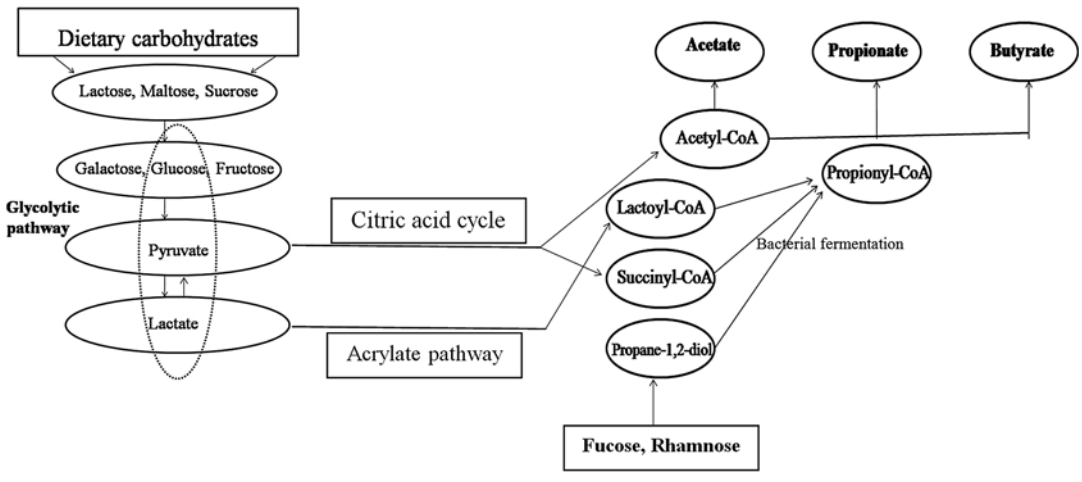

Fig. 6.3 Pathway showing the production of short-chain fatty acids in the colon

dietary component that promotes the growth of specific bacteria with definite metabolic functions.

Population survey indicated that SCFA are produced in the order as acetate > propionate ≥ butyrate (Fleming et al. 1991; Cummings and MacFarlane 1991; Takahashi et al. 1993; Segal et al. 1995; Muir et al. 1998; Topping and Clifton 2001). In infants fed with breast milk, acetate is predominant, propionate is very low, and butyrate is absent in the feces. In those fed with formula, butyrate may be found (Sigur et al. 1993; Edwards et al. 1994; Topping and Clifton 2001). The SCFA profile is important in gut development. In premature infants maintained in incubators, enhanced butyrate during the period between 14 and 21 days is critical as it may be related to the development of necrotizing enterocolitis (Szylit et al. 1998; Topping and Clifton 2001). In a healthy infant, fermentation is slower and butyrate production is established slowly but in 2 years, the adult SCFA profile emerge (Midtvedt and Midtvedt 1992; Topping and Clifton 2001). SCFA in general and specifically butyrate enhance the growth of lactobacilli and bifidobacteria. They play a crucial role in colon physiology and metabolism (Roy et al. 2006). Total SCFA and regional differences in SCFA concentration are of great concern in colon diseases, especially in cancer and gastrointestinal disorders, where disease often occurs distally (Roediger 1980; Roediger and Moore 1981; Jenkins et al. 1999; Floch and Hong-Curtiss 2002). Therefore, an increased SCFA production and their distal delivery, especially butyrate, may have a role in preventing noncommunicable diseases. This has led to the concept of colonic health and development of functional foods, such as "probiotics, prebiotics, and synbiotics" and other dietary components that target the colon and affect its environment, the composition of the microflora, as well as its physiology (Bomba et al. 2002). Dietary carbohydrates escaping digestion/absorption in the small bowel and prebiotics undergo fermentation in the colon and enhance SCFA production.

SCFA are absorbed by each intestinal segment as observed *in vivo* and in human volunteers (Binder 2010). Apical membrane and active transport are the modes of SCFA uptake (Fig. 6.4). The former includes nonionic diffusion, $SCFA^-/HCO_3^-$ exchange, while the latter includes SCFA transporters. The latter are monocarboxylate transporter isoform 1 (MCT1, coupled with

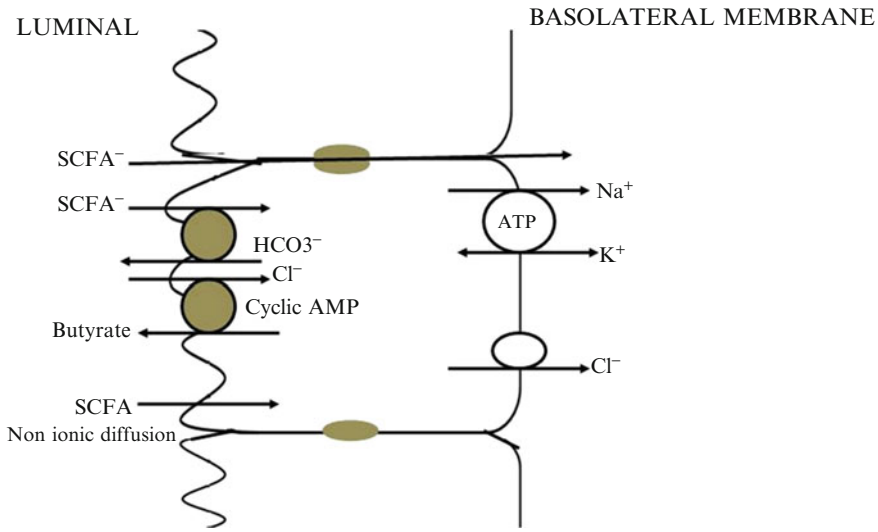

Fig. 6.4 Cellular model showing the absorption of SCFA. Absorption is chiefly through nonionic diffusion and paracellular absorption in the proximal colon. $SCFA^-/HCO_3^-$ luminal membrane exchangers help in SCFA transportation. Butyrates collected in luminal membrane undergo recycle. Basolateral membrane K^+ channels are inhibited during SCFA absorption, and increased proton concentration activates Cl^- channels in basolateral membrane

transmembrane H^+ gradient) and SLC5A8 (Na^+ coupled cotransporter) (Hamer et al. 2008; Binder 2010). The absorption of SCFA has a significant impact on the absorption of minerals and on electrolyte balance (Kunzelmann and Mall 2002). Butyrate exerts a powerful proabsorptive stimulus on NaCl transport and antisecretory effect towards Cl^- secretion in the gut (Binder and Mehta 1989). These effects of butyrate happen through two mechanisms:

1. stimulation of NaCl absorption by the action of two coupled transport systems that involves Cl^-/HCO_3^- and Na^+/H^+, and Cl^-/butyrate and Na^+/H^+ and
2. inhibition of Cl^- secretion by blocking the activity of the co-transporter Na-K-2Cl on the basolateral membrane.

In vitro studies showed that butyrate inhibits Cl^- secretion by inducing prostaglandin E2, cholera toxin, and phosphocholine. This inhibition could be due to the reduced production of intracellular cAMP secondary to the expression and regulation of adenylate cyclase (Fig. 6.4) (Binder 2010). The administration of butyrate is also known to alleviate the symptoms associated with antibiotic use, including diarrhea (Clausen et al. 1991).

Increase in SCFA results in the decrease of colonic pH, which indirectly influences the composition of the colonic microflora, decreases the solubility of bile acids, increases the absorption of minerals (indirectly), and reduces the ammonia absorption by the protonic dissociation of ammonia and other amines (formation of the less diffusible NH_4^+ when compared to the diffusible NH_3) (Vince et al. 1978; Jackson 1983; Jenkins et al. 1987).

6.2.1 SCFA Determination in Colon

Intubation has been used in humans to quantify the intestinal digestibility of carbohydrates, including starch. But SCFA cannot be determined by this method; hence, autopsy and surgery are adopted for gut content and portal venous blood (Cummings et al. 1987). Due to the limitations of the former, the new method involving dialysis sacs in gelatine capsules has been used to determine SCFA *in situ* in normal subjects (Wrong et al. 1965) with varying dietary interventions (Fredstrom et al. 1994). These have been adopted in clinical ulcerative colitis, where the severity of inflammation has been correlated to butyrate (18.9 mM) than normal (14 mM). Continuous sampling is impractical in this case.

Elsden et al. (1946) showed that there is a progressive decline in the volatile organic acids along the large bowel of herbivorous and omnivorous animal species. In pigs, depending on the dietary profile, total SCFA concentration in the proximal colon was ~70–140 mM, which fell to 20–70 mM in the distal colon (Marsono et al. 1993; Martin et al. 1998; Bird et al. 2000). This concentration may also vary in the distal colon with the loss of water and digesta mass. However, the availability of SCFA changes with the rate of digesta passage and is independent of the rates of production. These regional differences in SCFA may have implications on large bowel diseases, specifically colon cancer and ulcerative colitis (IBD).

6.3 Butyrate Paradox

Dietary fibers escaping digestion in the small intestine reach the large intestine in compact and undergo bacterial fermentation to produce SCFA, specifically butyrate, which has significant health benefits. Butyrate is metabolized by colonocytes but inhibits colon carcinoma cells. Though butyrate seems to be a useful agent in colon cancer therapy, its rapid metabolism and ineffective concentration *in vivo* influence its success as an inhibitor for colon cancer. Butyric acid and propionic acid inhibit growth and promote apoptosis of human colonic carcinoma cell lines (Fukuda et al. 2011), while antiinflammatory properties are reported as the cumulative effects of acetic, propionic, and butyric acids (Fukuda et al. 2011; Zanten et al. 2012). Recently, it is reported that acetic acid produced by bifidobacteria stimulates epithelial cell

defense against infection by *Escherichia coli* O157:H7 (Fukuda et al. 2011).

Butyrate is not produced by the lactic acid bacteria; however, certain probiotics may modify the ratio of SCFA in the colon, which is considered as a likely mechanism of anticarcinogenic action within the colon. Butyrate also stimulates immunogenicity of cancer cells. In a study of HCT116, colon cancer cells with wild-type p53 or p53 mutant and treatment with drug, vorinostat, indicated that 144 out of 275 miRNAs demonstrated several-fold changes in transcription and most were strongly affected by vorinostat (Shin et al. 2009). In colon cancer xenografts with wild-type p53, valproate has also been established to enhance the response to radiotherapy (Chen et al. 2009). Butyrate also show similar effects at molecular level (Zhou et al. 2011). Nevertheless, the chemopreventive effect of butyrate has not been confirmed till date, and this disagreement of butyrate and colon cancer has been termed as "butyrate paradox."

Colon bacteria that aid in producing butyrate belongs to the clostridial clusters I, III, IV, VI, XIVa, XV, and XVI, with cluster IV bacteria related to *Faecalibacterium prausnitzii* and cluster XIVa bacteria related to *Eubacterium rectale* and *Roseburia* spp. (Barcenilla et al. 2000). Butyrate plays a key role in maintaining a normal colonocyte population. It is metabolized by normal colonocytes for energy metabolism and promotes healthy colonic epithelial cells. However, it serves as an inhibitor for the proliferation and differentiation of cancer cells. Approximately, 70–90 % of butyrate is metabolized by the colonocyte (Zoran et al. 1997; Basson et al. 2000; Della Ragione et al. 2001). The addition of butyrate *in vitro* leads to the inhibition of cell proliferation, induction of apoptosis, or differentiation of tumor cells (Comalada et al. 2006; Hinnebusch et al. 2002). The anticarcinogenic effects of butyrate on normal enterocytes are not similar to that on cancer cells. Butyrate possibly stimulates the physiological pattern of proliferation in the colon basal crypt but reduces the number and the size of aberrant crypt foci (ACF), which are the initial detectable neoplastic lesions in the colon (Alrawi et al. 2006). This "butyrate paradox" is an important mechanism in which butyrate causes significant biological effects (Comalada et al. 2006).

6.4 Short-Chain Fatty Acids, Gut Microbiota, and Health

Carbohydrates entering the large bowel may alter the colonic physiology, firstly, by its physical presence and, secondly, by undergoing fermentation. Undigested monosaccharides, disaccharides, and oligosaccharides induce osmotic diarrhea on excess consumption (Cummings 1997). Fecal bulking, speed transit, and laxation are important components of the fiber hypothesis (Topping and Clifton 2001). Their effects are divided into those occurring in lumen and those arise due to their uptake and metabolism by the colonic cells.

SCFA are promptly absorbed in the cecum and colon with only 5–10 % excreted through the feces. This is also associated with enhanced sodium absorption and bicarbonate excretion. The possible mechanism of absorption has been postulated as the diffusion of protonated SCFA (60 %) and anion exchange (Cook and Sellin 1998). SCFA uptake is related to the transport of water, which is high in the distal end than in proximal. Acetate, propionate, and butyrate are absorbed at comparable rates throughout the colon (Fig. 6.5). Once absorbed, these are metabolized at three main spots:

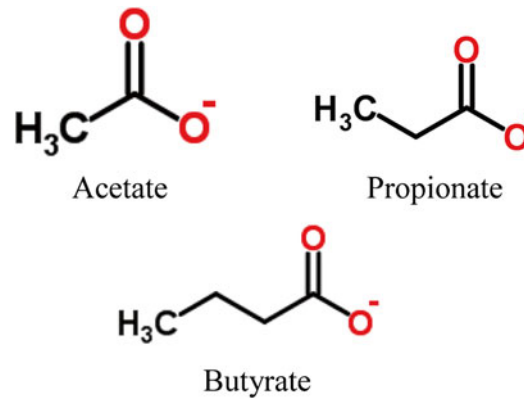

Fig. 6.5 Major short-chain fatty acids formed during bacterial fermentation of bioactive carbohydrates

1. ceco-colonic epithelial cells use butyrate as a major substrate for the maintenance of energy-producing pathways;
2. liver cells metabolize residual butyrate, together with propionate, for gluconeogenesis, while 50–70 % of acetate is taken up here; and, finally,
3. the muscle cells generate energy from the oxidation of residual acetate.

Acetate, propionate, and butyrate are found in the gastrointestinal tract at concentrations of about 13 mM/l in the terminal ileum, 130 mM/l in cecum, and 80 mM/l in the descending colon (Cummings et al. 1987). Acetate is readily absorbed and transported to the liver and, hence, is not metabolized in the colon. Acetyl-CoA synthetase in adipose and mammary glands use this acetate for lipogenesis after it enters the systemic circulation. It is also the primary substrate for cholesterol synthesis that might be absorbed and utilized by peripheral tissues (Pomare et al. 1985). Further, human intestine bacteria utilize acetate for the production of butyrate (Duncan et al. 2005). Other SCFA are formed by the microbial degradation of lactate, formate, ethanol, and acetoacetate (Fig. 6.6), which are produced from other dietary components, including proteins. In the intestinal level, butyrate is potentially effective in ion absorption, cell proliferation, cell differentiation, intestinal barrier function, immune regulation, oxidative stress, intestinal motility, visceral perception, and rectal compliance. In extraintestinal level, it potentially effects insulin sensitivity, cholesterol synthesis, energy expenditure, ammonia scavenger, stimulation of β-oxidation for very long chain fatty acids, cystic fibrosis transmembrane conductance (CFTR) function, neurogenesis, and fetal hemoglobin production (Canani et al. 2011).

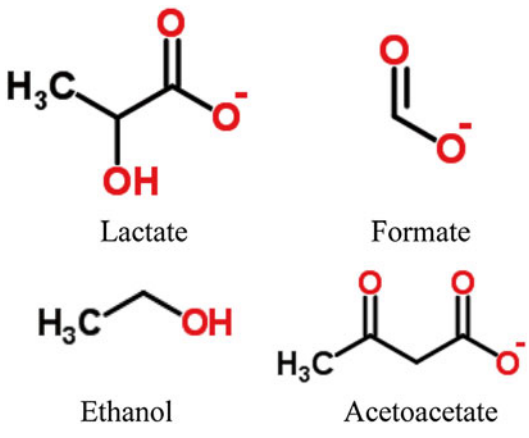

Fig. 6.6 Minor short-chain fatty acids formed during bacterial fermentation of bioactive carbohydrates

Generation of propionic and butyric acids involves specialized bacterial groups. Acetate forms the major bulk of SCFA and is generated by many of the bacterial groups. Decarboxylation of succinate and the acrylate pathway aids in the production of propionic acid (Cummings 1981). However, the intestinal glucose uptake is minimal in ruminants as the digestion and microbial fermentation of carbohydrates take place in the rumen. Several biochemical pathways for propionate formation have been reported (Macfarlane and Macfarlane 2003; Hosseini et al. 2011). The succinate route is generally used by *Bacteroides* species, and the acrylate route from lactate involves fermentation by clostridial cluster IX group. Bacterium *R. inulinivorans* is also involved in propionate formation from fucose (Scott et al. 2006) (Fig. 6.3). Hooper et al. (2002) observed that SCFA account for the major source of ruminant energy with propionate as a primary precursor for gluconeogenesis. It has been reported that increased propionate production may inhibit hepatic cholesterol synthesis. One of the possible determinants could be the ratio of propionate to acetate (Wolever and Bolognesi 1996).

6.4.1 Anticancer and Apoptosis

Colorectal cancer (CRC) is the fourth most common cause of cancer-related mortality in the world (Jemal et al. 2010). Many factors have been associated with CRC, which include genetic and environmental factors such as low levels of physical activity, smoking, alcohol consumption, obesity, low intake of dietary fibers, and high consumption of meat. Diet plays a significant role in the aetiology of CRC (Gill and Rowland 2002).

SCFA have been considered as gut-microflora-associated biomarker for CRC. Cancer is a multistep process, including progression of carcinogenesis from hyperproliferative epithelium to preinvasive and metastatic carcinoma through ACF (Young and Gibson 1995). At each step, genetic alterations occur. Most of the anticarcinogenic effects of butyrate are reported *in vitro* carcinoma cell lines. Butyrate paradox has different effects on apoptosis in normal and tumor cell lines. In normal cell lines, the absence of butyrate causes apoptosis within 150 min, along with a fivefold increase in the expression of Bax (Hass and Wickner 1996), whereas it leads to growth arrest, differentiation, and apoptosis in tumor cell lines (Deng et al. 1992; Gamet et al. 1992; Hague et al. 1995). Increased expression of biomarkers such as brush-border glycoproteins, alkaline phosphatase, and carcinoembryonic antigen are observed during differentiation in tumor cell lines. Normal colonic cells show decreased expression of these biomarkers. Heerdt et al. (1994) demonstrated that alkaline phosphatase level is elevated in cancer cells treated with butyrate, mainly in the floating apoptotic cells, suggesting that apoptosis occurs following differentiation. One possible mechanism for differentiation could be the reduction in the nuclear levels of the proto-oncogene c-Myc (Collins et al. 1992; Taylor et al. 1992), which is important in controlling tumor growth. SW620 and HT-29 cells exhibit different cell cycle factors during G_0/G_1 and G_2/M phases (Heerdt et al. 1994; Hass and Wickner 1996). Treatment of HT-29 tumor cells with butyrate reduces the cytoskeleton-associated tyrosine protein kinase activity, which plays a key role in cellular responses to cytokines (transforming growth factor-b1, TGF-β1) that participate in the growth of tumors (Barnard and Warwick 1993; Reimer and McBurney 1996). During G_2/M phase, inhibition of DNA synthesis occurs probably through histone deacetylase inhibition. Removal of histones is a vital step in DNA replication (Kruh 1982). Apoptosis may include mutation in p53; however, apoptosis induced by butyrate does not involve the p53 gene (Heerdt et al. 1994; Hague et al. 1993, 1995; Rubio and Rodensjo 1996; Sameshima et al. 2000) discussed in detail in Sect. 6.6.5.

Acetate stimulates the proliferation of normal crypt cells *in vivo* (Ono et al. 2004). It improves ileal motility and colonic blood flow and plays a significant role in adipogenesis and host immune system by interacting with G-protein-coupled receptors (GPCR43, 41) (Brown et al. 2003; Hong et al. 2005). Acetate lessens the lipopolysaccharide-stimulated tumor necrosis factor (TNF), interleukin-6, and nuclear factor-κB (NF-κB) level and enhance the peripheral blood antibody production in different tissues (Tedelind et al. 2007).

Propionate and acetate are also involved in inducing apoptosis but to a lesser extent than butyrate (Hague et al. 1995). Propionate induces apoptosis in adherent cells, and butyrate induces apoptosis in floating cells (Heerdt et al. 1994; Hague et al. 1995). Hence, the three fatty acids exhibit differential effects in inhibiting proliferation and inducing differentiation (Whitehead et al. 1986; Gamet et al. 1992). Butyrate and acetate also inhibit DNA oxidative damage due to free radicals *in vitro* (Abrahamse et al. 1999). Butyrate, though a trophic factor for the small intestinal epithelia and important fuel for colonocytes (Agarwal and Schimmel 1989), induces cell cycle arrest and apoptosis in colon carcinoma cells (Freeman 1986). Butyrate inhibits NF-κB activity in the human colon cancer HT-29 cell line (Inan et al. 2000).

6.4.2 Inflammation

The emergence of adaptive immune system in human beings indicates an advanced symbiotic relationship between SCFA and the intestinal microbiota.

Increase of fats in the diet is observed to amplify asthma and body weight. However, standard diet rich in dietary fiber is observed to reduce the inflammatory diseases. This aggravation is attributed to the activation of inflammasome, and its reduction caused by dietary fibers is attributed to dendritic cells (DC_R) (Kim et al.

2014). Analysis of gut and lung microbial communities of mice fed with dietary fibers indicates an increased proportion of Bacteriodaceae and Bifidobacteriaceae that ferment the fiber into SCFA. However, low-fiber diet led to the microbiome with increased Firmicute (Trompette et al. 2014). SCFA have also been linked to the generation of T_{reg} cells, which is essential for the control of adverse inflammation in the gut. The cells of the immune system sense the metabolic by-products and function of commensal microorganism and influence the balance between pro- and antiinflammatory cells.

Butyrate produced from dietary fibers, including cellulose and pectin, facilitates the extrathymic generation of T_{reg} cells in mice, which are essential for immunological tolerance. T_{reg}- cell generation in the periphery is also potentiated by propionate. These results indicate that bacterial metabolites mediate communication between the commensal microbiota and the immune system (Arpaia and Rudensky 2014). So it could be concluded that both butyrate and propionate have distinct immunological roles to play in the cross talk between the bacteria and the immune system (Arpaia and Rudensky 2014).

Butyrate has beneficial effects on the trophic and antiinflammatory effects on the gut epithelial cells. Butyrate transporter is downregulated in the colonic mucosa in patients with inflammatory bowel disease (IBD) (Thibault et al. 2007). It is also observed that in these patients, butyrate-producing bacteria, Bacteriodaceae and Bifidobacteriaceae, are considerably reduced in the gut mucosa and the fecal samples. Consumption of diets composed of animal or plant products alters microbial community structure and overpowers interindividual differences in microbial gene expression. Thus, gut microbiome rapidly responds to altered diet, which explains the varied responses to food selection and ingestion in terms of health (David et al. 2014). Butyrate and its derivates have potential application in preventing and treating metabolic syndromes in humans. Butyrate enemas are used to treat inflammatory bowel diseases, including Crohn's and ulcerative colitis (Steinhart et al. 1996).

6.5 Metabolic Regulation

Carbohydrates, including prebiotics and dietary fibers, are fermented (Fig. 6.1) by saccharolytic bacteria primarily in the proximal colon producing linear SCFA (chiefly acetate, propionate, and butyrate), CO_2, and H_2 (Macfarlane and Macfarlane 2003), which alter the colonic physiology. Rate and degree of fermentation depend on the type of dietary fiber. Insoluble fibers contribute mainly to the fecal bulk.

SCFA are organic fatty acids with 1–6 carbon atoms and are the principal anions that arise from bacterial fermentation of polysaccharide, oligosaccharide, proteins, peptide, and glycoprotein precursors in the colon (Cummings and Macfarlane 1991). SCFA arise from bacterial metabolism of "malabsorbed" carbohydrates that enter the colon, along with hydrogen gas (Cook and Sellin 1998). This hydrogen gas is consumed by three bacterial reactions in the colon, namely

(i) methanogenic bacteria that reduce CO_2 to CH_4 (50 % of population carries these bacteria);
(ii) sulfate-reducing bacteria that reduce SO_4 to sulfides, including H_2S (seen in nonmethane producers); and, finally,
(iii) acetogenic bacteria that reduce CO_2 to acetic acid by the use of hydrogen.

Fermentation involves a variety of reactions and metabolic processes, including

(i) anaerobic microbial breakdown of organic matter, yielding metabolizable energy for microbial growth and maintenance; and
(ii) other metabolic end products for host use; the chief end products are SCFA together with gases (CO_2, CH_4, and H_2) and heat (Topping and Clifton 2001).

Lactic acid is an intermediary product of carbohydrate fermentation (Table 6.1). The foremost SCFA involved in mammalian physiology are the straight chin fatty acids, including acetate (C2), propionate (C3), and butyrate (C4). Valerate (C5), hexanoate (C6), isobutyrate (C4) and isova-

Table 6.1 Metabolic fate of short-chain fatty acid (SCFA) and other by-products formed during the metabolism of bioactive carbohydrates in the colon

Components	Metabolic site	Outcome
Acetate	Muscle, kidney, heart, brain	–
Propionate	Liver	Possible gluceogenic precursor and suppresses cholesterol synthesis
Butyrate	Colonic epithelial cells	Regulate cell growth and differentiation
Lactate, succinate, pyruvate, ethanol	Colon	Fermented to short-chain fatty acids and absorbed
Gases	–	Partial excretion through breath

lerate (C5) are produced in the colon in minor amounts (5–10 % of total SCFA) (Canani et al. 2011) (Figs. 6.5 and 6.6). SCFA production in different populations is in the order of acetate > propionate > butyrate in a molar ratio of approximately 60:20:20 or 3:1:1, respectively, in the proximal and distal colon (Cummings 1981; Cummings et al. 1987; Topping and Clifton 2001). The molar ratios of acetate, propionate, and butyrate vary between the right colon (1:0.38:0.36, respectively) and the left colon (1:0.36:0.37, respectively) (Canani et al. 2011). The average concentration range of these in the entire intestine is 70–100 mM, with a relative ratio of 1:0.31:0.15 (Canani et al. 2011). Lactic acid accumulates at acidic pH (<5.5) when SCFA production is inhibited (Canani et al. 2011).

6.6 Mechanism and Effects of Short-Chain Fatty Acids on Health

Diet has significant impact on colon health. Different mechanisms have been elaborated to discuss the action of SCFA actions in inhibiting colon cancer.

6.6.1 Luminal Effects of SCFA

SCFA are the chief luminal anion in humans and are weak acids with pK_a value ~4.8. Ingestion of low fermentable nonstarch polysaccharide such as cellulose and wheat pericarp-seed coat at levels of 50–150 g/kg increases the cecal pH to 6.7–8.2 (Campbell et al. 1997; Choct et al. 1998). However, cellulose lowers the SCFA concentration from 102 to 70 mM. A negative correlation between the cecal SCFA and pH is established by few researchers (Campbell et al. 1997; Choct et al. 1998). *In vitro* incubation studies indicated that lowering pH values, thence raising SCFA, prevent the overgrowth of pH-sensitive pathogenic bacteria (Prohaszka et al. 1990). *E. coli* and *Salmonella* spp. are killed by propionate and formate at high pH (pH 5). However, greater SCFA production by rapid feeding of high fermentable carbohydrates (water-soluble polysaccharides) has been associated with the colonization of *Serpulina hyodysenteriae* and appearance of diarrhea *in vivo* (Pluske et al. 1998). Human studies have been few, but SCFA can assist in the management of antibiotic-induced and infectious diarrhea (Ramakrishna et al. 2000). Fermentable carbohydrates alter the microbial ecology significantly by acting as substrates or supplying SCFA.

6.6.2 Absorption and Metabolism of SCFA by Colonocytes

Less than 5 % of bacterially derived SCFA appear in feces due to colonic uptake (McNeil et al. 1978; Roediger and Moore 1981; Roediger and Nance 1986). This is the major cause for the decline in the concentration of SCFAs in the large bowel. SCFA are absorbed from the perfused human large bowel in a concentration-dependent manner (Rupin et al. 1980). Most of the SCFA uptake (60 %) happens due to simple diffusion (protonated SCFA) through the hydration of the luminal CO_2. The remainder is absorbed by cellular uptake of ionized SCFA involving the cotransport of sodium and potassium ions (Fleming et al. 1991; Engelhardt et al. 1995). Its

uptake has been associated with the transport of water that is high in distal end than proximal colon (Bowling et al. 1993). SCFA stimulate colonic fluid and electrolyte transport (Clausen et al. 1991). Retention of calcium and magnesium ions is observed in animals fed with oligosaccharides (Bird et al. 2000). Studies in human indicate the enhanced absorption of calcium ions after the consumption of fermentable carbohydrate, including inulin and beet fiber (Trinidad et al. 1996; Choudary et al. 1997).

SCFA are absorbed at similar rates in various regions of the large bowel (Engelhardt et al. 1995). Acetate, propionate, and butyrate are absorbed at comparable rates in humans and rats (Umesaki et al. 1979; Fleming et al. 1991). While in guinea pigs, the acetate clearance is high in the proximal colon and low in the cecum and distal colon (Engelhardt et al. 1995). At pH 5.5–7.5, more than 50 % of SCFA are available in dissociated form. It has been observed *in vitro* that putative unstirred layer where reassociation of SCFA may occur may affect the absorption rate. This could be the reason for the regional difference in colonocyte metabolism and SCFA absorption in the colon.

SCFA are the major respiratory fuels for the small bowel and colon (Yang et al. 1970; Windmueller and Spaeth 1977), and they supply 60–70 % of the energy needs (Roediger and McDermott 1995). SCFA suppress glucose (Ardawi and Newsholme 1985; Roediger and McDermott 1995) and spare pyruvate metabolism (Butler et al. 1990). Butyrate is the major intestinal fuel even when competing substrates such as glucose and glutamine are available (Roediger and Moore 1981). Butyrate is oxidized more in the proximal than in the distal colon. During ulcerative colitis, a defect in butyrate metabolism is observed. These patients have low butyrate and low pH and high lactic acid levels (Vernia et al. 1988). Intracolonic infusion of SCFA reduces the degree of inflammation (Agarwal and Schimmel 1989; Harig et al. 1989). Cell proliferation in the upper crypt, measured with proliferating cell nuclear antigen (PCNA), is reduced by treatment with butyrate (Scheppach et al. 1999).

6.6.3 Effect of SCFA on Colonic Blood Flow and Muscular Activity

In vitro studies show that acetate and propionate are more effective, even at low concentrations (3 mM) and dilated precontracted colonic arterioles in human colonic segments (Mortensen et al. 1990). SCFA also cause greater blood flow (1.5–5.0 fold). The mechanism of action of SCFA on blood flow does not involve prostaglandins and α- or β-adrenoreceptor-linked pathways (Mortensen et al. 1990). SCFA increase short-duration contraction at low molarity (0.1–10 mM) in rats in the following order: acetate << butyrate < propionate (Squires et al. 1992); and this could be the combined effects of local neural network and chemoreceptors. Manometric studies in human show that ingestion of fermentable carbohydrate or infusion of SCFA causes a decrease in the gastric tone and an increase in the volume. SCFA possibly activate the ileocolonic brake in a dose-dependent manner. Modulating the upper gastrointestinal passage of food improves nutrient digestion. Nevertheless, rapid transit of food through the colon is believed to improve laxation. Greater blood flow caused by SCFA enhances tissue oxygenation and transport of absorbed nutrients.

6.6.4 Trophic Effects of SCFA (Butyrate and Propionate) in Maintaining Normal Colonic Cell Phenotype

Growth of colorectal and ileal mucosal cells is observed *in vivo*, when SCFA is delivered colorectally or intraperitonealy (Sakata and Yajima 1984; Reimer and McBurney 1996). This could be due to the raise in ileal and cecal glucaogon-like peptide-1 mRNA levels. Butyrate lowers the risk of malignant transformations in the colon. In normal rats, butyrate enhances proliferation only at the crypt base. However, this is blocked by secondary bile acid (deoxycholic acid) (Velazquez et al. 1997). Secondary bile acids are cytotoxic and are associated with

greater susceptibility to the development of cancer (Deschner et al. 1983; Lipkin et al. 1983; Terpstra et al. 1987). Normal mucosa from colorectal carcinoma patients resists bile-acid-induced apoptosis, indicating the role of high levels of bile acids in resisting apoptosis (Payne et al. 1995). SCFA may aid in preventing cancer by lowering the intracolorectal pH, as bile acids are protonated and insoluble at pH 6 and could not be absorbed by the colonocytes. Lower pH also inhibits the bacterial conversion of primary bile acids to secondary, thus lowering the cancer potentiality (Nagengast et al. 1988).

6.6.5 Molecular Mechanism of Short-Chain Fatty Acid in Preventing Colon Cancer

At a molecular level, butyrate affects the gene expression via the phosphorylation and acylation of histone proteins (Archer and Hodin 1999). It is recognized as an inhibitor of histone deacetylase (HDAC) activity. Despite its rapid metabolism, it can have a prolonged effect on histone acetylation and the induction of differentiation markers in colon cancer cells, including alkaline phosphatase. It is not clear if this extended action reflects protection on binding with HDACs or if it is an independent action. In NCM460 cells, butyrate is the only HDAC inhibitor that causes a consistent increase in alkaline phosphatase activity (Lee and Workman 2007). However, this contradicts with the results in Caco-2 cells where a variety of HDAC inhibitors induces alkaline phosphatase activity. HDAC inhibitors in clinical trials include vorinostat, phenylbutyrate and romidepsin. It is postulated that inhibitors of HDAC activity cause changes in the expression of genes coding for proteins and microRNAs. Similar to vorinostat, sodium butyrate has also been reported to exert antiproliferative activity on many cells types, thus demonstrating the preventive effects of butyrate on colon cancer and adenoma development (Bornet et al. 2002) (Fig. 6.7).

Histone hyperacetylation results in chromatin relaxation, which makes DNA more accessible to transcriptional factors. It also causes an increase in the cell cycle inhibitor, p21 gene, which harbors butyrate-responsive elements at the promoter region. Upon butyrate treatment, this gene was reported to be induced due to HDAC inhibition resulting in G1 phase arrest (Blottiere et al. 2003) (Fig. 6.7).

Butyrate activates tissue inhibitor matrix metalloproteinase (TIM) -1 and −2 and inhibits the activity of metalloproteinases (MMPs), which in turn reduce the adherence of cancer cells to the basement membrane protein laminin substrate through fibronectin or type IV collagen and, thus, prevent the proliferation of cancer cells. Inflammatory cytokines (IL-4, TNF-α) aid butyrate in preventing colon carcinogenesis (Andoh et al. 2003).

G-protein-coupled receptors (GPCRs) consist of proteins involved in the transduction of extracellular stimuli to intracellular signals. The family of GPCR protein is activated on binding with a ligand or agonist that leads to a conformational change and activation of the G-protein heterodimer. Recently, acetate, propionate, and butyrate were reported to act as ligand for GPR41 and GPR43 that were expressed in colonic mucosa, suggesting their role in the normal development or function of the colon tissue (Tazoe et al. 2008). Tang et al. revealed that exposure of GPR43 in human colon cancers to SCFA causes its reduction, thus making cells more sensitive and leading to apoptosis by cell cycle arrest at G0/G1 phase. Thus, SCFA may influence GPR43 expression and prevent colon cancer development and progression (Tang et al. 2011) (Fig. 6.7).

Rapamycin (mTOR) negatively regulates autophagy, and the autophosphorylation at Ser2481 is considered as indicator of autophagy. Propionate treatment of colon cancer cells (HCT116) showed a strong time-dependent reduction in the phosphorylation state at Ser2481, while no change was observed in the total mTOR levels. Inactivation and activation of p70S6K (at Thr389, Thr421/Ser424) influence mTOR. Propionate treatment reduced the phosphorylation of p70S6K at Thr389, causing downregulation of the mTOR signaling pathway and inducing autophagy. Propionate also mediates AMPK activation causing a reduction in ATP lev-

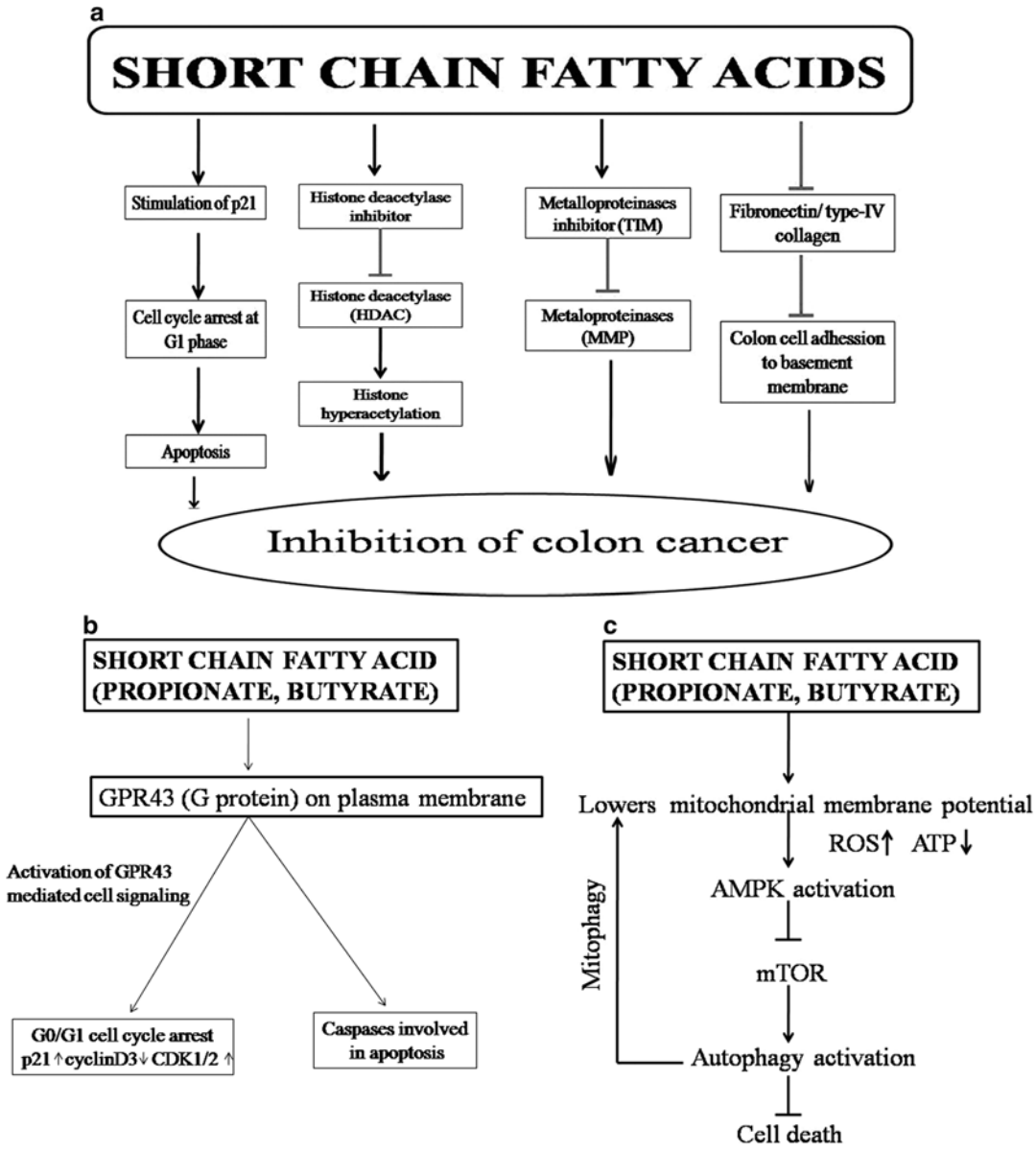

Fig. 6.7 Molecular mechanism of inhibition of colon carcinogenesis by SCFA. (**a**) Major mechanisms by which SCFA inhibit colon carcinogenesis. Propionate and butyrate induced (**b**) cell cycle arrest and apoptosis, and (**c**) increased ROS

els in colon cancer cells due to the falling out of mitochondrial membrane potential and releases reactive oxygen species (ROS) (Tang et al. 2011). Excess ROS induces autophagy.

Mitochondrial ATP depletes, and excess ROS is produced when cell environment changes, causing release of proteins that promote cell death. Fascinatingly, the cells can adapt to its own defense mechanism (mitophagy), where increased mitochondrial confiscation was observed in lysosomes following the stimulation of hepatocytes catabolism with glucagon (Tang et al. 2011). Mitophagy plays a key role during cellular quality control. Mitochondrial fission, fusion, and mitophagy are all important for mitochondrial homeostasis. Mitophagy has also been

shown to be required for steady state turnover of mitochondria, for the adjustment of mitochondrion numbers to changing metabolic requirements and during specialized developmental stages in mammalian cells such as during red blood cell differentiation (Youle and Narendra 2011). HCT116 colon cancer cells treated with propionate showed mitophagy, which selectively targets mitochondria with a depolarized membrane potential (Tang et al. 2011) (Fig. 6.7).

Further, treatment with propionate altered the lipid metabolism in HCT116 cells. Lipids are strongly involved in cell structure and metabolism. Propionate treatment reduced the expression of fatty acid synthase, involved in the catalytic synthesis of long-chain fatty acids from acetyl-CoA (ACC) and malonyl-CoA. GSK-3β (involved in inhibiting endergonic glycogen synthesis and activation of glycogen synthase) was also downregulated. AMPK kinase, which was upregulated during propionate treatment, inhibits acetyl-CoA, thus lipid synthesis (Tang et al. 2011).

Autophagy and apoptosis might be interconnected and interregulated and could act as partners in inducing cell death in a coordinated manner; however, the effects on cell fate are different. Propionate treatment in HCT116 induces ATP depletion by downregulating anabolic processes (glycogen and lipid synthesis) but stimulates mitochondrial biogenesis to resume cellular energy homeostasis (Tang et al. 2011). Inhibition of autophagy, in many studies, has been indicated to lead to apoptosis. Treatment of HCT116 and SW480, cancer cell lines with 3-methyladenine (3-MA), an inhibitor of autophagy, significantly reduced the percentage of GFP-LC3 formation. However, after 12 h treatment with propionate/3-MA increased the number of apoptotic cells via increased cleavage of caspase 3 and 7. AMPKα depleted cells with autophagy silencing showed a significant cytotoxicity with propionate treatment. Further, knocking down ATG5 expression (an important protein for autophagy) reduced the ability of propionate to induce GFPLC3 punctae formation. Thus, the finding suggests that autophagy confers a protective role in propionate-induced cell death in colon cancer cells (Tang et al. 2011).

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Conclusion

7

Abstract

Colorectal cancer (CRC) is the third most common cause of mortality in developed countries and is growing in developing countries. The International Agency for Research on Cancer and World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) have accounted that colorectal cancer (CRC) ranks third (approximately 8 % of all cancer deaths worldwide) among the list of other deadliest cancers. A large proportion (nearly 85 %) of colorectal cancers are related to environmental factors. Diet, dietary habits, and lifestyle factors are considered one of the prime environmental factors reported to be responsible for the high incidence of CRC. Red and processed meat cooked at elevated temperature, refined starch, sugar, chocolate, coffee, and saturated and trans-fatty acids and poor fiber intake are closely linked to the increased risk of CRC. Environmental risks contain various types of heterocyclic amines and polycyclic aromatic hyrdrocarbons that are formed due to the reaction between free amines and sugars causing DNA mutation, leading to cancer. Other contributing factors include excess body mass and sedentary behaviors. Chemotherapy, radiotherapy, antibiotics, and surgical removal of cancers or tumors are the current key modes of treating most CRCs. Nevertheless, the occurrence of cancer could not be spared by these techniques. Thus, this new concept of diet manipulating CRC has been introduced several years before. This has been reported to stimulate fermentation in the gut and encourage the production of short-chain fatty acids that have anticarcinogenic properties at molecular level.

Keywords

Fusobacterium • *Porphyromonas* • Probiotics • Prebiotics • Synbiotics

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Human gut and microflora inhabiting it play a key role in determining the host health and functions of the organs. An imbalance in the gut microbiome may affect the health and could activate colon carcinogenesis. This may also lower the host immunity. The role of gut microbes in inhibiting carcinogenesis and the role of beneficial microbes in preventing colon cancer have been reported earlier. Probiotics, prebiotics, dietary fibers, and synbiotics, as functional food, are known to improve the gut health and act as anticarcinogenic agents.

In synbiotics, the synergistic association of probiotics and prebiotics causes cumulative benefits rather than individuals. Prebiotics and dietary fibers form substrate for the probiotics that ferment the former and aid in the formation of short-chain fatty acids, including acetate, propionate, and butyrate. Fecal bulking, laxation, and reduction in the contact time with the intestinal lining are the other physiological means by which dietary fibers protect the colon. Reductions in colon pH, improved immune system, etc. are other benefits of dietary fiber.

Microbial colonies throughout the length of the digestive system vary in density and composition. Based on anatomical site, transit rate, host secretion, environmental condition, substrate, and the structure of gut wall, this microbiome varies. The stomach and proximal small intestine support low number of microorganism, in contrast to the large intestine that favors dense microbial community dominated by obligate anaerobes. A majority of bacteria belong to Bacteroides, Prevotella, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia. The human gut and its complex microbiome comprising of trillions of bacteria contribute significantly to the maintenance of the colon's health and its proper function. Long-term diet manipulates the structure and activity of gut microbes. Such diet-induced disruptive changes (gut dysbiosis) to gut-associated microbial environment could cause chronic illness and are strongly associated with improper colon health. This could be considered as a biomarker of colorectal carcinomas. Disturbance of the symbiotic relationship between the gut microbes and environmental changes interrupt metabolism, the immune system, etc. and may promote carcinogenesis. Patients with colorectal cancer are reported to have highest levels of *Fusobacterium* and *Porphyromonas* bacteria. The beneficial good bacteria that support the normal epithelial cells in the colon and inhibit adenomas and carcinomas are lost with tumor growth. Manipulation of gut microbiome with antibiotics may dramatically decrease the number and size of tumor; however, it will affect the good bacteria of the system too.

Elie Metchnikoff in 1907 established the scientific basis for the health benefits of lactic acid bacteria suggesting that dependence of intestinal microbes on the food makes it possible to exchange harmful microbes with beneficial microbes and could be adopted as a preventive measure to improve health conditions. Hence, lactic acid fermented foods and other cultured milk products became an integral part of human diet and led to the development of the term "probiotics." Probiotics are defined as live microorganisms, which when administered in adequate amount confer a health benefit on the host. Probiotics alleviate lactose intolerance, control diarrhea and urogenital infection, reduce cholesterol level, and prevent IBD and other colon disorders. They also act as antioxidants and inhibit gut pathogens. *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, and yeast such as *Saccharomyces boulardii* are recognized as probiotics. Scientific evidence has implicated a strong association between diet and gut microflora composition, thus inhibiting colon cancer. Probiotics could be delivered in the form of capsules or tablets and as functional food rich in probiotic bacteria. Bacterial enzymes prevent the conversion of procarcinogens to carcinogens. Short-chain fatty acids (SCFA) are an additional mechanism in preventing colon carcinogenesis. SCFA, specifically butyrate, alleviate colon cancer. Increased production of interferon-γ, tumor necrosis factor-α, and interleukin-1β thwarts the proliferation of cancer cells.

The mechanism of action of probiotics, together with bioactive carbohydrates (prebiotics and dietary fibers) and the development of the concept of synbiotics, has introduced a new arena for cancer therapy.

Dietary fibers are defined as the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine and with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber exhibits one or more properties, including laxation, fecal bulking and softening, increased frequency and/or regularity, blood cholesterol attenuation, and blood glucose attenuation. Dietary fiber sources include terrestrial and marine plant sources, such as cellulose, wheat bran, oat bran, pectin, guar gum, sulfated galactans, carrageenan, agaran, fucoidan, laminarin, ulvan, etc. Marine dietary fibers have not been explored in detail as cereal, fruit, and vegetable dietary fibers. The type of dietary fiber affects the gut microbiome and flora. The dietary fibers are categorized into insoluble and soluble dietary fibers based on their water solubility. Soluble fiber with its high water-binding capacity contributes to fecal bulking. The mechanism of action of dietary fiber in preventing colon carcinoma could be grouped as physiological and molecular mechanism. The former includes binding of mutagens or carcinogens and reducing their contact time with the intestinal lumen, fecal bulking, laxation, mineral absorption, and decrease in colon pH. The molecular mode of cancer prevention by the dietary fibers could be attributed to their fermentation to SCFA, namely acetate, propionate, and butyrate. Other mechanism of action includes enhanced bile acid deconjugation, and modulation of inflammatory bioactive substances.

Prebiotics are defined as a selectively fermentable nondigestible oligosaccharide or ingredient that brings specific changes, both in the composition and/or activity of the gastrointestinal microflora, conferring health benefits. It also prevents colorectal cancer by modifying human gut microflora and promotes the growth of beneficial probiotics. These are oligomers comprising of 4–10 monomeric hexose units. Common prebiotics include inulin, fructo-oligosaccharide, galacto-oligosaccharide, xylo-oligosaccharide, lactulose, lactitol, soy-oligosaccharides, polydextrose, etc. Inulin is reported to reduce the growth of potential pathogenic bacteria community, including *E. coli*, *Salmonella*, and *Listeria*. Prebiotics selectively stimulate the growth or activity of beneficial native bacteria or probiotics. They are nonviable. As they are regularly consumed as a part of food, they are highly stable; however, safe consumption is of great concern. Anticarcinogenic properties of prebiotics are due to SCFA production and the enhanced immunity. Modification of gene expression in the colon, cecum, and feces enhanced micronutrient absorption, and modulation of xenobiotic metabolizing enzymes is another means of action to prevent colon cancer. Synbiotics are probiotics and prebiotics taken in combination. The anticarcinogenic properties of probiotics and prebiotics, as synbiotics, could be utilized due to their improved combination event rather than from a single event.

The molecular mechanism of action of probiotics, prebiotics, synbiotics, and dietary fibers ultimately involves the fermented product, SCFA. Short-chain fatty acids are low molecular weight fatty acids that are found in the human intestine in different concentrations. Microbial fermentation of bioactive carbohydrates by probiotics, *Bifidobacterium* and *Lactobacillus* help in the formation of SCFA. Major short-chain fatty acids include acetate, propionate, and butyrate, of which propionate and butyrate are concerned with cell differentiation, growth arrest, and apoptosis of cancer cells. Acetate makes up around 60–75 % of total short-chain fatty acids, followed by propionate (15–20 %) and butyrate (15 %). Carbon dioxide and hydrogen gases are also formed along with these organic acids. Acetate (C2), propionate (C3), and butyrate (C4) released in the intestinal lumen are readily absorbed and utilized by colonocytes and other tissues as energy sources. Acetate improves ileal motility and immunity and increases blood flow in the colon. It is also involved in adipogenesis.

In addition, it lessens the lipopolysaccharide-stimulated tumor necrosis factor, interleukin, and nuclear factor-κB level, thus playing a key role in affecting host immunity. SCFA adjust the colonic and intracellular pH, cell volume, and ion transport and regulate cell proliferation, differentiation, and gene expression. About 70–90 % is utilized by the colonocytes, and the remaining is excreted through feces. Butyrates play a noteworthy role in inhibiting histone deacetylase resulting in histone hyperacetylation and growth inhibition in the colonic epithelial cells. Propionate and butyrate also exhibit strong antiinflammatory properties by inhibiting the production of TNF-α, NF-κB, and IL-8, IL-10, IL-12 in immune and colonic epithelial cells.

The anticarcinogenic effects of pro-, pre-, and synbiotics and dietary fibers are an emerging area and are of immense prospect. The functional foods and nutraceuticals comprising of these would thwart not only cancer but also other non-communicable diseases. The mechanism of action of these is yet to be investigated and studied in detail.

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