Indu Pal Kaur *Editor-in-Chief*
Kanwaljit Chopra · Mahendra Bishnoi
Kanthi Kiran Kondepudi *Editors*

Probiotic Research in Therapeutics

Volume 5: Metabolic Diseases and Gut Bacteria

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Foreword by J. V. Yakhmi

The saying attributed to Hippocrates, the Father of Medicine, that "Let food be thy medicine, and let medicine be thy food" never felt more valid than now when we are challenged by a variety of lifestyle diseases. The relevance of holistic healing has increasingly been related, in recent years, to the gut microbiome, composed of bacteria, archaea, viruses and eukaryotic microbes, all of which reside in our gut, and together have a strong potential to impact our physiology, both in health and in disease. When faced with a variety of diseases, our present-day knowledge lays emphasis on the importance of a healthy microbiome, not only limited to gut health but also to metabolic disorders, cancers, immunity, brain health and skin health. Can we manipulate the gut microbiota by probiotic intervention towards disease prevention and treatment? That is precisely what is receiving the attention of a large number of scientists engaged in research on human health. The growing market interest in health benefits of probiotics has intensified research and investments in this area. With an overwhelmingly large number of new products based on probiotics on the shelves of the supermarkets and pharmacies, it can be inferred that the research in this area is at a very exciting stage. Though the intricate mechanisms involved in the importance of gut flora may require some basic scientific expertise, surfing through scientific claims on usefulness of probiotic therapy can catch the fancy of even a general reader.

I have known Prof. Indu Pal Kaur, chief editor of this book series, for the past 12 years and have been closely following her research interests which essentially hover around being a formulation scientist, be it for small and large molecules, phytochemicals and probiotics. I have noticed her deep interest in trying to complement the observational data compiled in the traditional system of medicine with scientific rationale from currently available information. I have myself discussed with her, several times, the human microbiome and its manipulations for useful therapeutic options. She has been active in the topic of probiotics for a long time, and had, in fact, published her first review on Potential Pharmaceutical Applications of Probiotics way back in 2002, which has been cited over 500 times till date. Her passion to bring probiotics into mainstream therapeutics is not limited only to the ailments of the gut, *viz.* inflammation, ulcers and cancers, but is also aimed to extend it to other lifestyle diseases, such as depression, chronic fatigue syndrome, vaginal candidiasis, wound healing and skin health.

The present e-book series, comprising five volumes, brings latest information and key insights on application of probiotics in cancer and immunological disorders, gut inflammation and infection, skin ailments, neurodegenerative disorders, and metabolic disorders. The contributing authors are recognized experts which ensures that each chapter affords a critical insight into the topic covered, with a review of current research, and a discussion on future directions in order to stimulate interest. Each volume itself covers a broad theme in detail by including chapters disseminating basic information in the field in such a manner that it would attract the attention of even a stray reader or intending consumers. Of course, the whole series of five volumes is designed with care so as to not only ignite the minds of graduating students for future research but also boost the confidence of health professionals, physicians, dieticians, nutritionists and those practicing naturopathy by underlining the integrity of the data documented in the chapters of these volumes from well-established labs and groups. All in all, a very thoughtful compendium of probiotics research in therapeutics!

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Foreword by B. Sesikeran

Our lifestyle and dietary habits have changed significantly due to rapid economic growth and improvement of living standards, accelerating the incidence and prevalence of metabolic disorders such as diabetes, obesity and other non-communicable diseases. The World Health Organization described obesity as a global epidemic hazard way back in 1997. According to recent statistics, 11% of men and 15% of women are obese globally. From the last 2 decades, the diabetes prevalence has significantly increased with 420 million people affected worldwide, and it is predicted to double by 2030. Over the years, the significant role that microbes play in the intestinal ecosystem and thereby have a bearing on the health and well-being of the host was well established through animal studies as well as observations made in humans. The human gastrointestinal tract consists of nearly 100 trillion microorganisms, referred to as the gut microbiota or gut microbiome. The microbial colonization in the human gut begins at the time of birth and it keeps changing with age, diet, health status and antibiotic treatment. These microbes play several vital physiological and metabolic functions in the human body. Further, it is observed that alteration in the human gut microbiota can cause various chronic and acute metabolic diseases such as obesity, hypertension, neurogenic diseases and diabetes. Hence, re-establishment of normal microbial population, with the help of commensal probiotic bacteria, to undo the gut dysbiosis, has always been a topic of great interest.

The fifth volume of the e-book series "*Probiotic Research in Therapeutics: Metabolic Diseases and Gut Bacteria*", by Prof Indu Pal Kaur and her competent team of editors, is an essential addition to our growing understanding of the role of the gut microbiota in a wide range of metabolic diseases. The volume is a comprehensive compilation describing scope and application of probiotic and prebiotic therapies in the treatment of metabolic disorders right from obesity to diabetes to other metabolic and liver disorders, with each chapter demonstrating hope and scope of application. Readers will gain great insights on how probiotics are not confined to the microbial technology industry but hold great promise in medical therapeutic and pharmaceutical sector. The volume provides an authoritative and timely overview of the field. This e-book may serve as one of the riveting reference materials for times to come!

Hyderabad, Telangana, India — B. Sesikeran

Preface

In the early twentieth century, a Russian scientist and Nobel laureate, Metchnikoff, for the first time, linked consumption of "good" bacteria with superior host health. He suggested that the dysbiotic human gut microflora can be replaced by beneficial bacteriotherapy, i.e. probiotics. In the recent years, the faulty gut microbiome is implicated in etiopathogenesis of multiple diseases not limited to intestinal issues (inflammatory bowel diseases, colon cancer) but is also being alluded in metabolic disorders. However, the exact role of microbiome in metabolic diseases has remained elusive, primarily because the central drivers of dysmetabolism also influence the microbiome composition: diet and lifestyle. While it is conceptually intuitive that the gut microbiome and host metabolism would be interrelated, disentangling cause and effect remains a challenge.

The fifth volume of the present e-book series entitled "Probiotic Research in Therapeutics: Metabolic Diseases and Gut Bacteria" focusses on the potential scope of manipulating gut microbiome by probiotic therapy for management of metabolic disorders including diabetes mellitus, metabolic acidosis and obesity. The volume comprises 13 chapters. Introductory chapter highlights the importance of gut–brain axis and its important role in hunger and satiety. Mechanism of appetite regulation by gut microbiota and their role in obesity control is also elaborated in detail in this chapter. Chapter 2 unravels the anti-obesity action of probiotics and dairy based ingredients. Chapter 3 describes the connect between gut dysbiosis and metabolic endotoxemia and scope of its redressal by active probiotic/prebiotic supplementation. Next four chapters elaborate on the amelioration of diabetes mellitus, one of the most prevalent metabolic disorder, by probiotic and/or prebiotic therapy and the importance of healthy gut microbiota in ameliorating diabetes. Clinical and preclinical evidence of a close association of disrupted gut microbiota with the high incidence of diabetes are also discussed in these chapters. Chapters 8 and 9 portray the scope of probiotic therapy in prophylaxis and management of alcohol-induced liver diseases. The authors describe underlying mechanism of action and provide a consolidated overview on the preclinical and clinical evidence supporting probiotic therapy in the management and control of alcoholic liver disease. Next in line is the chapter discussing the effect of probiotic supplementation in modulation of serum lipids. Chapter 11 discusses the concept of prebiotics, microbiota modulation by prebiotics and the impact of prebiotics in various metabolic disorders. Chapter 12

envisages a brief introduction on designer probiotics, their developmental strategies and applications in regulating metabolic diseases, and finally the challenges in the path of their development. Last chapter of the volume describes the advantages and challenges of current animal models used in the evaluation of probiotics for metabolic disorders. Emphasis is laid on describing the future directions in designing better animal models for these evaluations.

With the state-of-the-art commentaries on all aspects of probiotic research for metabolic disorders, from contributors across the globe, the e-book provides an authoritative and timely overview of the field. It is envisioned that this book will be a useful educational and scientific tool to academicians, health professionals, students, and pharma/ biotech businessmen worldwide. As editors of the book, we express our sincere thanks to all the authors for their excellent contributions to the book.

Chandigarh, India — Indu Pal Kaur
Chandigarh, India — Kanwaljit Chopra
Punjab, India — Mahendra Bishnoi
Punjab, India — Kanthi Kiran Kondepudi

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About the Editors

Indu Pal Kaur is presently working as the professor and chairperson at the University Institute of Pharmaceutical Sciences (UIPS). She has more than 25 years of teaching and research experience. Her research forte enhances the performance of small and large biomolecules, including probiotics using active-tailored delivery systems. The emphasis of her work lies in industrial and clinical translation. She has delivered 50 invited talks, published more than 145 high-impact international publications and 21 book chapters; and edited four books and four journal special issues; more than 150 presentations have been made by her research group, with 30 sentenced as best presentations.

Kanwaljit Chopra the former chairperson of the University Institute of Pharmaceutical Sciences, has prolific experience of 28 years in research and holds distinguished expertise in neuropharmacology, diabetes and metabolic diseases, pain, inflammation, and menopausal complications. She has more than 215 international and national publications in high-impact journals, acclaimed globally for her research work. She has received 11 prestigious national and international awards. She has fetched grants of over 1.5 crores from the government as well as industry. She has been an active member of national and international societies. Her "Reverse Pharmacology" approach has provided strong leads to the scientific community for the translational potential of various moieties of natural origin.

Mahendra Bishnoi is working as Scientist E, Food and Nutritional Biotechnology, National Agri-Food Biotechnology Institute, Mohali. He is presently leading a group of young researchers dedicated to metabolic disorders (obesity and type-2 diabetes) research with a particular focus on nutrition and diet, the development of molecular evidence-based functional foods, and nutraceuticals. His scientific work is well recognized in more than 120 peer-reviewed and high-impact factor scientific journals, with around 4000 citations. Dr. Bishnoi is an Alexander von Humboldt Experienced Fellow, Early and Mid-Career Research (EMCR) fellow of Indian National Science Academy (INSA), Melvin Yahr awardee from World Federation of Neurosciences.

Kanthi Kiran Kondepudi is presently working as Scientist E in the Food and Nutrition Biotechnology Division at National Agri-Food Biotechnology Institute, an autonomous institute under the Department of Biotechnology, Government of India. Dr. Kondepudi is actively working on developing dietary interventions that could beneficially modulate the gut bacteria and could protect from proinflammatory stress underlying many diseases with an overall goal to develop functional foods based on millets and their bioactive ingredients, dietary fibers, prebiotics, probiotics, and other functional ingredients from different plant sources. His group uses rodent models, nutrigenomic and metagenomic approaches for evaluating the efficacy of bioactive ingredients and functional foods.

Gut–Brain Axis: Role in Hunger and Satiety

1

Kondapalli Vamsi Krishna, Shruti Malviya, Debaditya Bhattacharyya, and Alok Malaviya

Abstract

The human gastrointestinal tract consists of nearly 100 trillion microorganisms, referred as gut microbiota or gut microbiome. The microbial colonization in the human gut begins at the time of birth and its colonization increases with age which is influenced by factors like age, diet, and antibiotic treatment. Gut microbiota is believed to play a major role in human health as well as various physiological activities like metabolism, nutrition, physiology, etc. Imbalance of the normal gut microbiota has been linked with gastrointestinal conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) as well as wider systemic manifestations of disease such as obesity, type 2 diabetes, and atopy. Gut–brain axis, a two-way (bi-directional) connection and communication between the gut and the brain has potentially huge influence over our health which integrates neural, hormonal, and immunological signaling between the gut and the brain. There is growing evidence on the influence of gastrointestinal (GI) microbiota that modulates appetite, feeding, and metabolism as well as regulates the mechanisms of digestion. Gut hormones like Ghrelin, Cholecystokinin (CCK), Pancreatic Polypeptide (PP), Peptide YY (PYY), Glucagon-Like Peptide 1 (GLP-1), Oxyntomodulin (OXM), Glucagon, Gastric Inhibitory Polypeptide (GIP), and Amylin have been identified in the gastrointestinal system which have a fundamental role in coordinating digestive process within the

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gastrointestinal system, thus regulating feeding behavior and energy balance. Studies have indicated that the modulation in gut microbiota regulates the production of ghrelin and PYY in overweight and obese patients and helps in promoting weight loss and improves glucose regulation. Considering the importance of the role of gut microbiota on hunger and satiety, this chapter was written where we have discussed the gut–brain axis and its role in hunger and satiety. Further, mechanism of appetite regulation by gut microbiota and their role in obesity control have also been discussed. Finally, future perspectives on application of gut microbiota as potential probiotic solutions for obesity and related metabolic disorders will be discussed.

Keywords

Metabolic disorder · Microbiome · Hunger · Obesity · Probiotics · Satiety

1.1 Introduction

The human gastrointestinal tract harbors nearly 100 trillion microorganisms referred to as gut flora or gut microbiome. Their number outnumber the host's cells by a factor of 10. The total number of genes of the gut microbiome exceeds that of the human genome by at least 100-fold. Almost all of these organisms are bacteria, and minorities are achaeans, eukaryotes, and viruses. The gut microbiota consist of both strict anaerobes and also facultative anaerobes. The strict anaerobes outnumber the facultative anaerobes which can grow both aerobically and anaerobically. *Bacteroidetes* (Grams negative) and the *Firmicutes* (Grams positive) are the 2 major phyla (80–90%) and *Proteobacteria, Fusobacteria, Cyanobacteria, Verrucomicrobia,* and *Actinobacteria* are the other different minor phyla that represent the gut microbiota. A total number of more than 1000 different species can be identified in the human gut distributed in the different parts (Lin et al. 2014; Musso et al. 2010; Bull and Plummer 2016; Million et al. 2013; Guinane and Cotter 2013; Dahiya et al. 2017; Sharma and Tripathi 2019; Calum et al. 2014).

1.1.1 Development and Distribution of Microbes in the Gastrointestinal Tract

The human gut is believed to be microbe free (Sterile) or might contain a very low number at the time of birth. However, soon after the delivery, microbial colonization starts as the neonate passes through the birth canal from the mother. Infants who were delivered vaginally have more microbes compared to the infants delivered through cesarean during the first month. The microbial composition of the gut is simple and less complex during the initial years of life. They include *Enterobacter* and *Enterococci* followed by anaerobes *Bifidobacteria, Clostridia, Bacteroides,* and *Streptococci*. Thereafter the infants' gut microbiome slowly undergoes a shift in the

development and diversification due to the change in feeding habitats from breast or formula feeding to solid food. By the age of 2, the gut microbiome shows the community structure similar to the adult gut. External factors such as type of food, feeding habits, temperature related stress, etc. as well as internal factors like intestinal pH, microbial interactions, bile salts, peristalsis, host secretions and immune responses, drug therapy, and bacterial mucosal receptors influence the gut microbiota composition and diversity in a healthy gut (Bull and Plummer 2016; Guinane and Cotter 2013; Dahiya et al. 2017).

The microbial composition varies along the various parts of the gut. In the stomach and small intestine (duodenum), approximately 10^2–10^4 CFU per mL of microbes can be observed. *Lactobacilli, Streptococci, Staphylococci, Veillonella, Actinobacilli,* and Yeasts are predominantly in the stomach region. Due to a change in the pH and reduction of oxidation reduction potentials, the microbial load in the ileum increases to 10^6–10^8 CFU per mL. Microbes belonging to the *Firmicutes* phyla prevail in this region. 10^7–10^{12} CFU per mL of microbial cells that are strictly anaerobic and obligate anaerobes can be observed in the large intestine and bowel (Colon) where the pH is fairly neutral (Fig. 1.1). The maximum number of microbes are found in the colon region, which is predominated by the species belonging to *Bacteroidetes.* The microbial composition along the gut varies widely at the level of bacterial species than that of phylum level (Lin et al. 2014; Musso et al. 2010; Bull and Plummer 2016; Sharma and Tripathi 2019).

Nearly 80% of the gut microbiome cannot be grown in vitro. Hence the use of new molecular techniques plays an important role in understanding the gut microbial composition, its genetic information and functions. Molecular biology based

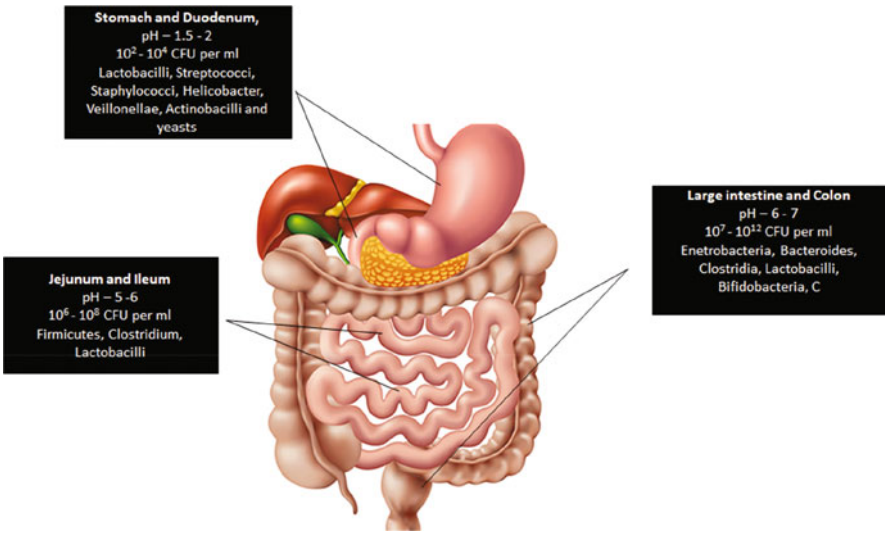

Fig. 1.1 Gut microbiota—distribution, nature as well as respective concentrations of various organisms present in different parts of gut varies depending on its local microenvironment

technologies like 16S r-RNA and 18S r-RNA sequencing by 454 Roche sequencing, pyrosequencing platforms, DNA microarrays, fingerprint techniques, fluorescent in situ hybridization (FISH), etc. have helped the researchers to study and understand the bacterial communities in the gut. Advances in various related fields such as bioinformatics, metagenomics, metatranscriptomics, and metabolomics have also aided in broader understanding of the gut microbiota. Advancement in the field of NMR and MS helped to analyze 1000s of different metabolites in the gut. MetaHIT (Metagenomics of human intestinal tract) and HMP (US human microbiome project) are large scale sequencing projects working towards understanding the gut microbiota. These various platforms are set to provide diverse information about the gut microbiota and will aid in understanding their role in human well-being (Musso et al. 2010; Guinane and Cotter 2013; Sharma and Tripathi 2019; Calum et al. 2014).

1.2 Gut–Brain Axis

The gut–brain axis is a complex bidirectional communication signaling network between the gut and the brain. It integrates neural, hormonal, and immunological signals between gut and brain which ensures proper maintenance of gastrointestinal homeostasis (Fig. 1.2). This offers the gut microbiota and its metabolites a potential route to access the brain (Bull and Plummer 2016; Mayer et al. 2015; Buhmann et al. 2014; Carabotti et al. 2015).

1.2.1 Morphology of the Gut–Brain Axis

The Central Nervous System (both brain and spinal cord) and the Peripheral Nervous System (PNS) together constitute the nervous system. The gastrointestinal tract (GIT) receives both excitatory and inhibitory innervation by the Autonomous Nervous System (ANS) which is a part of the PNS along with the Somatic Nervous System (SNS). The excitatory innervation to the gut is provided by the Parasympathetic Nervous System (PSNS) division through the vagal and pelvic nerves. The Sympathetic Nervous System (SNS) sends the inhibitory innervation to the gut through its splanchnic nerves. The Enteric Nervous System (ENS) is the third division of ANS. The very existence of ENS was postulated by Langley (Konturek et al. 2004), which consists of a network of neurons localized in two different areas: (i) between the outer longitudinal and middle circular muscle layer called Myoelectric (Auerbach) plexus and (ii) located between middle circular layer and the mucosa called submucous (Meissner) plexus. The ENS contains about 100 million of nerves serving as sensory neurons, interneurons, and effector motor neurons. ENS can be considered as an integral part of CNS which retains two-way communication pathways both parasympathetic and sympathetic, each of which includes efferent and afferent nerves. Both the branches of ANS regulate various functions in the gut. The efferent extrinsic parasympathetic nerves end on cholinergic ENS neurons in the

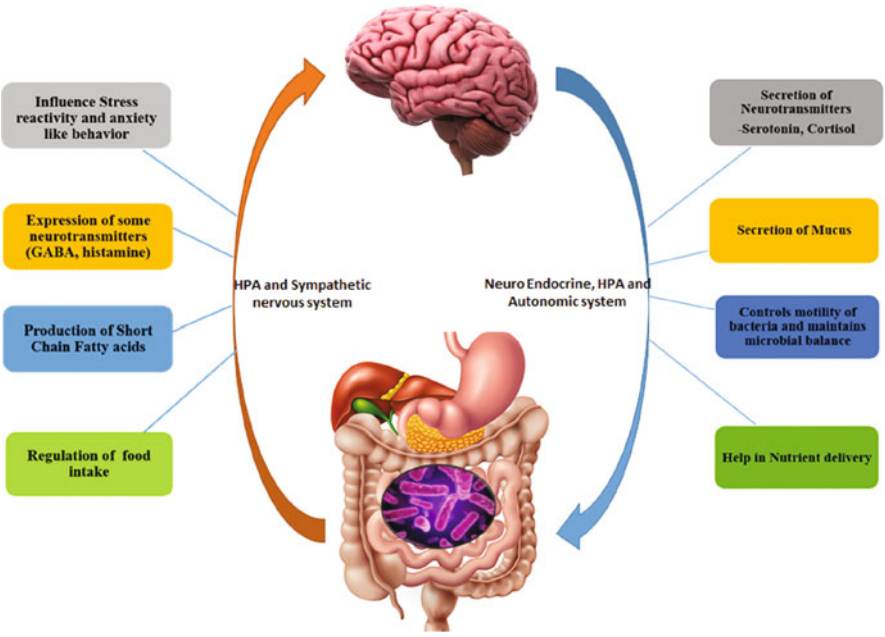

Fig. 1.2 Gut–Brain Axis—interconnection between gut and brain. Gut communicates with the brain via HPA and sympathetic nervous system while brain communicates with gut via neuro endocrine, HPA, and autonomic nervous system

myenteric plexus to control the motor activity in the gut and secretory activity of glandular cells or visceral circulation in the submucosal plexus. These nerves are pre-ganglionic fibers. The post-ganglionic nerves, i.e., the efferent extrinsic sympathetic nerves terminate on the post-ganglionic neurons of the ENS. They activate the α2-presynaptic receptors, which inhibit acetylcholine release. These might directly affect the motility of the gut or the vasoactivity of visceral vessels that influence the visceral circulation through the intestinal smooth cells (Bull and Plummer 2016; Mayer et al. 2015; Buhmann et al. 2014; Konturek et al. 2004).

1.2.2 Gut–Brain–Gut Signaling

In the gut–brain signaling system, 2 types of fibers control the gut–brain axis, namely: (i) the efferent fibers (brain–gut) and (ii) afferent fibers (gut–brain). Efferent fibers run through the pre-ganglionic parasympathetic vagal and pelvic nerves which represent the activities of ENS controlled by CNS during the interdigestive and digestive phases. Exogenous and endogenous secretion, motility patterns, and circulations are controlled through this way. Efferent fibers through the post-ganglionic sympathetic splanchnic efferent pathways are active during the stress, adaptation, and nociception (sensory nervous system's response to certain harmful

or potentially harmful stimuli). Afferent fibers run through the afferent vagal (parasympathetic) and spinal (sympathetic) nerves to the CNS. These signals from the gut respond to various mechanical stimuli such as contraction, distention, and various chemicals in the gut lumen. They also respond to neural-hormonal stimuli like gut hormones, neurotransmitters, and neuromodulators for cytokinesis along with inflammatory mediators produced by the gut microbiota (Fig. 1.2).

1.2.2.1 Gut–Brain–Gut Signals

These signals from the gut may be conveyed to ENS by short way from the sensory neurons to interneurons and then to the effector neurons through various mechanical, chemical, and nociceptive stimuli for intramural motor, secretary, and vascular reflexes. The other way (long way) includes the vagal and spinal afferent fibers, hypogastric and pelvic nerves to enter the brain stream for the vago-vagal reflexes and the spinal cord for the spinal reflexes. The afferent vagal fibers pass through the nodal ganglia to terminate on Nucleus Tractus Solitarii (NTS) that shows the viscerotropic representation with the fibers from the esophagus and the stomach. Splanchnic afferent nerves pass through the intervertebral ganglia and dorsal roots to terminate on lamina I and V of the spinal cord to trigger the spinal reflexes and thus controlling the motor and circulatory functions in the gut. The splanchnic nerves synthesize various neuropeptides like calcitonin gene related peptide (CGRP) or Substance P (SP) in their cell bodies. These will be transported along the peripheral afferent terminals to release and cause the vasomotor functions called as axonal reflexes. Information passed by the afferent vagal nerves to the brainstem terminate in the NTS, which is adjacent to Dorsal Motor Nucleus (DMN) within the Dorsal Vagal complex area (DMV). This completes the vago-vagal reflex pathways and controls various functions of the digestive system. Signals from NTS to paraventricular nucleus (PVN), Nucleus Arcuatus (ARC), Central Nucleus to Amygdale (CAN), the Bed Nucleus of the Stria Terminalis (BNST), and the ventral thalamus in the hypothalamus influence appetite behavior of individual (Bull and Plummer 2016; Calum et al. 2014; Konturek et al. 2004; Carabotti et al. 2015).

1.2.2.2 Hypothalamus

The Hypothalamus Axis (HPA) is an important zone in the brain involved in memory and emotional responses. Corticotrophin Releasing Factor (CRF) is released from hypothalamus due to environmental stress which stimulates the Adrenocorticotropic Hormone (ACTH) from the pituitary gland. This leads to cortisol release from the adrenal glands that has an effect on the human organs and brain. All these complex networks between the gut and CNS play a major role in hunger, satiety, and energy homeostasis in a healthy human gut (Buhmann et al. 2014).

1.3 Gut–Brain Axis and the Molecules Involved in Hunger and Satiety

The gut–brain axis has an important role in the control of food intake. Hypothalamus belongs to the central melanocortin system and plays a major role. The hypothalamus has a number of nuclei called as Arcuate Nucleus (ARC), the Paraventricular Nucleus (PVN), Ventromedial Nucleus (VMN), the Dorsomedial Nucleus (DMN), and the Lateral Hypothalamic Area (LHA). All of them are interconnected with energy homeostasis regulatory networks.

The hypothalamus integrates signals from the brain and the cortical centers and deduces information about the food intake. Two types of mechanisms arise from the gut that affect the feeding behavior–one under the control of lateral hypothalamic area, termed as "feeding or appetite center" and (ii) the other called "satiety center" under the control of the ventromedial hypothalamic area. In the feeding or appetite center, the ARC receives peripheral signals and co-expresses neuropeptide Y (NPY) and agouti related peptides. An interplay of these two increases the appetite and thus simulates the eating and body weight gain. On the other hand, in the satiety center, the ARC co-expresses two molecules pro-opiomelanocortin (POMC) and cocaine and Amphetamine Regulated Transcript (CART) responsible for reducing the hunger and appetite, which in turn reduces the food intake and causes the body weight loss. Once the food is taken, the sensory information reaches the NTS from the GIT, either via vagal and somatosensory afferent fibers or by the gut hormones through the bloodstream. The gut hormones also play an important role in hunger and satiety by influencing the brain areas after being released into the bloodstream from the GIT (Buhmann et al. 2014; Carabotti et al. 2015; Konturek et al. 2004).

Gut Hormones: The gastrointestinal tract secretes various hormones into the bloodstream that influence the food intake. These are released during fasting, feeding, and also during the digestion. The gut hormones released into the bloodstream directly access the brain through the Area Postrema (AP) situated above the NTS (Sanger and Lee 2008). Some of the associated molecules have been indicated in Table 1.1 and discussed in the following section:

Cholecystokinin (CCK): CCK is the first gut secreted peptide released from the I cells in the duodenum and jejunum. This is known to be involved in appetite control mainly as a satiety factor. It exists in three major forms—CCK-8, CCK-33, and CCK-39 in the mucosa. Fats and proteins in the food stimulate the CCK formation and its levels in the plasma increase after 15 min of meal intake. CCK via CCK1 receptors affects the gallbladder motility and via CCK2 receptors affects the gastrointestinal motility. CCK through the vago-vagal reflexes triggered by the activation of CCK 1 receptors results in various functions of GIT such as gastric emptying, pancreatic and biliary secretion, and pancreatic growth (Buhmann et al. 2014).

Glucagon-like peptide (GLP)-1: In response to the meal, GLP 1 is a neuropeptide released from L cells present on the ileum. It is formed by cleaving the proglucagon (post-translational modification). It acts through a GLP-1 receptor belonging to the family B of G Protein coupled receptors that can be seen in the brain, gastrointestinal tract, and pancreas. GLP 1 stimulates insulin secretion

Table 1.1 Different hormones and their roles in the human body

Hormones	Cells involved in secretion	Receptors	Function	References
Cholecystokinin	I cells of duodenum and jejunum	CCK1, CCK2	Reduces gastric emptying, induces pancreatic secretion and gallbladder contraction.	Buhmann et al. (2014)
Glucagon-like peptide (GLP)-1	L cells of ileum	GLP-1R	Increases insulin secretion, beta-cell proliferation, beta gene expression, reduces gastric acid secretion, gastric emptying and apoptosis.	Buhmann et al. (2014), Ahima and Antwi (2008)
Pancreatic polypeptide	PP cells in pancreatic islet	Y4	Induces relaxation of gallbladder and reduces appetite.	Buhmann et al. (2014)
Peptide YY	L cells of ileum	Y2-R	Reduces gastric emptying in elderly people, role in development of obesity.	Sanger and Lee (2008), Hussain and Bloom (2013)
Amylin	Beta cells of pancreas	AMY1-3	Delays gastric emptying, inhibits gastric secretion, improves blood sugar level, and reduces appetite and body weight.	Buhmann et al. (2014), Ahima and Antwi (2008)
Ghrelin	Gastric epithelial cells and pituitary gland	GSH-R	Major role as neurotransmitter, increases growth hormone secretion, gastric motility, vasodilation, and cardiac contractility.	Sanger and Lee (2008), Hussain and Bloom (2013), Buhmann et al. (2014)
Oxyntomodulin	Endocrine L cells	GLP-1	Decreases gastric secretion and dietary intake.	Hussain and Bloom (2013), Buhmann et al. (2014)
Glucagon	Alpha-cells of pancreas	GCGR	Maintains blood glucose level during fasting and exercise, promotes hepatic glycogenesis, and increases energy expenditure.	Hussain and Bloom (2013), Buhmann et al. (2014)
Gastric inhibitory peptide (GIP)	K cells of duodenum and jejunum	–	Promotes energy storage, increases fat deposition, triglyceride accumulation, insulin secretion, and decreases apoptosis.	Buhmann et al. (2014)

depending upon the glucose levels after the intake of carbohydrates in the diet, upregulates the pancreatic B cell gene expression, promotes B cell neogenesis, and inhibits apoptosis. The target neurons in the area postrema, NTS, and paraventricular hypothalamic nucleus thus induce satiety and decrease hunger (Buhmann et al. 2014; Ahima and Antwi 2008).

Pancreatic Polypeptide: Pancreatic polypeptide is a 36 amino acid peptide belonging to Pancreatic Peptide (PP) family, released by PP cells in the pancreatic islets. It has an affinity towards Y4 belonging to Y family receptors. It induces relaxation of gallbladder which in turn delays gastric emptying (time it takes for food to empty from the stomach and enter the small intestine) and reduces appetite (Buhmann et al. 2014).

Peptide YY: Peptide YY is also a 36 amino acid peptide belonging to PP family, released by the endocrine L cells of the gastrointestinal tract which also releases the GLP 1. It is found throughout the gastrointestinal tract with highest concentrations in colon and rectum. PYY is released based on the caloric intake. The levels are low during the fasting time and increase post-prandially for several hours. Along with CCK, PPY levels are associated with delayed gastric emptying in the elderly people. It has a pathophysiological role in the development of obesity (Sanger and Lee 2008; Hussain and Bloom 2013).

Amylin: Amylin is co-synthesized and released with insulin by the β-cells of the pancreas. Like CCK, it also enhances the satiety effect. It delays gastric emptying, inhibits gastric secretion, improves blood sugar, and reduces the appetite and body weight (Ahima and Antwi 2008; Buhmann et al. 2014).

Ghrelin: Ghrelin is a 28 amino acid peptide released by the gastric epithelial cells and also by the pituitary gland. It is called "hunger hormone." Ghrelin plays a major role as a neurotransmitter. It's level in the plasma increases before the food intake and decreases immediately after having the food. It is expressed in the ARC and periventricular area of the thalamus. It acts via the growth hormone secretagogue receptor (GHS-R). The regulation of the appetite is mediated via stimulation of the NPY/agouti related peptide (AgRP) co-expressing neurons (Sanger and Lee 2008; Hussain and Bloom 2013; Buhmann et al. 2014).

Oxyntomodulin (OXM): OXM, a gut peptide is released by the endocrine L cells, which also releases GLP 1 and PYY in correlation with the food intake. It has a weak affinity to the GLP-1 receptor. OXM decreases gastric acid secretion and dietary intake (Hussain and Bloom 2013; Buhmann et al. 2014).

Glucagon: Glucagon is a pancreatic hormone produced by the alpha-cells of the pancreas. Its main effect is to maintain blood glucose levels during fasting and exercise by promoting hepatic glycogenolysis and gluconeogenesis. Their levels rise after the protein diet. The vagus nerve has been implicated in transducing glucagon signaling to the brainstem, so as to mediate the satiating effects (Hussain and Bloom 2013; Buhmann et al. 2014).

Gastric inhibitory peptide (GIP): Gastric inhibitory peptide (GIP) is a 42 amino acid peptide released by the K cells in the duodenum and proximal jejunum. It is released after fat and carbohydrates intake through food. It also promotes energy storage by direct actions on adipose tissue (Buhmann et al. 2014).

Peripheral adiposity signals: Insulin and leptin are long term mediators of energy balance which is circulated by the adipose tissue. Insulin is secreted by the pancreatic beta cells after carbohydrate intake and regulates the glucose homoeostasis. Insulin receptors are widely distributed in the hypothalamic nuclei of the brain and contribute to the regulation of dietary intake. Similarly, leptin is secreted by the white adipose tissue wherein the levels correspond with the fat mass of the individual. Leptin mediates its anorectic effects via the ARC where it inhibits NPY/AgRP neurons and activates POMC/CART neurons, leading to reduced food intake and increased energy expenditure. These numerous neuropeptides in the body affect the food intake either by stimulation of the hormones like ghrelin, OXM or inhibiting expression of hormones like CCK. By stimulating the NPY and AgRP expression in the hypothalamus, they also help to initiate the food intake (Ahima and Antwi 2008; Konturek et al. 2004).

Eating is driven by various reasons beyond physiological needs like energy deficiency following fasting to physical exercise when the food is presented in a palpable way and also social reasons. Each of these reasons is mediated by different neural signals and normal mechanisms regulating food intake. Food also provides visual, smell, and taste signals that can override satiety and stimulate feeding. Taste receptors including sweet, salt, sour, bitter tastes are expressed by taste cells in the tongue as well as oral cavity and transmit the information to the NTS and parabrachial nucleus in the brain stem. Taste sensation is then relayed to the thalamus and lateral frontal cerebral cortex, central nucleus of the amygdale, and lateral hypothalamic area. In summary, the entry of nutrients in the small intestine stimulates the release of the peptide which acts as a negative feedback signal to reduce the meal size and stop the feeding. Hormones secreted by the peripheral organs exert long-term effects on energy balance by controlling the food intake and energy expenditure. The neurons located in the hypothalamus and brainstem are involved in the homeostatic regulation of feeding (Dahiya et al. 2017; Mayer et al. 2015; Buhmann et al. 2014; Konturek et al. 2004; Hussain and Bloom 2013; Ahima and Antwi 2008; Sanger and Lee 2008).

1.4 Gut Microbiota Influencing Gut–Brain Axis

Clinical and experimental studies indicate that the enteric microbiota has a major impact on the gut–brain axis. In humans, evidence of the gastrointestinal microbe–brain interaction was first recognized 20 years ago when the health conditions in the hepatic encephalopathy patients were improved after oral administration of probiotics (Mayer et al. 2015). Later, many experimental works have been conducted in the last few years on animal models to understand the role and contribution of the microbiota in controlling and modulating in the brain axis. Technical strategies like germ free (GF) animal models, gut manipulations with probiotics, prebiotics, antibiotics, and infection studies were being used for these studies. Sudo and his colleagues were the first to make some considerable progress in animal models. They showed that the absence of the normal gut microbiota can

significantly affect adult stress responsiveness and these can be partially reversed by the colonization of the gut. Later various studies in the GF animals revealed that the bacterial colonization is very critical in the development and maturation of ENS and CNS (Carabotti et al. 2015). Conditions like altered expression and turnover of neurotransmitters in both nervous systems, gut sensory motor functions like delayed gastric emptying and intestinal transit, etc. are associated with absence of microbial colonization. It was found that all these anomalies could be restored once the bacterial colonization starts. In the next few sections, we will discuss the various studies conducted on GF animals.

1.4.1 Animal Model Studies

It was observed that microbiota influences the stress reactivity and anxiety-like behavior and regulates the set point for HPA activity in the studies conducted in GF animals. Decreased anxiety and an increased stress response with augmented levels of ACTH and cortisol were shown by these model animals. Normalization of the axis with reversibility of exaggerated stress response was observed after the microbial colonization in the gut starts irrespective of the age. This led to the conclusion that microbiota play an important role in establishing neural regulation. Several studies showed that expression of brain-derived neurotrophic factor (BDNF), one of the most important factors involved in memory which is located in the hippocampus and cerebral cortex, is reduced in the brains of GF animals and increased in the infected models. BDNF regulates various aspects of brain activities including cognitive functions as well as muscle repair, regeneration, and differentiation (Carabotti et al. 2015; Mayer et al. 2015). Other studies showed changes in the receptor expression, including GABA receptor A and B subunits. These mediate the effects of the major inhibitory neurotransmitter in the brain (Mayer et al. 2015). Treatment with *L. rhamnosus* (JB-1) induced region dependent alterations in GABA mRNA in the brain. It was observed that GABA (B1b) receptor mRNA expressions increased in the cortical regions (cingulate and prelimbic regions) and decreased in the subcortical regions (hippocampus, amygdala, and locus coeruleus). Similarly GABA (Aα2) mRNA expression was also found to be decreased in the prefrontal cortex and amygdala but increased in the hippocampus. These mice showed reduced anxiety and depression like behaviors. Studies indicated that the microbiota communicates with the brain through the vagus nerve that transmits the information from the luminal environment to CNS. Neurochemical and behavioral effects were absent in the vagotomized mice which identifies the vagus as a major modulatory constitutive communication pathway between the gut and the microbiota (Carabotti et al. 2015). Microbiota interacts with GBA through the modulation of the intestinal barrier, whereas perturbation can influence all the underlying compartments. Pretreatment of animals with probiotics combined with *L. helveticus* R0052 and *Bifidobacterium longum* R0175 restored tight junction barrier integrity and attenuated HPA axis along with ANS. Probiotics also prevented changes in hippocampal neurogenesis and expression in hypothalamic genes involved in synaptic

plasticity. The gut microbiota affects the mucosal immune activation as well. In one such study, the mucosal inflammation was induced in mice by oral administration of antimicrobials which increased the substance P in the ENS. This effect could be normalized by the administration of bacteria *L. paracasei* (Carabotti et al. 2015).

1.4.2 Influence of Molecules Released by Microbiota

Furthermore the ENS can be influenced by molecules produced by the microbiota such as GABA, serotonin, histamine, and acetylcholine which can act as local neurotransmitters. One of the major roles of gut microbiota is to assist in complete digestion of the dietary fibers that are incompletely digested by the host enzymes during the process of digestion. During this process, metabolites called short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate are released. These SCFAs are said to be host energy sources and also act as signal transduction molecules via G protein coupled receptors such as Free Fatty Acid Receptor 2 and 3 (FFAR2 and FFAR3), OLFR78 - Olfactory receptor 78, GPR109A - G protein Receptor 109 A (Kasubuchi et al. 2015). These SCFAs are capable of stimulating sympathetic nervous systems. They exert multiple beneficial effects on host energy metabolism by improving the intestinal environment. However, the exact signaling mechanisms and physiological functions of SCFAs in the host peripheral tissues are unclear. The microbial protease mediates the immune activation. These are upregulated in intestinal-immune mediated disorders and become the end-stage effectors of mucosal and enteric nervous damage (Carabotti et al. 2015).

All these studies suggest that gut microbiota along with its metabolites play an important role in the bidirectional interaction between gut and nervous system. They interact with CNS by regulating the brain chemistry and influencing the neuro endocrine systems. Most of these studies on GF animal models suggest that many of the effects are strain specific and they can be used as potential probiotic strains (Mayer et al. 2015; Carabotti et al. 2015; Kasubuchi et al. 2015).

1.5 Mechanism of Appetite Regulation by Gut Microbiota

Microbiota in the human gut plays an important role in host physiology such as immune modulation, digestion of various food materials, production of vitamins, bile salts, and metabolites. Underlying mechanisms for some of these functions are explained in the following section.

1.5.1 Carbohydrate Metabolism

Regular diets in humans include a large amount of carbohydrates that include mono, di, and polysaccharides. Monosaccharides like cane sugar and fruit sugars are readily absorbed in the intestine. Disaccharides like sucrose, lactose, maltose, and complex

polysaccharides such as starch, cellulose, xylan, hemicellulose, etc., need to be broken down into simpler forms in the intestine. As our digestive tract is deprived of the enzymes to digest these polysaccharides, these are fermented by the gut microbiota. Bacteroides, the dominating anaerobes, help in digesting these polysaccharides and convert them into simple sugars. The sugars thus converted would be utilized through glycolytic pathway to generate ATP (Adenosine Triphosphate) (Calum et al. 2014; Dahiya et al. 2017; Sharma and Tripathi 2019).

1.5.2 Role of Short-Chain Fatty Acids (SCFAs)

As discussed earlier, SCFAs such as acetate, propionate, and butyrate are the end products of the microbial fermentation process. These are absorbed into the body by partial diffusion. Almost 10% of the energy required by the host colonic epithelial cells and nearly 70% of the energy required for cellular respiration is obtained from SCFAs. However, there is a controversy in the concept of production of SCFAs from the indigestible food depending on various factors like availability of substrate, mucosal absorption, transit time of food, and also the interactions between different gut microbes (Calum et al. 2014; Kasubuchi et al. 2015; and Brusaferro et al. 2018). SCFAs function as ligands for G protein coupled receptors such as Free Fatty Acid Receptor 2 (FFAR2) and Free Fatty Acid Receptor 3 (FFAR3), Olfactory receptor 78 (OLFR78) and G protein Receptor 109 A (GPR109A). FFAR3 is triggered by the presence of propionate, followed by butyrate and acetate and similarly, FFAR2 is triggered by all the three SCFAs at same rate. These receptors are present in the different peripheral tissues and thus it indicates the role of SCFAs in regulating food intake and satiety by modulating the intestinal enteroendocrine L cells derived peptides PYY and GLP1 (Kobyliak et al. 2016; Kasubuchi et al. 2015). As we mentioned earlier, the PYY role is to reduce the appetite by acting on neuropeptide Y (NPY), and thus inhibiting the gastric movements and food intake. Similarly, GLP1 also regulates the appetite and inhibits gastric emptying and secretion of insulin (Kasubuchi et al. 2015). In summary, the SCFAs produced from the fermentation of polysaccharides by the gut microbiota have direct influence on L cells, which in turn results in the rise of GLP1 and PPY in plasma (Nøhr et al. 2013). This is supported by several experimental studies where FFAR2 and FFAR3 gene knockout mice had reduced levels of GLP1 and found to be impaired with glucose tolerance in vivo and in vitro (Tolhurst et al. 2012). In another related study, the mice without FFAR2 gene became obese even with a normal diet, while the mice over expressing the FFAR2 gene in the adipose tissue remained lean. In addition to this, FFAR2 also helps in suppressing insulin mediated fat accumulation that in turn regulates the energy balance by inhibiting the deposition of excess energy and including fat consumption (Kimura et al. 2013).

1.5.3 Fatty Acid Oxidation

Adenosine monophosphate kinase (AMPK), an important enzyme that helps in maintaining the cellular energy homeostasis, is expressed in the liver and muscles. Activation of AMPK enzyme triggers the activation of carnitine palmitoyltransferase-1 (Cpt-1) through acyl-Coa carboxylase (Acc). This enhances fatty acid oxidation of the mitochondria and inhibits the anabolic pathways like glycogen storage and improved insulin sensitivity (Angin et al. 2016). The gut microbiota inhibits the AMPK enzyme where this negatively influences the fatty acid oxidation in the target organs. Eventually, it promotes the synthesis of cholesterol and triglycerides thus favoring lipogenesis. This in turn leads to excess fat storage and ultimately to obesity (Boulangé et al. 2016). This was supported by a study where the GF mice on a Western type diet had higher levels of AMPK, ACC, and CPT 1 in the liver and muscles than the conventionally raised mice. This explains that gut microbiota has a suppressive effect on the AMPK activity which in turn affects fatty acid oxidation and thus leads to obesity (Bäckhed et al. 2007).

1.6 Role of Gut Microbiota in Obesity Control

Latest advances in different molecular techniques and animal model studies have established the role of gut microbiota in regulation of hunger and satiety via the gut–brain axis. The gut microbiota is an integral part of human health and plays an important role in the diseases related to GI. Recent rapid expansion in the disease states has variously been linked with changes in the gut microbiota (Calum et al. 2014). Hence it is reasonable to assume that modulation of gut microbiota can be used as a therapeutic approach to treat GI diseases.

Obesity, a complex metabolic syndrome developed from prolonged imbalance of energy intake and expenditure which is marked by accumulation of body mass in the abdominal region. It is a major metabolic health disorder associated with increased morbidity (the condition of being diseased) and mortality (the state of being subject to death) as well as aggravating personal, social, and economic consequences. It is often said that obesity is the mother of many other diseases like diabetes, cardiovascular, nonalcoholic fatty liver disease, and some types of cancers (Kobyliak et al. 2016; Dahiya et al. 2017; Brusaferro et al. 2018). It affects the well-being of a person and also a lot of burden on the society. About 500–650 million people around the globe are living with the stigma of obesity which shows the severity of this disease (Kobyliak et al. 2016; Dahiya et al. 2017; Brusaferro et al. 2018).

Several factors like lifestyle, diet and exercise, host genetics, metabolisms are considered to be the major factors leading to obesity. However, in-depth mechanisms that lead to the development of obesity have to be understood. Enormous research efforts have been made in the last few years that aim to develop successful weight loss therapies. Hence, knowledge in neuroendocrine biology of eating, body weight regulation, and mainly the role of gut hormones has been significantly augmented. However, these insights did not lead us to develop any

new therapies. At present orlistat is the only available drug for long term treatment of obesity (Hussain and Bloom 2013). However, it causes a number of gastrointestinal side effects and also its cost makes this drug an impractical solution. Bariatric surgical procedures such as gastric banding, biliopancreatic diversion, and Roux-en-Y Gastric Bypass (RYGB) are found to be more effective in the treatment of obesity. But these options are also limited by their cost, operative risks, and limited availability of the procedure which makes them an impractical solution as well (Hussain and Bloom 2013).

These issues highlight the need for the development of new drugs that could be used as potential obesity control solutions. It is a bigger challenge for the researchers to develop a safe and effective drug for obesity and application of gut microbiota-based probiotics as a potential healthcare solution, which could be applied to address this problem.

1.6.1 Role of Gut Microbiota: Animal Model Studies

Recent studies have suggested that the gut microbiota plays an important role in the onset and establishment of obesity (Tables 1.2 and 1.3). The gut microbes adhered to the gut affect the nutrient acquisition and energy homeostasis in the host by influencing the effector molecules which decides the fat storage in the adipocytes. Hence, the changes in the microbial count in the gut might be a possible cause for obesity.

Various animal model studies have revealed the role of microbes in obesity (Table 1.2). The first evidence for the role of gut microbiota in the role of energy homeostasis and adiposity came from group experiments performed by Bäckhed et al. (2004). They observed that there is 40% less total body fat in the GF mice than the conventionally raised mice even if their caloric take was 29% more. In a similar kind of experiment by Bäckhed et al. (2007), GF mice significantly gained weight and fat than the conventional ones after feeding both of them with a high fat, high carbohydrate western diet for 8 weeks. The identification of key populations/taxa that may be associated with weight gain has also been the subject. In one such study, *Firmicutes* were found to be more abundant in obese mice and those fed on the western diets were found to have a lesser number of *Bacteroidetes*. Class Mollicutes belonging to phylum *Firmicutes* were the most common in the obese mice. However, the genes for enzymes involved in lipid and carbohydrate metabolism were found to be more in the *Bacteroidete*s than the *Firmicutes* (Turnbaugh et al. 2009). *Bacteroides thetaiotaomicron* from the *Bacteroidetes* phylum was found to improve nutrient absorption and processing in the host. However, a varied *Firmicutes/Bacteroidetes* ratio has been reported in humans. In few studies, the *Firmicutes/Bacteroidetes* ratio is found to be high while in some other studies it was found to be reversed. In some other studies, no correlation between the BMI and the *Firmicutes/Bacteroidetes* ratio was observed (Mai et al. 2009). In one of the studies, sequencing of the gut microbiota of lean mice and obese mice revealed the differences in the ratio of *Bacteroidetes* and *Firmicutes.* A 50% reduction of *Bacteroidetes* and

Table 1.2 Examples of animal model studies to study the impact of various probiotic strains on obesity

Sl. no	Strain used as Probiotic	Model	Effects	References
1	*Bifidobacterium pseudocatenulatum* SPM 1204kk, *Bifidobacterium longum* SPM 1205 and *Bifidobacterium longum* SPM 1207	Sprague-Dawley rats	Reduction of body weight gain and fat weight, TC, HDL-C, LDL-C, triglyceride, glucose, leptin, AST and ALT levels. Increment of fecal LAB counts.	An et al. (2011)
2	*Pediococcus pentosaceus* LP28	C57BL/6Jcl (SPF)	Reduction of visceral fat amount, hepatic triglycerides, and cholesterol contents. Downregulation of hepatic gene expressions (CD36, SCD1, and PPARc).	Zhao et al. (2012)
3	*Bacteroides uniformis* CECT 7771	C57BL-6 mice	Reduction of body weight gain, liver cholesterol, and triglyceride concentrations. Reduction of serum cholesterol, triglyceride, glucose, insulin and leptin levels.	Gauffin et al. (2012)
4	*Lactobacillus salivarius* UCC118 Bac+	C57BL/J6 mice	Increment of Bacteroidetes and Proteobacteria. Reduction in Actinobacteria. Reduction in weight gain. Reduction in blood glucose and TG levels.	Murphy et al. (2013)
5	*Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032	C57BL/6 J	Decrease in Proteobacteria. Reduction in body weight gain and fat accumulation. Reduction of plasma insulin, leptin, and total cholesterol. Downregulation of pro inflammatory genes (TNFα, IL6, IL1B, and MCP1) Downregulation of fatty oxidation related genes (PGC1a, CPT1, CPT2, and ACOX1)	Park et al. (2013)
6	De Simone Formulation	C57J/B67 HFD and ob/ob mice	Increase in Bacteroidetes and bifidobacteria. Decrease in Firmicutes. Suppression of body weight and insulin resistance. Reduction of food intake. Improvement of glucose tolerance. Increment of SCAFs (butyrate) levels - change on gut flora.	Yadav et al. (2013)
7	*Lactobacillus plantarum* KY1032 and *Lactobacillus curvatus* HY7601	C57BL/6J mice	Reduction in fat accumulation in liver and adipose tissue. Reduction of the expression of genes involved in fatty acid transport (Ppara) and fatty acid b-oxidation (NR1H3). Reduction of lowered cholesterol in plasma and liver.	Yoo et al. (2013)

(continued)

Table 1.2 (continued)

Sl. no	Strain used as Probiotic	Model	Effects	References
8	*Lactobacillus coryniformis* CECT5711	C57BL/6J mice	Reduction in LPS in the plasma and metabolic endotoxemia. Reduction of TNFα expression in the liver.	Toral et al. (2014)
9	*Saccharomyces boulardii* Biocodex	db/db mice	Affects the gut microbial community at different taxonomic levels. Reduction in body weight gain, fat mass, hepatic and systemic inflammation.	Everard et al. (2014)
10	*Lactobacillus casei* NCDC 19	C57BL/6J mice	Increment of Bifidobacteria population. Reduction of body weight gain, epididymal fat weight, blood glucose, plasma lipids and expression levels of leptin.	Rather et al. (2014)
11	*Lactobacillus paracasei* CNCM I-4270, *Lactobacillus rhamnosus* I-3690 *Bifidobacterium animalis* subsp. lactis I-2494	C57BL/6J	Improvement of glucose—Insulin homeostasis and reduced hepatic steatosis. Reduction of weight gain, macrophage infiltration.	Wang et al. (2015)
12	*Lactobacillus brevis* OK56	C57BL/6J mice	Reduction in Bifidobacteria count. Inhibition of NF-κB activation and TNFα, IL-1β, and IL-6 expression in LPS-stimulated macrophages. Suppression of body weight and epididymal fat weight gain.	Kim et al. (2015)
13	*Lactobacillus salivarius* Ls33; *Lactobacillus rhamnosus* LMG S-28148 & *Bifidobacterium animalis* subsp. lactis LMG P-28149	C57BL/6J	Increment of Bacteroidetes. Reduction in the fasting glucose and insulin levels. Reduction of total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels.	Alard et al. (2016)
14	*Lactobacillus plantarum* HAC01	C57BL/6J	Increment of Lachnospiraceae family (phylum Firmicutes). Decrement of Deferribacteres population. Reduction in body weight gain and mesenteric fat mass. Changes in gene expression associated with lipid metabolism. Reduction of serum glucose and triglycerides levels.	Park et al. (2017)

Table 1.3 Examples of human clinical trials to study the impact of various probiotic strains on obesity

Sl. no	Probiotic used	Duration	Study type	Effects	References
1	*Lactobacillus gasseri* SBT2055	12 weeks	Multicenter, double-blind, randomized, placebo-controlled intervention trial.	Decrease in abdominal visceral and subcutaneous fat areas. Decrease in body weight, BMI, and other body measures (hip, waist).	Kadooka et al. (2010)
2	*Lactobacillus rhamnosus*	8 weeks	Double-blind, placebo-controlled pilot study.	Decrease in alanine aminotransferase and antipeptidoglycan-polysaccharide antibodies irrespective of changes in BMI z score and visceral fat. TNFα remains unchanged.	Vajro et al. (2011)
3	Microbiota from lean individuals to male recipients with metabolic syndrome or autologous microbiota	6 weeks	Randomized, double-blind, parallel, placebo-controlled trial.	Increase in insulin sensitivity, and butyrate producing bacteria.	Vrieze et al. (2012)
4	*Lactobacillus salivarius* Ls-33	12 weeks	Double-blinded intervention.	Increase in the ratio of Bacteroides–Prevotella–Porphyromonas group to Firmicutes with Clostridium cluster I, Clostridium cluster IV, *Faecalibacterium prausnitzii,* Enterobacteriaceae, and Enterococcus. No change in the production of SCFAs.	Larsen et al. (2013)
5	*S. thermophilus* (KCTC 11870BP), *L. plantarum* (KCTC 10782BP), *L. acidophilus* (KCTC 11906BP), *L. rhamnosus* (KCTC 12202BP), *B. lactis* (KCTC 11904BP), *B. longum* (KCTC 12200BP), and *B. breve* (KCTC 12201BP).	8 weeks	A randomized, double-blind, placebo-controlled study.	Increase in *Bifidobacterium breve, B. lactis, Lactobacillus rhamnosus, and L. plantarum.* Decrease in Firmicutes/Bacteroidetes ratio Decrease in the weight and waist circumference.	Lee et al. (2014)

proportional increase of *Firmicutes* was observed in the obese mice when compared with lean mice (Ley et al. 2005). Hence, the ratio of *Firmicutes* to *Bacteroidetes* in case of obesity is still not clear.

Several studies investigated the association between specific bacterial species and obesity in humans. Turnbaugh et al. (2006) found a higher proportion of *Actinobacteria* in the obese individuals. They also demonstrated more environmental gene tags of Archaeans such as methanogenic organisms that increase the bacterial fermentation efficacy, in the caecal microbiome of obese mice than in that of lean mice. *Methanobrevibacter smithii* is a methanogenic archaeon found in the human gut (Turnbaugh et al. 2006). In one such study, the mice are colonized with the archaeon *Methanobrevibacter smithii* and other organism *B. thetaiotaomicron*. The results showed that the co-colonization of these microbes can increase the polysaccharide fermentation efficiency that leads to increase in adiposity when compared to mice colonized with only one of the organisms (Samuel and Gordon 2006). *Lactobacillus* species (from the phylum *Firmicutes*) was found significantly higher in obese patients than in lean individuals. Higher levels of *L. refuter* and lower levels of *L. casei/paracasei* and *L. plantarum* have been associated with obesity (Million et al. 2012). *Bifidobacterium* from the phylum *Actinobacteria* was also found to be closely associated with obesity in humans (Mai et al. 2009; Million et al. 2012). Also, there number was found to be higher in infants with age 4–6 weeks and in the intestinal microbiota of breast-fed infants as compared to those of formula-fed infants (Stark and Lee 1982; Kallus and Brandt 2012). Similarly, in a study that includes 28 mixed participants of whom 15 were obese and 13 non-obese, levels of *Faecalibacterium prausnitzii* from the phylum *Firmicutes* were significantly higher in the obese children than the other participants (Balamurugan et al. 2010). In a study, where 39 overweight and obese adolescents were given a calorie-restricted diet and increased physical activity, it was found that the proportions of *Bacteroides–Prevotella* were increased after weight loss in obese adolescents. In the same study it was observed that there exists a correlation between reductions in *Clostridium histolyticum* and *Eubacterium rectale–Clostridium coccoides* proportions and weight loss (Nadal et al. 2012). In another study by Zhang et al. (2009), *Methanobacteriales* were found only in obese individuals as compared to normal-weight or post-gastric-bypass participants (Zhang et al. 2009). All these studies clearly indicate that gut microbiota plays a crucial role in the etiology of obesity and offer an opportunity to prevent or treat obesity by therapeutic modulation.

SCFAs (acetate, butyrate, and propionate), the end products of microbial fermentation, produced by gut microbes which have direct influence on L cells. These molecules result in the rise of GLP1 and PPY in plasma. GLP1 regulates appetite, inhibits gastric emptying, and stimulates insulin secretion and PYY reduces appetite and thus inhibits gastric motility and reduces food intake which results in maintaining the energy homeostasis through the receptors FFAR2 and FFAR3. In this way, presence or absence of SCFAs affects obesity indirectly (Dahiya et al. 2017: Karra et al. 2009; Steinert et al. 2016). Similarly, gut microbiota influences inhibition of AMPK activity, thus affecting fatty acid oxidation and making the host

susceptible to obesity. Gut microbiota also influences fasting induced adipose factor produced by tissue liver, skeletal muscle, and intestine in response to fasting (Bäckhed et al. 2004; Dutton and Trayhurn 2008). The main role of this factor is to inhibit the activity of lipoprotein lipase (LPL) which in turn restricts TG accumulation in the adipocytes. The gut microbiota suppresses the expression of FIAF thereby increasing the LPL activity and fat storage in the adipocytes causing obesity (Wang and Eckel 2009). The primary bile acids (Cholic acid and Chenodeoxycholic acid) are synthesized in the liver from cholesterol and are converted to bile salts after conjugation with either taurine or glycine, prior to release in bile. They are converted into secondary bile acids by the action of gut microbiota. Through, a transporter called ileal bile acid transporter (IBAT), these are passively transported into the upper small intestine and colon then to liver via blood circulation where they recalculate and inhibit the bile acid synthesis which is known as enterohepatic circulation. The gut microbiota inhibits bile acid synthesis in the liver by elevating the levels of Foresaid X factor (FXF) in the ileum. Foresaid X receptor (FXR) plays a major role in the feedback inhibition, that negatively regulates two genes Cholesterol 7-alpha hydroxylase (CYP7A1 - Cytochrome P450 Family 7 Subfamily A Member 1) and Cytochrome P450 family 27 subfamily A member 1 (CYP27A1) that are required for classic and alternate bile acid synthesis pathways, respectively, with the help of fibroblast growth factor 15 (FGF15) (Chiang 2009; Dahiya et al. 2017). Another mechanism that bile acids activate receptors like G protein coupled bile acid receptor1 (GPBAR1) which in turn helps in glucose homeostasis by activating the secretion of GLP1 (Jasirwan et al. 2019). Thus, the gut microbiota modulates the bile acid metabolism through FXR/GPBAR1 regulation and indirectly contributes towards obesity (Chiang 2009; Dahiya et al. 2017; Jasirwan et al. 2019).

1.6.2 Role of Gut Microbiota: Human Clinical Trials

The avoidance and managing the onset of obesity is proposed to begin in the babyhood itself when a variety of environmental factors put forth a long-term effect on the risk for obesity in the later stages of life (Kobyliak et al. 2016). The majority of the human studies relating to the impact of probiotics on body weight were limited to the analysis of biochemical (inflammatory markers) and physical parameters (Table 1.3). In a study by Kadooka et al. (2010), fermented milk containing *L. gasseri* SBT2055 was given for 12 weeks in a double-blind, randomized, placebo-controlled trial. Considerable reduction of abdominal visceral and subcutaneous fat areas were observed along with decrease in body weight, BMI, and other body measures (hip and waist) (Kadooka et al. 2010). Vajro et al. (2011) evaluated the effects of *L. rhamnosus* strain GG for 8 weeks in children with obesity-related NAFLD. They reported a decrease in alanine aminotransferase and antipeptidoglycan-polysaccharide antibodies irrespective of changes in Body Mass Index (BMI) z score and visceral fat. On the other hand, TNFα remains unchanged. Randomized, double-blind, Parallel, placebo-controlled trial conducted by Vrieze et al. (2012), increase in insulin sensitivity, and butyrate producing bacteria was seen

when the microbiota was infused from lean individuals to male recipients with metabolic syndrome (Vrieze et al. 2012). In a double-blinded intervention study performed by Larsen et al. 2013, 50 individuals were given *L. salivarius* Ls-33 or placebo for a period of 12 weeks. Afterwards the fecal microbiota was assessed by real-time quantitative PCR and compared for samples taken before and after intervention. They found that there was an increase in the ratio of *Bacteroides–Prevotella–Porphyromonas* group to *Firmicutes* with *Clostridium* cluster I, *Clostridium* cluster IV, *Faecalibacterium prausnitzii*, *Enterobacteriaceae*, and *Enterococcus*. The population of *Lactobacillus* sp. and *Bifidobacterium* sp. also changed remarkably post feeding. Yet there was no change in the production of SCFAs. Based on these results, the authors concluded that fecal microbiota was modulated by the probiotic administration (Larsen et al. 2013). In one such study, the combined effect of probiotic capsules having *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus, Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium* breve, and *Streptococcus thermophilus* in combination with herbal medicine was studied in the obese patients (Lee et al. 2014). They found a major decline in body weight and waist circumference. However, no remarkable differences in body composition and metabolic biomarkers were observed. It was finally concluded that correlations between gut microbiota and change in body composition indicate that probiotics may influence energy metabolism in obesity. The correlation between endotoxin level and weight reduction indicates that probiotics may play an important role in prevention of endotoxin production, which leads to microbiota dysbiosis associated with obesity (Lee et al. 2014).

Considering all the available data, manipulation of the gut microbiota through probiotics-host metabolism has gained considerable interest nowadays. However, none of the reported clinical studies could clearly state that these alterations are solely responsible for reduction in body weight or obesity. The comparative effect of different strains has to be studied and even the probiotic effects may vary amongst individuals. Further research is needed for understanding the gaps in the research and their advantageous effects in the management of obesity in humans.

1.6.3 Metabolic Endotoxemia

Lipopolysaccharides (LPS) derived from the cell wall of Gram-negative bacteria circulate in the blood stream of healthy individuals. A substantial two- to –threefold increase in the LPS concentration, a condition called metabolic endotoxemia, is caused because of consumption of a high fat diet. This alters the composition of gut microbiota like *Bacteroides, Eubacterium rectale, Clostridium coccoides group,* and *Bifidobacterium* species in humans. Endotoxemia is thought to contribute to low grade inflammation and insulin resistance. In a study, antibiotic treatment in high fat fed mice and obese mice reduces the levels of LPS in the blood and feces, thus indicating that the gut microbiota contributes to obesity by inducing chronic inflammation (Brusaferro et al. 2018; Dahiya et al. 2017; Cani et al. 2007).

1.6.4 Gut Microbiota Manipulation and their Effects

Since it is well known that the gut microbiota modulates the host energy homeostasis and adiposity through different mechanisms, it is quite possible that positive modulation of the gut microbiota by external sources may provide a beneficial effect on the host. Among the different intervention options like diet, antibiotics, and surgery, dietary strategy is much preferred by medical practitioners because of its lesser cost and safety issues (Dahiya et al. 2017). Nowadays, probiotics (live bacteria given in oral quantities that can colonize the gut) and prebiotics are being projected as promising because of their direct influence on the gut microbiota (Sanders 2008; Gibson et al. 2004).

Prebiotics: Fructo-oligosaccharides, galacto-oligosaccharides, lactulose and non-digestible carbohydrates inulin, cellulose, hemicelluloses, resistant starch, gums, and pectins are the most commonly used prebiotics. In a study, it was found that inulin type of prebiotics promoted the growth of *Firmicutes* and *Actinobacteria* and inhibited *Bacteroidetes* (Dahiya et al. 2017; Gibson et al. 2004). Prebiotics can be classified into 2 types: (i) Inulin type dietary fructans (ITF) and (ii) Galacto-oligosaccharides based on their chemical structure (Lin et al. 2014). In one of those studies in the obese women, the ingestion of ITF led to increase in the population of *Bifidobacterium* species and decrease in the production of SCFAs, which eventually reduced the host metabolic parameters associated with obesity (Dahiya et al. 2017).

Probiotics: Probiotics is an alternative dietary approach which is used to alter the gut microbiota. Members of lactic acid bacteria, namely *Lactobacillus* and *Bifidobacterium* sp. are the two extensively studied probiotics that have provided anti-obesity effects in animal models and also in human beings. The beneficial effects of probiotics are related to their capacity to produce vitamins, antioxidants, and defenses against the pathogenic competitors. They are characterized by the production of SCFAs and absence of toxins. Tables 1.2 and 1.3 shows the results of animal model studies and human trials to understand the effect of probiotics on obesity.

There is growing evidence that dietary substances, especially probiotics and prebiotics can help in the alteration of host gut microbiota in a positive way and are therefore considered as important assets in the management of obesity (Lin et al. 2014; Musso et al. 2010; Million et al. 2013; Dahiya et al. 2017; Calum et al. 2014; Helena et al. 2014; Hussain and Bloom 2013).

1.7 Conclusion

The gut–brain axis is a complex communication network, which is meant for maintaining metabolic homeostasis in the body. In addition to the gut microbiota, various components of gut–brain axis include the CNS, enteric nervous system (ENS), the autonomic nervous system (ANS) and its associated sympathetic and parasympathetic branches, neuroendocrine and immunological systems (Grenham

et al. 2011). Gut–brain axis communication is shaped through sensory data being translated into neural, hormonal, and immunological signals, which are handed-off to and fro from the CNS to the gut (Mayer et al. 2015).

The gut–brain axis has a fundamental role in coordinating the digestive process within the gastrointestinal system and thus regulating feeding behavior and energy balance. Imbalance in this coordination leads to conditions like obesity, which is one of the major public health problems due to its high prevalence and comorbidities. It has variously been suggested that the gut microbiota may influence adiposity and weight gain through several interdependent pathways, including energy harvest and subsequent generation of metabolites such as short-chain fatty-acids (SCFA), modification of host behavior, and inducing satiety through the gut–brain axis (Moran and Shanahan 2014). Surgical intervention achieved through gastric bypass surgery is currently the most effective and sustained treatment option for obesity (Grima and Dixon 2013). However, in the recent past, manipulation of the gut microbiota has been proposed to be a potential novel therapeutic strategy that could be applied for combating obesity and its comorbidities (Bauer et al. 2016). Although prebiotics and probiotics have provided promising insights regarding the role of microbiota within the gut–brain axis and the obesogenic state (Moran and Shanahan 2014), currently, the understanding of the complex interactions associated with the gut–brain–microbiota axis and obesity is in their infancy and need to be developed more with new insights. More studies are required to elucidate the "ideal" microbiota phenotype with respect to the "healthy" state. This will help to develop a probiotic based anti-obesogenic treatment. With all its limitations, we conclude that the gut–brain axis and associated microbiota provide a promising solution space as preventive healthcare solution as well as future treatments strategy for obesity and related problems.

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Anti-Obesity Activities of Probiotics and Dairy Based Ingredients

2

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Abstract

Obesity is a condition of medical in which excess body fat has accumulated to the extent that it may have a negative impact on human health. Obesity is a serious global epidemic and poses a significant health threat to humans. Probiotics are live microorganisms that are beneficial for host health when administered in appropriate amounts. The use of probiotic and prebiotics are efficient ways to improve the efficacy of gut microbiota and treating obesity. The effect of probiotics on body weight is dependent on the particular species and strains. Different food ingredients play significant role in better management of obesity. A variety of natural milk ingredients, e.g., calcium, protein and functional fatty acids, and other natural dietary compounds have been used in different anti-obesity products. Many studies have shown the beneficial effects of consuming dairy products on metabolic risk factors in overweight and obese individuals.

Keywords

Short-chain fatty acids · Polyunsaturated fatty acids · Symbiotic · Phytochemicals · Non-alcoholic fatty liver disease

2.1 Introduction

The word "obesity" comes from the Latin "obesitas," which means stout, fat, or plump. Medically, blubber may be a condition during which excess body fat has accumulated to the extent that its going to have associate adverse result on health, resulting in reduced anticipation and/or increased health problems. Obesity may be a

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medical condition during which excess body fat has accumulated to the extent that its going to have a negative result on health (WHO 2015).

Obesity and overweight primarily happen either due to excess calorie intake or insufficient physical activity or both. Furthermore, various genetic, behavioral, and environmental factors play important role in obesity (James 2008). At individual level, the combination of higher food energy intake and lack of physical activity is thought to explain most of obesity causes. Nowadays societies become more and more dependent on big-portions, energy-dense, and fast-food meals. The association between fast-food consumption and obesity becomes more concerning (Rosenheck 2008). Obesity increases the likelihood of various diseases and conditions, particularly type 2 diabetes, certain types of cancer, cardiovascular diseases, obstructive sleep apnea, depression, and osteoarthritis (Luppino et al. 2010). Lactic acid bacteria produce essential vitamins like riboflavin, folate, cobalamin, and short chain fatty acid (SCFA) which have health impacts (anti-obesity, anti-diabetics, anti-microbial, and other chronic diseases prevention) to the host (Hati et al. 2019).

The use of probiotics (i.e., *Lactobacillus* and *Bifidobacterium*) and prebiotics are efficient ways to improve the efficacy of gut microbiota intervention by selectively increasing the amount of microbes that promote human health. The appropriate application of prebiotics and probiotics is an essential and efficient way to improve the efficacy of gut microbiota in treating the obesity. In the research studies have shown the beneficial health effects of eating dairy products on metabolic risk factors in obese individuals and overweight (Martinez-Gonzalez et al. 2012). It has been thought that the slenderizing effects of yogurt are due to a probiotic bacteria-mediated anti-obesity mechanism. Among the various studied potential determinants of obesity, the intestinal microbiota has been proposed to have an impact on the energy balance in people (Forslund et al. 2015). Specific bacterial populations, such as *Prevotellaceae*, *Lactobacillus*, *Bifidobacterium*, and *Blautia coccoides* group have been reported to be related to anti-obesity. Consequently, it is accepted that modulation of the intestinal microbiota toward a healthier "non-obese" profile might present a promising tool for prevention (Sanchez et al. 2014). Yoo and Kim (Yoo and Kim 2016) have indicated that probiotics and prebiotics affect cardiovascular diseases and type 2 diabetes (T2D) by changing gut microbiota, lowering cholesterol, and regulating insulin signaling.

Different food ingredients play significant role in better management of obesity. A variety of natural milk ingredients, e.g., calcium, protein, and functional fatty acids (e.g., polyunsaturated fatty acids and conjugated fatty acids), and other natural dietary compounds have been used in different anti-obesity products. Recent evidence has highlighted the role that dietary protein may play in weight control (Larsen et al. 2010). Natural supplements products, i.e. dietary fiber, polyphenols, etc., which primarily helping consumers to fight the battle against obese people have been widely explored.

2.2 Status of Obesity

Based on data analysis of body mass index (BMI), the World Health Organization (WHO) described obesity as an epidemic hazard in the world in 1997. According to worldwide 11% of men and 15% of women were obese where about 13% of the world's adult population were obese (WHO 2015). In India, 13 to 50% of the urban population and 8 to 38.2% of the rural population suffer from obesity (Behla and Misraa 2017). In 2008 the WHO estimates that at least 500 million adults (greater than 10%) are obese, with higher rates among women than men. The percentage of adults affected in the USA as in 2015–2016 is about 39.6% (37.9% of males and 41.1% of females) (Hales et al. 2017). The rate of obesity also increases with age at least up to 50 or 60 years old and severe obesity in the USA, Australia, and Canada is increasing faster than the overall rate of obesity (Howard et al. 2008). Once considered a problem only of high-income countries, obesity rates are rising worldwide and affecting both the developed and developing world. These increases have been felt most dramatically in urban settings (Tsigos et al. 2008). India is a fast growing economy, currently undergoing higher epidemiological, nutritional, and demographic transitions. These transitions tend to promote obesity in all age groups. Obesity is more commonly seen in women compared to men and is increasing in children and adolescents (Behla and Misraa 2017). Urban India's greatest comforts are the cause of a supersize health problem: Obesity. Easy access to sedentary lifestyles, high calorie packaged foods, and a predilection for gizmos have resulted in almost 70% Indians in mega cities such as Mumbai, Delhi, Bangalore, and Chennai being overweight and obese says a new multi-city survey. In Gujarat, 23.7% (BMI $\geq$25.0 kg/m^2) women are overweight or obese (excludes pregnant women and women with a birth in the preceding 2 months) and about 19.7% men are overweight (National Family Health Survey-4 2015–2016).

2.3 Classification

Obesity is a one type of medical condition in which higher body fat has accumulated to the extent that it may have an adverse effect on health. It is known as BMI and further evaluated in terms of fat distribution via total cardiovascular risk factors and the waist–hip ratio. BMI is closely related to both percentage total body fat and body fat (WHO 2015). Table 2.1 gives the classification of obesity based on BMI.

BMI = m/h^2 where m and h are subject's weight and height, respectively.

2.4 Factors Associated with Obesity

Excessive food energy intake, lack of physical activity (Lau et al. 2006), medical reasons (Bray 2004), genetics, or psychiatric illness (Bleich et al. 2008) can all be one or the other reasons for initiating obesity.

Table 2.1 Classification of obesity according to BMI

BMI (kg/m^2)		
From	Up to	Classification
<18.5		Under weight
18.5	25.0	Normal weight
25.0	30.0	Overweight
30.0	35.0	Class I obesity
35.0	40.0	Class II obesity
>40.0		Class III obesity

2.5 Disadvantages of Obesity

Obesity is the mother of many deadly diseases, particularly cardiovascular, diabetes, non-alcoholic fatty liver disease (NAFLD), and some form of cancers (Vucenik and Stains 2012). Obesity facilitates the development of metabolic disorders such as hypertension and chronic diseases such as osteoarthritis, sleep apnea, stroke, some cancers, and inflammation-based pathologies. In mechanical effects on the body because of the extra weight placed on the skeleton, obesity is associated with a maximum incidence of several pathologies. The risk of overweight and obesity is maximum in patients with psychiatric disorders than in persons without psychiatric disorders (Chiles and van Wattum 2010).

2.6 Probiotics and Obesity

The term probiotic means "for life" (pro: for and bios: life). Fuller defined that a probiotic as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2002). Probiotics are live microorganisms that are beneficial for host health when are administered in appropriate amounts. The effect of probiotics on body weight is dependent on the particular species and strains. *Lactobacillus* and *Bifidobacterium* can alter the abundance of most beneficial gut bacteria and differentially attenuate obesity of high fat diet-fed mice (Sanchez et al. 2017). Prebiotics are non-digestible but fermentable ingredients. They are widely used to alter the composition and/or the function of gut microbiota. Prebiotics can reduce the numbers of pathogens, strengthen the gut barrier, and stimulate host immune response. However, high-fat diets lacking prebiotics can increase the amount of LPS containing microbiota and subsequently cause the inflammatory reaction that is accompanied by obesity (Sanchez et al. 2017).

Gut microbiota is said to play an integral role in the development of obesity (Rouxinol-Dias et al. 2016). Research evidence so far demonstrates that the gut microbiota affects nutrient absorption and energy regulation and that its composition differs in an obese person when compared to a lean one. Hence modifying the gut microbiota through a diet rich in probiotics can become an important treatment

option for obesity (Dror et al. 2017). The proposed mechanism of action of probiotics against obesity includes alteration in the gut microbial community, production of bioactive compounds by probiotic strains, reduction in fat storage, alterations in serum lipid profiles, induction in fatty acid oxidation genes, reduced expression of pro-inflammatory cytokines, and stimulating the production of satiety-inducing peptides (Dahiya and Puniya 2017).

Bindels et al. (Bindels et al. 2015) reported that prebiotics can improve the efficacy of gut microbiota intervention for obesity treatment by the following mechanisms: amelioration of metabolic disorders through the improvement of gut microbiota, selectively increasing the levels of probiotics, such as *Bifidobacterium* in guts and altered production of SCFAs by gut microbiota. Butyrate, one of the SCFA, is confirmed to have the ability to minimum the incidence of obesity. However, the benefits of prebiotics are significantly influenced by the original gut microbiota. Studies using *Bifidobacterium longum* AH1206 revealed that long-term bacterial colonization depended on factors such as the starting abundance of resident *B. longum* and the absence of specific carbohydrate utilization genes. Clinical studies performed on rats indicated that the composition of resident gut microbiota was associated with the persistence of *Lactococcus lactis* CNCM I-1631 from a fermented milk product in gut, indicating that the introduced bacteria had subject-specific effects on host gut microbiota (Sanchez et al. 2014).

The effects of *Lactobacillus* species on weight control were also analyzed by Yoo et al. (Yoo et al. 2013) in a study in which C57BL/ 6J mice fed a high fat/cholesterol diet were provided with either *L. plantarum* KY1032 (PL) (1×10^{10} CFU/day), *L. curvatus* HY7601 (CU) (1×10^{10} CFU/day), or a combination of the two probiotics for a period of 9 weeks. Mice were divided into five groups and fed a normal diet (ND), high fat high cholesterol diet (HFCD), HFCD and probiotic PL alone, HFCD and probiotic CU alone, or HFCD and probiotic PL + CU combination. Final body, liver, and adipose tissue weights were significantly increased in HFCD-fed mice. However, probiotic supplementation with CU alone or the PL + CU combination effectively suppressed a gain in final body weight and reduced liver and adipose tissue weights. Furthermore, adipocyte size was significantly decreased by all probiotic supplementation groups compared to HFCD group. Plasma cholesterol, hepatic cholesterol storage, hepatic FFAs, and triacylglycerols were all significantly increased in HFCD-fed mice, but significantly decreased by probiotic supplementation with CU or PL + CU (Yoo et al. 2013).

Effects of probiotics are strain dependent, and hence not all strains are considered to be effective in treating or preventing obesity. Arora et al. (Arora et al. 2013) showed that *L. acidophilus* NCDC13 did not have an effect on cumulative body weight gain in C57BL/6 mice. Most of the data available originates from experiments performed in rodent models. However, Sanchez et al. (Sanchez et al. 2014) investigated the impact of *L. rhamnosus* CGMCC1.3724 (LPR) supplementation on weight loss and maintenance in obese men and women over a 24 week period. In phase 1 (weight loss period), supervised dietary restriction with or without probiotic LPR supplementation was followed over 12 weeks. Phase 2 was an amount of weight maintenance with superintendence of dietary habits while not restriction

over 12 weeks throughout that LPR or placebo supplementation was continuing. Women within the LPR cluster continuing to lose weight and fat mass throughout the weight-maintenance amount. In addition to its effect on weight and fat mass loss, LPR reduced circulating leptin concentrations by about 25% at the end of the weight-maintenance phase. There were no effects observed in man. Considering these data, Sanchez et al. (2014) demonstrated that LPR supplementation can accentuate body weight loss in women submitted to energy restriction. Thus, LPR supplementation seems to help obese women to maintain a healthy body weight.

The effect of *L. gasseri* SBT2055 was examined in 2 studies employing a cohort of Japanese adults with massive visceral fat areas (VFA). The participants were every which way appointed to a few teams receiving increasing colony-forming units (CFUs) of *L. gasseri* SBT2055 for 12 weeks. The results showed a reduction in body mass index (BMI), waist, abdominal VFA, and hip circumferences (Kadooka et al. 2013). Additionally, a hypocaloric diet added with a probiotic-enriched cheese using true bacteria *plantarum* reduced the BMI, the ptomaine content and therefore the internal organ lactobacilli in Russian adults with fat and cardiovascular disease. Similarly, there was a reduction in the blood pressure (BP), namely a lower diastolic BP and a tendency toward lower systolic BP at the end of the intervention in the obese hypertensive patients that received the hypocaloric diet supplemented with the probiotic cheese (Kadooka et al. 2013).

The administration of *L. acidophilus* La5, *B. lactis* Bb12, and *L. casei* DN001 was evaluated in individuals with high BMI who were randomly assigned to three groups depending on particular intervention diets: one group was established with a regular yogurt with low calorie diet (RLCD), the second one received a probiotic yogurt with low calorie diet (PLCD), and the third one received probiotic yogurt without low calorie diet (PWLCD) for about 2 months. A reduction in BMI, fat percentage, and leptin level was shown that was more obvious in groups who received the weight loss diet including probiotic yogurt. Additionally, a reduction in the serum levels of C-reactive protein (CRP) was more evident in the PWLCD group than in the PLCD and RLCD groups after the 8-week intervention. The expression of the FOXP3, T-bet, GATA3, TNF-α, IFN-γ, TGF-β, and ROR-gt genes in peripheral blood mononuclear cell (PBMCs) was assessed before and after intervention. In the three groups, ROR-gt expression was reduced and FOXP3 was increased. The expression of the TNF-α, TGF-β, and GATA3 genes did not change. Interestingly, T-bet gene expression was down-regulated in the PLCD and PWLCD groups. However, the IFN-g expression was down-regulated in all groups. The authors recommended that weight loss diet and probiotics yogurt had effects on organic phenomenon in PBMCs among overweight and rotund people (Rajkumar et al. 2014).

Senan et al. (Ipar et al. 2015) evaluated whole-genome based validation of the adaptive properties of Indian origin probiotic *Lactobacillus helveticus* MTCC 5463. They found Cbsh (conjugate bile salt hydrolase), FOF1ATP-c, FOF1ATP-b, FOF1ATP-delta, FOF1ATP-alpha, FOF1ATP-epsilon and FOF1ATP-beta anti-obese genes from *Lactobacillus helveticus* MTCC 5463 by genome analysis which are beneficial for anti-obesity.

An 8-week, randomized, double-blind, placebo and compliance-controlled parallel study in overweight and obese subjects was conducted to evaluate the effects of one strain of *E. faecium* and two strains of *S. thermophilus*. The patients were arbitrarily divided into 5 groups: (1) a food hard with 2 strains of *S. thermophilus* and two strains of *L. acidophilus*; (2) a placebo food hard with delta-acid-lactone; (3) a food hard with 2 strains of *S. thermophilus* and one strain of *L. rhamnosus;* (4) a food hard with one strain of *E. faecium* and two strains of *S. thermophilus;* and finally (5) two placebo pills daily (Agerholm-Larsen et al. 2000). After adjustment for small changes in weight, beta-lipoprotein cholesterol (LDL-C) remittent and clotting factor inflated considerably when 8 weeks within the cluster that received the yogurt soured with one strain of *E. faecium* and two strains of *S. thermophilus* compared to the cluster intense with chemicals hard food and therefore the placebo pill cluster. Additionally, when 8 weeks, the heartbeat BP was considerably a lot of reduced in cluster one and therefore the cluster that received the food hard with one strain of *E. faecium* and two strains of *S. thermophilus* than in group 3.

The administration of capsules with *Lactobacilli*, *S. thermophilus*, and *Bifidobacteria* was evaluated in overweight subjects. The probiotic mixture had a major improvement in their lipid profiles, reducing total cholesterol (TC), LDL-C levels triacylglycerols (TAG) and increasing high-density lipoprotein cholesterol (HDL-C) levels. The probiotic mixture improved insulin sensitivity and decreased C-reactive protein (CRP) which is useful for obese people (Zarrati et al. 2014). Figure 2.1 describes the mechanism involved in role of probiotic in weight loss.

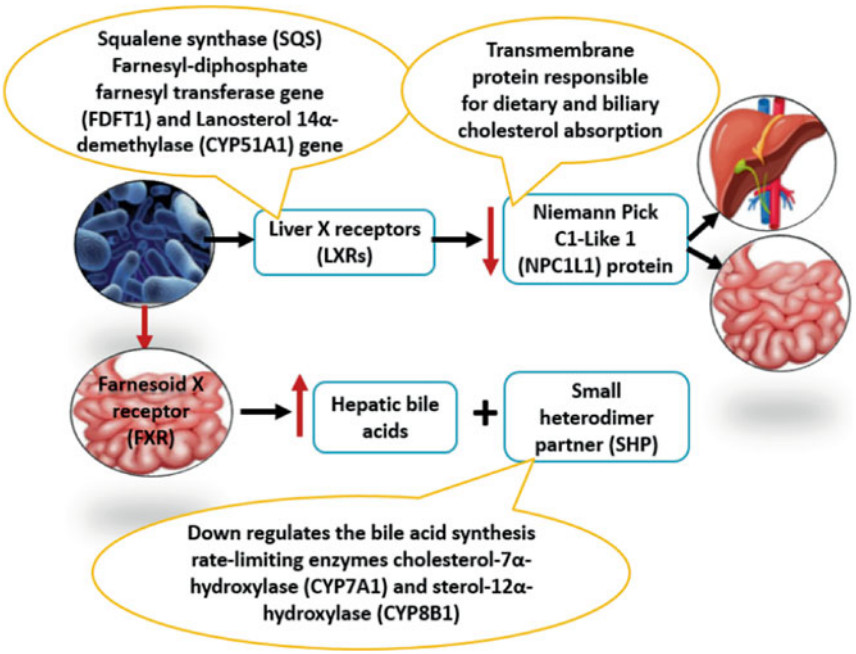

Fig. 2.1 Mechanisms of probiotic and weight control (Ishimwe et al. 2015)

2.7 Synbiotics and Obesity

The term synbiotic is employed once a product contains each probiotics and prebiotics. Because the word alludes to synergism, this term ought to be used for product during which the prebiotic compound and probiotic compound.

The impact of *L. rhamnosus* CGMCC1.3724 with oligo-fructose and inulin supplementation was investigated on weight loss and maintenance in obese men and women during 24 weeks (Sanchez et al. 2014). The mean weight loss in girls within the *L. rhamnosus* group was significantly higher than in women in the placebo group after the first 12 weeks, whereas it was similar in men in the two groups. The *L. rhamnosus* induced weight loss in girls was associated not solely with important reductions in fat mass and current leptin concentrations however additionally with the relative abundance of bacterium of the *Lachnospiraceae* family in the feces, this family belongs to the *Firmicutes* phylum, a taxonomic group that has previously been reported to be positively associated with obesity (Sanchez et al. 2014).

In high fat youngsters, two studies evaluated the effect of synbiotic supplementation on cardiometabolic risk factors, serum lipid profile, anthropometric measurements, and oxidative stress levels. The intake of synbiotics resulted in a significant reduction in the BMI score and waist circumference, as well as in some cardiometabolic risk factors, such as TC, LDL-C, and TAG, and also changes in anthropometric measurements (% reduction comparing to baseline) were significantly higher in the children receiving synbiotics. After synbiotic supplementation, total oxidative stress serum levels significantly decreased (Senan et al. 2015).

2.8 Different Food Ingredients Added in Natural Anti-Obesity Products

2.8.1 Calcium

Calcium is one of the major components of dairy products which may be related to obesity. Although recent reviews reported that dietary calcium intake could lead to weight loss (Dougkas et al. 2011), dietary calcium is an important factor in the maintenance of blood calcium level, skeletal integrity, and modulation of chronic diseases risks. Several authors have reported that a high intake of calcium may increase energy expenditure and fecal fat excretion. The mechanism of increasing fecal fat excretion is most probably due to the formation of insoluble calcium-fatty acid soaps and/or binding of bile acids (Bendtsen et al. 2013). Another study has demonstrated that intracellular calcium plays a key role for regulating adipocyte metabolism. It is supposed that more dose of dietary calcium could modulate circulating calcitriol levels. It in turn decreases intracellular calcium and affects fat metabolism in human adipocytes based on the findings from different cellular studies, animal studies, epidemiological studies, and clinical trials (Trigueros et al.

2013). A threshold of Ca intake of ~600–800 mg/day has been proposed for the beneficial effects on weight regulation.

2.8.2 Protein

Many protein supplements such as whey protein, soy protein, and casein protein have been sold and also marketed as an anti-obese product for a long while. Going on a high-protein daily diet could help people lose weight and prevents weight gain rebound (Khoury et al. 2013). Protein is more satiating component than carbohydrate and is also associated with a higher diet-induced thermogenesis. The individual dairy proteins (whey and casein) may enhance satiety via increases in circulating appetite regulating hormones including glucagon-like peptide-1 (GLP-1).

Protein is the most satiating macronutrient and it is a dairy whey protein. It is more satiating than other protein sources. There is a general consensus that proteins slow lipid absorption and synthesis and promote lipid excretion (Khoury et al. 2013). A recent randomized controlled trial showed that whey protein supplementation appeared to have a positive and acute postprandial effect on satiety and fullness compared with casein and carbohydrate supplementation in overweight and obese individuals (Pal et al. 2014). In particular, their effects are due to enterohormonal changes CCK, GLP-1, and PYY-1 observed after their exclusive ingestion. A recent study suggested that a mixture of Whey Protein (WP) plus glucomannan (Glucomannan has important satiety property due to volume increase following gelification) exerted a decrease in the desire to eat which is correlated to enterohormonal modification (GLP-1 increase) despite the low content of protein (8 g) and the presence of glucomannan, which could reduce the fast absorption of WP in relation to the net forming during the gelification of the gastric environment (Sukkar et al. 2013). In the study, supplementation with approx. 56 g/day whey protein concentrate without any dietary supplement for 6 months resulted in significantly lower body weight, waist circumference, and fat mass in overweight and obese individuals compared to an isoenergetic carbohydrate (CHO) control (Baer et al. 2011). Figure 2.2 illustrates mechanism underlying role of proteins in weight loss.

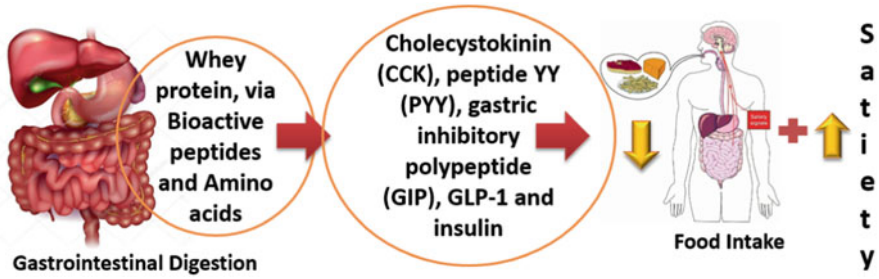

Fig. 2.2 Mechanisms between protein and weight control

2.8.3 Short-Chain Fatty Acids (SCFAs)

Obesity and amount of short-chain fatty acids (SCFA) in gut have direct relationship. SCFAs are saturated aliphatic organic acids. It consists of one to six carbons of which acetate (C2), propionate (C3), and butyrate (C4) are the most abundant (≥95%) (Cook and Sellin 1998). Each of the individual SCFA affects health differently. For example, acetate acts as a precursor for lipogenesis and cholesterol synthesis, propionate has been reported to inhibit acetate incorporation into cholesterol (Hellerstein et al. 1991). SCFAs are delivered to the liver and converted into triacylglycerols; these de novo synthesized lipids are then deposited in adipocytes through a process that involves, in part, microbial suppression of intestinal epithelial production of the circulating lipoprotein lipase (LPL)-inhibitor, Angptl4 (or Fiaf) (Backhed et al. 2007). LPL on the surface of the endothelium of the capillaries hydrolyzes the triacylglycerols in chylomicrons and very low density lipoprotein, providing free fatty acids (FFAs) and glycerol, which are reassembled into new triacylglycerols in the fat cells. A higher activity of the LPL clearly entails to a higher storage of triacylglycerols and indeed LPL is increased in the adipose tissue of humans and rodents in obesity (Mattijssen et al. 2014). Chaudhry et al. (Chaudhary 2019) studied the LAB strains on the basis of their SCFA production, bile deconjugation ability, and anti-oxidant activity. Results showed that cultures differed significantly ($P < 0.05$) in their ability to produce SCFAs (acetate, propionate, and butyrate) in skim milk medium. Hence, the LAB strains V3, MD2, NS4, and NS6 give best probiotic potential activity, were selected for further study, and were evaluated for their probiotic potential. Hati et al. (Hati et al. 2019) evaluated potent Lactobacillus isolates from traditional fermented foods of Garo Hills, Meghalaya, India (North East Part of India) showed maximum production of B2, B9, andB12 as well as short-chain fatty acids and could be used for their application as health beneficial functional fermented dairy products.

2.8.4 Polyunsaturated Fatty Acids (PUFAs)

The potential anti-obesity effects of PUFAs might be explained by their performance in the following aspects: a balance between energy intake and energy expenditure, lipid metabolism, status of adipocytes, and neuroendocrine system. It has been demonstrated that the PUFAs could reduce the activity of the key enzymes responsible for lipid synthesis, such as stearoyl-CoA desaturase-1 and fatty acid synthase. Thus, they might avoid free fatty acids entering adipocytes for lipogenesis and also improve lipid oxidation and thermogenesis (Trigueros et al. 2013). Differentiated diets for dairy cows with polyunsaturated fat (n3) and polyphenols can naturally enrich milk with PUFA and polyphenols (Santos et al. 2016). In a recent study, the administration of this enriched milk as a supplement to obese rats has resulted in increased muscle mass and reduced LDL values (Santos et al. 2017). Thus, whole common milk and PUFA-rich milk have shown to be beneficial in a normal

metabolic condition, whereas common milk and milk enriched with PUFA and polyphenols improve metabolic effects of obesity.

2.8.5 Dietary Fiber

Some anti-obesity products are rich in various types of dietary fiber, such as pectin, cellulose, gum, and soluble dietary fiber. In 1970, Heaton summarized the anti-obesity functions of dietary fiber. He has invested that dietary fiber could act like a physiologic obstacle to reduce energy intake by three mechanisms: (1) displacement of other nutrients in the diet by the dietary fiber, (2) providing satiety and decrease appetite, and (3) inhibiting food absorption in small intestine. Furthermore, the studies have shown that fermentability and viscosity are two important physico-chemical properties, that properties are closely related to the beneficial physiological effects of dietary fiber (Chaudhary 2019). Many soluble dietary fibers (e.g., gums, pectins, and B-glucans) become thicken while mixing with liquids. Viscosity is a major contributor to physiological effects in the small intestine. An increase in viscosity may present a barrier to slow gastric emptying and delay nutrient absorption. Dietary fiber could also be a fermentable substrate for the colon microbiota, which supporting higher the microbial mass increase and production of short-chain fatty acids. A lot of prospective studies have indicated that long-term consumption of fiber-rich diet has a negative correlation with body weight gain (Astrup et al. 2010). Champagne et al. (Champagne et al. 2011) studied the effect of alterations in consuming dietary products on losing weight/maintenance and they concluded that an increment of fruits, vegetables, and low-fat dairy food containing dietary fiber may help achieve weight loss and maintenance. According to recommended dietary allowances (RDA), the dietary fiber of 35 g/day is recommended for healthy adults (Sarker and Rahman 2017). Probiotic fermented milks with finger millet and without finger millet are sensorily acceptable products having adequate level ($>10^8$cfu/g) of probiotic count in it. The products are found to have good functional properties in terms of its anti-microbial, anti-oxidant, bile deconjugation, and lipase inhibition properties. Both products showed very promising anti-obesity effect proven through in vivo animal study, indicating the potential role of our LAB strains as well as finger millet in preventing obesity. Hence, it can be concluded that both products can be potential functional foods for prevention and management of obesity (Chaudhary 2019).

2.8.6 Phytochemicals

It is well known that the consumption of phytochemicals can have a major contribution to biological effects. The mechanisms of action of phytochemicals include: (1) inhibition of proliferation of precursor cells; (2) increase of apoptosis effect; (3) inhibition of pancreatic lipase activity; and (4) increase in energy expenditure (Birari and Bhutani 2007). Polyphenols are functional compounds that have anti-

carcinogenic, anti-oxidant, anti-bacterial, and anti-viral activities. In the past two decades, polyphenols have also been reported to have beneficial effects against obesity. For example, dietary polyphenols could regulate adipocyte metabolism to inhibit the growth of adipose tissue (Baboota et al. 2013). Phenolic acids, flavonoids, and stilbenes are the common polyphenols, which being used in the development of different natural weight management products. Phytosterols, which encompass plant-derived sterols and stanols, are compounds structurally similar to cholesterol. They occur in high concentrations in vegetable oils such as corn, soybean, and sunflower oil. Plant stanols and sterols have been proved capable of blocking the absorption of reducing body weight gain and intestinal fatty acid in animal tests (Gupta et al. 2015). Lee et al. (2013) studied the anti-obesity activity of *Lactobacillus paracasei subsp. paracasei* NTU 101 and *Lactobacillus plantarum* NTU 102 and their soy milk fermented products (SM101 and SM102). They found that SM101 and SM102 both improved obesity in Wistar rats fed with a high fat diet (HFD) and that this improvement was stronger than that observed for unfermented soy milk. Soy food products are recognized as healthy food and are considered an important part of the diet. The genistein and soy protein supplementation in soy yogurt can decrease triglyceride, total cholesterol, and LDL cholesterol levels in the serum and liver of mice which helps in anti-obesity (Vij et al. 2011).

2.9 Future Research on Anti-Obesity

Probiotic bacteria and/or some additives have role to play in modulation of obesity so more research is needed to understand the mechanism about potential probiotic cultures are expressing on different anti-obese genes. More studies have required enrichment of probiotic fermented milk with prebiotic which can improve the nutritional and functional aspects of the resultant product especially in terms of its dietary fiber and mineral contents and functional properties such as anti-microbial, anti-oxidant, and anti-diabetic aspects and may exert anti-obesity effect. Further studies to evaluate the best dose-response effect of probiotics and synbiotics are needed, including following up with patients after the probiotic intervention to evaluate the persistence of their potential beneficial effects in obesity.

2.10 Summary and Conclusion

Obesity is one of the most important public health problems worldwide which affecting both developed and developing countries. Physiological, behavioral, and environmental contributions all influence the development of obesity. A major advance in obesity therapy, since modifying the gut microbiota, through a diet enrich with probiotics and prebiotics or food ingredients could be an alternative approach for controlling the obesity. Novel dairy products with probiotics and prebiotics for obese people have to be developed. Further clinical studies on obesity are required to claim before marketing the anti-obese food products. However,

functional dairy foods with probiotics and prebiotics could be a novel approach for the obese population.

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Effect of Pre/Probiotic Supplementation on Metabolic Endotoxemia

3

Seema Bansal and Nitin Bansal

Abstract

Metabolic endotoxemia (ME) refers to a condition in which endotoxin levels (lipopolysaccharide) are significantly elevated in the blood, without the evidence of an infection. ME may activate toll-like receptor 4 (TLR-4) mediated inflammatory reactions and enhance a low-grade chronic inflammatory and oxidative stress resulting in damage of target organs. ME plays an important role in the pathophysiology of different chronic inflammatory diseases especially cardiovascular disorders (obesity, type 2 diabetes mellitus), non-alcoholic fatty liver disease (NAFLD), pancreatitis, and brain diseases including Alzheimer's disease. Various studies reported that gut biota alteration leads to leakage in intestinal membrane which results in translocation of microbial components (endotoxin) in systemic circulation and escalate pro-inflammatory cytokines. Thus manipulation of gut microbiota can prove to be an important strategy to treat ME. Probiotics are alive microorganisms which, when administered in sufficient amounts, shows favorable effects during gut dysbiosis. Prebiotics are dietary fibers that increase the count of beneficial bacteria via selectively stimulation of their growth and nourishment while decrease the count of pathogenic bacteria that release endotoxins. This chapter will enlighten upon possible mechanisms via which gut dysbiosis leads to metabolic endotoxemia and role of probiotic/prebiotic supplementation in metabolic endotoxemia.

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Probiotics · Lipopolysaccharide · Metabolic endotoxemia

3.1 Introduction

The term metabolic endotoxemia (ME) refers to a situation in which microbes and microbial fragments such as lipopolysaccharides (LPS), flagellin, peptidoglycan enter the systemic circulation from the gut causing low-grade inflammation and lipolysis (Moludi et al. 2020). The term ME was defined by Cani et al. (2007) when they detected increased LPS levels in the blood of mice fed with high-fat diet-induced type 2 diabetes and non-alcoholic fatty liver disease. They reported that LPS (Abbreviation should come before) concentration within the blood is undetectable during normal physiological conditions, it can be detected in blood only during disease conditions such as infection or inflammation of inner lining of colon. During ME, LPS levels are increased 2–three fold in the blood stream as compared to normal control. However, during septic shock LPS levels are increased 1000 folds as compared to normal control. Thus during ME LPS levels are 10–50 times lower than those observed during septic shock. Lipopolysaccharide is a constituent of outer cell wall of gram negative bacteria and plays a key role in induction of various inflammatory reactions via triggering Toll-like receptor (TLR) signaling pathways. LPS binds with LPS-binding protein (LBP) to form complex LPS-LBP and presented to cluster of differentiation 14 (CD14) on innate immune cells, which is expressed mainly by macrophages, neutrophils, and dendritic cells; this subsequently mediates signal transduction, including nuclear factor kappa B (NF-κ B), which then activates transcription of many proinflammatory genes that encode cytokines and chemokines which eventually leads to various chronic diseases (Suganami et al. 2007). Rise in blood LPS as a consequence of metabolic endotoxemia was observed as a major risk factor in various chronic diseases such as non-alcoholic fatty liver diseases, metabolic syndrome, insulin resistance, Alzheimer and pancreatitis (Moludi et al. 2020). Thus detection and modulation of ME can be an important strategy to treat various chronic diseases. Large number of preclinical and clinical studies have reported that gut dysbiosis plays a key role in metabolic endotoxemia and associated cardiovascular side effects. Probiotics are living microbes that, when administered in adequate amounts, confer beneficial effect in gut biota. There are different groups of probiotic microorganisms including Bifidobacterium, Lactobacillus, etc. Along with this, prebiotics are nondigestible dietary ingredients that indirectly benefit the host via selectively stimulating the growth or activity of a limited number of bacteria in the colon. It includes mainly oligosaccharides, inulin, galacto-oligosaccharides, insoluble dietary factors, etc. Both probiotics and prebiotics modulate the gut biota and reduce metabolic endotoxemia via multiple mechanisms including maintenance of gut permeability, enhanced immunity, and release of different metabolites (Fuke et al. 2019). In this chapter, the potential link between gut dysbiosis and onset of metabolic endotoxemia

along with an evidence on the potential role of probiotics/prebiotics in gut biota dysbiosis and associated development of metabolic endotoxemia will be reviewed.

3.2 Reasons of Metabolic Endotoxemia

Data from previous literature has revealed that diet plays an important role in the regulation of endotoxemia (Cândido et al. 2018). This statement is supported by previous studies that plasma LPS levels are increased in mice fed with high energy diet (Kapoor et al. 2015). An increase in postprandial serum endotoxin levels is observed in healthy adults taking high-fat diet, especially saturated fat diet when compared with adults taking diet rich in polyunsaturated fatty acids (Bonnabry et al. 2005). Along with this, postprandial LPS levels are also reported to be increased in men who received high lipid meal as compared to fasted individuals (Lyte et al. 2016). Possible reason behind this may be LPS incorporation in to the micelles and chylomicrons. Intestinal-epithelial cells can internalize LPS from the apical surface and transport LPS to the golgi. The golgi complex also contains newly formed chylomicrons, the lipoproteins that transport dietary long chain fat through mesenteric lymph and blood. As LPS has affinity for chylomicrons, thus it may be possible that chylomicron formation promotes LPS absorption (Ghoshal et al. 2009). Further study by Erridge et al. (2007) reported that intake of high-fat diet accentuates LPS absorption through the intestinal barrier, increasing plasma LPS levels. Mechanism behind increase in LPS absorption through intestinal membrane may be formation of quilomicron which promote LPS absorption and internalization of intestinal microfold cells and enterocytes. In addition, increase in local pressure or loosening of tight junctions between enterocytes and even rupture of basement membrane due to excess chylomicrons generated from high-fat diet may result in compromised intestinal barrier and increasing intestinal permeability especially for LPS (Moreira et al. 2012). A high fatty diet also influences phylum gut biota and reduces number of beneficial gram-positive bifidobacterium species hence leading to increase in plasma LPS concentration (Cani and Delzenne 2011). The type of fatty acid ingested also affects induction of endotoxemia. In a study with mice fed omega-6-rich meals reported that mice exhibited elevated levels of endotoxemia and low intensity inflammation, however, mice fed with omega-3 fatty acids had decreased LPS production with marked reduction in metabolic endotoxemia (Kaliannan et al. 2015).

3.3 Metabolic Endotoxemia and Gut Dysbiosis

Gut microbiota is the complex community of microorganisms in the intestinal mucosa. Mostly it includes Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (Fuke et al. 2019). All these microbes participate in most of the metabolic activities and show a profound effect on immune system and metabolism. During normal physiological conditions, gut microbes play a variety of functions

such as aid in energy harvesting, nutrition, and fermentation of indigestible food components of the host and vitamins production. They also maintain intestinal epithelial cells, homeostasis, drug metabolism, and activate immune system for protection against pathogens. Further, gut microbiota protects the gut health via strengthening of gut integrity by release of mucin. Furthermore, gut microbes either directly impede colonization of pathogens that compete for space and nutrients or indirectly produce antimicrobial substances such as chemically modified bile acids and volatile fatty acids. Thus gut microbiota can perform defensive functions via impeding inoculation and development of enteric pathogens via colonization resistance. Thus, balanced gut biota plays an important role in maintenance of normal metabolic functions. However, alteration of microbial composition also known as gut dysbiosis make intestinal membrane leaky which results in infiltration of bacteria and their components (lipopolysaccharide) into the systemic circulation which stimulate immune cells leading to production of inflammatory cytokines, and this increase in cytokines signaling further decreases protein synthesis and enhances catabolism. This low-grade systemic inflammation leads to development cardiometabolic risk factors including hypertension, impaired lipid profile, diabetes, insulin resistance, and obesity (Fig. 3.1). Various animals and human studies have reported that intake of high-fat diet changes the intestinal flora (imbalance in bacteroides, firmicutes, and bifidobacterium levels) (Radilla-Vázquez et al. 2016; Fuke et al. 2019). Study by Ferrer et al. (2014) reported that relative proportion of

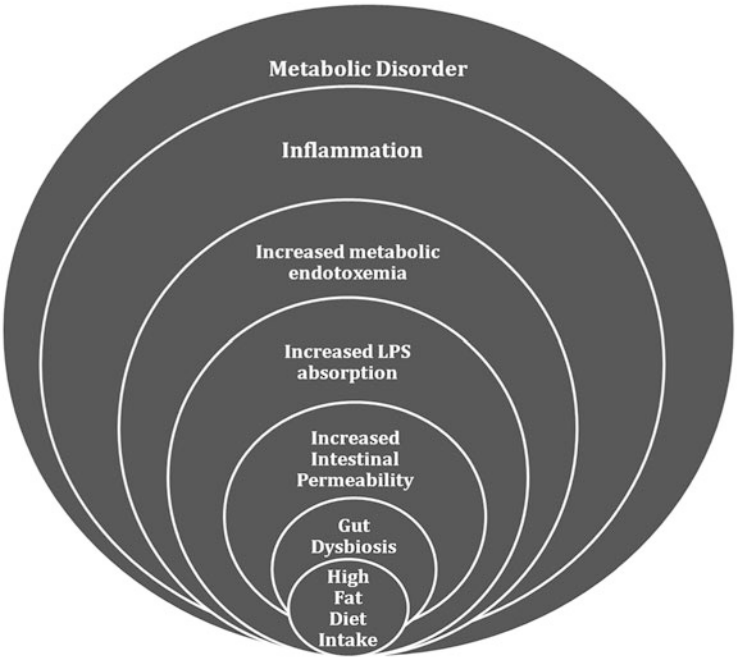

Fig. 3.1 Metabolic endotoxemia from high-fat diet intake to metabolic disorders

different microbes like lower abundance of bacteroidetes and more of firmicutes are found in metabolic syndrome. However, opposite to it, in another human study, it has been reported that high-fat diet intake results in increase of bacteroidetes and decrease of firmicutes and proteobacteria. One possible reason behind difference of animal and human flora may be different type of fat intake. Study by (Ohlsson et al. 1996) reported that LPS exposure enhanced oxidation of lipoproteins and oxidized LDL plays a key role in atherogenesis.

3.4 Role of Pro/Prebiotics in Metabolic Endotoxemia

The term probiotics was given by Elie Metchnikoff in the twentieth century and is defined as living microbes which modulates the gut health via harmonizing intestinal microbes. Most commonly used probiotic products contain bifidobacteria/lactobacilli, streptococci, and lactococci. Other than this Bacillus, Enterococcus, Propionibacterium, Escherichia, and Saccharomyces are also play an important role as probiotics. In addition to probiotics, prebiotics can be administered to modulate gut health. The concept was given by Gibson and Roberfroid in 1995. Prebiotics are non-digestible, fermentable carbohydrates that modulate gut health via varying the activity and composition of gut microbes. Most commonly used prebiotics are Inulin-type fructans (ITF) and galacto-oligosaccharides (GOS). Prebiotics selectively enhance bifidobacteria/lactobacilli growth, and markedly change composition of gut microbiota. Other than this cyclodextrins, palatinose, xylo-oligosaccharides, fructooligosaccharides, and pectic oligosaccharides are key prebiotics. *Lactobacillus*, *Bacteroides*, *Akkermansia*, *Roseburia*, and *Prevotella* are possible bacterial genera that may contribute to the reduction of blood LPS levels. Large no of preclinical and clinical studies reported that probiotics decrease LPS in the cecal content, improve inflammation, enhance glucose intolerance and insulin sensitivity, decrease body weight, and improve metabolic endotoxemia via modulation of gut health hence prevented development of many chronic diseases (Table 3.1). Moreover, prebiotic-induced changes in the gut microbiota improve metabolic endotoxemia and decrease incidence of metabolic syndrome. Cani et al. (2007) reported that Bifidobacterium spp. content is inversely proportional to plasma LPS levels and supplementation of Bifidobacterium spp. decreased intestinal endotoxin levels and improved mucosal barrier function. Further, Cani and Delzenne (2009) reported that prebiotic dietary fiber treatment to high-fat fed mice normalizes endotoxemia. Although the mechanisms via which prebiotic treatment reduces ME are not much clear but main mechanism behind this seems to be enhancement of Bifidobacterium spp. via prebiotics. Bifidobacterium supplementation lowers bacterial translocation and endotoxemia leading to decreased activation of inflammatory cascade (Horiuchi et al. 2020). In vitro study by Mokkala et al. (2016) reported that Bifidobacterium lactis 420 and fish oil enhance intestinal epithelial integrity in intestinal epithelial cell model (Caco-2 cells). Further results were verified by in vivo studies by Stenman et al. (2014) that bifidobacterium 420 reduce epithelial translocation of *E. coli* resulting in lower circulating LPS levels in diet-induced obese mice.

Table 3.1 Most relevant studies showing effect of Pro/Prebiotics in Metabolic Endotoxemia

Reference	Title	Type of study	Treatment	Results and conclusion
Xue et al. (2017)	Probiotics may delay the progression of non-alcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia	In vivo studies	Probiotics administered to eight-week-old male SD non-alcoholic fatty liver disease (NAFLD) rats	Both alteration in gut flora and endotoxemia resulted in the development of NAFLD. Probiotics administration may delay the development of NAFLD via LPS/TLR4 signaling.
Kim et al. (2019)	Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 simultaneously alleviate high-fat diet-induced colitis, endotoxemia, liver steatosis, and obesity in mice	In vivo studies	Oral administration of lactobacillus plantarum LC27 and/or Bifidobacterium longum LC to mice with high-fat diet-induced obesity	Probiotic supplementation decreased fecal lipopolysaccharide production and Firmicutes and Proteobacteria counts in HFD mice. LC27/LC67 administration alleviated liver steatosis, obesity, and colitis by inhibition of gut microbiota lipopolysaccharide production.
Rios et al. (2019)	Protective effect of prebiotic and exercise intervention on knee health in a rat model of diet-induced obesity	In vivo study	Male Sprague-Dawley rats with metabolic knee osteoarthritis in a high-fat/high-sucrose (HFS) diet-induced rat model of obesity.	Prebiotic fiber administration along with aerobic exercise, prevented knee joint damage via improving gut microbiota, endotoxemia, insulin resistance and dyslipidemia, in the HFS rats
Li et al. (2019)	Dietary inulin alleviates diverse stages of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in db/db mice	In vivo study	Inulin administered to diabetic mice	Dietary inulin suppresses inflammation, lipopolysaccharide levels and modulate gut microbiota resulting in alleviation of T2DM

(continued)

Table 3.1 (continued)

Reference	Title	Type of study	Treatment	Results and conclusion
Neyrinck et al. (2012)	Wheat-derived arabinoxylan oligosaccharides with prebiotic effect increase satietogenic gut peptides and reduce metabolic endotoxemia in diet-induced obese mice	In vivo study	Wheat-derived arabinoxylan oligosaccharides (AXOS) was administered in diet-induced obese mice	AXOS constitute a promising prebiotic nutrient in the control of obesity and related metabolic disorders via reducing ME and macrophage infiltration in adipose tissue.
Chen et al. (2019)	Fucoidan and galacto-oligosaccharides ameliorate high-fat diet-induced dyslipidemia in rats by modulating the gut microbiota and bile acid metabolism	In vivo study	Fucoidan (FUC) and galacto-oligosaccharides (GOS) administered to high-fat diet-induced dyslipidemic rats	In rats, GOS and FUC administration significantly reduced lipopolysaccharide, serum total bile acid, serum high-density lipoprotein cholesterol, cholesterol 7-alpha hydroxylase expression in the liver.
Chan et al. (2016)	High-fat diet-induced atherosclerosis is accompanied with low colonic bacterial diversity and altered abundances that correlates with plaque size, plasma A-FABP, and cholesterol: A pilot study of high-fat diet and its intervention with lactobacillus rhamnosus GG (LGG) or telmisartan in ApoE −/− mice	In vivo study	Lactobacillus rhamnosus was administered to six-weeks-old female ApoE−/− mice	Lactobacillus rhamnosus treatment significantly decreased HFD induced rise in endotoxin levels
Kikuchi et al. (2018)	Sterilized bifidobacteria suppressed fat accumulation and	In vivo study	Sterilized bifidobacteria was administered to male C57BL/6J	The present study indicates that sterilized bifidobacteria suppressed fat

(continued)

Table 3.1 (continued)

Reference	Title	Type of study	Treatment	Results and conclusion
	blood glucose levels		mice orally for 4 weeks	accumulation, improved insulin resistance, and lowered blood glucose levels in high-fat diet fed mice via reducing LPS levels and modulation of gut biota
Cani et al. (2009)	Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability	In vivo study	*Ob/Ob* mic were treated with prebiotic	Prebiotic-treated mice exhibited a lower plasma lipopolysaccharide (LPS) levels, lower intestinal permeability and improved tight-junction integrity resulting in the improvement of obesity-associated hepatic and metabolic disorders
Dewulf et al. (2013)	Insight into the prebiotic concept: Lessons from an exploratory, double-blind intervention study with inulin-type fructans in obese women	A double-blind, placebo-controlled, intervention study	Dietary inulin-type fructans (ITF prebiotics) in obese women	Treatment with ITF prebiotic results in enhancement of Bifidobacterium and Faecalibacterium prausnitzii; both bacteria negatively correlated with serum lipopolysaccharide levels. ITF prebiotics selectively modulates the gut microbiota composition, decreases metabolic endotoxaemia in obese women
Pedersen et al. (2016)	Host–microbiome interactions in human type 2 diabetes following prebiotic	Human study	Randomized, double-blind, placebo-controlled parallel study. Prebiotic (galacto-	The current study does not provide any evidence for the role of prebiotics in

(continued)

Table 3.1 (continued)

Reference	Title	Type of study	Treatment	Results and conclusion
	fiber (galacto-oligosaccharide) intake		oligosaccharide) supplementation for 12 weeks on patients with T2D.	the management of type 2 diabetes.
Parnell et al. (2017)	Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity.	Human study	Randomized, double-blind, placebo-controlled trial were used for analysis. Probiotic oligofructose was administered to obese adults for 12 weeks	Oligofructose administration mitigates obesity-associated inflammatory markers via decreasing plasma LPS levels and metabolic endotoxemia.
Dehghan et al. (2014b)	Oligofructose-enriched inulin improves some inflammatory markers and metabolic endotoxemia in women with type 2 diabetes mellitus: A randomized-controlled clinical trial.	Human study	Randomized-controlled clinical trials. Oligofructose-enriched inulin was administered to type 2 diabetic women	Oligofructose-enriched inulin caused a significant decrease in the plasma lipopolysaccharide levels resulting in decrease of metabolic endotoxemia and modulation of glycemic parameters in type 2 diabetic women
Morel et al. (2015)	α-Galacto-oligosaccharides dose-dependently reduce appetite and decrease inflammation in overweight adults.	Human study	Double-blind, randomized, placebo-controlled trials, α-galacto-oligosaccharides were administered to overweight adults	α-Galacto-oligosaccharides administration results in significant decrease in lipopolysaccharide levels, reduced appetite and diet intake, and inflammation in overweight adults. It revealed that α-GOS promote weight loss and mitigate metabolic disorders.
Farhangi et al. (2018)	A randomized-controlled trial on the efficacy of resistant dextrin, as functional food, in	Human study	Triple blind study to investigate effect of resistant dextrin in women with type 2 diabetes mellitus	Supplementation of resistant dextrin results in decrease in lipopolysaccharide

(continued)

Table 3.1 (continued)

Reference	Title	Type of study	Treatment	Results and conclusion
	women with type 2 diabetes: Targeting the hypothalamic-pituitary-adrenal axis and immune system			levels, metabolic endotoxemia, and boosting immunity in women with T2DM.
Dehghan et al. (2014a)	Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial.	Human study	A randomized-controlled clinical trial. Inulin was administered to women with type 2 diabetes	Inulin-supplemented patients exhibited a significant decrease in inflammatory markers including LPS. It can be concluded that inulin supplementation may modulate inflammation and metabolic endotoxemia in type 2 diabetes.

Furthermore with preclinical study was supported by clinical study by Le Barz et al. (2015) that administration of bifidobacterium 420 lowers circulating Zonulin a potential biomarker of intestinal permeability, decrease circulating inflammatory markers in obese adults. Modulation TLR4 signaling via probiotics is a very interesting target in metabolic endotoxemia. In vitro study by Takanashi et al. (2013) reported that in Caco-2/TC7 cell lines lactobacilli strains such as Lactobacillus casei negatively regulate TLR4 via inhibiting the NF-κB and p38 pathways and upregulating negative regulators Tollip and Bcl-3 in bovine intestinal epithelial cells. Similarly, Bifidobacterium 420 and its cell-free metabolites down-regulate TLR4 gene expression in intestinal epithelial cells.

Lactobacillus is a gram-positive bacterium that produces large amounts of lactic acid during carbohydrate fermentation and plays an important role in decreasing LPS and associated metabolic endotoxemia. Study by Plaza-Diáz et al. (2017) reported that administration of probiotic containing *Lactobacillus rhamnosus* decreased the mRNA expression levels of endothelin receptor type B (*Ednrb*) in the intestinal mucosa, and reduced the blood LBP level to obese Zucker-Lepr^{fa}/^{fa} rats. Further study by Jang et al. (2019) reported that *Lactobacillus sakei* is reported to suppress fecal *Proteobacteria* population and LPS levels in mice fed high-fat diet-induced colitis. In addition to this study Cui et al. (2017) reported that oral administration of *Lactobacillus* maintains the intestinal barrier function via suppressing LPS-induced apoptosis of intestinal epithelial cells (Cui et al. 2017). Furthermore, study by Huang

et al. (2014) reported that supplementation with *Lactobacillus acidophilus* reduces cholesterol absorption in atherosclerotic mice. All the above data reported that Lactobacillus decreases systemic LPS levels via modulation of intestinal permeability and via decreasing LPS levels in feces.

Bacteroides are obligatory gram negative anaerobes found in human and mice intestinal flora modulating expression of genes involved in strengthening of mucosal barrier. Study by Tan et al. (2019) reported that administration of *Bacteroides fragilis* HCK-B3 and *Bacteroides ovatus* modulates cytokines production and attenuate LPS-induced intestinal inflammation in mice. In contrary to this, *Bacteroides* degrade the mucin layer of the intestinal tract and enhance inflammation during low dietary intake, which shows Bacteroides may act as pathobionts (Desai et al. 2016). Study by Cartmell et al. (2017) reported that *Bacteroides intake with sulfate polysaccharide* nourishes *Bacteroides* in gut flora and suppresses metabolic endotoxemia via its anti-inflammatory and barrier function-enhancing effect. *Akkermansia* is a mucin-adherent intestinal bacterium which produce short chain fatty acids like propionic acids. These short chain fatty acids enhance function of intestinal barrier. Further study by Chelakkot et al. (2018) reported that *Akkermansia*-derived extracellular vesicles administered in mice are directly enhance intestinal barrier function by increasing epithelial cell expression of tight junction proteins. Furthermore, administration of Akkermansia to obese mice reduced thinning of mucin layer and blood LPS concentration.

3.5 Potential Mechanisms Via Which Pro/Prebiotic Modulates Metabolic Endotoxemia

Probiotics modulate intestinal environment either directly via inhibiting effect on gut permeability or indirectly via release of bacterial metabolites that effect on epithelial barrier. Study by Yan et al. (2007) reported that probiotic produces bacteriocins, which normalize intestinal epithelial cells survival and growth via inhibiting pathogenic bacteria. Further Everard and Cani (2013) reported that administration of Akkermansia muciniphila to diet-induced obese (DIO) mice due to probiotic effect reduces metabolic endotoxemia and adipose tissue inflammation via improving thickness of intestinal mucus membrane. Furthermore, Forsyth et al. (2009) reported that Lactobacillus GG treatment secretes bacterial metabolites that defend intestinal epithelial cells from oxidative stress via inducing cytoprotective heat shock proteins. Prebiotic supplementation (oligofructose) to obese mice results in decrease of various inflammatory markers (TNF-α, IL-1β, IL-1α, IL-6, IFN-γ) in serum and metabolic endotoxemia. Study by Menard (2004) reported that intestinal barrier functions can be improved by secreting metabolites of lactic acid bacteria (*Bifidobacterium breve and Streptococcus*) which may modulate intestinal barriers function.

Another possible mechanism of probiotics is maintenance of balance in the composition gut microbes. Imbalance of gut biota composition especially decreased ratio of Bifidobacterium and Lactobacilli enhances endotoxin release (Wang 2004).

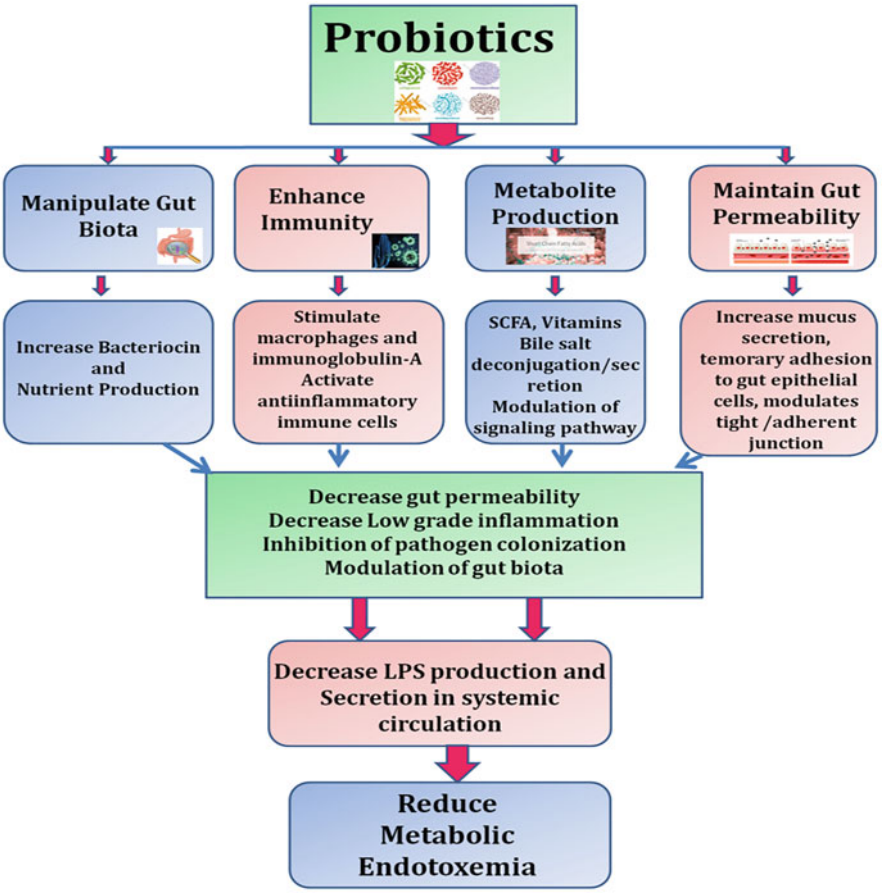

Fig. 3.2 Potential mechanism of action of Probiotics in Metabolic endotoxemia (Adapted from: Le Barz et al. 2015)

Study by Bernini et al. (2016) reported that supplementation with probiotic containing Bifidobacterium and lactobacilli to patients with impaired lipid profile and metabolic syndrome results significant decrease in lipopolysaccharide levels in plasma. Probiotics also maintain gut barrier permeability. Study by (Ruseler-van Embden et al. 1995) reported that Lactobacillus casei strain GG and Bifidobacterium bifidum do not mortify intestinal mucus glycoproteins and improve microvillus environment by preventing bacterial translocation and intestinal permeability (Fig. 3.2).

Prebiotics treatment (Fructans) causes alteration in intestinal mucosal architecture via increase of villus height and crypt depth, improves thickness of mucosal layer in the colon (Kleessen et al. 2003). Cani et al. (2009) reported that prebiotic administration to obese mice led to increase in endogenous GLP-2 production and enhancement of mucosal barrier function, decreasing plasma LPS concentrations, oxidative

stress, and inflammation as a result of improving tight junctions. These results indicate that enhancement of GLP-2 production decreases metabolic endotoxemia. Use of newer prebiotics such as arabinoxylans (AX) and arabinoxylan oligosaccharides (AXOS) in obese mice reduced adiposity and metabolic endotoxemia. Further AX administration in rodents modulates gut biota by increasing Bifidobacterium and Roseburia in diet-induced obese mice (Neyrinck et al. 2012) and also shifting mucin degradation from caecum to the colon where A. muciniphila (mucolytic) enhances favorable metabolites such as propionate (Le Barz et al. 2015). In an in vitro study by Johnson-Henry et al. (2008) the probiotic strain (*Lactobacillus rhamnosus* and *casei*) improved epithelial barrier function via decreasing *Escherichia coli*-induced endotoxin. In addition to all these studies, effect of various pre/probiotics in metabolic endotoxemia has been summarized in Table 3.1.

3.6 Conclusion

Accumulating evidences indicate that metabolic endotoxaemia is the leading risk factor and valuable marker of various cardiovascular and metabolic diseases. Alteration of gut biota plays a key role in the pathogenesis of metabolic endotoxaemia and associated diseases. Administration of pro/prebiotics modulates endotoxaemia via direct inhibition of gut permeability, restoring composition of gut microbes and via improving effect of active bacterial metabolites on epithelial barrier. Thus pro/prebiotic supplementation can prove to be an important treatment strategy to improve metabolic endotoxaemia, however, further clinical and molecular studies are warranted to establish their detailed mechanism of action.

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Probiotics in the Management of Diabetes

4

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Abstract

Probiotic is an expedient dietary supplement which is having a positive effect on the host in the intestinal tract. In recent years, there has been increased attention towards probiotics due to their role to improve health conditions, particularly in diabetic patients. As diabetes is a metabolic disease associated with an increase in blood sugar level. Probiotics which are a class of microorganisms have the ability in controlling the hyperglycemia as well as its various complications by modifying the glucose utilization before its absorption. The current review discusses the potential role of probiotics in the amelioration of diabetes both type 1 and 2 in both human and animal models.

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Keywords

Probiotics · Diabetes · Diabetes mellitus · Gut microbiota · Diabetes management

4.1 Introduction

4.1.1 Diabetes

Diabetes mellitus (DM) or simply diabetes indicates a metabolic syndrome of numerous etiology portrayed by hyperglycemia and glucose intolerance as their hallmark that results from deformities in the body's ability to secrete insulin and/or insulin activity or both (DIAMOND Project Group 2006; Blair 2016). It is a condition which is associated with the level of the hyperglycemia which escalates chances of both microvascular (nephropathy, retinopathy, and neuropathy) and macrovascular (stroke, ischemic heart disease, and peripheral vascular disease) related complications, which results in reduced life expectancy, marked morbidity, and decreased quality of life (Delahanty and Halford 1993; Papatheodorou et al. 2016; Papatheodorou et al. 2018).

From the last two decades, the diabetes prevalence has adequately increased with 420 million people affected worldwide and as revealed by the World Health Organization (WHO), the worldwide pervasiveness was calculated at 2.8% in 2000, with up to 4.8% increase in 2030 (World Health Organization 2016).As per the International Diabetes Federation (IDF), the total diabetic's number is predicted to increase by about 650 million by the year 2045 (Cho et al. 2018). DM in this regard is considered a leading global economic and health burden in the aging population and is presently the eighth major factor of death worldwide (Seuring et al. 2015). Currently, India alone presents more than 40 million diabetics and the number will be around 90 million by 2030 which will be the largest population of world diabetics. Its preponderance in the last three decades in India has raised from 1.2% to 11% (Ramachandran et al. 2006; Ramachandran and Snehalatha 2009).

The three main types of Diabetes are type 1, type 2, and gestational diabetes. Type 1 diabetes (T1D/IDDM, also called insulin dependent, juvenile, or childhood-onset) which constitutes about 10% of all instances of diabetes (Association 2010; Association 2015) involves impairment of insulin secreting β-cells and requires daily administration of insulin for survival (World Health Organization 2016; Lamichhane et al. 2018; Bhat et al. 2020). Due to the unsuccessful preventive and therapeutic strategies, the pervasiveness of T1D is increasing worldwide. So there is a need for comprehensive knowledge of the pathophysiology of T1D. Besides, for the T1D progress, environmental factors alongside with genetic factors impart a vital role (Rewers et al. 2018; Battaglia and Atkinson 2015).

Type 2 diabetes (T2DM/NIDDM, also called non-insulin dependent, adolescent or maturity-onset diabetes) is distinguished by abnormal lipid and glucose metabolism due to inadequate secretion of insulin or due to its insensitivity and is found to represent 90–95% of those with diabetes (Blair 2016). Even though T2DM is mostly

found in older adults, but due to obesity and physical inactivity, the incidence has been observed to increase in children (Cho et al. 2018). The major risk factors for T2DM are smoking, genetic factors, high caloric intake, and sedentary lifestyle along with gut microbiota as one of the reasons with related comorbidities (Lyssenko et al. 2008).

The gestational diabetes mellitus (GDM) is a typical issue featured by elevated glucose level during the second and third trimester of pregnancy constituting about 2–5% of all pregnancies. It happens in people who have an acquired inclination to develop diabetes and may appear as either type I or II diabetes. It requires cautious therapeutic supervision amid the pregnancy (Bellamy et al. 2009).Women with GDM have heightened chances of complications during pregnancy and preterm delivery and are prone to develop type 2 diabetes in the future (World Health Organization 2016; Asemi et al. 2013; Kubo et al. 2014).

4.1.2 Gut Microbiota and Diabetes

It has been found that there is an absence of consistency in gut microbiota description of diabetics compared to healthy patients. In Europe and China, a human metagenome-wide association study was conducted which showed that in a diseased state function framework of gut microbiota changes in T2D patients showing remarkable correlations with specific bacterial genes, gut microbes, and digestion process (Larsen et al. 2010). Such patients as compared to non-diabetics showed increased levels of *Lactobacillus spp*. which are positively associated with glycated hemoglobin (HbA1c) levels and fasting glucose (Lê et al. 2013), whereas *Clostridium spp* showed a negative correlation with HbA1c, fasting glucose and plasma triglycerides, and positive association with cholesterol and adiponectin (Derrien et al. 2004).

In one of the study it was observed that amount of genus *Faecalibacterium* and *Prevotella* gets decreased in diabetics and thus microbiota plays a part in T1DM and in diabetics (Brown et al. 2011). Further, the elevation of *Akkermansia muciniphila* in the mucous layer is seen after the administration of metformin (Karlsson et al. 2012). Some studies have reported that the *Firmicutes* and *Clostridium spp.* proportions were decreased markedly in diabetics in comparison with controls (Lau et al. 2011). In the same manner, the *Bacteroidetes* to *Firmicutes* and *Bacteroides/Prevotella* group's ratios to *C. coccoides/Eubacterium rectale* groups were found in positive correlation with plasma glucose levels. Also, it has been proclaimed that inflammation-induced effect of the gut microbiota might be responsible for autoimmune diabetes, T1D development (Wen et al. 2008). The progression of autoimmune diabetes has been correlated with the microbiota of gut due to the common receptors in inflamed pancreas as well as in the gut (Cani et al. 2007).

4.1.3 Probiotics Potential Sources and Health Benefits

Probiotics as the name suggests are the denotation for life. Lilly and Stillwell described probiotic in 1965 as antibiotics like substances specifically manufactured by few microorganisms which tend to enhance the development of other microorganisms (Lilly and Stillwell 1965). In 1974, Parker further added that probiotics include microorganisms as well as their products which provide health benefits to the host organism by revamping its microbial balance (Fuller Afrc 1989; Fuller 1989). In the year1989, the probiotics were regarded as microorganisms in living form producing beneficial outcomes in the intestine (Havenaar and Huis 1992). In 1992, live microorganisms in a mix or pure form were termed probiotics having useful ramifications on the host.

The World Health Organization (WHO) has recommended that probiotics are the products or preparations containing live, specified microorganisms in adequate numbers which grant beneficial effects on the host by affecting its gut microflora (Vandenplas et al. 2015). Moreover, they also impart an important role in boosting the endogenous flora. Probiotic preparations are considered safe since they are health-promoting food products. Several non-pathogenic bacteria have been found to go through antipathetic interplay with other strains of bacteria and thereby can influence bacteria which are pathogenic in nature (Kaur et al. 2009; Scholz-Ahrens et al. 2007).

Nowadays, probiotics are entirely consumed in the form of fermented products like yogurt besides in vegetables and meat in the time ahead (Vandenplas et al. 2015). The basis for selecting a probiotic microorganism includes antimicrobial activity against pathogenic bacteria, the capacity to confront acidity and digestive enzymes, besides they must be safe for human consumption and should confer beneficial effects on the host in conjunction with recognition up to strain level (Swain et al. 2014). Currently, probiotics include both the genera of bacteria and that of the yeast (Saccharomyces). Through immune, hormonal, and neuronal controls, probiotics are supposed to neutralize pathogenic microorganisms like *Enterica serotype*, *Enteritidis*, *Listeria*, and *E. coli* once administered into the host (Carabotti et al. 2015; Fijan 2014).

Traditionally, fermented foods like yogurt, sauerkraut, and tempeh serve as the regular source of probiotic strains (Chilton et al. 2015; Swain et al. 2014). Among the fermented dairy products, yogurt is considered to be as an ideal medium for the distribution of probiotic bacteria, besides provide health benefits by imparting additional nutritional physiological values (Awaisheh 2011; Stanton et al. 2001). Several constituents with different properties are found in soyabean that has a beneficial effect on human health (Choi et al. 2011). Kimchi, a traditional Korean fermented food can be regarded as a vegetable probiotic food that provides beneficial effects to health in the same way as provided by yogurt and have been recommended to carry probiotic bacteria, and also the consumer acceptability (Yoon et al. 2006; Pereira et al. 2011). Meat is one of the most nutritious food as it is loaded with a wide range of nutrients and its taste, flavor, and texture are liked by the consumers. Because of its composition and structure, meat had been presented as an exceptional

probiotics vehicle. Moreover, meat was found to give protection to LAB to confront the dangerous action of bile (Gänzle et al. 1999).

Consumption of probiotics may be associated with stimulation of immune system, decreased cholesterol levels, suppression of inflammation which results in anti-tumorigenic effects (Pintado et al. 2014). Lately, probiotics are proclaimed for exerting a controlling influence on the immune system, through the fixing of gut homeostasis (Gill and Prasad 2008). Clinically, probiotics have been used for various health benefits prompting an interesting area of research that is yet to explore currently. Some of the best properties of probiotics studied in various models include hypercholesterolemia (Nguyen et al. 2007), anti-diabetic (Tonucci et al. 2015; Talacchi et al. 2010), anti-pathogenicity, anti-obesity (Karimi et al. 2015), anti-inflammatory, anti-cancer (Awaisheh et al. 2016), anti-allergic, and angiogenic activities and their effect on the brain and central nervous system (CNS) (Song et al. 2016; O'sullivan et al. 2005).

4.2 Current Therapeutic Approach to Treat Diabetes

Currently, for T2DM various pharmacological agents are available, whereas for T1DM only a few drugs have been approved. The anti-diabetic drugs due to their route of drug administration present a marked barrier for their use. The quality of life of patients had been much better if oral preparations could be available. Current remedial treatment for type II diabetes is constrained and includes insulin and four fundamental classes of oral anti-diabetic operators that invigorate pancreatic insulin discharge, decrease hepatic glucose generation, defer processing and retention of intestinal starch, or enhance insulin activity (Gough and Narendran 2010; Bastaki 2005; Association 2019).

The hyperglycemia in diabetes mellitus is the after effect baffled between the amount of insulin important to direct metabolic procedures and the proportion of insulin being discharged by the β-cells. Insulin replacement therapy is the backbone for type I diabetes mellitus patients while eating regimens and way of lifestyle alterations are the steps for management of type II diabetes mellitus in its underlying stages. Insulin is additionally imperative in type II diabetes mellitus when blood glucose levels cannot be controlled by abstaining from food, weight reduction, exercise, and oral hypoglycemic drugs (Gough and Narendran 2010; Bastaki 2005; Tripathi and Srivastava 2006). Oral hypoglycemics are imperative in the therapeutics of type II diabetes mellitus where there are lingering working pancreatic β-cells. Be that as it may attributable to the dynamic nature of the sickness, oral anti-diabetic drugs when utilized frequently are unfit to control the hyperglycemia (Krentz and Bailey 2005; Wallace and Matthews 2003). Main oral anti-diabetic drugs are sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, and one more new class meglitinide analogs (Krentz and Bailey 2005; Krentz et al. 2008). Sulphonylureas act specifically on the islet β-cells to close ATP-sensitive potassium channels, which invigorate insulin discharge and are among the widely used drugs to treat T2DM (Reimann and Gribble 2002). Metformin is the most

usually utilized biguanide. Its mechanism of activity is not completely comprehended even though it is anti-hyperglycemic and not hypoglycemic. At the point when utilized alone or with a sulfonylurea, metformin enhances glycemic control and lipid levels in patients who are nonresponsive to sulphonylureas (Bailey 2008; Grant 2003). The insulin resistance diminished by metformin in the liver, skeletal muscle, and adipose tissue is well documented (Zhou et al. 2001). Moreover, it does not weight gain or low blood glucose levels (Bastaki 2005; Cusi and DeFronzo 1998). Currently two thiazolidinediones, viz. rosiglitazone and pioglitazone are being used. They act by decreasing insulin resistance in peripheral tissue, yet an impact to bring down glucose generation by the liver has been reported (Staels and Fruchart 2005). Their blood glucose-bringing down ability is reliant on typical circling levels of insulin and viability is more noteworthy when administered with insulin or an insulin releaser (Kahn et al. 2006). A new class of drugs recently introduced for the treatment of diabetes is meglitinide analogs. They were created from sulphonylureas (meglitinide segment). Repaglinide, nateglinide, and meglitinide are examples of meglitinide analogs, their action is to enhance early-stage insulin discharge (Blicklé 2006). The meglitinides are quick-acting insulin secretagogues that have a quick beginning and brief span of activity bringing about more physiological discharge of insulin from the β-cell without causing proceeded with level of insulin in the post absorptive stage, hence lessening glycemia without hypoglycemia (Blicklé 2006). α-amylase and α-glucosidases are responsible for breakdown of sucrose and polysaccharides to absorbable form glucose in brush border membrane of the small intestine, their inhibition diminishes sucrose and polysaccharides digestion and decreases post-prandial hyperglycemia. They are effective with other oral anti-diabetic drugs and used alone for strict glycemic control. Acarbose, miglitol, and voglibose are three examples of clinically used α-glucosidase inhibitors (Bailey and Day 2009; Holman et al. 1999).

The important adverse reactions of insulin and most oral anti-diabetic drugs are extreme hypoglycemia, lactic acidosis, eccentric liver cell damage, perpetual neurological deficit, stomach related inconvenience, cerebral pain, unsteadiness, and mortality. So there is much-needed demand for some safe and effective medications (Watkins et al. 2008).

4.3 Role of Probiotics in Diabetes Management

4.3.1 Probiotics and Type 1 Diabetes (T1DM)

Probiotics impart a variety of benefits when given in appropriate quantity to host (Hill et al. 2014; de Oliveira et al. 2017). Probiotics represent an essential part of the microbiota of the human gut besides maintaining a balance of gut microbiota, regulation of metabolic activities, and even release of (SCFAs) (Nagpal et al. 2018; Zheng et al. 2018). The utilization of some strains of probiotics reduces the release of cytokines such as IL-1β, IL-6, and TNF-α and elevating the levels of anti-inflammatory cytokines including 1 L-10 and (TGF-β) which help in the prevention

Table 4.1 Summary of major animal studies of probiotics related to type 1 diabetes (T1DM)

Probiotics	Model involved	Mechanism of action	References
Probiotics (Oral)	NOD mice	Promote inhibition of cytokines like IL-1β	Dolpady et al. (2016)
Bacterial LPS or Zymosan	NOD mice	Suppression of autoimmunity and elimination of inflammatory immune cells	Karumuthil-Melethil et al. (2015)
HMOS prebiotic	NOD mice	Increase the concentration of small chain fatty acids in the gut	Xiao et al. (2018)
Dietary resistant starch	Sprague-Dawley rats	Enhances the proliferation of β-cell as well as the synthesis of insulin	Koh et al. (2016)
CARF extracted from PV	Swiss Webster mice	Reduces the activity of α-amylase as glucosidase	Raafat et al. (2016)
Lactobacillus reuteri	C57BL/6J Diabetic mice	Inhibition of TNF-α signaling	Zhang et al. (2015)
Lactococcus lactis	NOD mice	Prevent β-cell destruction and suppress insulitis	Takiishi et al. (2012)
Dietary fibers	NOD mice	Alters the production of cytokine in pancreas and spleen	Chen et al. (2017)

of T1DM (Zheng et al. 2018; Mariño et al. 2017). The production of (SCFAs) by some probiotics plays a principal role in the regulation of the immune system as well as the pathogenesis of type 1 diabetes which is an autoimmune disease by activation of free-fatty acid receptors (FFAR2 and FFAR3) (Ang and Ding 2016). The (SCFAs) arbitrated activation of FFAR2/3 receptors enhances the release of glucagon-like peptide-1(GLP-1) form intestinal cells. The GLP after its release simulates the pancreatic β-cells to release insulin, thus decreases the blood sugar level. All the above factors are in support of probiotics in regulating as well as preventing T1DM or restoring the homeostasis of the gut microbiota–immune axis (Priyadarshini et al. 2018; Psichas et al. 2015; Christiansen et al. 2018). Various animal and human studies (Mishra et al. 2019) on probiotics have been carried out related to T1D- shown in Tables 4.1 and 4.2.

4.3.2 Probiotics and Type 2 Diabetes (T2DM)

Probiotics are considered as an alternative and supportive form of medicine along with other health supplements including minerals, vitamins, and various other food materials (April et al. 2012). Probiotics impart a very important role in various metabolic diseases including type 2 diabetes which is also a metabolic disorder (Panwar et al. 2013). They also alter the intestinal microbiota in a positive way which is having a beneficial impact on various diseases. On administration of

Table 4.2 Summary of major human studies of probiotic related to type 1 diabetes (T1DM)

Probiotics	Model involved	Mechanism of action	References
Lactobacillus rhamnosus GG andBifidobacterium lactis Bb12	Children in the age group between 9 and 18	The mucosal barrier of the gut gets improved	Groele et al. (2017)
Dietary fiber intake	Adult human with T1DM	Reduce systole and diastolic pressure of heart Reduction in the use of medicines for diabetes treatment	Beretta et al. (2018)
Dietary fiber	Patients with T1DM	Development of anti-inflammatory properties	Bernaud et al. (2014)
Adjunct therapy with DAPA	Patients aged between 13–22 years with T1DM	Decrease in the requirement of insulin Increase in the excretion of glucose via the urine	Biester et al. (2017)

Table 4.3 Summary of major animal studies of probiotic related to type 2 diabetes (T2DM)

Probiotic	Model involved	Mechanism of action	Reference
Lactobacillus casei CCFM419	Male mice (C57BL/6J	Decrease in PBG FBG levels and increase in short chain fatty acids	Li et al. (2017)
Lactobacillus plantarum Ln4	Male C57BL/6J mice	Decrease in triglyceride level	Lee et al. (2018)
Lactobacillus rhamnosus	Swiss mice	Decrease in TNF-α, IL-6 and LPS levels	Bagarolli et al. (2017)
Lactobacillus casei CCFM419	Male C57BL/6J mice	Increase in GLP-1 and IL-6	Wang et al. (2017)
Lactobacillus paracasei TD062	Rats induced with T2DM	Decrease in insulin level and increase in glucose tolerance	Dang et al. (2018)
Saccharomyces boulardii	Rats induced with T2DM	Control glycaemia and decrease in IL-6 and TG level	Brandao et al. (2018)
Lactobacillus plantarum MTCC5690	Mice	Increase in GLP-1	Balakumar et al. (2018)

various probiotics, an improvement in the various symptoms of T2DM has been observed including reduction in the levels of LPS, augmentation in intestinal integrity, reduction in endoplasmic stress, enhancement in sensitivity to insulin peripherally (Park et al. 2015; Lim et al. 2016; Balakumar et al. 2018). It has been reported that on administration of various strains of probiotics in animal and clinical models, Tables 4.3 and 4.4 showed an advancement in T2DM (Salgaço et al. 2019) including decrease in plasma lipid levels and genes mediating inflammation (TNF-α,

Table 4.4 Summary of Clinical trial studies of probiotic related to type 2 diabetes

Probiotic	Model involved	Mechanism of action	Reference
Lactobacillus casei	People with T2DM ($n = 20$)	Decrease in TNF-α, IL-6 and 1L-1β	Khalili et al. (2019)
Multiprobiotic "Symbiter"	People with T2DM ($n = 50$)	Decrease in HOMA-IR and HbA1c	Kobyliak et al. (2018)
Lactobacillus reuteri DSM 17938	People with T2DM ($n = 46$)	Increase in ISI and DCA	Mobini et al. (2017)
Lactobacillus acidophilus La 5	People with T2DM ($n = 64$)	Increase in erythrocyte SOD and GPx	Ejtahed et al. (2012)
Lactobacillus casei	People with T2DM ($n = 68$)	Improvement in bowel Dysbiosis	Sato et al. (2017)

IL-6, IL-β) and amelioration in production of short chain fatty acids (SCFA). Some studies reported that amalgam administration of different strains of probiotics is having much more health benefits as compared to individual strains (Bagarolli et al. 2017; Ejtahed et al. 2012; Kobyliak et al. 2018; Razmpoosh et al. 2019).

4.4 Mechanism of Action Through Which Probiotics May Improve Glucose Homeostasis

Probiotics acted via multiple pathways in maintaining the glucose homeostasis, one of the pathways is altering the intestinal microbiota which results in the suppression of the inflammatory process (Fåk et al. 2008; Fooks and Gibson 2002; Delzenne et al. n.d.; Burcelin et al. 2011). Moreover, altering gut microbiota also results in the release of gut hormones in a disorganized manner. The gut hormones play an important role in maintaining glucose homeostasis as they control the growth of β-cells as well as its survival. As a matter of fact, probiotics enhance the antioxidant system of β-cells and in turn, improves the glucose homeostasis via a reduction in insulin resistance (Yadav et al. 2008). It has been reported in various animal studies that supplementation of gliclazide drug with probiotics increases the bioavailability of the drug in the blood which results in the homeostasis of blood glucose (Al-Salami et al. 2008). Besides another possible way by which probiotics maintain homeostasis is the maintenance of insulin sensitivity (Fig. 4.1) (Andersson et al. 2010). Moreover, the gut microbiota alters the glucose metabolism via the transformation of polysaccharides which is indigestible by human enzymes making the availability of gastrointestinal absorbable glucose (Zhang et al. 2009).

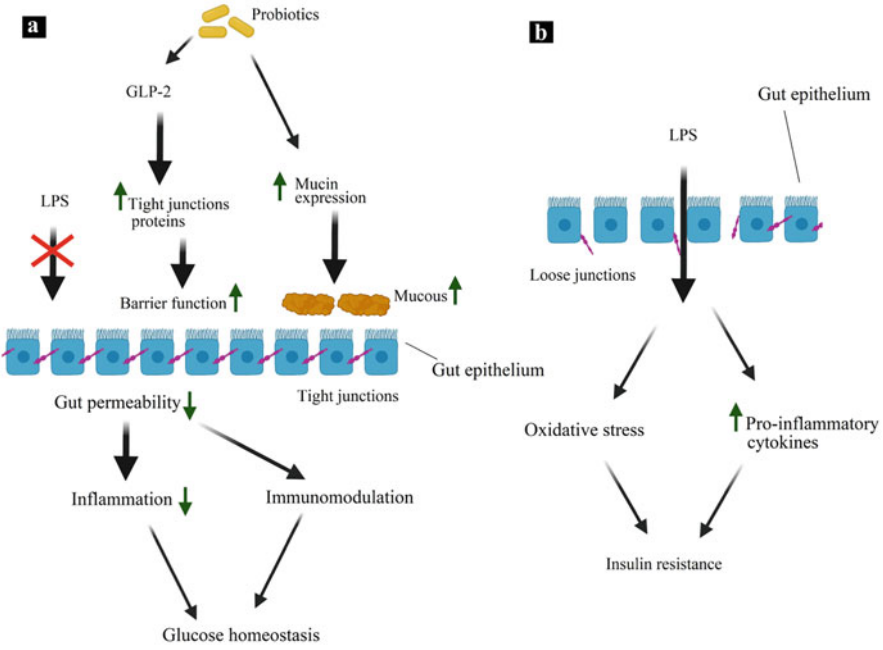

Fig. 4.1 Schematic representation of the mechanism of action of probiotics. (**a**) Probiotics induce the secretion of GLP-2 (glucagon-like protein) and increase the expression of mucin which in turn result in increase in expression of tight junction proteins and mucous secretion over gut epithelium, respectively. These activities result in the enhancement of the barrier function of the epithelium, decreased gut permeability and inhibition of the passage of LPS (lipopolysaccharides); (**b**) Mechanism of action of LPS that cause insulin resistance

4.5 Conclusion

The dynamic interactions between diet and gut microbiota and their metabolic consequences play a key role in the pathogenesis of diabetes. Modulating the gut microbiota by the use of probiotics, especially *Lactobacillus* and *Bifidobacterium* strains may have benefits in improving glucose metabolism and insulin resistance. Although clinical and experimental studies have revealed the important potential of these probiotic strains in the management of diabetes, further investigations are still required to elucidate the molecular mechanisms involved to develop more effective strategies against diabetes and its complication.

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Intestinal Microbiota Modulation for Type 1 and Type 2 Diabetes Prevention

5

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Abstract

Over the last decade, the use of probiotics in the treatment of different metabolic disorders especially diabetes has spectacularly improved with special note on the beneficial role of gut microbial community that resides inside us. Probiotics are beneficial constituents of functional foods. Now, they are recognised as nutraceuticals that may improve the intestinal microbial ecology and body's metabolism. The main intent of present chapter is to accumulate and give mechanistic exploration of beneficial effects of probiotics on diabetes mellitus (as main metabolic diseases in various disease models). Human and animals' studies showed that selection of probiotics has advantageous in DM patients with obesity by distressing the BMI and fat ratio. Probiotics also had a positive impact on insulin receptor substrate (IRS), downregulation of the cell adhesion molecule-1, whereas synbiotic decreases the insulin resistance and lipid concentration in plasma. Furthermore, probiotics also enhance the carbohydrate metabolic pathway, regulate fasting blood sugar level, insulin sensitivity, improve antioxidative potential, and distress the metabolic stress in diabetic patients. This chapter elucidated that the oral consumption of probiotics helps in prevention and management of diabetes, supported with the various clinical and experimental reports. However, there is still a huge void for the investigation of these healthy dietary nutraceuticals known as probiotics as crucial modulators of metabolic disorders.

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Diabetes · Probiotics · Gut microbiota · Prebiotics · Management

5.1 Introduction

In relation to major metabolic diseases, probiotic have been studied with respect to diabetes. Diabetes is a condition of having originated from various factors but have the main problem with high blood sugar due to the lack of insulin secretion or less insulin sensitivity. There were lack of dose dependent studies and especially in case of probiotics various culture parameters may affect their efficacy also. Besides there are several positive results where well identified probiotics strains showed positive response on metabolism. Therefore, there is urging of more studies for mechanistic exploration of their effects. Interaction of diabetes and obesity particularly in type II diabetes, there is resemblance of systemic inflammation like conditions, which includes obesity, induces the phosphorylation on the serine amino acid residues in its main insulin receptors substrate-1, which immensely deteriorates the insulin sensitivity. Also, the same sub-type microbial components induce the autoimmune response. Various recent studies evidently showed that some probiotics strains have capability to decline the host inflammatory response and also gut permeability. These effects enhance the insulin sensitivity and reduce autoimmune responses (Sáez-Lara et al. 2016; Plaza-Díaz et al. 2015; Backhed et al. 2005; Hooper et al. 2002). Likewise, prebiotics may affect the composition of gut microbiota and their activity and through this way also controlling the diabetes. Importantly, more research is needed to investigate if probiotics can be used against both types of diabetes and if so, what is the complete mechanistic process underlies this (Ferrario et al. 2014; Kim et al. 2013).

5.2 Gut Microbiota and Diabetes

5.2.1 Intestinal Microbiota

The management and complications with studies with diabetes mainly possess hyperglycaemia as highest risk factor. In humans, cellular energy comes from glucose especially specific cells including neurons and red blood cells are dependent on glucose, but with very special note the high glucose level above with certain threshold value could be very detrimental to the health (Asemi et al. 2013). A homeostasis of blood sugar has been maintained within very fine range and through the hormonal regulation of glucose production and consumption/storage. It is a bidirectional communication between the metabolic organs like gastrointestinal lining, liver, brain, muscles, adipose tissues, and pancreas. Following intake of food, digestion takes place in gut and the nutrients absorption takes place through intestinal lining which results into the upregulation of glucose level in blood. During

fasting condition, blood sugar level falls off and then liver starts to produce glucose via endogenous glucose production a process known as gluconeogenesis, which helps to maintain optimum blood sugar level. Increasing blood sugar levels causes the pancreas to secrete more insulin, which increases glucose absorption in muscles and adipose tissue and decreases gluconeogenesis. While in type II diabetes, insulin is not able to increase the glucose transfer in muscles and diminishes gluconeogenesis that leads to increase of blood sugar level a condition known as hyperglycaemia. There are several other illnesses closely associated with diabetes include such oxidative stress, hyperlipidaemia, low immunity, hormonal imbalance, gestational diabetes, and impaired metabolism (Lyssenko et al. 2008).

5.2.2 Diabetes Mellitus (DM)

DM is a metabolic disease demonstrated by high blood glucose level which is the resultant of insulin resistance or deficient in insulin secretion (Ahola et al. 2017). Diabetes is categorized into four key types as follows: type 1 which is insulin dependent, type II-insulin independent, gestational diabetes, and others. Among all of these, type II commonly known as type II diabetes mellitus (T2DM) occupied as the more than 90% of total diabetes case which evidently represent the major threat (Masharani and German 2011; Cavan et al. 2015; WHO 2016; Shaw et al. 2010). Type II represents 90% of diabetes cases.

Nowadays, the management of diabetes has become a global issue, and effective treatment is needed to be found. Medical treatment for diabetes such as insulin injections and oral hypoglycaemic agents caused adverse side effects such as liver problems, lactic acidosis, and gastrointestinal problem (Carrascosa et al. 1997; Kono et al. 1999; Carrascosa et al. 2001).

5.2.3 Type I Diabetes Association of Intestinal Microbiota

First kind of diabetes known as Type 1 diabetes (T1D) is an autoimmune disease featured by immune dysregulation of pancreatic beta (β)-cells. Various genetic and environmental factors play crucial role in this dysregulation of immune response through preconditioning of a hyperactivated immune profile of β-cells. The microorganisms residing in the human intestine influence the enteric mucosal immune response very affectively. Therefore, anomalous pattern in the gut microbiota (dysbiosis) is strongly associated with the pathology of Type 1 Diabetes (T1D) especially to the subjects detected with autoimmune hyperactivity due to a vigorous and robust immune response (Drexhage et al. 2016). The immune cells of an individual are significantly affected by the disproportion of microbiota composition (dysbiosis) which is frequently linked with the progression of T1DM. The development of T1D includes the hyperactivation of self-reactive T-cells which

leads to the execution of β-cells by CD-8+T-cells, which evidences that gut microbes strongly interact with the immune cells (Pushalkar et al. 2018; Knip and Honkanen 2017). The enrichment of gut microbial community by supplementation of probiotics and prebiotics has been reported to strongly link with downregulation of the autoimmune response with very low grade inflammation and the permeability of intestinal lining is also diminished with the simultaneous augmentation of tight junction proteins. This section of the chapter specifically explores the possible role of intestinal microbiota and the immune system which involves the development of T1D with the help of animal and human studies.

Dysbiosis of gut microbiota and/or its metabolism which activates anomalous immune responses in the gut-associated lymphatic tissues (GALT) for example upregulated the expression level of Ig A and proliferation of T regulatory cells (Tregs) (Pabst and Mowat 2012). The disruptions in the microbiota-immune lead to the impairments in systemic infection induced inflammation which is controlled through mainly two pathways. Firstly, activation of innate immune system via Toll-like receptors (TLRs) and secondly, by activation of FFAR2/3 via microbial metabolites such as short-chain fatty acids (SCFAs) (acetate, propionate, and butyrate) and lactic acid as well. Particularly in these SCFAs, butyrate is reported to be linked with the differentiation of naïve T-cells into Tregs, whereas acetate and propionate are responsible for the relocation of Tregs to the intestine (Wen et al. 2008; Shi et al. 2014; Scott et al. 2018). Upregulated expression of TLRs and a significant decline in the secretion of SCFAs, including butyrate, during gut dysbiosis, make them potential therapeutic candidates for the treatment of T1D.

Association of bacterial machinery, for instance, LPS or other metabolic molecules with adaptive and innate immune responses could endow with protective gauges against T1D. Hence, various animal and human studies have been reported the advantageous effect of probiotics and prebiotics on the gut microbiota–immune axis and their impact on the development, progression, and prevention of T1D. Remarkably, the important animal models used to analyse the impact of probiotics, prebiotics on T1D are NOD mice (Chen et al. 2018), Streptozotocin-induced T1D rats and mice (Abdelazez et al. 2018; Yadav et al. 2018), Alloxan activated T1D Swiss Webster mice (Raafat et al. 2016), BBDR rats, and Bio-Breeding Diabetic Pathogen rats (Lau et al. 2011; Sarmiento et al. 2015). There are some significant findings in recent years which revealed the beneficial/positive effects of gut microbes as follows: including Lactobacillus rhamnosus HN001 and Bifidobacterium longum BB536 in several clinical trial in human subjects (Toscano et al. 2017;); Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 (Messaoudi et al. 2011). There are more clinical trials underway for T1DM, mainly using Lactobacillus salivarius, Lactobacillus johnsonii, Bifidobacterium lactis, Lactobacilli plantarum, and Lactobacillus paracasei (Table 5.1).

Table 5.1 Clinical trials underway on T1DM

S. No	Study design (Multistrain)	Type	Age group, subjects	Duration	Link
1.	L. plantarum Heal 9 and L. paracasei 8700:2	Randomized, controlled trials	10–18 years. both gender 200 subjects	12 months	ClinicalTrials.gov (n.d.-c) https://www.clinicaltrials.gov/ct2/show/NCT04014660
2.	L. salivarius, L. johnsonii, and B. lactis	Randomized, controlled trials	06–18 years. both gender 80 subjects	6 months	ClinicalTrials.gov (n.d.-e) https://www.clinicaltrials.gov/ct2/show/NCT03880760
3.	L. paracasei, L. plantarum, L. acidophilus, L. helveticus, B. lactis, B. breve, S. thermophilus	Randomized, controlled trials	5–17 years 60 subjects	3 years	ClinicalTrials.gov (n.d.-d) https://www.clinicaltrials.gov/ct2/show/NCT04141761
4.	L. johnsonii N6.2	Randomized, controlled trials	8–18 years 57 subjects	6 months	ClinicalTrials.gov (n.d.-a); https://www.clinicaltrials.gov/ct2/show/NCT03961854
5.	L. johnsonii N6.2	Randomized, controlled trials	18–45 years 52 subjects	12 months	ClinicalTrials.gov (n.d.-b) https://www.clinicaltrials.gov/ct2/show/NCT03961347

5.2.4 Type II Diabetes Association of Intestinal Microbiota

The intestinal microorganisms are now frequently designated as a secreted organ consisting of trillions of microorganisms which are very crucial for our metabolic health (Patterson et al. 2016). In adults, intestine comprised of approximately 500–1000 various bacterial species with a mass weight of 2 kg appro. Recent metagenomics studies have represented that chiefly two bacterial phyla Bacteroidetes and Firmicute occupied the 90% of the total bacterial species present in the intestine. Also, phyla refer to as Actinobacteria, Proteobacteria, and Verrucomicria are also present but in low amount (Human MICROBIOME PROJECT C 2012a, 2012b; Kalinkovich and Livshits 2019). The microbial composition and volume may vary with the particular region or anatomy and abiotic environmental milieu. The gut microbiota is differentiated by an individual's variability and the variations in hereditary, diet, health status, and hygiene and by age as well (Kalinkovich and Livshits 2019).

A health of an individual is affected by its gut health which is associated with almost all the imperative activities including digestion, vitamins (water soluble) production, energy production from food, toxins degradation metabolites production. These crucial functions enhance the barrier integrity and maintain the functional competence of the epithelial cells of gut which protects from the pathogenic agents (Van de Wiele et al. 2016). While on the other side, dysbiosis is an imbalance of the host microbiota community due to any type of indurations, use of antibiotics, gut inflammation, stress or anxiety, menopause, high toxin secretion, and several other stimulants (Hegde et al. 2018). Moreover, dysbiosis has been associated with a series of disease including cardiovascular (Battson et al. 2018), autoimmune diseases (Opazo et al. 2018), autism (Sgritta et al. 2019), obesity (Bianchi et al. 2018), and T2DM (Karlsson et al. 2013).

Among all the above -mentioned metabolic diseases, T2DM is more likely to link with gut health (Roager et al. 2017; Sabatino et al. 2017). Also, as gut microbiota imbalance is directly responsible for the chronic as well as low grade inflammation. The compositional disturbance of intestinal microbial species is directly related with development of prediabetic conditions like insulin resistance. In the same array, plethora of studies in the recent past demonstrated the impact of gut microbiota of human population with T2DM and also the evaluation of probable link between some specific microbial special and metabolism measures which are important for strengthening the role of the microbiota in the diabetic condition. The main effect of microbiota on T2DM are decline butyrate-producing bacteria (especially Roseburia intestinalis and Faecalibacterium prausnitzii); restrained dysbiosis; pro-inflammatory conditions with amplified expression of microbial genes involved in mounting oxidative stress, downregulated of genes responsible in vitamin production expression, enhanced LPS concentration in serum and also enhanced intestinal permeability (Roager et al. 2017; Sabatino et al. 2017).

Significantly, the supplementation of probiotics and prebiotics has been noticed to be one of the major used therapies to modulate gut microbiota and following this exposure prevention and delay in progression of diabetes observe (Yadav et al. 2006, 2007a, 2007b). These findings were confirmed by animal studies in which mice represented upregulated levels of Bifidobacterium associated with the enhanced glucose tolerance and reduction in inflammation when fed with prebiotics (Cani et al. 2007). Hence, probiotics specifically lactobacilli and bifidobacteria gained significant role as efficient bio-therapeutics for anticipation for diabetes and other metabolic diseases (Panwar et al. 2013).

The term prebiotics is referred as a non-digestible food ingredient that advantageously affects the host by distinguishingly activates the expansion of bacterial species in the gut having very positive effect on the enrichment of gut microbiota composition (Schrezenmeir and de Vrese 2001). Chiefly, there are two bacterial genera used in probiotic preparations are Lactobacillus and Bifidobacterium. Several clinical trials investigating the impact of numerous probiotic strains (Ritchie and Romanuk 2012).

Clinical and experimental studies demonstrated the influence of gut microbiota on diabetes and assess the promising associations between the abundance of definite

bacterial species and metabolic facet, which are basic to elucidate and reinforce the role of intestinal microbiota on the clinical conditions. Various studies have investigated that peoples with T2DM have a high number of opportunistic pathogenic bacteria including Clostridium clostridioforme, Bacteroides caccae, Clostridium hathewayi, Clostridium ramosum, Clostridium symbiosum, Eggerthella sp., and Escherichia coli. Also a low number of butyrate-producing bacteria like Clostridiales sp. SS3/4, Eubacterium rectal, Faecalibacterium prausnitzii, Roseburia intestinalis, and Roseburia inulinivoran. There is a strong correlation between the upregulated levels of Roseburia spp. and butyrate levels with enhanced insulin sensitivity (Vrieze et al. 2012; Karamali et al. 2016; Larsen et al. 2010; Qin et al. 2012). These studies evidently recommend the significance of butyrate-producing bacteria in regulating blood sugar level (Qin et al. 2012). There are various clinical trials which have proved the efficacy of probiotics in T2DM subjects (details are given in Table 5.2).

5.3 Gestational Diabetes Association of Intestinal Microbiota

Gestational diabetes is a transitory condition of hyperglycaemia diagnosed during pregnancy. Echoing hormonal metabolic and immunological alteration occurs during pregnancy to maintain the strain of the developing foetus (Mor and Cardenas 2010). During the first trimester, anabolic processes take place due to the increased insulin production and glucose uptake by adipose tissue to compensate the ample energy supply for the growth of foetus. Hence, the pregnancy often results into the weight gain of pregnant women. Simultaneously, with each trimester, pro-inflammatory cytokines rise, whereas placental and metabolic hormones decrease, resulting in decreased insulin sensitivity. In the last trimester, the gluconeogenesis and lipolysis occur due to high insulin insensitivity and this leads to heightened levels of plasma glucose and FFAs levels in women refer as catabolic state via which foetus receives optimum energy for proper development. Although, women who are unable to recompense for insulin resistance are at high risk of getting the hyperglycaemic condition and developing gestational diabetes (Kim et al. 2014; Plows et al. 2018).

Several studies have found a link between compositional changes in gut microbiota and the pathogenesis of GDM by comparing gut microbiota throughout the first, second, and third trimesters utilising 16S r RNA sequencing. According to studies, the third trimester has the highest concentrations of Proteobacteria and Actinobacteria, with a steady decrease in Faecalibacterium. The Bacteroidetes and Firmicutes, on the other hand, remained throughout the pregnancy. In addition, bacterial abundance decreased as the pregnancy progressed towards the third trimester. (Koren et al. 2012; Avershina et al. 2014; DiGiulio et al. 2015).

Moreover, investigations of GDM in pregnant women revealed a vast array of gut microbiota dysbiosis, which was linked with various pathobionts originated from Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria phyla including Ruminococcaceae, Desulfovibrio, Enterobacteriaceae, P. distasonis, Prevotella,

Table 5.2 Randomized clinical trials conducted in T2DM with different Probiotics

S. No	Study design Multistrain	Groups	Study duration	Diabetes	Reference
1.	(Multistrain probiotics) A randomized, double-blind, placebo-controlled trial	$n = 31$; 14 males and 17 females	Twice-a-day for 6 months	T2DM	Sabico et al. (2019)
2.	UB0316, (L. salivarius UBLS22, L. casei UBLC42, L. plantarum UBLP40, L. acidophilus UBLA34, B. breve UBBr01, B. coagulans Unique IS2) A double-blind, randomized, placebo-controlled study	Total of 126 participants, either sex, age 18–65 years	Twice-a-day for 12 weeks	T2DM	Madempudi et al. (2019)
3.	*L. plantarum* HEAL9 and *L. paracasei* 8700:2 A randomized, double-blind, placebo-controlled clinical trial	118 children	Daily oral administration for 6 months	Peripheral immune response in children with celiac disease autoimmunity	Håkansson et al. (2019)
4.	Lactobacillus rhamnosus GG and Bifidobacterium lactis BB12 Maternal obesity on pregnancy weight-Gain and Birthweight: Healthy Mums and Babies (HUMBA) randomized trial	230 women	≤28 weeks	(Pregnant women) healthy Mums and Babies (HUMBA)	Okesene-Gafa et al. (2019)
5.	Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium lactis, and Bifidobacter longum A randomized controlled trial	120 adults (35 and 70 years)	24 weeks	Prediabetes (Metabolic Syndrome in Prediabetic)	Kassaian et al. (2019)
6.	Randomized, double-blind, placebo-controlled trial; selenium plus;	54 diabetic people	200 μg/day for 12 weeks	Type 2 diabetes mellitus (T2DM) With Coronary Heart Disease	Raygan et al. (2019)

Table 5.3 Total meta-analysis performed in the Diabetes subjects depicting the role of various Probiotic therapy

S. No	Study design (Multistrain)	Groups (Gender biasness)	Study duration	Diabetes	Reference
1.	Randomized placebo-controlled trials Multistrain probiotics	614 subjects		Diabetes	Sun and Buys (2016)
2.	Randomized placebo-controlled trials *Lactobacillus* and *Bifidobacterium*	288 pregnant women	6–8 week	Gestational diabetes mellitus	Taylor et al. (2017)
3.	Randomized placebo-controlled trials *Lactobacillus*, *Bifidobacterium*, or *streptococcus*,	2575	<37 weeks.	Gestational diabetes mellitus	Jarde et al. (2018)
4.	Randomized placebo-controlled trials Multistrain probiotics	1196 pregnant women,		Gestational diabetes mellitus	Peng et al. (2018)
5.	Randomized placebo-controlled trials Lactobacillus	719 participants	4–8 weeks	Gestational diabetes mellitus	Zhang et al. (2019)
6.	Randomized placebo-controlled trials Lactobacillus and Bifidobacterium	$n = 1060$ Age >18 years	6–12 weeks	Gestational or pre-diabetes	Zheng et al. (2019)

and Collinsella, whereas advantageous butyrate-producing bacterial species including Faecalibacterium and Bifidobacterium were reduced. Dysbiosis of intestinal microorganisms in pregnant females is allied with several other malformations including increase in adipose tissue (fat storage), glucose intolerance, and enhanced inflammatory processes which are very much alike to condition of gut microbiota compositional alteration occurred in diabetic patients. The assortment of a suitable and favourable probiotics strain or consortium is very important as the impact of different probiotics is depending on the type of probiotics strain. Details of some clinical trials conducted on gestational diabetes are given in Tables 5.2 and 5.3.

5.4 Nutritional Therapy for Diabetes

More recently various nutritional therapies for diabetes have emerged due to the vast side effects of allopathic medicines. These includes mainly herbal medicines and probiotics which are more specifically using for chronic diseases rather than acute forms as chronic diseases have more deleterious throughout the life span. Several nutraceuticals functional foods or natural herbal medicines in different regimes have tested on diabetic population to validate their positive metabolic effect related to

glycaemia, lipid profiling, and oxidative stress parameters (Dham et al. 2006; Adams and Standridge 2006).

A recent study demonstrated that Dietary Approaches to Stop Hypertension (DASH) diet with probiotics combination known as Pro-DASH diet recovers the blood glucose levels. The bacterial are capable of secreting butyrate compound which is essential for insulin sensitivity thus results into the improvements in glycaemic levels due to the consumption of probiotics enriched diet (Pandey 2016). These results should also examine on the larger subjects and thus might be beneficial for the management and treatment of diabetes. Probiotics have many functions, including anti-oxidation, anticancer, anti-inflammation, and improved metabolism and immunological function. In mice model, it was evident that that some probiotics bacterial species secrete the butyrate and compound linked with the insulin sensitivity by glycaemic index of the animals improved with the probiotics supplemented diet. Although more investigations with large human subjects produce more validated results, DASH diet could be more established as the nutritional therapy for diabetes.

5.5 Factors Help in Establishing Chronic Inflammation

5.5.1 Oxidative Stress

A plethora of studies postulate the crucial role of oxidative stress in the pathophysiology of diabetes and suggests antioxidant activity of lactic acid bacteria on the metabolic regulation (Grajek et al. 2005; Zommara et al. 1994). The whey as the by-product of fermented milk has shown as suppressing activity on the upregulation of lipid hydroperoxide which is activated by bile duct ligation. In this study, rodent supplemented with milk whey and its fermented product presents lower levels of mitochondrial hydroperoxide activity, whereas animals with bile duct ligate feed rated as control counterparts. Also, Lactococcus lactis has antioxidative potential due to having superoxide dismutase (SOD) activity (Sanders et al. (1995). Similarly whey accumulated from cultured skim milk demonstrated heightened antioxidants enzymatic in liver and RBCs of rat animal model (Zommara et al. 1994). The SOD and glutathione peroxide activity is enhanced on diet containing lactobacillus acidophilus in comparison to controlled/normal diet. These outcomes suggest that fermented milk whey formulations produce antihyperoxide and antioxidative potential. Apart from this, some other investigators recognized that most of the lactic acid bacteria demonstrate known as Lactobacillus acidophilus ATCC 4356 and Bifidobacterium longum downregulates the bad cholesterol level through oxidation process (Lin and Chang 2000). Likewise there are reports of postulating antiatherogenicity, strong resistant of lipoprotein longstanding resistance against oxidative pathway enhancing the overall oxidative activity in goat's milk (Terahara et al. 2000; Kullisaar et al. 2003).

Diabetes mainly T2DM is a life threatening metabolic diseases grounded from multiple factors like genetic factors, behavioural alteration, and also environmental

conditions. Since the last two decades, the importance of promising risk factors including various inflammatory mediators, intestinal microorganisms, and oxidative stress in the pathogenesis of T2DM has been elucidated (Bordalo et al. 2017; Gomes et al. 2014; Hu 2011). The compositional changes in the gut microbiota have been reported to affect metabolism of glucose and its bidirectional influence on lipid and insulin levels and these abnormalities induce diabetes (Hartstra et al. 2015) (Fig. 5.1). Some studies in recent years evidence that generous ingestion of probiotics in food as in the form of living microorganisms and prebiotics as non-digestible food constituents could pass positive impact on overall health of an individual by convalescing the gut health encompassing various parameters like total amount of beneficial bacterial species, intestinal integrity, homeostasis of total gut bacteria (Asemi et al., FAO and WHO 2002; Schrezenmeir and de Vrese 2001). Also, biomarkers for glucose and insulin resistance also transformed by gut microbiota (Rajkumar et al. 2014: Ejtahed et al. 2012).

The oxidative stress is referred as a discrepancy between the free oxygen species and regulation by antioxidants which can be altered by enzymes and other regulators. The advantageous impact of probiotic supplementation and the antioxidative agents on blood glucose markers and insulin sensitivity in diabetic patients have been particularly shown in various researches (Tabatabaei-Malazy et al. 2012, 2014; Khodaeian et al. 2015). The bacterial microorganisms specifically Lactobacillus rhamnosus, Lactobacillus lactis, and Lactobacillus plantarum have been reported to be most beneficial in case of increased oxidative stress (Mikelsaar and Zilmer 2009; Uskova and Kravchenko 2009). Some other investigations suggest the probiotics positive induction of metabolic regulators in the form of free oxygen reactive species (Yadav et al. 2008). However this view needs more researches in order to validate this. A recent meta-analysis critically evaluated the interaction between gut microbiota, oxidative stress and T2DM in 13 RCT comprised of 840 subjects. Authors revealed that the intake of probiotics significantly enhanced the serum levels of Fasting Blood Sugar (FBS), antioxidative activity, and total amount of glutathione and malondialdehyde with no significant data of nitric oxide and Hb A1C. These results present that gut microbial community put forth its positive effects in diabetic populations through deteriorating the oxidative stress (Ardeshirlarijani et al. 2019).

5.6 Probiotics + Fermented Foods

Today with huge diabetic population the management of this deadly disease has become a warning concern worldwide and successful treatment is still yet to be established. Hence there is needful urge to search for complementary therapies for diabetes. Many plants have immense anti-diabetic properties which could be used as herbal and probiotics therapies for diabetes and moreover their anti-diabetic properties were enhanced by the fermentation via addition of pro or/ prebiotics (Hartajanie et al. 2018). These fermented products contain increased number of phytomedicines like charantin, peptides, and alkaloids of high anti-diabetic

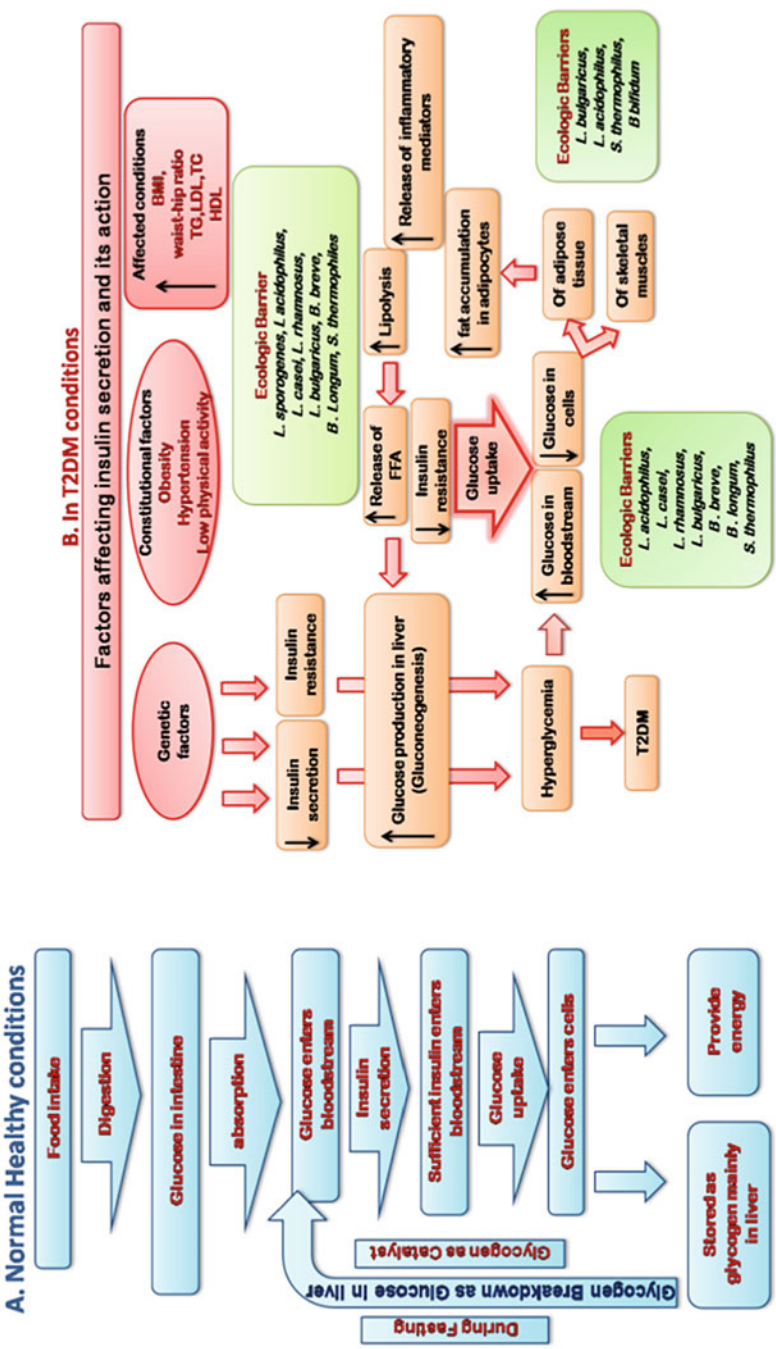

Fig. 5.1 (**a**) Normal healthy conditions and metabolism of glycogen. (**b**) Various probiotic regulated the insulin secretion and helps in maintenance of glycogen during T2DM conditions

potential. These foods are helped to enhance the restraint to blood sugar without upregulation the insulin level and also activating adenosine monophosphate-activated protein kinase which has crucial role in energy consumption in metabolic syndrome (Kumar et al. 2010; Chaturvedi 2012; Joseph and Jini 2013).

Fermented vegetables have improved efficacy than as compared to non-fermented foods. In this array Hartajanie et al. 2020 very first time demonstrated the anti-hyperglycaemic effect of fermented Momordica charantia (MC) juice in combination of Lactobacillus fermented LLB3 (15% enhanced antioxidant activity) which is very high in antioxidants which leads to improved metabolism. However the anti-hyperglycaemic impact of probiotic fermented foods still in its infancy, more mechanistic explorative investigations should have conducted. The cumulative anti-diabetic effect of fermented MC juice is due to the presence of peptides, alkaloids, and antioxidants which decreases the level of blood sugar levels without affecting insulin level (Hartajanie et al. 2020). There are no side effects reported with these fermented foods till now.

5.7 Conclusion

The application of gut microbial community acts as an important regulator in the development and progression of T2D, nevertheless moreover, more investigation needs to be done to the impending influences of probiotics on glucose metabolism. However, results from the animal studies promote the fact that probiotics do have very significant role to prevent avert and decline the severity of diabetes and other associated metabolic diseases probably via transforming gut microbiota composition and inflammation causing factors. In medical studies, the implication of probiotics in modulation of glucose level of diabetic patients has evidenced various outcomes and very few investigations have afforded to examine the different markers of oxidative stress and inflammation which might be exploited apparent links between glucose level control and gut microbiota. Additionally, no clinical trials have been performed on the assessment of GLP-1 and LPS. The analysis of probiotics effectiveness in human subjects is much complex in comparison to experimental models so, accommodating large subjects with regular interval for probiotics therapy should be done since various issue including diet, bad lifestyle, use of medicine or drugs, and endotoxin consumption surely affects the gut microbiota and its control of sugar in our body.

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Mechanisms of Beneficial Effects of Probiotics in Diabetes Mellitus

6

Vivek Kumar Sharma, Thakur Gurjeet Singh, Sonia Dhiman, and Nikhil Garg

Abstract

Diabetes is an ailment of multifactorial origin where metabolic, genetic, and environmental factors have a dominant role to play. The ever-increasing prevalence and complications associated with diabetes mellitus have expanded the research domain and have encouraged the out of the box thinking. In this direction the nutritional physiology and gastrointestinal tract associated control of biological mechanisms has gained much attention. The human gastrointestinal tract contains more than one thousand microorganisms that majorly include bacteria belonging to the bacteroidetes phyla. The intestinal microbial arrangement has found close association with several pathologies including peptic ulcer, inflammatory bowel diseases, asthma, cardiovascular complications, and endocrinal abnormalities including diabetes and dyslipidemia. The gut microbiota constitutes a dynamic environmental aspect that modulates the multifaceted network between genetic and environmental interfaces and influences the pathological progression of diabetes mellitus. The altered state of intestinal microbiota is characterized by increased intestinal permeability, decreased mucosal defense and reduction in tight junction proteins that favors translocation of bacterial lipopolysaccharides and inflammatory mediators into systemic circulation leading to altered immune and inflammatory responses detrimental for diabetes. The composition, properties, and integrity of microbiota may be influenced by probiotics. Probiotics are animate microbes that when used in appropriate quantities are well documented for their health promoting profits.

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Probiotics are investigated for a broad range of malfunctions including obesity, allergy, cardiovascular diseases, hepatitis, and cancer. Although use of probiotics offers a viable and dynamic approach to target molecule pathogenesis of diabetes mellitus (both type 1 and type 2) yet the major concerns include origin, safety, diagnostic identification, absence of genes that may be responsible for antibiotic resistance. The present work gives an insight into the molecular mechanism of probiotics in diabetes and includes reduction of oxidative stress, immunomodulation, improvement in absorption of antioxidants, anti-inflammatory action, and intervention in insulin resistance.

Keywords

Diabetes · Microbiota · Probiotics · Inflammation · Oxidative stress · Antioxidants · Anti-inflammatory · Insulin resistance

6.1 Introduction

According to an estimate, there were approximately 451 million people suffering from diabetes and approximately almost 850 billion dollars have been spent on associated healthcare (Liu et al. 2020). Diabetes mellitus (DM) is among a primogenital ailment reported in Egyptian manuscript about 3000 years ago. The terms "Diabetes" and "Mellitus" have Greek origin where "diabetes" signifies "a passer through; a siphon" while "mellitus" denotes "sweet." The Greeks termed it so owing to the disproportionate urination by diabetics which attracted flies and on the same principle Chinese used it as a method of diagnosis by observing engrossment of ants to a person's urine and in primitive ages, European physicians used to analyze the illness by noticing the urine themselves, an act depicted in Gothic theories (Ahmed 2002; Patlak 2002; Sharma and Singh 2020a, b). DM has arisen as one of the major threats to human well-being the instances of which are increasing exponentially in India (Bommer et al. 2018; Sharma and Singh 2020a, b). DM is an amalgamation of diverse complexities that include episodes of hyperglycemia, lack of insulin, glucose intolerance, defective insulin action, or combination of all. These complications come up due to defective insulin action or signaling which adversely affect storage, mobilization, and metabolism of carbohydrates, lipids, and proteins (Piero et al. 2014). The DM complications comprised of major organ failures, bioenergetic and metabolic complications that may proceed to fatality (Gupta et al. 2020). DM is escorted by risk of peripheral, vascular, cardiovascular, and cerebrovascular complications owed to autoimmune destruction of β cells in pancreas leading to decreased insulin secretion and lowered sensitivity of body cells and tissue to insulin action (Fu et al. 2013). Heredity, lack of physical activities, dietary behavior, obesity, use of medications, stress and environmental factors are the major factors associated with DM. DM is characterized by a chronic state of low-grade inflammation. Over the years, substantiation evidences are there which confirm the role of human gut and associative deformities which leads to a state of dysbiosis and

affect development of insulin resistance and chronic inflammatory process detrimental for DM (Vallianou et al. 2018, Singh et al. 2019).

6.1.1 DM: Major Types

The classification of DM is based on etiology and clinical presentation of the patient. The most important subtypes of DM include Type 1, Type 2, and gestational diabetes. The distinction between the two types (Type 1 and 2) is based on age of onset, gradation of insulin resistance, and loss of β cell function and presence of diabetes-associated auto-antibodies. This classification does not distinguish one type with the other type of diabetes and also does not account for the whole range of diabetic phenotypes (World Health Organization 2019). Type I diabetes (childhood-onset diabetes, insulin-dependent) involves pancreatic β cell destruction and aggravated and comprehensive insulin inadequacy. The pathological outcome of type 1 diabetes (T1DM) is the result of an autoimmune reaction in which insulin producing pancreatic β cells undergoes an innate (inborn) and adaptive immune response which leads to destruction of insulin producing cells. The clinical signs appear when majority of β cells are affected. The total absence of insulin forming cells imposes the daily administration of insulin to optimize the normal glucose levels and to prevent the otherwise possible impairment of homeostasis. This management protocol requires frequent monitoring of glucose levels to escape risk of hypoglycemia (McCall and Farhy 2013; King 2012; Mathieu et al. 2017). Although pathogenesis of T1DM is partially understood, yet the presence of continual inflammatory progression triggered in genetically prone individuals is accepted and this is accompanied by dysfunctional state of oral tolerance to ingested proteins and reformed bile acids digestion which is arbitrated through G.I.T. The most common subtype of T1DM (Type1a) involves autoimmune pathogenesis and detection and presence of auto-antibodies (IA-2, ZnT8, IAA, and ICA) points to a recurrent genetic predisposition. Further, there are reports of less incidences of T1DM in Caucasian Europeans and increased incidence in monozygotic twins which proposes the part played by environmental and genetic factors (Gomes et al. 2014; Simran et al. 2019). The dysfunctional status of intestinal barrier increases the susceptibility of T1DM due to increased intrusion of immune cells which may provoke and make the immune system hostile for pancreatic destruction. This inadequacy of intestinal barrier is prevalent in patients of T1DM (Neu et al. 2005) which results in over exposure of immune system to antigens that initiate an immune-inflammatory cascade proving detrimental for pancreatic framework (Vehik 2011). The leaky gut theory of T1DM is strengthened by the finding that T1DM patients show trepidations in the framework and structural integrity of tight junctions for which inefficient zonulin expression may be responsible to an extent. Zonulin is a protein that regulates permeability of intestine and paracellular space between intestinal epithelial cells thus inefficient expression of this protein may have far reaching consequences for a well-organized intestinal structure (Lam et al. 2012). Zonulin thus not only modulates tight junctions but also influences epithelial

permeability thus may be a potential biomarker to predict intestinal dysfunction. The intestinal microbiota through modulation of immune response influences pathogenesis of T1DM as antigens of dietary or pathogenic origin trigger inflammation and defective intestinal permeability facilitate this infiltration (Atkinson and Chervonsky 2012; Vaarala et al. 2008; Singh et al. 2020a). Type 2 diabetes mellitus (T2DM) affects more than 400 million people and unfortunate predictions are that by 2040 there will be more than 640 million diabetics worldwide. The occurrence of T2DM is influenced by increasing age, obesity, cardiovascular disease, end-stage renal disease, and other metabolic complexities (Singh et al. 2020b, c). The correction of dysregulated metabolism is the aim of treatment which requires combination of corrected lifestyle and pharmacological interventions (Marín-Peñalver et al. 2016). T2DM which is responsible for majority of incidences of DM and is categorized by hyperglycemia which result from insulin resistant and insulin deficient states complimented by secretory defects and inefficient use of insulin physiologically (Cannon et al. 2018). In T2DM the physiological production of insulin may be normal but insulin is ineffective due to development of insulin resistance and there is rise in blood glucose level. The malfunctioned state of T2DM is generally less noticeable thus diseased state continues until complications are ascended. T2DM prevention and delay in progression of pathological cascade can be achieved through lifestyle and pharmacological interventions. Now to intervene glycemic control, use of food supplements, lifestyle modifications, and dietary interventions has increased which may work by modulating and diversifying the microbiota (Tiderencel et al. 2020). Gestational diabetes mellitus (GDM) is not clearly overt diabetes and is noticed in second or third trimester of pregnancy. GDM endures danger of T2DM and affects almost 10 percent of pregnant women. GDM leads to slight elevation of blood sugar levels during pregnancy and if blood glucose is considerably high it may be classified as diabetes mellitus in pregnancy. GDM is diagnosed when women have a fasting sugar level of 5.6 mmol/L or above or 2 h plasma glucose level of 7.8 mmol/L or above (Okur et al. 2017). For diabetes measurement polyuria, polydipsia, frequent weigh loss besides fasting plasma glucose level 2 h postprandial after a 75 g oral glucose tolerance test; HbA1c; and random blood glucose in the presence of signs and symptoms of diabetes are most frequently used. As per WHO people the fasting plasma glucose values of $\geq$7.0 mmol/L (126 mg/dL), 2-h post-load plasma glucose $\geq$11.1 mmol/L (200 mg/dL), a random blood glucose $\geq$11.1 mmol/L (200 mg/dL), and HbA1c $\geq$6.5% (48 mmol/mol) in the presence of signs and symptoms are considered to have diabetes (World Health Organization 2019).

6.1.2 Existing and Novel Therapies

The maintenance of optimal glucose levels is the major desired outcome of DM therapy for which various different oral hypoglycemics are used. These drugs work through different mechanisms. Sulfonylureas and other secretagogues normalize the glycemic state through up-regulation of endogenous insulin secretion while

α-glucosidase inhibitors adjourn carbohydrate absorption through intestine. Thiazolidinediones (TZDs) enhance sensitivity of insulin by increase in peripheral glucose disposal and suppression of hepatic glucose production (Rines et al. 2016). Sulfonylureas (Glipizide), glinides (Repaglinide, Nateglinide), α-Glucosidase inhibitors (e.g., Acarbose, Miglitol), TZDs (Rosiglitazone, Pioglitazone,), incretin-based therapies (Dipeptidyl Peptidase-4 Inhibitors, GLP-1 receptor agonists), amylin agonists (amylinomimetics), and sodium-glucose transporter 2 (SGLT-2) blockers are the major targets in therapy. These are agents which are tried and are being tried in pharmacotherapy of diabetes but majority of them are associated with side effects (GI disturbance, contraindication in renal insufficiency, fluid retention, CHF, weight gain, bone fractures, expensive, potential increase in MI). Also, the novel areas in DM management include stem cell therapy, use of statins, and applications of nanotechnology. Although the list of effective therapies and interventions may be acceptable and successful to an extent yet the associated side effects, recurrence of complications and threat of secondary failure is the major concern of physicians and researchers (Rines et al. 2016; Kumar and Singh 2020). There is a variety of pharmacological agents to treat T2DM while the list is shorter and scarce for the T1DM. For T1DM the only feasible way is to administer the insulin externally as the β cells cannot produce desired insulin thus T1DM patients necessitate lifelong insulin therapy (rapid-acting, long-acting, and intermediate options) (Fig. 6.1). For T2DM too, FDA has approved an injectable medication, pramlintide which is an injectable analogue of amylin hormone and is released like insulin. The gut microbiota primarily influences metabolic conditions and endocrinal functions including diabetes thus manipulation of gut microbiota has emerged as an interesting and feasible area to investigate the underlying pathobiology of metabolic

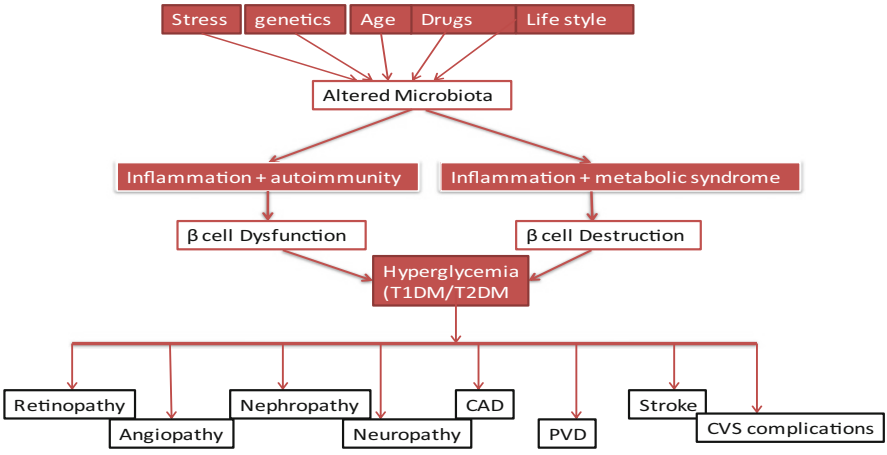

Fig. 6.1 Microbiota–diabetes axis: Dysbiosis constitutes as the major underlying cause of type 1 and type 2 diabetes mellitus
T1DM Type 1 diabetes mellitus, *T2DM* Type 2 diabetes mellitus, *CAD* Coronary artery syndrome, *PVD* Peripheral vascular disease, *CVS* Cardiovascular

complications. In this course use of probiotics is a potential zone that involves modification of clonal flora which has shown encouraging outcomes (Vallianou et al. 2018). These finding gets support form scientific advances that shows prevalence of T2DM is related to swift environmental changes and its undesirable influence on etio-pathogenesis of diabetes. The undesired environmental changes include sedentary lifestyle, stress lack of exercise which may affect and alter gut microbiome composition. Probiotics influence plethora of biological functions including absorption of micronutrients and nutrients, regulation of hormonal levels, immune responses, changes in pH, and antimicrobial functions of gut microbiota (Liu and Lou 2020).

6.2 Host Microbiome

The human gastrointestinal tract (GIT) is a dynamic organ that influences almost all biological processes. It has high density of immune cells and the constituting microorganisms are affected by a variety of genetic and environmental factors. More than 70% of microbes living in intestine establish mutual relationships with the host. The vast bacterial microbiome illustrates divergence in the concentration gradient from proximally to distally and mucosa to lumen and among individuals. The novel advances show that ever-changing adaptive capabilities of gut microbiome can be programmed to prevent and treat T2DM (Liu and Lou 2020). Besides bacteria, the gut microbiota comprised of wider variety of flora that includes protozoa and fungi although bacterial population is prevalent. Five phyla (firmicutes, proteobacteria, Bacteroidetes, actinobacteria, and verrucomicrobia) consist of majority of bacteria which has a physiological role that includes protection against pathogenic microbes and modulate immunity and metabolic processes (Cani 2014; Tremaroli and Backhed 2012). The physiological role of human microbiota includes regulation of lipids, bile acids, proteins, immune system maturation, and resistance against pathogens through activation of host immune system then grooming of immune cells (Baquero and Nombela 2012). The expanded microbial community is extremely sensitive to external triggers thus modulate the physiological and biological response of an individual (Biagi et al. 2016). A newborn's intestinal microbiota consists mainly of Enterobacteria and Bifidobacteria and gets modified to more complex prototype over the years (Zoetendal et al. 2008). These microorganisms and microbial metabolites specifically interrelate with epithelial cells of GIT (intestine) owing to the anatomical significance and mucus production by goblet cells. Mucus functions to insulate bacteria at intestinal barrier level but cannot obstruct the diffusion of bacterial fragments across intestinal barrier but still the binding pattern of recognition receptors influence the process (Wells et al. 2011). Also, the gut microbiome carries millions of genes which have the ability to regulate intestinal processes according to environmental changes. The diverse metabolic activities performed by the intestinal microbiota have led to the emergence of a new term called metagenome which includes genomes of all microorganisms which can live in various organs. The gut microbiota in response to varied ecological and

environmental factors regulates the versatile genetic and environmental network which finally influence the metabolic status by having influence over insulin production, signaling, and resistant states. These physiological and pathological states ultimately decided outcome for T1DM and T2DM (Tonucci et al. 2015). This assumption originates from the results that reveal influence of microbiota on inflammatory states (Cani et al. 2005). The majority of bacterial species constituting microbiota includes Gram -ve, bacteroidetes, and Gram +ve Firmicutes phyla and most of the bacterial population are obligate anaerobes. As, the gut microbiota has great influence on nutritional metabolism and states of metabolism thus it will have a definite impact on metabolically dysregulated states like DM. In several reports, the beneficial effects of *Lactobacillus acidophilus* and *Lactobacillus casei* have been found on fructose induced glucose intolerance and plasma glucose levels. The increased microbiota proportion of *Bifidobacterium* species has also improved the glucose intolerance and escalated the insulinotropic action. *Firmicutes* and *Bacteroidetes* improve the obesity related pathology of diabetes by decreasing body weight and improvement in glucose tolerance concurrently modifying the gut microbiota. Thus, optimizing and diversifying microbiota (through probiotic use) may be potential and feasible area to target metabolic complications including DM. (Naydenov et al. 2012). The altered microbiota (dysbiosis) is characterized by augmented permeability changes in intestine and over mucosal response that has found close association with diabetic progression. The increase in intestinal permeability is the primary event which starts with the abridged and aberrant expression of tight junction proteins. These proteins are of utmost significance as their expression is highly desirable to regulate the integrity and structural framework of intestine. The different or dysfunctional expression of these proteins may favor translocation of bacterial lipopolysaccharide (LPS) and other etiological factors to promulgate metabolic endotoxemia and insulin resistance. Besides the protein expression dietary intake has a major influence on intestinal properties, e.g., high intake of saturated and polyunsaturated fatty acids or scarcity of oligosaccharides modifies the bacterial metabolic action. A diet rich in fatty acids (saturated) has undesired consequences for microbiota that includes increased intestinal permeability, increased prevalence of intestinal infections which propagates metabolic endotoxemia and state of insulin resistance (Cani et al. 2007). The adult microbiota differs in composition and function in normal and diabetic individuals which have a massive impact on the status of energy absorption of ingested foods, mucosal resistance (systemic inflammation), intestinal permeability, and transit-time of gastrointestinal tract (Larsen et al. 2010; Gravitz 2012; Jandhyala et al. 2015). The degraded tight junctions due to dysbiosis lead to increased translocation of whole bacteria and their metabolites through the gut epithelium to the circulation and leads to the consequential increase in intestinal absorbency (permeability) that aggravates insulin resistance mediating metabolic dysfunction and inflammation (Carvalho and Saad 2013; Halmos and Suba 2016). T2DM patients have a greater number of opportunistic pathogens (*Bacteroides caccae, Clostridium clostridioforme*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*) and abridged number of advantageous microbes, especially bacteria producing short chain fatty acids (butyrate) (Qin

et al. 2012; Karlsson et al. 2013; Kesika et al. 2019). The increased content of Roseburia species and resultant optimized levels of butyrate have found close association with insulin responsiveness (Vrieze et al. 2012). Additionally, these bacteria also arouse antioxidant potential (Qin et al. 2012). In this discussion it becomes perceptible that dysbiosis and malfunctioned state of microbiota shares a direct relationship with diabetic progression and correction of this malfunctioned state may be a feasible area for the treatment of DM. The correction of dysbiosis through administration of probiotics has been recognized as an approach with tremendous potential through amending intestinal microbiota may afterward avert the pathological progression of diabetes (Stee et al. 2000).

6.3 Probiotics

The physiological regulation and maintenance of microbiota can be achieved through administration of advantageous live bacteria, i.e., probiotics which confer a health benefit to the host (Adeshirlarijaney and Gewirtz 2020) (Fig. 6.2). According to Élie Metchnikoff probiotics are the beneficial bacteria which can enhance health and prolong life. The concept came into limelight while noticing that high consumption of western diet has disastrous health outcome as it may aggravate production of life-shortening autotoxins by intestinal organisms which hamper the physiological homeostasis in many ways. The administration of probiotics mitigates these effects (Chugh and Kamal-Eldin 2020) and to emphasize their microbial origin, Fuller (1989) defined probiotics as the viable microorganisms that exert a beneficial effect on their host (Fuller 1989) when consumed in appropriate amounts (Guarner and Schaafsma 1998). Probiotics promote health by restricting pathobiont bacterial growth, modification of GI tract, production of short chain fatty acids (SCFAs), pH balance, and stimulation of the immune system (Kechagia et al. 2013). Many of these effects are strain definite thus credentials of a potent and target strain are important to fully unleash the therapeutic application of probiotics. Probiotic strains most often searched for glycemic control include Lactobacillus, *Akkermansia muciniphila, Bifidobacterium*, and *Streptococcus* (De La Cuesta-Zuluaga et al. 2017). Probiotics prove beneficial to the host through maintaining a ratio of beneficial to pathogenic bacteria and also through countering harmful bacteria through secretion of various antimicrobial proteins. Likewise, probiotic microorganisms such as Bifidobacterium adolescentis and Lactobacillus plantarum are natural producers of B group vitamins, enhance the absorption of vitamins, generation of organic acids which finally impact the efficiency of the immunological system. Probiotics also produce enzymes required for various biological processes. Many probiotics have antibiotic properties and immunosuppressive properties. Probiotics antagonize the production of antimicrobial substances, impede the adhesion of pathogens to epithelium, and also support the host immune system while dealing with neoplastic host cells (Markowiak and Slizewska 2017). All these beneficial effects are not only strain specific but individualistic too. The interaction and cross talk of GIT bacteria, epithelial and mucosal lymphoid elements positively

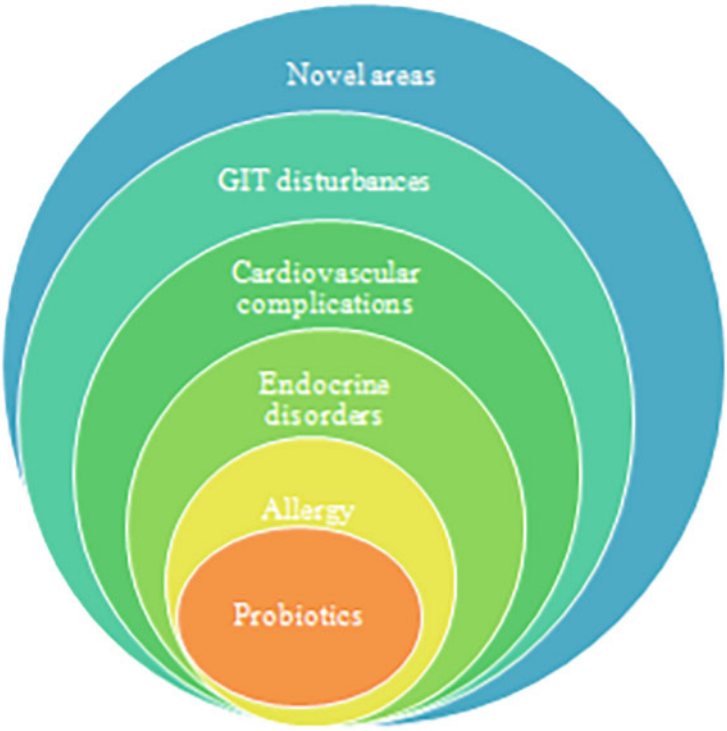

Fig. 6.2 The dynamic and ever-expanding domain of Probiotics
Probiotics offer novel and safer treatment approaches in major organs systems including cardiovascular, endocrinal, and gastrointestinal complication. Novel areas include cancer, peptic ulcer, and immunomodulatory disorders

regulate the host defense and this interaction involves close proximity of microbial molecular patterns involved as Toll-Like Receptors (TLRs) (Kawai and Akira 2006). On this interaction mesenteric lymph nodes up-regulate IgA antibodies against intestinal pathogens and *Lactobacillus* strains stimulate up-regulation of mucous genes in intestinal goblet cells (Macpherson and Uhr 2004; Andreasen et al. 2010; Mack et al. 1999). Probiotics thus by enhancing the mucosal secretion alter the attachment and antigen presentation mechanisms which inhibits the colonization of pathogenic microorganisms (*E. coli*). Besides immune-modulatory effects probiotics also lower blood cholesterol, help to rectify state of insulin resistance, and also act as adjuvant in management of angiogenesis and intestinal diseases owing to their anti-inflammatory and antitumorigenic effects (Moroti et al. 2012; Ejtahed et al. 2012). Furthermore, probiotics also influence the absorption of antioxidants positively and also reduce the postprandial lipid concentrations. These actions directly improve the antioxidant defense mechanisms and thus may be helpful to combat stressful and altered physiological changes (Mikelsaar and Zilmer 2009).

6.3.1 Probiotics in DM

Bacterial phyla have different mechanism of action and influence on carbohydrate metabolism thus influence glycemic control in different and individualistic manner. Carbohydrates are the primary source of energy and also the major dietary component of mammals. They also decide the composition of microbiota by influencing metabolism of carbohydrates and other essential components. Mammals have limited ability to hydrolyze polysaccharides but simple sugar can be absorbed in proximal jejunum. Disaccharides can be hydrolyzed to monosaccharides (Hooper et al. 2002). Bacterial enzymes play an important role to digest undigested polysaccharides and partially digested starches when they reach in vicinity of microbiota (Musso et al. 2011). Bacteria convert monosaccharides to pyruvate through glycolysis and generate ATP. This pyruvate generation undergoes microbial fermentation in the extremely anaerobiotic atmosphere of the lumen and generates energy providing an extent of nutritional value. The recovery of nutritional value of degraded polysaccharides involves various pathways that ensure uptake and utilization of products of bacterial fermentation like SCFA. Butyrate a major constituent of SCFA in colonial epithelial cells is transformed to ketone bodies or is oxidized to CO_2 (Louis et al. 2007). More than 60 percent of energy is provided to colonic epithelium by butyrate while propionate and acetate get absorbed into hepatocytes and execute process of lipogenesis and gluconeogenesis. The production and concentration of SCFA are pH dependent and vice versa. In presence of SCFA, the pH of proximal part of colon is lowered due to increased production and presence of ethyl butyrate while at pH 6.5 higher concentration of propionate is observed. (Louis and Flint 2009; Walker et al. 2005). The microbial composition influences several endocrinal processes and also impacts the antioxidant and anti-inflammatory status of GIT responses. Probiotic consumption regulates these processes by regulating the oxidative stress and inflammatory state as evident in diabetic pathogenesis (Stephens et al. 2009). LPS which is the active component of cell membranes of gram-negative bacteria acts as an endotoxin which may induce inflammatory responses and aggravate oxidative stress. This state of metabolic endotoxemia aggravated by LPS changes the intestinal permeability through derangement of intestinal cell junctions (Cani et al. 2007). The unrestrained potential of probiotics in T1DM can be gauged from the fact that there is a vast difference of microbial composition among normal subjects and patients of diabetes. This difference in microbiota also extends to autoimmune and T1DM patients susceptible for diabetic complications. Innate immunity which is recognized as basic etiological factor in T1DM influences intestinal commensal bacteria and in this response toll-like receptors (TLR) have an important role to play. TLRs are highly expressive receptors on immune and non-immune cells that recognize pathogen-associated molecular patterns and initiate the proceedings of innate immune system. There is a divergent class of microbes that smoothen or inhibit autoimmunity of T1DM through TLR family. The altered state of intestinal microbiota impacts T1DM pathogenesis by disturbing the normal bacterial taxa, bacterial metabolites, and pattern of immunological maturation (Brown et al. 2019) (Fig. 6.3). The leaky gut hypothesis is also applicable to

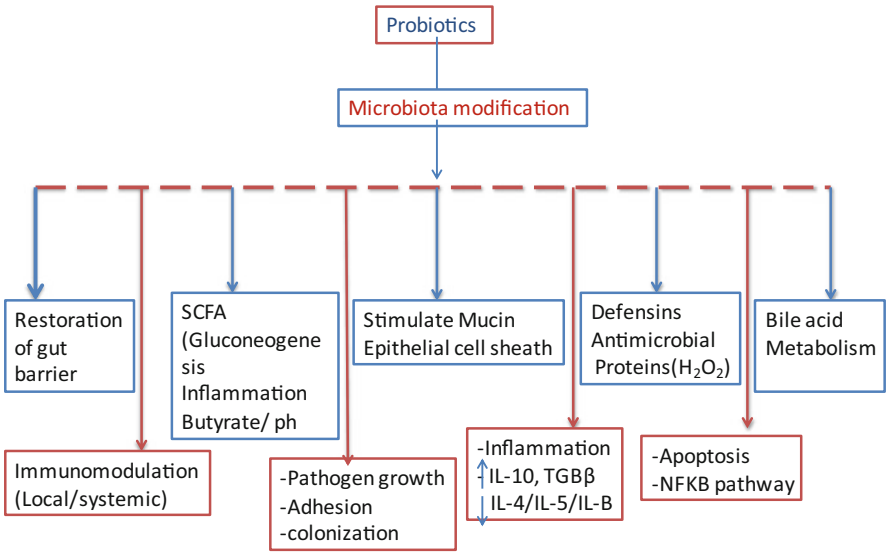

Fig. 6.3 Probiotics and their major mechanisms of action in diabetes mellitus
SCFA Short chain fatty acids, *IL* Interleukins, *NF-κB* Nuclear factor kappa B, *TGF* Transforming growth factor

T1DM as the intestinal barrier dysfunction is common in T1DM. The butyrate producing bacterial species influence the maintenance of intestinal barrier integrity. Butyrate upregulates the expression of claudin-2, occludin, and cingulin which are tight junction proteins thus tightens the epithelial junctions and reduces membrane permeability, leakage, and also induces mucin synthesis. Bacteroides which share a positive correlation with T1DM and allow fermentation of glucose and lactate to propionate, acetate, and succinate also restrict the mucin biosynthesis**.** Rather, they reduce the assembly of tight junctions and increase gut permeability and promote T1D-associated autoimmunity. The bacteria which convert lactate to butyrate increase mucin synthesis, formation of tight junctions and thus facilitate gut health while bacteria metabolizing lactate to other SCFAs impair gut permeability, thus bacteria may also be of great significance for the intestinal barrier function (He Zhou et al. 2020). Probiotics increase the gene expression profile of the intestinal tight junction markers and also prevent translocation of bacterial LPS into the systemic circulation and in this connection Lactobacillus paracasei subsp. paracasei NTU 101 and Bifidobacterium spare of prime significance and improvement of the intestinal environment. This preserves the intestinal integrity and also restricts the infiltration of LPS into systemic circulation (Holst 2007; Balakumar et al. 2018). T2DM is also influenced by environmental factors and LPS mediated low-grade inflammation which ultimately promulgates a state of insulin resistance (Noble et al. 2011; Adeshirlarijaney and Gewirtz 2020). T2DM is characterized by an imbalanced ratio of Firmicutes to Bacteroidetes and the microbes producing lactic acids are also reduced. These species are among the major producer of SCFAs and incretins that

regulate postprandial insulin secretion. T2DM shows an augmented existence of pathogenic bacteria (enterobacteriaceae) (Allin et al. 2015) which increases and propagates a state of inflammation and dysbiosis. Probiotics (Lactobacillus, Bifidobacterium, and Streptococcus) induce the favorable shifts in microbiota composition and prevent the unfavorable metabolic alterations. They also regulate glycemic control via satiety signaling, antioxidant property, and maintenance of gut integrity (Yadav et al. 2007, 2008; Ruan et al. 2015). In its normal state gut microbiota improve insulin sensitivity through activation of bile receptors (TGR5) and also attenuate activation of proinflammatory expression. The resultant decrease of proinflammatory molecules also improves insulin resistance and prevents destruction of pancreatic β cells via decreased activation of NF-κB pathway (Wang et al. 2012; Hsieh et al. 2013).

6.3.2 Mechanisms of Probiotics

6.3.2.1 Anti-Inflammatory

Dietary intake influences and alters plasma concentration of lipopolysaccharides which normally varies with circadian cycle but dietary influence has a major role to play in provocation of inflammatory response. High fat diet induces metabolic endotoxemia through increasing circulating LPS levels which has a well-established role in impeding insulin signaling and development of insulin resistance. This indicates that LPS levels may influence and interact with normal insulin signaling pathways. Thus, metabolic endotoxemia regulates glycometabolic and inflammatory states and that may be by activation of LPS. This LPS–TLR interaction is the main pathway which induces production of proinflammatory cytokines (IL-6 and TNF-α) which have a well-established role in insulin resistance and diabetogenic cascade. Rosiglitazone, which is a proven antidiabetic also, shows its action by lowering plasma LPS levels and serum insulin levels (Noce et al. 2019). The inflammatory response in GIT is mediated by several proteins including high-mobility group protein, flagellin, peptidoglycans, and damage-associated molecular pattern molecules (DAMPS) (Medzhitov 2008). The recognition of these proteins is mediated by specific receptors, including TLR, C-type lectin receptors, and nucleotide binding oligomerization domain-like receptor. Activation of these receptors phosphorylates c-Jun N-terminal kinases (JNKs) and Ikappa B kinase complexes (IKKβ) and this phosphorylation amplifies the inflammatory response (Hirabara et al. 2012). In the similar way TLR4 activates NFκB via toll-interleukin 1 receptor domain-containing adapter protein (TIRAP) and of the TIR domain-containing adaptor-inducing interferon-β [TRIF]-related adaptor molecule (TRAM). TIRAP and TRAM activation triggers the myeloid differentiation factor 88 (MyD88) and the TRIF pathways, respectively. The MyD 88 recruits' proteins of the families Interleukin-1 receptor-associated kinase (IRAK) and TNF-α receptor-associated factor 6 (TRAF6). TRAF6 activates transforming growth factor β-activated kinase 1 (TAK1) which promotes phosphorylation of the kappa beta kinase (IKK) inhibitors α, β, and γ (Akashi-Takamura and Miyake 2008). Phosphorylated IKK

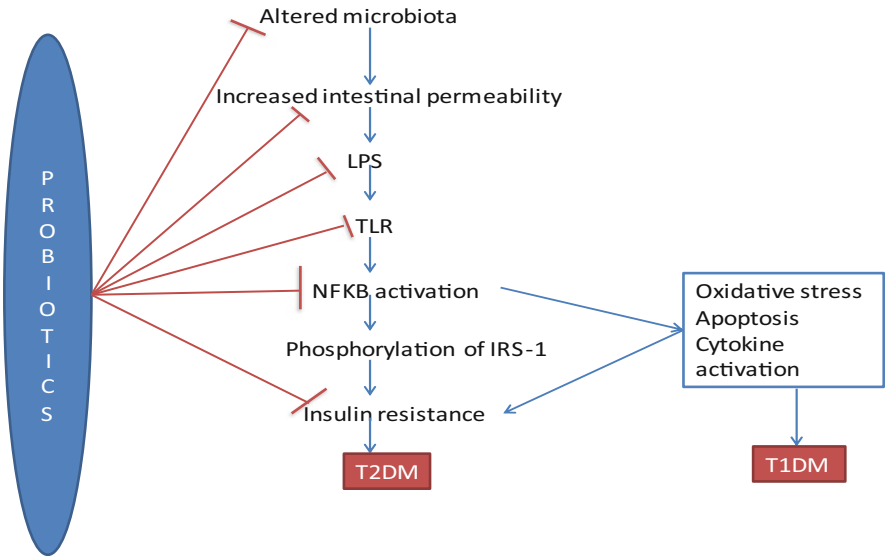

Fig. 6.4 Probiotics and inflammatory cascade in Diabetes mellitus: Probiotics target almost every step of inflammatory cascade in diabetes mellitus (Type 1 and type 2)
LPS Lipopolysaccharides, *TLR* Toll like receptor; *IRS* Insulin receptor substrate; *NF-κB* Nuclear factor kappa B; *T1DM* Type 1 diabetes mellitus; *T2DM* Type 2 diabetes mellitus

complexes degrade the inhibitory kappa B (IkB), translocating the NFκB to the nucleus which induces the expression of proinflammatory cytokines and inducible nitric oxide synthase (Stamler et al. 1997; Sugita et al. 2002). The resultant phosphorylation events inhibit the insulin transduction signal via phosphorylation of insulin receptor 1 substrate (IRS-1) and leads to insulin resistance in hepatic, muscle, and adipose tissues (Ovadia et al. 2011). Other studies have shown phosphorylation of IRS-1 by proinflammatory cytokines (Konner and Bruning 2011) and this activation arbitrates reticence of insulin receptor tyrosine kinase and protein kinase B (AKT) signaling (Saltiel and Kahn 2001). *Lactobacillus kefiranofaciens M* and *lactobacillus kefiri K* are reported to mitigate progression of T1DM through inhibition of pro-inflammatory cytokines and elevation of IL-10 which prevents β cell destruction (Wei et al. 2015). Probiotics by increasing concentration of gram positive and decreasing gram-negative bacteria show desirable action by inhibition of metabolites like tri-nitrobenzene sulfonic acid which inhibits TNF-α gene expression. Figure 6.4 shows the impact of probiotics in inflammation and insulin signaling that converges to counter pathological outcome of both T1DM and T2DM (Fig. 6.4).

6.3.2.2 Probiotics Improve Mucosal Function

Mucus production has an important role to play in regulation of intestinal permeability and adhesion of endotoxins. The intestinal mucosa is the first and prime site for bacterial invasions thus its intact state is providing first line of defense. The mucus layer in GIT is in ever-changing state and it has huge importance for keeping

microbiota intact. The probiotic selection depends on the ability of theirs to adhere to intestinal surfaces and this adherence is provided by the secreted mucus. The attachment of probiotics is a prerequisite step that may decide their influence on GIT and their immune-modulatory effects. The mucus layer which covers the epithelial lining acts as a primary sheath that ingested microorganisms come in contact and are of prime consideration for attachment, adhesion, and colonization. Mucus is subjected to continuous hydrolysis and degradation with formation of novel glycoproteins. These mucin glycoproteins constitute the major building blocks of epithelial tissue which serves both physiologically and pathophysiologically. The bacterial/pathogens which get attached to mucin/mucus cannot react to the epithelial cell and get washed away through these secretions. Interestingly, the composition and degradation of intestinal mucin are transformed as per the changed dietary style. In pathological conditions and scarcity of mucus secretion, the undesirable substances such as acids and microbes can come in contact with the GIT lining and exaggerate harmful immune responses. Besides promoting anti-inflammatory and antibacterial action, probiotics also promote mucus secretory effects of GIT. Microbes like Lactobacilli mount the adhesion proteins (E-cadherin and β catenin) and stabilize the junctional complex through involving protein kinase assembly (Hummel et al. 2012). All these probiotic mediated changes increase the mucus production and aid in maintenance if intestinal integrity and defense mechanisms yet further studies are needed to verify these claims (Plaza-Diaz et al. 2018).

6.3.2.3 Defensins

Defensins are basically antimicrobial proteins and out of three categories (alpha, beta and theta) alpha and beta are expressed in human tissues. They not only have antimicrobial properties but have also shown anti-inflammatory actions. Probiotics also show intestinal protection and normalize microbiota through secretion of defensins from epithelial cells. These are the proteins which have antibacterial, antifungal, and antiviral properties. They also stabilize the gut barrier function and serve as first line of defense with antimicrobial proteins such as C-type lectins, defensins, ribonucleases, and cathelicidins. These proteins kill pathogenic bacteria secretion of hydrolytic enzymes which destroys cell walls and non-enzymatic action disrupts the bacterial membranes. Defensins by attaching to anionic phospholipid groups create the pores in membranes and disrupt the membrane permeability leading to cell lysis (Kagan et al. 1990). Cathelicidins also promote membrane disruption by binding through electrostatic interactions (Bals and Wilson 2003). Probiotic bacteria also fight harmful bacteria through some non-defined mechanisms including production of SCFA, intonation of the pH, and production of specific substances including bacteriocins and bacteriocin-like inhibitory substances. Bacteriocins are basically proteins (ribosomally synthesized peptides) obtained from one species and are active against closely related strain like antibiotics. These structurally and functionally diverse class of proteins kill bacteria by promoting competition among resident and pathogenic organisms and also kill malfunctional proteins by direct elimination. They may also act as signaling peptides or immunomodulators to residing microbiota. Bacteriocins also disrupt the

membrane permeability by pore formation and also disrupt the cellular division processes (Chugh and Kamal-Eldin 2020). Several microbiota including lactobacillus and Bifidobacterium have been reported to act like probiotics and also lead to production of bacteriocins.

6.3.2.4 NF-κB

Nuclear factor kappa B (NF-κB) has a well-established role in apoptosis. These proteins also upregulate cytokine levels and have a role in autoimmunity mediated demise of β cells in pancreas. The elevation of NF-κB closely relates with the pathogenic state in T2DM which is evidenced by the raised levels of NF-κB 1 variants in T2DM (Coto et al. 2018). NF-κB decreases differentiation of β cells and impairs insulin secretion, thus pharmacological modulation of this pathway can be an efficient way to prevent β cell death (Chen et al. 2018). NF-κB has a proapoptotic role in death of beta cells. NF-κB crucially regulates inborn as well as adaptive immunity by controlling more than hundred genes regulating diverse cellular processes. Although the physiological potential of this pathway is well known, but in case of diabetes the role is mainly pathogenic. In normal β cells the pathway is not activated and remains almost in inactive state but due to external triggers like oxidative and inflammatory stimuli, the activation and translocation of NF-κB lead to initiation of pathological cascade. The abnormal environmental triggers activate the phosphorylation event of NF-κB/ IκBα complex followed by ubiquitinoylation and proteasomal degradation leading to rupturing non-covalent interactions and activation of NF-κB. The activated NF-κB through its translocation to nucleus binds to its DNA binding site in the promoter or enhancer regions of specific genes. NF-κB is a prime regulator of genes encoding for proinflammatory, TNF-α, and COX-2. The expression of adhesion molecules and infiltration of IL-1 and IL-6 also depend on activated NF-κB (Karin and Ben-Neriah 2000; Zhou et al. 2001). All these changes promulgate and activate the inflammatory cascade which further devastates the pathological progression in DM (Serasanambati and Chilakapati 2016). As expected, the inactivated state of NF-κB leads to suppression of associated target genes which ultimately improve insulin sensitivity and halt the apoptotic progression in pancreatic islets. Probiotics modulate NF-kB signaling at different sites and processes that includes TLR activation, phosphorylation, transcriptional activation, and proteasomal degradation of IkBα. The probiotic bacteria (*L. reuteri, B. bifidum L. acidophilus, L. rhamnosus GG, B. infantis, and L. salivarius)* have been shown to inhibit NF-κB activation and also suppression of TNF-α and IL 8 gene expression. In this line the reported and other potential probiotics may influence intestinal permeability and insulin sensitivity through regulating NF-κB signaling and resultant anti-inflammatory action (Rabia et al. 2020).

6.3.2.5 Probiotics Modulate Immune Differentiation

Maintenance of GIT homeostasis is majorly influenced by the immune system which helps to establish balance between commensal, symbiotic, and opportunistic strains. To maintain this balance communication between immune system and microbiota is

of great importance as the altered state of immunity is primarily involved in diabetes pathogenesis. Immune system intonation by probiotics is the major areas of probiotics action in almost all types of diabetes. Most of the immune cells show intestinal existence thus probiotics have direct influence maintenance of the body's immune system. Innate immunity although has a protective and physiological role yet the exaggerated response may be harmful to the host. There are divergent classes of probiotics which stimulate and regulate numerous features of innate and acquired immune reactions (Gill and Prasad 2008). Probiotics are also reported to influence host immune response. The common probiotics of food industry (Lb. rhamnosus, S. cerevisiae Lb. casei, B. animalis, etc.) show physiologically protective response by up-regulation of anti-inflammatory genes (Plaza-Diaz et al. 2014). Lactobacillus johnsonii provides immune regulation and immunity to the development of T1DM while B. animalis proves beneficial through downregulation of proinflammatory markers and plasminogen activator inhibitor-1 (Amar et al. 2011). Probiotics maintain a sensitive balance between normal and excessive defense response including innate and adaptive immune response. The major targets of probiotic actions include points of interaction between bacterial and intestinal epithelial cells, infiltration of T and B cells. Apart from them, probiotics may also modify bacterial genome, host genes, and gene expression and signaling pathways. Overall probiotics work through modulating properties of mucosal layers and regulate tight junctions in epithelium which regulate immune reactions relevant to metabolic complications.

6.3.2.6 Effects on Oxidative Stress

In case of dysbiosis there is excessive proliferation of intestinal microbiota which induces metabolic endotoxemia and oxidative stress. Probiotics by lowering pH provide unsuitable environment for growth of bacteria and also produce substances like bacteriocins and biosurfactants which are harmful for pathogens. Hyperglycemia is consistently associated with increased oxidative stress which induces molecular, cellular, and organ damage. Oxidative stress mediated increased non-enzymatic glycosylation and decreased antioxidant potential contributing to pathogenesis of DM (Pasupathi et al. 2009). Probiotic supplementation (yogurt) has shown beneficial results in diabetes by decreasing fasting blood glucose, Hb1c and increasing levels of antioxidants superoxide dismutase and glutathione while malondialdehyde levels were decreased. L. casei has a potent antioxidant potential and it suppresses the activation and functional status of CD4+ T cells, accompanied by reducing the proinflammatory molecules. Also, other stains including L. paracasei, L. plantarum, L. rhamnosus, L. fermentum have shown antioxidant activities in vitro and in vivo. *Lactobacillus johnsonii* increases number of paneth cells which are important regulator of intestinal barrier and producer of antimicrobial proteins. Probiotics hydrolyze proteins and peptide metabolites thus produced also shows antioxidant, anti-inflammatory, and immune-modulating effects. Probiotics also directly increase activity of antioxidative enzymes, e.g., catalase, glutathione S-transferase, glutathione reductase, etc. Probiotics mediate these antioxidant actions by modulating different pathways that include Nrf2-Keap1-ARE (Nrf2 activation detoxify xenobiotics and ROS), PKC, MAPK, etc.

6.3.2.7 GLP Secretion

Presence of Nutrients in GIT Leads to Release of Gastric Inhibitory Peptide, GLP-1

There are diverse class of substances which are released in GIT in response to nutrients which includes incretins, gastric inhibitory polypeptide (GIP), and glucagon-like peptide-1 (GLP-1) which are responsible for more than 50 percent of postprandial insulin secretion. T2DM is characterized by impaired incretin effects and loss of insulin secretion in response to GIP and reduced potency of GLP-1. GLP-1 and glucagon-like peptide-2 (GLP-2) derived from enteroendocrine L-cells have emerged as important targets for the treatment of diabetes and other metabolic complications (Madsen et al. 2019). GLP-1 receptor agonists through their intestinotrophic and gastrointestinal effects have proven the antidiabetic potential. Besides GLP-1, the cosecreted GLP-2 also has beneficial and target specific effects for diabetes that include epithelial proliferation, stimulation of nutrients, improvement of mucosal integrity, nutrient absorption, improve mucosal integrity, and reduce gut permeability. Thus GLP-1/2 co-agonists can be supposed to have better effects through modulation of GIT than GLP-1 agonist alone (liraglutide). The gut microbiota modulates expression and secretion of GLP. The hypoglycemic and insulinotrophic effects of GLP are facilitated by SCFA, particularly, butyrate after the intake of prebiotics (Yadav et al. 2013). SCFA stimulates GLP-1 production from L-cells. In case of microbiota Firmicutes:Bacteroidetes ratio is important to be influenced by high fat diet and this ratio can be optimized by use of probiotics. Firmicutes being major SCFA producers contribute to host metabolism and adiposity by assisting efficient nutrient processing and energy conservation. The supplementation of probiotics to diabetic rats also increases bioavailability of gliclazide, an oral sulfonylurea antidiabetic drug (Al-Salami et al. 2008). GLP-1secretion impacts diabetes pathology by decreasing glucotoxicity, improving carbohydrate metabolism and also improves insulin responsiveness. GLP-1 inhibits postprandial hyperglycemia by increasing output of insulin from pancreatic β cells. These beneficial effects of GLP-1 can be mimicked by administration of probiotics (Maryam et al. 2017) while the dysbiosis produces contradictory results including resistant state of insulin and glucotoxicity. The state of GLP-1 resistance due to dysbiosis also leads to malfunctioning of gut–brain axis which has negative impact on metabolic functions including reduced gastric emptying and reduced insulin secretion (Yamane and Inagaki 2018). Lactobacilli also reprogram intestinal cells into glucose-responsive insulin-secreting cells and therefore ameliorate hyperglycemia and diabetes (Duan et al. 2015). Overall the dual GLP agonist shows various beneficial effects on metabolism by increasing abundance of members of Akkermansia muciniphila and Clostridiales among others.

6.3.2.8 Short Chain Fatty Acids

The indigestible carbohydrates are converted to hexose sugars and probiotics employ them to produce SCFA (butyrate, acetate, etc.), branched chain fatty acids (isovaleric, isobutyric acids), organic acids (lactic acid and acetic acid), etc. The

majority of energy produced is derived from butyrate that yields approximately 70 percent of energy which is utilized by brain cells, muscles, and colon. Lactic acids by inducing consumption of oxygen decrease the pH and make the environment unsuitable for growth of pathogens by promoting anaerobic conditions. The pathogen resistant atmosphere (decreased pH and anerobic environment) is mediated through mediation of G protein coupled receptors (GPR43). SCFA, mainly butyrate and propionate by binding to GPR43 escalates production of anti-inflammatory cytokines (IL-10) and also has immunomodulatory effects (Chugh and Kamal-Eldin 2020). When production of SCFA is decreased especially in metabolic malfunctioned state (DM/T2DM) there is also decrease in production of SCFA. The imbalanced intestinal microbiome and associated decrease in SCFA hamper several physiological pathways especially endocrinal/metabolic axis. SCFAs help to maintain intestinal and immune homeostasis by regulating pH, absorption of calcium, iron and are also beneficial for glucose and protein metabolism in the liver thus their reduction negatively impacts normal structure and integrity of intestine (Markowiak and Slizewska 2017). SCFA also inhibits activation of NF-κB by negatively impacting translocation of NF-κB complex to nucleus. They also encourage growth of beneficial microbes, increase intestinal blood flora, increase epithelial differentiation, and help to maintain integrity of intestinal membranes. SCFA also increases mucin and glycoprotein synthesis and thus aid in preventing pathogenic contact through reinforcement of mucosal defense. SCFA by stimulating synthesis acetyl-CoA may aid in fat biosynthesis guaranteeing the integrity of mucous membranes. SCFA impacts synthesis of incretins (GLP-1 secretion) and helps to maintain insulin sensitivity (Maryam et al. 2017). They are also primary source for enterocytes and promote intestinal epithelial growth by promoting cell proliferation; modifying expression of proteins that regulates tight junctions of intestine, enhancing barrier function. Only the intact epithelial barrier stop the entry of proinflammatory factors into bloodstream (Liu et al. 2020; Zheng et al. 2020).

6.3.2.9 Insulin Resistance

Insulin is a vital hormonal component that controls glucose homeostasis. Activation of insulin receptors through autophosphorylation leads to tyrosine phosphorylation of insulin receptor substrates (1 and 2). The phosphorylation of insulin receptors substrates activates PI3K leading to serine phosphorylation of protein kinase B (AKT) leading to muscular glucose transport, synthesis of glycogen in liver, and lipogenesis in adipose tissue. The proper functioning of this insulin signaling (insulin-insulin receptor -insulin receptor substrate-PI3K-AKT) is very important for proper maintenance of glucose homeostasis. This signaling may be disrupted by mechanism that include serine phosphorylation of insulin receptor substrates by decreased tyrosine phosphorylation of IRS-1, c-Jun *N*-terminal kinase (JNK), and by inhibitory κB kinase (IKK)-β. As discussed earlier, high fat diet induced elevation of LPS levels induces metabolic endotoxemia (Cani et al. 2007). LPS induced induction of TLR upsurges the actions of several phosphorylation dependent and independent proteins (JNK, TNF, IRAK, TRAF6, and TAK1). The activated JNK and IKK-b/NF-κB pathways promote macrophage infiltration and upregulate

proinflammatory cytokine mRNA expression, such as IL-1β, IL-6, and TNF-α in liver and muscle tissues. IFN-γ, IL-17 also lead to insulin resistance and induced by high fat diet. The JNK and IKK-b are entrusted with serine phosphorylation of insulin receptor substrate (IRS-1Ser 307), suppression of PI3-K, and downregulation of Akt/Ser473 serine phosphorylation thus their impaired state reduces insulin signaling and impairing glucose uptake in peripheral tissues (Cani et al. 2007; Amar et al. 2011). The altered state of microbiota influences insulin resistance through LPS-associated low-grade inflammation which has been confirmed by the use of multiple probiotic treatments (Amar et al. 2011; Hasain et al. 2020). In all forms of diabetes, immunity and inflammation have a decisive role to play. The low grade but a chronic inflammatory response is consistently associated with insulin resistance (T2DM). Among bacteria, E. coli is more closely associated with insulin sensitivity which is gram-negative bacteria and has higher levels of lipopolysaccharides and induces endotoxemia and inflammation. Interestingly the diet poor in carbohydrate and fiber content thereby supplying less amount of SCFA increases growth of Gram-negative bacteria thus may hamper insulin sensitivity. Microbe derived proinflammatory metabolites have a role in insulin sensitivity by having impact on regulation of proinflammatory genes and translocation of lipopolysaccharide from the gut into portal circulation (Adeshirlarijaney and Gewirtz 2020).

6.4 Existing Drugs Targeting Microbiota

Metformin is one of the most successful treatment options for T2DM and its mechanism of action is partially understood, it both has AMPK dependent and independent mechanisms to improve blood glucose levels. It also controls mitochondrial framework that influences hepatic gluconeogenesis by activating AMPK and ameliorating glucagon-induced cAMP. It also works by increasing synthesis of SCFA and affects mucus synthesis and hydrolysis by controlling microbial population. Moreover, there are ample evidences of its role in modulation of gut microbiota to improve glycemic control as it is reported to influence anaerobic glucose metabolism in enterocytes. The influence of metformin on microbiota is further proved by lack of potency of intravenous metformin to regulate hyperglycemia, indicating that intestine is the major site of action. It also normalizes intestinal microbiota and increases the abundance of E. coli and condenses the plenty of intestinibacter that impacts amalgamation of SCFA. It also influences secretion of GLP-1 and alleviates insulin resistance and improves glucose tolerance. All these effects are produced by normalizing microbiota and its probiotics mimicking effects (Liu et al. 2020). Acarbose is a α-glucosidase inhibitor and is an efficacious therapeutic option for T2DM. It delays absorption of complex carbohydrates through inhibition of pancreatic amylase and glucosides. Acarbose also alters microbiota composition and after acarbose treatment the abundance of beneficial bacteria (B. longum) and decrease in LPS mediated inflammatory mediators are noticed (Su et al. 2015; Zhang et al. 2017a, b). Acarbose positively regulates bile acid metabolism by increasing the

number of lactic acid bacteria and bifidobacteria in the intestinal flora (Liu and Lou 2020). Sitagliptin, a DPP-IV inhibitor also improves intestinal microbial structure and also reduces intestinal inflammation and also preserves intestinal integrity (Yan et al. 2016). Vildagliptin also improves balance of beneficial to pathogenic bacteria and normalizes the Bacteroides-Prevotella ratio (Zhang et al. 2017a, b).

6.5 Herbal Agents and Gut Microbiota

Herbal treatment is also an important aspect of antidiabetic therapy because of their diverse range of action. Microbiota absorbs and metabolizes herbs into pharmacologically active metabolites which have important pharmacological effects and they have diverse range of mechanism of action. Several potent and successfully tested herbal derived drugs and herbs like alliin, resveratrol, berberine, pectin, galactomannan, red pitaya, and cranberry proanthocyanidins have intestinal bioactivity and have reported antidiabetic effects. Majority of these herbs increase fecal butyrate concentration and produce desirable effects. Baicalein, oroxylin A, quercetin, ginsenoside, wogonin, sarsasapogenin, isopsoralenoside modulate the NF-κB and MAPK pathways and produce anti-inflammatory action in T2DM. Alliin from garlic also improves beneficial microbiota and aids in maintaining glucose homeostasis and insulin sensitivity. Berberine by increasing levels of SCFA decreases serum lipid level, decreases chemoattractant proteins; decreases LPS binding proteins; lowers blood lipid and glucose levels (Lin et al. 2019; Adeshirlarijaney and Gewirtz 2020). The active constituents of herbs including tannins, glycosides, steroids, coumarins influence microbial composition and maintain intestinal permeability, epithelial proliferation; attenuate LPS; mediate inflammatory state; and regulate insulin signaling avoiding reaching a state of insulin resistance (Lin et al. 2019). The metabolism of herbals drugs is influenced by several enzymes produced by microbiota that includes Azoreductase, β-D-glucuronidase, etc. which converts herbal drugs into their active and potent metabolites (Lin et al. 2019). The majority of these effects involve mucosal defense, intestinal permeability, and inflammation.

6.6 Microbiome, Probiotics, and DM: Epigenetic Connection

Epigenetic alterations involve heritable alterations in gene functions without a change in the nucleotide sequence. The major epigenetic changes include DNA methylation, histone modifications, and noncoding RNAs. DNA methylation is necessary for cell-specific gene expression and has a decisive role in embryonic development, establishment of imprinting and X chromosome inactivation (Dethlefsen and Relman 2011), The microbiome regulates host genes epigenetically and it has been proved that intestinal microflora is a epigenetic controller that may influence host metabolism through DNA methylation (Kumar et al. 2014). All the major epigenetic changes are evident in T2DM (Stitzel et al. 2010; Stoffers et al. 2003) and gut microbiota and modulate these changes and affect immune

homeostasis. Microbiota is the major producer of several metabolites that includes bile acids, vitamins, organic acids, etc. Microbiotas are also responsible for production of B group vitamins which by acting as enzyme cofactors contribute significantly to epigenomic processes. Nicotinamide adenine dinucleotide (active form of niacin) acts as cofactor of NAD-dependent histone deacetylases (HDACs) which catalyzes the deacetylation of histones. Niacin also interacts with microbiota and controls glucose metabolism. Pantothenate by donating acetyl group donor allows conversion of coenzyme A to acetyl-CoA that is prerequisite for histone acetylation. Folate from dietary sources as well as synthesized for colonic bacteria acts as a key methyl group donor in DNA methylation. Vitamin B2 serves as a cofactor of methylene tetrahydrofolate reductase which is a folate-dependent enzyme involved in DNA methylation. The dietary supplementation of methyl donors (betaine, folate, etc.) changes the microbial population indicating the putative role of microbiota-metabolized folate in epigenetic regulation. Also, firmicutes in the maternal gut and the differential methylation rates in *UBE2E2* and KCNQ1 share a positive relation for insulin sensitivity. These studies provided a possible connection between gut microbiota and epigenetic processes, particularly the methylation of T2DM associated genes. SCFAs produced by microbial fermentation of carbohydrates and their levels are dependent on microbial concentration. Butyrate and acetylate inhibit DNA acetylases and also influence post-translational modifications. They may also act as DNA acetylases thus impact decondensation and chromatin relaxation. The microbes or microbiota that positively regulates SCFA levels may have epigenetic effects on host (Lee et al. 2017). SCFAs also share epigenetic connection with obesity and DM. It has been found that butyrate as well as the methylation levels of its receptor (FFAR3 promoter gene) was lowered in diabetes and obesity cases compared to control. Also, the body mass index and methylation levels of FFAR3 share an inverse relation. In experimental set-up it has been found that dietary richness of SCFA content may protect animals from T1DM via immunomodulatory mechanisms that involve expansion of T cells and reduction in B cells, CD4+, and CD8+ T cells. The butyrate endowed protection mechanisms also involves DNA methylation and histone acetylation dependent mechanisms that promote gene transcription of podocytes related functions. Also diets rich in fibers enhance butyrate production and downstream mechanisms related to suppression tumor growth too (D'Aquila et al. 2020). The environmental and dietary modifications and challenges have potential to cause aberrant chromatin modification thus epigenetically modifications offer a therapeutic viability to treat DM.

6.7 Major Concerns

Through several studies probiotic supplementation has been showcased as putative supplementation to improve health status of DM yet there are contradictory results too. In various studies there was no impact on glycemic and metabolic status with the supplementation of several probiotics including (*L. salivarius, L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum*, etc.) (Kesika et al.

2019). The concept of consumption of microorganisms to induce health benefits has fascinated humans for centuries. At present probiotics have emerged as a multibillion industry which gets support of physicians, specifically gastroenterologists (Hoffmann et al. 2014; Draper et al. 2017). But various regulatory authorities, European Food Safety Authority and USFDA have yet to approve any probiotic formulation as a therapeutic modality thus the major concerns include safety, interaction, and viability in GIT. Probiotics modulate immune system, suppresses growth of pathogens, provides protection against physiological stress, and helps to maintain optimum microbiota but the experimental studies carried out till date utilize cell culture settings that may not account for exact physiological set-up which mimic inter-microbial and host–microbe interactions. Apart from that, GIT is a dynamic and continuously changing system and thus its replication is challenging for in vivo studies. Moreover, the present studies are mostly carried out in murine models and probiotics have different colonization capacity in these models as compared to human physiological scenario. The adverse effects of probiotics which may be affecting at level of transcriptome need to be comprehensively assessed. The long-term clinical effects of treatment also need consideration (Suez et al. 2019).

6.8 Conclusion

The recent technological advances supplemented by past studies have thrown a deeper insight into the biologically significant interactions between mammal hosts and indigenous microorganisms. The microbial dysbiosis shares a strong linkage with plethora of complications ranging from cancer to obesity and diabetes. The intestinal microbiome has emerged as a therapeutic viability in diabetes owing to their interaction and influence at various signaling pathways (antioxidant, anti-inflammatory, immunomodulatory, integration of intestinal membranes, etc.). Although use of probiotics is associated with bit of skepticism yet microbiota, modulation of microbiota and perseverance of microbiota through use of probiotics is ever-expanding dynamic area which offers numerous targets to treat diabetes.

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Preventive and Therapeutic Role of Probiotics in Type-2 Diabetes and Its Associated Complications

7

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Abstract

Our lifestyle and dietary structure have changed significantly due to rapid economic growth and improvement of living standards, accelerating occurrence of metabolic disorders such as type II diabetes and other non-communicable diseases. In recent decades, T2DM and its complications have increased dramatically worldwide. As per the recent report, 463 million peoples are living with diabetes, and it has been estimated that the number will rise to 700 million by 2045. T2DM is inferred from multifactorial sources, including genetic and environmental factors. Different therapeutic strategies have adopted, and several medicines developed that work in various ways to promote glycemic management in T2DM, current treatments for T2DM have some drawbacks. Nowadays, the role of microbiota in T2DM pathogenesis has taken into consideration. Some earlier evidences suggest that even the composition of the gut microbiome may lead to T2DM. Since then, tremendous efforts have made to explore the relation between the composition of gut microbiota and T2DM, as well as the role of probiotics in the modulation of gut microbiota. Our current food habits will disturb the gut microbiota composition. Ingestion of probiotics maintain the dysbiosis and produce some secondary metabolites like bacteriocins, short-chain fatty acids (SCFAs), and other organic compounds. These compounds are acting at various levels in controlling metabolic disorders. A recent study has also reported that the dead cells can also be working by maintaining the permeability

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of intestine barriers. In this chapter, we summarized the relevant results and addressed the close association between intestinal microbiota and T2DM. In this chapter, we summarized the beneficial effects of probiotics on improving glycemic control of T2DM with relevant results and addressed the close association between intestinal microbiota and T2DM in detail.

Keywords

Type-2 diabetes · Probiotics · Gut microbiota · Prebiotics · Diabetes

7.1 Introduction

Currently significant scientific evidence is available regarding the effects of microbiota on glucose metabolism in T2DM subjects. In this chapter, we briefly discuss disbiotic microbiome of T2DM patients and summarize the most reliable findings for use of probiotic for glycemic control. Probiotics not only control the glucose hemostasis but it also play a significant role in regulating the comorbidities associated with diabetes like obesity, hypertension, inflammation, oxidative stress lipid abnormality, and some brain disorder. All this diseases/ disorders are non-communicable and very much interlinked with the center point of gut environment; once the gut system is maintained properly all other abnormalities can be significantly controlled or improved. Here we also brief the benefits of probiotics in improving glycemic control in T2DM and its associated complication.

7.2 Brief of Gut Microbiota in Type 2 Diabetes (Dysbiosis and T2DM)

Dysbiosis do not directly cause diabetes but it can induce oxidative stress and inflammation, two most common factors in pathophysiology of diabetes.

Earlier, it is believed that the mammal's gastrointestinal tract is sterile at birth and gut microbial flora colonization in infants start during delivery and further develop during breastfeeding. (Abdul-Ghani and DeFronzo 2010). However, this belief has recently revised that original colonization usually starts during gestation (Walker 2017). Evidence suggests that bacteria of the maternal intestine are generally transferred through the blood circulation of the mothers and later travel into the placenta, and eventually enter the amniotic fluid (Aagaard et al. 2014; Cao et al. 2014). In the human gut, there are mainly five phyla present, and their composition varies based on age and food habits. Newborn intestinal microbial flora generally exhibits low diversity and has a comparatively large concentration of phyla proteobacteria and actinobacteria (Turroni et al. 2008; Rodríguez et al. 2015) and it slowly shifts into a more complex form in adults (Rajilić-Stojanović et al. 2007; Zoetendal et al. 2008). Metagenomics studies have shown that approximately 90%

of the bacterial phyla in the adult gut belonged to the phyla of Bacteroidetes and Firmicutes (Blaut and Clavel 2007; Ravel et al. 2014; Rinninella et al. 2019).

These complex microorganism and their metabolites interact differently, in the small and large intestines, with the intestinal epithelial cells (Hsiao et al. 2008). Mucus layers serve as a bacterial insulator at the level of the intestinal barrier. Still, it does not entirely inhibit the diffusion of bacterial fragments across the intestinal barrier and its binding to pattern recognition receptors. This mechanism not only contribute significantly to the defence of the intestinal barrier, but also to an innate and adaptive immune response (Wells et al. 2011).

Diet is essential for intestinal microbiota regulation. Due to our modern lifestyle, we are consuming mainly processed food with excess nutrients such as saturated (De La Serre et al. 2010) and polyunsaturated fatty acids (Kankaanpa et al. 2001) or less oligosaccharide (Shoaf et al. 2006) and phytochemicals (Carrera-Quintanar et al. 2018). These food patterns can alter the bacterial metabolic activity. High fat diets affect the gut microbiota resulting in greater intestinal permeability and vulnerability to microbial antigens. Reports have shown that decreased bifidobacterium due to high-fat dietary consumption has been linked with higher LPS concentrations in serum, one of the features of metabolic endotoxemia (Cani et al. 2012). (Metabolic endotoxemia elaborated in Chap. 9). In addition, this typical diet enhances the oxidation of fatty acids in the liver and adipose tissue. Research findings indicate that the reactive oxygen species (ROS) lead oxidative stress because of polyunsaturated fatty acid oxidation and it reduces mucous development. This directly damage the epithelial cell membranes, enhancing the permeability of the intestinal tight junction by stimulating proinflammatory signaling cascades (Muccioli et al. 2010) and indirectly via increasing barrier-disrupting cytokines [TNFα, interleukin (IL) 1B, IL6, and interferon γ (IFNγ)] and decreasing barrier-forming cytokines (IL10, IL17, and IL22) (Rohr et al. 2019). Mild chronic inflammation is one of the characteristic features of metabolic diseases such as obesity and T2DM, which may occur due to the activation of toll-like receptors by LPS, which are present in the cell wall of gram −ve bacteria. Toll-like receptors 4 (TLR4) are present in insulin targeted tissues (Boulangé et al. 2016; Rogero and Calder 2018).

Through activating cytokine-signaling cascades alongside the increased concentration of reactive oxygen species (ROS), these actions may be compromised upon stimulation of TLR4.

Inflammation levels are a key element in the development of insulin resistance, contributing to a deficiency in the action of insulin (Boulangé et al. 2016). Extending the duration of defects in insulin action causes the overproduction of insulin, which leads to a defect in the pancreatic cells, leading to a defect in insulin secretion (Boulangé et al. 2016). It is resulting in T2DM.

The Intestinal tight junction is a multi-protein complex that forms a selective permeable seal between adjacent epithelial cells and demarcates the boundary between apical and basolateral membrane domains.

7.3 Therapeutics for T2DM

Nowadays, T2DM management has become a worldwide epidemic, many therapeutic techniques have been adopted and a wide variety of drugs have been produced to enhance glycemic regulation through improved insulin production and utilization, decrease sugar production and absorption, inhibit glucose re-sorption, and enforce urinary glucose excretion. These are achieved by mainly 5 type of drugs, namely biguanides, sulfonylureas, a-glucosidase inhibitors, and thiazolidinediones (TZDs), which are used to treat hyperglycemia (Chaudhury et al. 2017). Generally, it is commonly accepted that new T2DM therapies have some adverse side effects such as liver disorders, lactic acidosis, and gastrointestinal issues (Manandhar Shrestha et al. 2017). Therefore, alternate methods focused on intestinal microbiota were investigated, indicating promising prospects for the future T2DM intervention (Gérard and Vidal 2019).

7.4 Studies on the Glycemic Control of Gut Microbiota in T2DM

In the last two decades, numerous studies reported the beneficial effects of gut microbiota on metabolic diseases, including T2DM. Meta-analysis of the reports suggest that effect of probiotics on glycemic control is stain specific. Among the widely published findings, the genes of Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia, and Roseburia have been identified as being negatively associated with T2DM and Ruminococcus, Fusobacterium, and Blautia were positively associated with T2DM. Although Lactobacillus genus is most frequently identified and reported still, most discrepancies in their effect in T2DM were reported (Gurung et al. 2020). Some of recent probiotic clinical trials conducted on T2DM given in Table 7.1.

The exact mechanisms used by probiotics for their advantages were uncertain. Nevertheless, a number of hypothesized processes describe many of their favorable effects. Probiotics control the T2DM in various ways, such as modulate the inflammation (Shen et al. 2018; Maldonado Galdeano et al. 2019), interact with dietary constituents, affect gut permeability (Tian et al. 2016) and lipid metabolism. Mainly the short-chain fatty acids (SCFAs) (acetate, butyrate, propionate) are the major anions in the colon and are largely produced by probiotic bacteria from indigestible polysaccharides (Geirnaert et al. 2017). SCFAs stimulate improvement of intestinal barrier function and upregulation of glucagon-like peptide-1 (GLP-1) (Macfarlane and Macfarlane 2003). GLP-1 is a gut incretin hormone that induces insulin production from the ß cells and inhibits the secretion of glucagon that contribute to glucose homeostasis (Lovshin and Drucker 2009). Probiotic improves the gut physiology and promotes epithelial cell growth by producing vitamins and hormones (Mach and Fuster-Botella 2017; Indira et al. 2019).

Table 7.1 Clinical trials on probiotic glycemic control in T2DM

Probiotic used	Design control Dose/ Duration	Participants, Age (year), Gender, Case/control (n)	Outcomes	References
Probiotic yogurt: *L. acidophilus* La5, *Bifidobacterium lactis* Bb12	**DB-RCT. 300 g daily/ 6 week Conventional yogurt**	T2DM, 30–60 years Both, 30/30	↓FBS, HbA1C, ↑FSI, Improved oxidative stress Biomarkers	Ejtahed et al. (2011)
Symbiotic shake containing *L. acidophilus B. bifidum* and fructooligosaccharides	**DB-RCT 200 ml daily/ 15 days Placebo**	50–60 years Both 10/10	↓FBS, HbA1C, ↑HDL-Cholesterol, TC & TG non-significant reduction	Moroti et al. (2012)
Capsule: *L. acidophilus*, *L. casei*, *L. rhamnosus*, *L. bulgaricus*, Bifidobacterium breve, B. longum, Streptococcus thermophiles FOS	Cross-over DB-RCT One capsule daily/ 8 week **Placebo**	T2DM, 35–70 years Both, 27/27	↓FBS, HOMA-IR, HbA1C, hs-CRP, ↑FSI, Improved oxidative stress biomarkers	Asemi et al. (2013)
L. acidophilus, *L. bulgaricus*, *L. bifidum*, *L. casei*	SB-RCT One capsule twice Daily 6 week Placebo	T2DM 25–65 years. Both 16/18	↓FBS, HOMA-IR ↓inflammatory markers hs-CRP ↑FSI, Improved oxidative stress biomarkers and lipid profile.	Asemi et al. (2013)
Synbiotic food Lactobacillus sporogenes, Inulin	Cross-over DB-RCT **3 times daily/ 6 w** Same food without probiotic bacteria & prebiotic inulin	T2DM, 35–70 years. Both, 62/62	↓FBS, HOMA-IR, HbA1C, hs-CRP ↑FSI, Improved oxidative stress biomarkers	Asemi et al. (2014)
Probiotic fermented milk (kefir) containing *L. casei, L. acidophilus* and *Bifidobacteria*	**DB-RCT** 8 week Milk	T2DM 35–65 years Both 30/30	↓FPG, HbA1c, Improved lipid profile.	Ostadrahimi et al. (2015)

(continued)

Table 7.1 (continued)

Probiotic used	Design control Dose/ Duration	Participants, Age (year), Gender, Case/control (n)	Outcomes	References
Synbiotic food: L. sporogenes, inulin, beta-carotene	Cross-over DB-RCT **3 times daily/ 6 w** Same food without probiotic, inulin, **and beta-carotene**	T2DM, 35–70, both, 51/51	↓FBS, FSI, HOMA-IR, hs-CRP, Improved oxidative stress biomarkers	Asemi et al. (2016)
L. acidophilus, L. casei, L. lactis, B. bifidum, B. longum B. infantis	DB-RCT 12 week Placebo	T2DM, 30–70, both, 48/53	↓FBG, insulin, HOMA-IR, HbA1c, Improved lipid profile	Firouzi et al. (2017)
Fermented milk: *L. acidophilus* La5, *Bifidobacterium animalis subsp. lactis* Bb12	**DB-RCT 120 g daily/6 w** Conventional fermented milk	T2DM, 35–60, both, 23/22	↓FBS, HOMA-IR, HbA1C, Improved oxidative stress biomarkers and and inflammatory markers	Tonucci et al. (2017)
Probiotic capsule: *L. acidophilus, Bifidobacterium bifidum, L. reuteri, L. fermentum*	**Parallel DB-RCT** One capsule daily/12 w **Placebo**	DN (T1DM & T2DM), 45–85, NA, 30/30	↓FBS, HOMA-IR, HbA1C, hs-CRP, BUN, Creatinine, Improved inflammatory markers and oxidative stress biomarkers	Mafi et al. (2018)
Probiotic capsule: *L. acidophilus, Bifidobacterium bifidum, L. casei, L. fermentum*	**Parallel DB-RCT** One capsule daily/12 w	DF (T1DM & T2DM), 45–85, both, 30/30	↓FBS, HOMA-IR, hs-CRP, HbA1C, Improved lipid profile, inflammatory Markers and oxidative stress biomarkers	Mohseni et al. (2018)
Probiotic capsule: *L. acidophilus, Bifidobacterium bifidum, L. casei, L. fermentum*	**Parallel DB-RCT** One capsule daily/12 w **Placebo**	T2DM, 40–85, Both, 30/30	↓FBS, HOMA-IR, hs-CRP, Inflammatory markers, Improved lipid profile and oxidative stress biomarkers	Mohseni et al. (2018)

(continued)

Table 7.1 (continued)

Probiotic used	Design control Dose/ Duration	Participants, Age (year), Gender, Case/control (n)	Outcomes	References
Lactobacillus spp Bifidobacterium spp Propionibacterium spp Acetobacter spp	**Parallel DB-RCT** One capsule daily/8 w **Placebo**	T2DM, 18–75, Both, 31/22	↓HOMA-IR, HbA1C, ↓Inflammatory markers	Kobyliak et al. (2018)
Probiotic honey: *Bacillus coagulansT4*	**Parallel DB-RCT 25 g daily/ 12 w Honey**	DN (T1DM & T2DM), 45–85, NA, 30/30	↓FBS, HOMA-IR, hs-CRP, BUN, Creatine, Improved lipid profile, inflammatory markers, and oxidative stress biomarkers.	Mazruei Arani et al. (2019)
Capsule: *L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, Bifidobacterium breve, B. longum, Streptococcus thermophiles* FOS	**Parallel DB-RCT** One capsule BD 6 week **Placebo**	T2DM, 30–75 years. Both, 30/30	↓FBS, HbA1C, ↑HDL-Cholesterol, no significant changes HOMA-IR, TC & TG	Razmpoosh et al. (2019)
L. casei	**Parallel DB-RCT** One capsule daily 8 week Placebo	T2DM, 30–60 years. Both, 20/20	↓FBS, HOMA-IR, Fetuin-A ↑ insulin Sirtuin1 no significant changes HbA1C,	Khalili et al. (2019)
Probiotic capsule: *L. acidophilus, L. rhamnosus, B. bifidum, B. longum S. bouladi,* FOS	DB-RCT One capsule twice Daily 12 week Placebo	T2DM, 30–65, Both, 30/30	↓FBS, HOMA-IR Improved oxidative stress biomarkers, lipid profile, kidney and liver markers	Our observation Un published data

Note: ↑ = increased, ↓ = decreased, *FOS* Fructooligosaccharide, *DB* Double blind, *RCT* Randomized clinical trial, *FBS* Fasting blood glucose, *FSI* Fasting serum insulin, *hs-CRP* High sensitive C-reactive protein, *IR* Insulin resistance, *TC* Total cholesterol, *TG* Triglycerides, *BUN* Blood urea nitrogen

Based on the reports, probiotics supplementation is not only reducing the glucose level, but also improving the other metabolic abnormalities linked with diabetes such as hypertension, BMI, lipid profile, oxidative stress markers. Some research has also

shown their beneficial effects on mental health. None of the studies has reported any toxic effects on liver and kidney like other synthetic drugs.

7.5 Beneficial Effects of Probiotic for Glycemic Control

Probiotics and their metabolites are involved in various pathways to improve glycemic control (Fig. 7.1) which is explained in the below sections.

7.5.1 Probiotic on Hypertension Associated with Diabetes

There are mainly two forms of hypertension: primary and secondary. Primary hypertension occurs mainly because of genetic variables and unspecific lifestyle, it is characterized as elevated blood pressure, around 95% cases belong to this category, whereas secondary hypertension is attributed to an identifiable cause such as Cushing's syndrome, obesity, and glucose sensitivity. However, it is still not clear about the etiology of hypertension (Sukor 2011). Indirect involvement of gut microbiota in the regulation of hypertension has been recognized in recent time. As we have seen earlier, in dysbiosis the diversity of microbes increases and Firmicutes/Bacteroidetes ratio changes. These changes accompanied by decrease in acetate and butyrate producing bacteria (Yang et al. 2015). SCFA plays an important role in maintaining blood pressure (BP). Short-chain fatty acid receptors are G-protein coupled receptors GPR41, GPR43, GPR109a, and olfactory receptor OLF79 in mice and OR51E2 in humans. Short-chain fatty acid receptors, such as GPR41 and OLF78, have shown to have inverse roles in blood pressure regulation (Pluznick 2014). Dysbiosis also leads to increased permeability of the intestinal wall. It is an essential factor that influences the bidirectional flow of microbes, cells, metabolites, molecules, and hormones that inevitably interfere

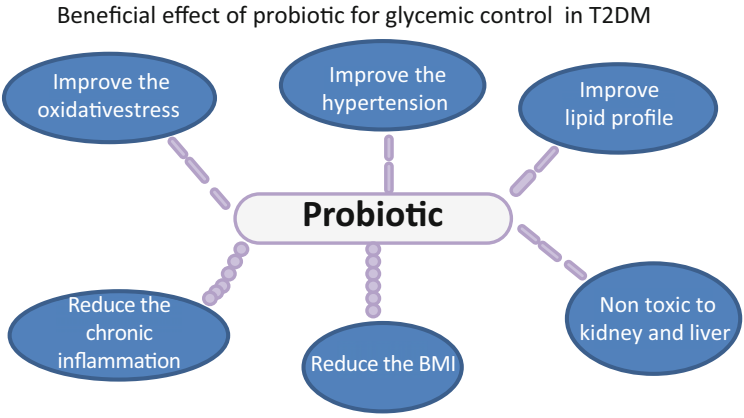

Fig 7.1 Beneficial effects of probiotic for glycemic control

with peripheral but also central BP control mechanisms. (Raizada et al. 2017). BP reduction was observed in the late phase of angiotensin II infused wild-type mice, suggestive of the favorable effect of propionate on hypertension (Bartolomaeus et al. 2019).

One study stated that the role of the gut microbiota in steroid enterohepatic circulation and its findings are consistent with the possibility that steroid metabolites contribute to the physiological response to exogenous steroids when reabsorbed in the enterohepatic circulation (Honour 1982). Some other studies reported differences in circulating inflammatory cells in hypertensive individuals compared to controls due to microbial diversity in hypertensive patients. Dysbiosis also contributes to increased T-helper 17 cell activation and is mediated by gut-intrinsic pathways (Kim et al. 2015).

7.5.2 Probiotic on Obesity Related with Diabetes

Obesity is typically associated with metabolic alterations related to glucose homeostasis and cardiovascular risk factors (Eckel et al. 2005). These metabolic alterations are associated with low-grade inflammation that contributes to the onset of these diseases (Olefsky and Glass 2010). Probiotics and prebiotics reduce gut inflammation, which leads to improvement in metabolic dysfunction in obese-insulin resistant model (Thiennimitr et al. 2018). Some studies found that gut microbiota conferred host resistance to high-fat diet-induced obesity through the production of polyunsaturated fatty acid metabolites (Miyamoto et al. 2019). Considering the effect of calorie restriction and weight loss on fetuin-A and SIRT1 levels it can be understood by reducing the appetite and dietary intake and body weight. Studies also found that probiotic supplementation significantly decreased total energy, carbohydrate, fat, and protein intake compared with placebo (Khalili et al. 2019). Other studies found that higher endogenous GLP-1 and GLP-2 production; prebiotic treatment increases the number of enteroendocrine cells producing GLP-1 and GLP-2 (L-cells) in the jejunum and colon (Cani et al. 2012).

7.5.3 Probiotic on Oxidative Stress

In diabetes, free radical formation by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation causes the damage of enzymes, cellular machinery, and increased insulin resistance. (Asmat et al. 2016). SCFA produced by the probiotic in the digestive system provide nicotinamide adenine dinucleotide phosphate (NADPH) for the synthesis of GSH, induces apoptosis and increases the expression of the pathway of oxidative pathogens. Probiotic supplementation plays a direct role in NO production and reduces the ROS (Asmat et al. 2016; Heshmati et al. 2018). Studies on beneficial effects of probiotic on oxidative stress are given in Table 7.1.

7.5.4 Probiotic on Lipid Management

Glucose and lipid metabolism are related in several ways. Diabetic dyslipidemia, characterized by high triglycerides, LDL particles, and low HDL-C are the most important clinical manifestation of this interaction and this is main cause of cardiovascular diseases (Parhofer 2015; Eid et al. 2019). Cholesterol is the precursor to bile acids. Bile acids are metabolized into secondary bile acids by gut microbiota. Probiotic controls the lipid metabolism by assimilation of cholesterol during growth, binding of cholesterol to cellular surface, disruption of cholesterol micelle, deconjugation of bile salt, and bile salt hydrolase activity (Lye et al. 2010; Jones et al. 2012, 2013; Huang et al. 2014). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist (Sayin et al. 2013). Mechanisms are explained in more detail in Chap. 8.

7.5.5 Probiotic on Comorbid Brain Disorders Associated with Diabetes

The most common causes of T2DM and brain disorders are poor sleep, lack of exercise, and diet habits (Watkins and Thomas 1998; Yoda et al. 2015). There are numerous studies in the past reporting the effects of diabetes on the brain. Certainly, high glucose levels can damage blood vessels in the brain and thereby increase the risk of stroke. However, its effects are more widely felt than that. High glucose and insulin resistance affect many neuronal processes and contribute to inflammation in the brain. T2DM also appears to increase the risk of Alzheimer's disease and other dementias (Li et al. 2015). The other ways stress stimulates the hypothalamus–pituitary–adrenal axis (HPA-axis) and the sympathetic nervous system (SNS) are: increased levels of cortisol in the adrenal cortex, and adrenalin and noradrenalin in the adrenal medulla (Smith and Vale 2006; Stephens and Wand 2012). Chronic hypercortisolemia and excessive SNS activity promote insulin resistance, visceral obesity and contribute to T2DM (Pickup and Crook 1998; Wang et al. 2013).

In addition, constant stress also causes immune dysfunction directly or via the HPA or SNS axis, enhancing the production of inflammatory cytokines. High levels of inflammatory cytokines interfere with the regular functioning of pancreatic β-cells, induce insulin resistance and other consequences. The other studies reported that pro-inflammatory cytokines have been found to interact with many of the pathophysiological domains that characterize depression, including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and behavior (Fig. 7.2).

Consumption of diet rich in high fats and refined carbohydrates mainly sugar has the ability to disturb the healthy microbiota composition, leading to dysbiosis. Studies showed that dysbiosis increases lipopolysaccharide (LPS) levels, which triggers the production of proinflammatory cytokines in the gut (Zeevi et al. 2015; Agus et al. 2016). Dysbiosis imposes regulatory roles on inflammation and oxidative stress and is a pathogenetic contributor associated with various diseases characterized by a pro-oxidative and pro-inflammatory disorder mainly AD,

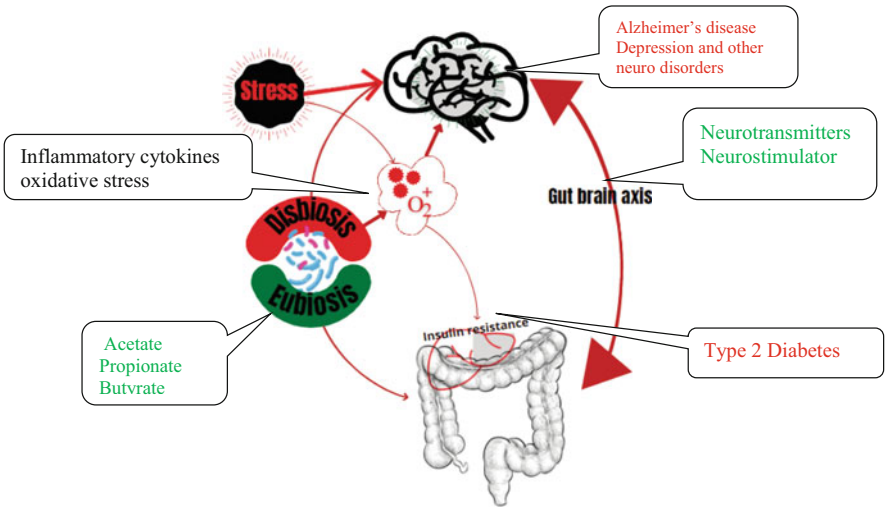

Fig. 7.2 Bidirectional interactions between the gut microbiota and the central nervous system

depression, and T2DM (Luca et al. 2019). The gut–brain axis involves a number of sophisticated channels of communication among many interconnected systems, including the CNS, the autonomic nervous system (ANS), the HPA axis, as well as the GI corticotropin-releasing factor system, and the intestinal immune response system featuring the intestinal mucosal barrier (Carabotti et al. 2015).

Dysbiosis is also confirmed by the high levels of comorbidity among depression and T2DM subjects. This may account for the genetic similarities related to these disorders and contribute to an increase in the risk of dementia. It is strictly associated with metabolism, cognition, and mood (Rowland and Bellush 1989; Hilakivi-Clarke et al. 1990; Thakur et al. 2013; Thakur et al. 2016). Gut microorganisms are capable of producing and delivering neurotransmitters such as serotonin and gamma-aminobutyric acid, which act in the gut–brain axis and modulate food intake and energy balance in the system (Cryan and Dinan 2012; Borre et al. 2014).

Cross talk between the brain and the gut involve many interacting pathways, including the autonomic, neuroendocrine, immune systems as well as bacterial metabolites and neuromodulatory molecules. Bacterial metabolites (SCFAs) like acetate and propionate are mainly produced by the bacteroidetes, while Firmicutes generate most of the butyrate. Butyrate also prevent inflammatory reactions by inhibition of NF-kappaB (NF-κB) (Segain et al. 2000). Propionate is usually utilized by the liver and has also been reported to inhibit NF-κB, as well as boost insulin sensitivity, while acetate is normally released into circulation so that it can enter peripheral tissues, including the brain (Guarner and Malagelada 2003; Al-Lahham et al. 2010; Iwanaga and Kishimoto 2015). Both propionate and acetate have been found to improve insulin sensitivity (Canfora et al. 2015; González Hernández et al. 2020). Acetate and butyrate are structurally related to ketone, acetoacetate, and d-β-hydroxybutyrate, all of which have positive effects in neurological conditions

(Stilling et al. 2016; Courchesne-Loyer et al. 2017). Meta-analysis suggests that modulating the composition of the gut microbiota using prebiotics and probiotics may produce beneficial effects on brain disorders associated with diabetes (Schachter et al. 2018).

7.5.6 Other

The beneficial effect of probiotics also extends to chronic liver and kidney disease (Lo et al. 2014; Jia et al. 2018). Our lab observation also finds that the probiotic supplementation has improved the kidney and liver function markers of the diabetic subjects with metabolic syndrome (unpublished observation). Detailed effect of probiotic on liver diseases is mentioned in Chap. 10.

7.6 Conclusion

The available evidence from experimental studies and clinical trials supports that the modulation of the intestinal microbiota by probiotics uptake may be effective towards prevention and management of T2D and other related complications.

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Protective Effect of Probiotic in Alcohol-Induced Liver Disorders

8

Role of Probiotics in Alcohol-Induced Liver Disorders

Onkar Bedi, Sudrishti Chaudhary, and Thakur Gurjeet Singh

Abstract

The major key culprit which produces burden on liver is concerned with abnormal dietary habits include high fat, high fructose rich products, and alcohol beverages. Thus, on the basis of dietary culprits, liver disorders are classified into two broad categories which include non-alcoholic fatty liver disease and alcoholic fatty liver disease. The alcohol consumption not only alter the physiological function of the liver but also affect the gut microbiota. The gut microbiota includes bacteria, fungi, and archaea which co-evolved to live in the human gut which helps in the regulation of various physiological activities and they together play a vigorous role in the management of numerous metabolic disorders. Alcohol produces deleterious effect on the natural gut microbiota which leads to microbial dysbiosis resulting into increased gut permeability to bacterial endotoxins. The chronic disruption of normal gut microbiota due to alcohol consumption produces various pathological effects like oxidative stress, inflammation and interferes with fasting-induced adipose factor (FIAF) and modulates lipid metabolism ultimately causes fatty liver, fibrosis, cirrhosis, and HCC. The therapeutic trends have now shifted towards the probiotics treatment which contains live microbial preparations that modify or restore the gut microflora and help in the treatment of alcohol-induced liver disorders.

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Keywords

Probiotics · ALFD · Microbial dysbiosis · Oxidative stress · Bacterial burden

8.1 Introduction

Alcoholic liver disease (ALD) is a canopy term covering a variety of disorders progressing from steatosis, steatohepatitis, fibrosis, and cirrhosis to hepatocellular cancer (Meroni et al. 2019). Most of the fatalities due to chronic liver disease are of alcohol-related aetiology. Despite intensive research in this field, there is currently no specific therapy or treatment to ameliorate ALD progression. Numerous studies have highlighted the critical role of the gut–liver axis and gut microbiome in ALD pathogenesis. It has been shown that alcohol induces change in gut microbiota composition, i.e. intestinal dysbiosis along with the increased intestinal permeability (Hartmann et al. 2015). The surge in the harmful bacteria results in increased levels of microbial products, i.e. bacterial translocation from the gut to the liver and elevated pathogen-associated molecular patterns, for example, lipopolysaccharide (LPS), which acts as inflammatory signals, produces inflammation observed in ALD by activating toll-like receptor-4 on the Kupffer cells (Ceccarelli et al. 2014). Previous reports reveal how compositional and functional changes in the intestinal microbiome are observed in patients with alcohol misuse along with increased intestinal permeability and eminent levels of gut-derived microbial products present systematically (Bajaj 2019). Therefore, pathological bacterial translocation and disturbed intestinal homeostasis appear vital for the alcoholic liver disease pathogenesis, thus suppressing cellular responses to microbial products, steadying the mucosal gut barrier and maintaining eubiosis, and protecting from alcoholic liver disease (Cassard and Ciocan 2018).

The gut–liver axis and related dysbiosis being established as important controllers in the pathophysiology of ALD might include new therapeutic tactics such as prebiotics, probiotics symbiotics, faecal microbiota transplantation (FMT), and bile acid regulation for gut microbiota modulation (Imani Fooladi et al. 2013; Sarin et al. 2019). Recently, role of probiotics has been recognized in alleviating and averting the advancement of ALD (Hong et al. 2019). A probable mechanism is that the probiotics restore gut eubiosis by altering the composition of pathogenic intestinal microbiota, leading to reduced bacterial translocation, intestinal permeability, endotoxemia, and thus hampering the ALD expansion and its progression to a stage of an irreversible damage.

Of late many preclinical studies and clinical trials have demonstrated that probiotics retreated hepatic steatosis and inflammation induced by alcohol and helped improve liver enzymes in animal models and in patients (Liu et al. 2020). Probiotics lessen the levels of pro-inflammatory cytokines and oxidative stress by reducing reactive oxygen species (ROS) production and augment immune responses induced by alcohol in both intestine and liver. Thus, for combating alcohol-induced

hepatic steatosis, probiotics tend to reduce lipogenesis and increase fatty acid β-oxidation (Hong et al. 2019).

Although alteration of gut microbiota through probiotics seems to be a favourable therapeutic approach for the management of intestinal barrier dysfunction, there is a paucity of research that focuses on role of probiotics from the ALD prospective. Although potential therapeutics are being identified against ALD in the form of a growing quantity of probiotic strains and their related products, the defined mechanisms stating the significance of probiotics in regulating gut microbiota, gut–brain axis, intestinal barrier function, and the pathogenesis of ALD still needs to be elucidated. Therefore, it will be worthwhile to investigate the mechanisms of probiotic action on alcohol-induced liver injury. This kind of study will have a major impact on the development of a probiotics-based new therapeutic strategy for the inhibition and treatment of alcoholic liver disease (Cassard and Ciocan 2018). Thus, here we summarize the existing knowledge about the mechanism of action of probiotics and their potential therapeutic applications to deduce the effects of alcoholic liver disease.

8.2 Pathological Background of Alcohol-Induced Liver Disorder and Its Impact on Microbiota

Worldwide, alcohol consumption constitutes 4% of all deaths and ranks third among the several risk factors for disease and debility (Meroni et al. 2019). Alcohol toxicity and metabolism have most impact on the liver which affects the disease pathogenesis ranging from steatosis to hepatitis, cirrhosis, and hepatocellular carcinoma. The ALD pathophysiology varies according to the presence of genetic and nongenetic factors and the stage of the disease that affect its beginning and clinical development (Fig. 8.1) (Liu et al. 2020). Ethyl alcohol upon oral ingestion diffuses through cell membranes and is metabolized to a highly reactive molecule, i.e. acetaldehyde (Zakhari 2006). As a consequence, formation of acetaldehyde produces reactive oxygen species, depletion of cofactors like NADP, activation of pro-inflammatory and other signalling pathways causing adverse effects of alcohol consumption in all tissues (Waris et al. 2020).

Triggered by alcohol metabolism, the aldehydes produced in the intestine increase the levels of reactive oxygen species that activate pro-inflammatory cytokines and cause leaky gut pathophysiology, foremost to increased translocation of bacterial products, endotoxins, pathogen-associated molecular patterns (PAMPs), and bacterial DNA, from the gut lumen to the liver, instigating liver injury. Moreover, alcohol considerably brings about changes in gut microbial diversity, there occurs increase in the intestinal mucosal permeability. Intestinal epithelial tight junction protein expression is reduced leading to barrier dysfunction. Thus, endotoxin translocation into the blood occurs, inducing inflammatory responses and ROS production affecting the gut–liver axis causing hepatic steatosis and inflammation (Tuma et al. 1998; Teare et al. 1993).

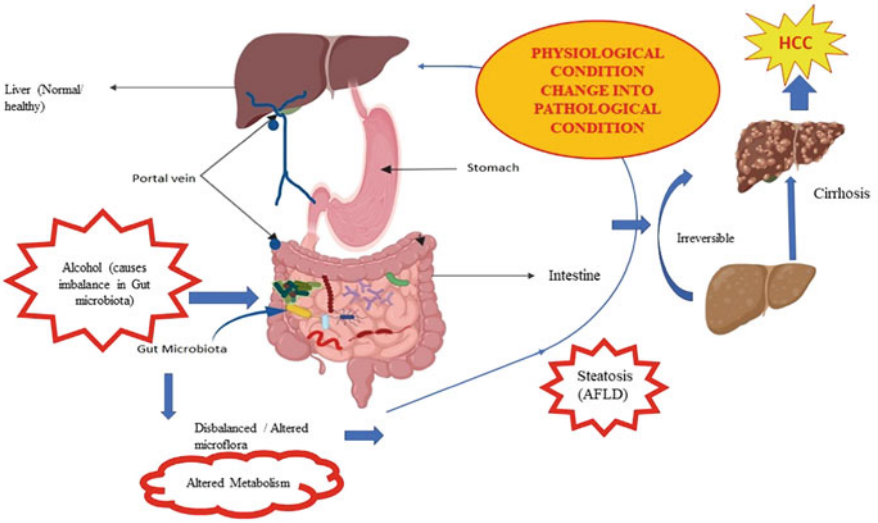

Fig. 8.1 Illustration of alcohol-induced abnormal metabolism causes ALD

ALD and gut microbiota correlation and association are not yet fully explored, however alterations in the microbiome both qualitative and quantitative have its sturdy impact on intestinal microbiota along with the increase in endotoxin levels, hepatic inflammation, and injury caused by ethanol and its vital role in ALD development. Due to intestinal bacterial dysbiosis which is a loss in balance of the different intestinal commensals, the gut homeostasis is perturbed. The consequences of dysbiosis both in the small and large intestine include loss of beneficial microbes, reduced bacterial diversity, and increase in the pathogenic species predominantly the overgrowth of Gram-negative bacteria, induced by both chronic and acute alcohol consumption (Bluemel et al. 2020). In human subjects with ALD, alcohol consumption led to leaky gut physiology with higher levels of systemic overload of detrimental bacteria as compared to healthy controls (Leclercq et al. 2014).

The development and propagation of liver injury are intricately involved with the gut microbiota in patients with chronic alcohol abuse. The alcohol induces change in microbial functions which are a consequence of human gut microbiota alteration and have potential to accelerate this injury. Previous studies have demonstrated the role of gut initiated LPS as the dominant mediator of inflammation in alcoholic steatohepatitis and its modulation via LPS pathways have been proposed to treat patients with ALD (Liu et al. 2017). Furthermore, it has been shown that the altered gut microbiota and LPS signalling can be reversed by TLR4 antagonists and probiotics (Mandrekar and Szabo 2009). In patient study, increased levels of faecal Bifidobacteria and Lactobacilli were observed by treating with probiotics therapy containing *L. plantarum* and *B. bifidum* which led to decrease in liver injury markers in serum like AST in ALD patients compared to standard therapy. In the same study they concluded that bowel flora was restored when given short-term oral

supplementation with the probiotics and was also associated with improvement in alcohol-induced liver injury when compared to the standard therapy alone (Kirpich et al. 2008). In severe liver disease due to alcohol, the integrity of the intestinal barrier along with reduction in endotoxin levels (LPS) was confirmed on probiotic administration. Hence gut intestinal barrier is affected at numerous levels upon both acute and chronic alcohol consumption, therefore the change in gut microbiota should be involved as a therapeutic approach in strengthening intestinal related barrier functioning and in regression of ALD.

8.3 Does Alcohol Produce Deleterious Effects on Gut Microbiota?

The gut microbiota varies with development of alcohol liver disease. Pathogenesis of alcoholic liver disease relevant to intestinal dysbiosis has very diverse consequences (Woodhouse et al. 2018); therefore, it would be valuable to mention the influence of gut microbiota on alcohol liver disease progression and vice versa. Crosstalk between bacterial components such as LPS and various hepatic receptors like TLR's affects the gut–liver axis. Modulation of this interaction leads to worsening of hepatic disorders due to dysbiosis and altered intestinal permeability. In one such study a decline in the abundance and richness of both Firmicutes and Bacteroidetes at phylum level was observed upon chronic ethanol feeding along with a relative increase in Actinobacteria and Proteobacteria phyla. Moreover bacterial genera comprising Gram-positive Corynebacterium and Gram-negative alkaline tolerant Alcaligenes showed the greatest growth (Bull-Otterson et al. 2013). Metagenomic and metabolomics studies reveal how chronic alcohol administration lowers the gut microbial capacity to form new saturated long-chain fatty acids (LCFA). Lower bacterial synthesis of LCFA results in decrease in the abundance of good bacteria, such as Lactobacillus species, used saturated LCFA as a rich energy source. The decrease in these LCFA producing bacteria causes tight junction barrier disruption (Chen et al. 2015). The eubiosis is restored by supplementation of the saturated LCFA, which normalizes the intestinal gut barrier, and diminishes ethanol induced liver disease in mice. Thus, gut microbiota contributes to the progression of alcohol-related liver disease through different mechanisms. In alcoholic hepatitis the altered gut bacteria and produces immune depression, increase in inflammation of hepatocytes and changes in metabolites of microbial by various ways (1) *By Pathological bacterial translocation*, which is the most commonly known mechanism linking intestinal dysbiosis to alcoholic liver disease progression. The dysfunction of the gut barrier is brought about by ethanol with its metabolite acetaldehyde, these microbial products translocate to the portal venous blood from the intestine which activate Kupffer cells and hepatic stellate cells, thus further damage the hepatocytes (Mazagova et al. 2015), thus causing progression of alcoholic liver disease as a consequence of hepatotoxic outcome of alcohol and its metabolites. (2) *Changes in intestinal metabolites*, its challenging to know which metabolites from the dysbiotic microbiota provoke intestinal inflammation. Similar studies in rats following chronic

ethanol administration, the changes in the propionate and short-chain fatty acids (SCFAs) butyrate, known as faecal lipid metabolites have been reported (Parada Venegas et al. 2019). These SCFAs, such as acetate, propionate, and butyrate produced from the digestion of carbohydrates by the gut microbiota were found in high abundance in the healthy colon. (3) *Bile acid metabolism:* Bile acids are important correspondents between the intestine and the liver. Bile acids conjugates are modified in the intestine by bacteria and are secreted into the duodenum from the hepatic biliary system. A reduced bile flow is observed in patients with liver cirrhosis. Bile acids lead to produce antimicrobial molecules by activation of FXR signalling in intestinal epithelial cells therefore reduced bile flow leads to overgrowth of intestinal bacterial (Raedsch et al. 1983; Inagaki et al. 2006).

For a well explanation of the liver–gut axis with respect to alcohol, identification of other pathways linking the alcoholic liver disease to gut microbiota is essential, which could be instrumental in designing interventional trials. To better define the bidirectional crosstalk of how the liver interconnects to the intestine, the interplay of gut microbiota and bile acids in ALD need to be explored for the development of novel pharmaceutical agents for treatment of patients with chronic alcohol abuse. Taken together, chronic alcohol administration leads to dysbiosis and is associated with a microbial shift, i.e. decline in healthy bacteria that are good for physiology such as Lactobacillus spp., whereas the pathogenic, i.e. bad bacteria such as Enterobacteriaceae increase (Yan et al. 2011). Thus, supplementing probiotics, prebiotics, or combination of both, i.e. symbiotic appear to be a usable option for treatment of alcoholic liver disease by inhibiting dysbiosis or restoring eubiosis.

8.4 Types and General Aspects of Probiotics

The term Probiotic was first introduced by Lily and Stillwell in 1965. The first probiotic species studied was lactic acid bacteria, the lactobacillus acidophilus identified by Hull in 1984 (Lilly and Stillwell 1965). Later in 1991, Holcombh identified *bifidobacterium bifidum*. Lactobacillae and Bifidobacterium are the main probiotics and other probiotics identified later were *Enterococcus, Escherichia, Saccharomyces, Bacillus, Propionibacteria,* and *Streptococcus* (Hamilton-Miller 2003). According to WHO, the probiotics are described as next most important elements in immune defence system following antibiotic resistance (Boden and Snapper 2008; Fonseca-Camarillo and Yamamoto-Furusho 2015).

8.4.1 Probiotics

Probiotics are the beneficial microorganisms with potential to maintain the healthy microbial balance, which are incorporated in the host to produce favourable effect. The administered probiotics have various health benefits as they lower the intestinal pH, decrease the abundance and colonization by pathogenic bacteria. Moreover the

probiotics make the microenvironment enriched with good microbes which have potential to modulate the host immune response (Williams 2010).

8.4.2 Composition of Probiotics

Most commonly used probiotics are comprised of yeast or bacteria which are marketed as dietary supplements and food in the form of dairy products like yoghurts, capsules, liquid drinks, and other fermented foods. These probiotic products constitute either a single strain or a mixture of several bacterial species. Probiotics are mostly available in the form of yeast, moulds, or bacteria; however, bacterial probiotics like lactic acid containing bacteria are more common (Hempel et al. 2011).

8.4.3 Criteria for Probiotics

In order to define probiotics, Fuller back in 1989 defined the important criteria for a probiotic to be classified as a good one. (1) A probiotic strain should have a beneficial effect on the host phenotype, such as probiotics should increase resistance to disease and proliferation capabilities. (2) A probiotic should be non-pathogenic in nature and no toxic effect on the host. (3) The probiotic organisms should be viable with a capacity to be given in large numbers. (4) The probiotics administered strains should be sustainable and have good storage properties (Markowiak and Slizewska 2017).

8.4.4 Commonly Used Bacterial Probiotics

(i) Lactobacillus*: rhamnosus, reuteri, acidophilus, and fermentum*; (ii) Bifidobacterium: *bifidum, longum, infantis, thermophilum*; (iii) Streptococcus: *lactis, intermedius, cremoris, salivarius*; (iv) Propionibacterium; (v) Pediococcus; (vi) Leuconostoc; (vii) Bacillus; (viii) Enterococcus; (ix) E. faecium (Doron and Gorbach 2006).

8.4.5 Important Functions of Probiotics

There are various health benefits probiotics provided to the host as being an essential component of the host gut microbiota. (a) Reduces disease progression particularly in chronic liver disease and others. (b) Increases calcium absorption from gut to prevent osteoporosis. (c) Probiotics protect from pathogenic microorganisms like Candida by competing for their colonization. Probiotics are also useful as they inhibit growth of harmful bacteria by producing various inhibitory substances

which include antibiotics and make the microenvironment acidic and unfavourable for pathogenic bacteria. (d) Liver toxicity reduction. (e) Promotion of healthy digestive tract colonization by maintaining peristalsis, better digestion of proteins, fats, carbohydrates, and nutrients re-absorption. (f) Maintain balance of oestrogen levels. (g) Boost levels of vitamin B and K. (h) Increase in host immunity and resistance to various infectious diseases. (i) Improves lactose intolerance (Rastogi et al. 2011; Madsen 2001; Ewaschuk et al. 2007; Singh et al. 2013).

8.4.6 Mechanism of Action of Probiotics

Clinical benefits produced by the probiotics are a consequence of the combined effect of several mechanisms. The probiotics most likely modulate immune system at both cell-mediated immune response and humoral immune functions. Probiotics produce organic acids and are important for both gut microbiota and probiotic–host interactions. They improve the barrier function and produce various small metabolites with local and non-local effects (Sanders et al. 2019).

8.4.7 Role of Probiotics

In many previous studies, the role of probiotics in the modulation of various physiological processes like immunological, respiratory, and gastrointestinal functions has been defined (Floch et al. 2011). Probiotics have been shown to release antibacterial substances such as bacteriocins to decrease the harmful bacterial growth playing a protective role by competing with intestinal pathogens (Cotter et al. 2005). Probiotics produce various metabolites such as acetic acid and lactic acid having favourable effect on host health (Servin 2004). Earlier Metchnikoff discovered that healthy bacteria in the form of lactic acid bacteria (LAB) were reported to have a marked influence on both digestive and the immune system (Perdigon et al. 1995). In current times gram-positive probiotic strains such as Lactobacillus and Bifidobacterium are being used as treatments of intestinal dysfunctions (Marco et al. 2006). However, Escherichia coli Nissle 1917 (EcN), a gram-negative bacteria are also used as probiotics in the treatment of chronic bowel disease and colitis (Mollenbrink and Bruckschen 1994). Besides, engineering these natural probiotics may aid in escalating the benefit to the host by producing various immunomodulatory molecules. The gut microbiota thus has an essential role in many disorders and its modulation in the form of probiotics could be an effective therapy to treat various diseases. Therefore, probiotic intervention increases the community of beneficial microorganisms and its product could serve better therapeutic option for various disorders including chronic liver disease.

8.4.8 Role of Probiotics in Liver Disease

Probiotic intervention modulates gut microbiota and is known to alleviate progressive liver disease. Previous preclinical studies in rats with ALD probiotic and prebiotic treatment prevented dysbiosis in colon lumen (Mutlu et al. 2009). In the same line several other studies have also supported the role of probiotics by its supplementation which alleviated alcohol-induced liver injury by restoring gut microbiota homeostasis (Loguercio et al. 2002; Dhiman et al. 2014; Lata et al. 2007). It was shown that 2 week treatment with LGG positively changed the alcohol-induced dysbiosis in mice with continued alcohol intake (Wang et al. 2011). Importantly, the LGG supplementation prevented ethanol-persuaded pathogenic variations in the microbiome and decreased the liver disease (Wang et al. 2012) thereby establishing the beneficial effects of probiotics by restoration of gut microbiota in ALD highlighting the important function of microbiota in gut-liver pathophysiology.

8.5 Mechanism of Action of Probiotics for the Management of Alcohol-Induced Liver Disorders

Probiotics are preparations containing living microorganisms that play a vital role in altering the balance of gut microflora (Lambert et al. 2003). Favourable physiological conditions can be attained by adjusting the composition of the host microflora (Lata et al. 2007). It is already established that excessive intake of alcohol leads to dysbiosis, that damages gut mucosa and results into increased intestinal permeability, which in turn increases bacterial translocation across epithelium. As a result of increased bacterial translocation, bacterial products such as lipopolysaccharides (LPS) increase in circulation and enhance the production of free radicals or reactive oxygen species (ROS) and various pro-inflammatory cytokines, leading to ROS mediated liver injury (Thurman et al. 1998). Endotoxins derived from the superficial membrane of Gram-negative bacteria such as LPS act via recognition of CD4T mediated receptors like toll-like receptors (TLRs), articulated in Kupffer cells and induce the release of various cytokines and chemokines which are responsible for activation of TNF-α and NFκB and these are the major involved in liver injury. A study showed that reduction in TLR4 complex or CD14 proved to protect mice from alcohol-induced liver injury (Mutlu et al. 2009). Here, probiotics come in role, they restrain the altered gut microflora and improve alcohol-induced gut dysfunction thereby decreases gut permeability and prevents intestinal bacterial translocation.

As a result, level of alcohol-induced pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS) decreases in the intestine and liver. Probiotics are known to reverse the inhibited fatty acid β-oxidation due to alcohol, i.e. reduced lipogenesis, thus beneficial in the management if alcohol-induced hepatic steatosis (Gu et al. 2019). Another pathway involved in alcohol-induced liver injury is

AMP-activated protein kinase (AMPK) signalling pathway, responsible for regulation energy balance by lipid metabolism via alteration of various transcription factors such as sterol regulatory element-binding protein 1 (SREBP-1) and peroxisome proliferator-activated receptor-α (PPARα), which are involved in lipogenesis and fatty acid oxidation (Li et al. 2011). Excessive alcohol intake decreases acetyl-CoA carboxylase (ACC) and AMPK phosphorylation and increases malonyl-Co-A (MCA) production, which is a primary cause of abnormal lipid uptake in the liver. Most often used probiotic strain, Lactobacillus rhamnosus GG (LGG) has been proved to be beneficial in treating ALD, it increases fatty acid oxidation and decreases lipogenesis in liver (Zhang et al. 2015).

8.6 Preclinical Evidences of Probiotics for the Management of Alcohol-Induced Liver Disorders

The favourable properties of probiotics have been studied in various animal models in the management of non-alcoholic steatohepatitis (NASH) and ALD (Kirpich and McClain 2012). Nowadays the most commonly used probiotic strains are Bifidobacteria, Lactobacillus rhamnosus GG (LGG), lactic acid bacteria (LAB), and Saccharomyces boulardii. Various strains of Lactobacilli such as Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus helveticus are widely being used. Numerous probiotics are reported to be used for the treatment and management of a various disorders. Among them, the most frequently used strain is Lactobacillus rhamnosus GG (LGG) (Lee and Salminen 2009). In some of the ALD models of rats and mice, administration of LGG revealed significant improved liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST), reduction in plasma endotoxin level. Wang et al., in 2011 showed that LGG supplementation in mice causes significant reduction in alcohol-induced hepatic steatosis and endotoxemia (Wang et al. 2011). Nanji et al., in 1994 were one of the groups experimentally demonstrated the efficacy of LGG in treating ALD (Nanji et al. 1994). They administrated LGG concentrate (10^{10} CFU per mL) to Wistar rats and there was a significant reduction in alcohol-induced liver endotoxemia. In another study, rats with alcohol pancreatitis-related liver damage were fed with a combination of Lactobacillus helveticus, Lactobacillus acidophilus, and Bifidobacterium which effectively protected the rats against bacterial translocation and liver damage during acute pancreatitis (Marotta et al. 2005). Additional studies on LGG in rats showed significant reduction in alcohol-induced intestinal permeability, oxidative stress, and inflammation (Forsyth et al. 2009) and improve intestinal dysbiosis by restoring gut microflora (Mutlu et al. 2009). We have represented several preclinical experimental studies on probiotics for the treatment of ALD in Table 8.1.

Table 8.1 Preclinical evidences associated with the use of probiotics in the management of alcohol-induced liver disorders

S. No	Animal model	Intervention	Observation	Pathological outcome	Reference
1.	57BJ/6N mice (Lieber DeCarli diet containing 5% alcohol for 8 weeks). [protein (17%), corn oil (40%), carbohydrate (7%) and alcohol (35%)]	Lactobacillus rhamnosus GG (LGG) supplementation (2 weeks). (10^9 CFU/mouse per day)	– Histology of liver and intestine – Barrier function analysis and Caco-2 monolayer cell culture	Significant reduction in alcohol-induced hepatic steatosis and endotoxemia	(Wang et al. 2011)
2..	Male Wistar rats (diet containing corn oil and ethanol)	Ethanol with lactobacilli GG concentrate (10^{10} CFU per mL)	Measurement of plasma endotoxin and severity of pathological variations in the liver.	Mean ± SE of the pathology score was significantly higher in the CO + E group compared to the CO + E + L group.	(Nanji et al. 1994)
2.	Male Sprague–Dawley rats were gavaged with alcohol twice daily (8 gm/kg) for 10 weeks.	Rhamnosus Gorbach–Goldin (LGG) or vehicle (V) [Once a day]	Analysis of hepatic tissues for oxidative stress and inflammatory biomarkers.	Significant reduction in alcohol-induced intestinal permeability and oxidative stress.	(Mutlu et al. 2009)
3.	Sprague–Dawley rats (alcohol-rich diet for 2 weeks)	Pre-treatment for 1 week with a mixture of synbiotics (Lactobacillus acidophilus, Lactobacillus helveticus, and Bifidobacterium).	Measured transaminase and endotoxemia levels before treatment, after 6 h, after 24 h and 2 weeks later, at the time when rats were sacrificed.	Synbiotics improved the acute pancreatitis-induced increase in endotoxemia and transaminase levels.	(Marotta et al. 2005)
4.	Mice (ethanol containing diet for 4–5 weeks)	Oral administration- Heat-killed L. brevis (dose −100 or 500 mg/kg once a day for 35 days)	Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, triglyceride content (TG) and total cholesterol	Inhibited an increase in the level of serum ALT and AST, TG, and total cholesterol. Suppression of overexpression of TNF-α, SREBP-1, and SREBP-2 mRNA in the liver	(Segawa et al. 2008)

8.7 Clinical Evidences Associated with the Use of Probiotics for the Management of Alcohol Induced Liver Disorders

Till date, several studies have reported the beneficial effects of probiotics in experimental ALD, still there is lack of clinical data and limited clinical trials. Some of the clinical evidences and clinical trials associated with the use of probiotics for the treatment of ALD have been shown in Tables 8.2 and 8.3. In a clinical study,

Table 8.2 Clinical evidences associated with the use of probiotics for the management of alcohol-induced liver disorders

Disease/ condition	No. of patients/ subjects (each group)	Intervention	Pathological outcomes	Reference
Alcoholic cirrhosis	10	De Simone Formulation (Lactobacillus rhamnosus, Plantarum, Bifidus, Salivarius, Lactis, acidophilus, Casei, Bulgaricus, Breve, Fructooligosaccharides, folic acid, Fe gluconate, and Zn oxide) for 3 months.	Reduced plasma ALT, AST, and GGT levels; Normalized plasma TNF-α, IL-10, and IL-6 levels and decreased 4-HNE, MDA, and S-NO levels	(Loguercio et al. 2002)
Alcoholic cirrhosis	20	*Lactobacillus casei* Shirota treatment for 4 weeks.	Reduction in sTNFR1, sTNFR2, TLR4, and IL10 levels	(Loguercio et al. 2005)
Alcoholic psychosis and liver disease	66	*Lactobacillus plantarum* 8PA3 and *Bifidobacterium bifidum* treatment for 5 days.	Increased lactobacilli and Bifidobacteria; reduction in AST, ALT, LDH, GGT, and total bilirubin	(Kirpich et al. 2008)
Alcoholic cirrhosis	12	A combination of different strains of lactic acid bacteria for 2 months	Improvement in gut microflora, reduced ALT, γ-GT, and TNF-α levels	(Stadlbauer et al. 2008)
Alcoholic and non-alcoholic cirrhosis And hepatic encephalopathy patients	89	De Simone Formulation treatment for 6 months	Lesser risk of hospitalization for hepatic encephalopathy, improvement in CTP (Child-Turcotte-Pugh) and MELD (model for end-stage liver disease) scores	(Dhiman et al. 2014)

Table 8.3 Clinical trials on probiotics for the management of alcohol-induced liver disorders

S. N	Study title	Study type	No. of subjects	Intervention/ treatment	Recruitment status	Phase	Primary outcome measures	Secondary outcome measures	Clinical trials.gov identifier:
1.	Effect of Probiotics on gut–liver axis of alcoholic liver disease (EPALD)	Interventional (Clinical Trial)	130	Drug: hepatitis, alcohol, probiotics Drug: alcohol, hepatitis, Placebo	Completed	4	Liver Enzymes (ALT) [Time Frame: 7 days after probiotics]	Lipopolysaccharide (LPS) and Pro-inflammatory Cytokines [Time Frame: 7 days after probiotics]	NCT01501162
2.	Profermin®: Prevention of progression in alcoholic liver disease by modulating dysbiotic microbiota (SYN-ALD)	Interventional (Clinical Trial)	40	Dietary Supplement: Profermin Plus, FSMP, probiotics Dietary supplement: Fresubin, dietary supplement	Recruiting	NA	Hepatic stellate cell activity [Time Frame: 24 weeks]	1. Hepatic a-SMA acti vity 2. Alfa-smooth muscle actin concentration [Time Frame: 24 weeks]	NCT03863730
3.	Effect of Probiotics on gut–liver axis of alcoholic hepatitis	Interventional (Clinical Trial)	140	Drug: Probiotics (Lacidofil®) Drug: Placebo	Unknown	4	Liver enzymes [Time Frame: 7 days after probiotics]	1. LPS and pro-inflammatory cytokines. 2. Stool culture and stool Polymerase chain reaction denaturing gradient gel electrophoresis [Time Frame: 7 days after probiotics]	NCT02335632

(continued)

Table 8.3 (continued)

S. N	Study title	Study type	No. of subjects	Intervention/ treatment	Recruitment status	Phase	Primary outcome measures	Secondary outcome measures	Clinical trials.gov identifier:
4.	Novel therapies in moderately severe acute alcoholic hepatitis (NTAH-Mod)	Interventional (Clinical Trial)	130	Dietary Supplement: Lactobacillus rhamnosus GG Drug: Placebo for probiotic	Recruiting	NA	MELD score [Time Frame: 180 days] Improvement in MELD score over 180 day study duration.	Gut mucosal permeability [Time Frame: 180 days] Gut mucosal permeability will be measured by changes from baseline in the gut mucosal integrity as assessed by the lactulose/ mannitol test.	NCT01922895

Loguercio et al., in 2002 stated that a probiotics mixture, known as De Simone Formulation (Lactobacillus rhamnosus, Plantarum, Bifidus, Salivarius, Lactis, acidophilus, Casei, Bulgaricus, Breve, Fructooligosaccarydes, folic acid, Fe gluconate, and Zn oxide) has protective effects in liver disease. De Simone Formulation treatment showed significant improvement in plasma levels of 4-hydroxynonenal (4-HNEand) malondialdehyde (MDA) in NAFLD and alcoholic cirrhotic (AC) patients (Loguercio et al. 2002). Kirpich et al., in 2008 demonstrated the effectiveness of probiotics in the management of ALD patients and reported that probiotics (L. plantarum 8PA3B and bifidum) have shown significant increase in Bifidobacterium and Lactobacillus in human faecal matter and improved the levels of low-density lipoprotein (LDL), ALT, and total bilirubin (STB) (Kirpich et al. 2008). In an open-labelled study, Loguercio et al. assessed the efficacy of the probiotic containing Lactobacillus casei Shirota on healthy controls and patients suffering from alcoholic cirrhosis (AC). As compared to control group, patients receiving probiotic treatment of 4 weeks had a lesser TLR4 expression with a decrease in level of sTNFR1 (soluble TNF receptor 1) and sTNFR2, which clearly suggests that probiotic therapy is effective as well as safe for the patients with weak immune system (Loguercio et al. 2005). Another study revealed that treatment with a symbiotic mixture of different strains of bacteria with a prebiotic significantly improved liver function in AC patients. In a study Stadlbauer and co-workers used a combination of different strains of lactic acid bacteria for the treatment of alcoholic cirrhosis and observed a significant decrease in γGT (Gamma Glutamyl Transferase) and ALT levels (Stadlbauer et al. 2008).

8.8 Future Prospective and Conclusion

In preclinical studies, excessive consumption of ethanol causes dysbiosis leading to bacterial overgrowth in gut, which subsequently causes impairment of gut mucosa layer along with the damage of. The damaged tight junctions result into endotoxemia. Elevated endotoxins cause activation of Kupffer cells and stimulate inflammation and hepatic steatosis. Probiotics and prebiotic supplements prevent from alcohol-induced intestinal and liver injury via multiple mechanisms: (a) Alteration of gut microbiota; (b) reduction of free radicals or ROS production in liver and intestine; (c) improvement in mucosal layer and CRAMP, antimicrobial peptide, and expression of claudin-1 protein through increased HIF signalling; (d) inhibition of miR122a expression leading to upregulation of occludin; and (e) activation of hepatic AMPK. There is still requirement of exhausted research in the field of probiotics for the management of alcohol-induced liver disorders. From the above discussion, it was proposed that probiotics exert anti-inflammatory, antioxidant properties and help in the normal functioning of various vital organs involved in metabolism.

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Molecular Mechanism of Beneficial Effects of Probiotics in Alcohol-Induced Liver Disorder

9

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Abstract

Regular consumption of alcohol remains a predominant cause of a variety of hepatic disorders. There exist many alcohol-induced liver diseases including steatosis, steatohepatitis, and cirrhosis. Microflora of the gut has been considered as important in the pathophysiology of different disorders and long-term alcohol consumption significantly disrupts the intestinal flora composition and gut microbiota in liver disorders. Recent research studies have shown that probiotics significantly modulate the gut microbiota as well as ameliorate alcohol consumption-induced intestinal barrier dysfunction. Thus, targeting the gut–liver axis may be beneficial in the above-mentioned disorders. Interestingly, the investigations on the use of probiotics in alcohol-induced liver diseases are gaining more clinical importance. It has been shown that probiotics bring about

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an improvement in the responses of the immune system and significantly bring down the generation of free radicals induced by alcohol. Further, they reduce the inflammatory cytokines in the liver and intestine. Besides, studies have shown that the use of probiotics significantly increases fatty acid β-oxidation and decreases lipogenesis which is beneficial in the management of hepatic steatosis induced by alcohol. The current book chapter will focus on the use of probiotic species in preventing and treating alcohol-induced liver disorders along with the underlying potential mechanism of action.

Keywords

Probiotics · Alcohol-induced liver disease · Steatosis · Cirrhosis · Molecular mechanism

9.1 Introduction

Alcoholic liver disease (ALD) is defined as a chronic disorder of the liver that is a consequence of consumption of alcohol in large amounts. In its early stages, alcohol ingestion causes fatty liver disease, which further progresses to hepatitis, liver fibrosis, and cirrhosis.

According to statistical analysis, 80% of habitual drinkers show alcoholic liver injury. In about 10%–35% of such subjects, it may graduate to hepatitis while in 10%–20% of such people, signs of liver cirrhosis may be visible (Gao and Bataller 2011). According to the FAO/WHO, probiotics can be defined as live microbes that impart a great health advantage to the recipient when given in sufficient quantity (Hajela et al. 2014). A sizeable number of preclinical and clinical trials have investigated the useful outcomes of probiotic species in a variety of liver-related diseases including nonalcoholic steatohepatitis (Forsyth et al. 2009), cirrhosis (Sánchez et al. 2014), alcoholic liver disease (Sánchez et al. 2014; Segawa et al. 2008). Studies have shown the ability of probiotics to strengthen the gut barrier, keep the gut healthy, and decrease the translocation of bacteria (Tsai et al. 2019).

Intake of alcohol beyond a certain limit initiates the generation of free radicals in large amounts and significantly inhibits oxidation of fatty acids in the liver, which ultimately leads to ROS-mediated liver injury (García-Villafranca et al. 2008). Further, excessive alcohol ingestion induces significant changes in the intestinal barrier function. This can be attributed to the release of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukins (IL-1β) (Wang et al. 2013). An alteration in the gut barrier function results in bacterial translocation. Consequently, a sizeable proportion of liver Kupffer cells get accumulated, and further toll-like receptors (TLRs) present on their surface combine with the bacterial endotoxin to cause activation of signaling pathway including mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB), production of inflammatory cytokines, i.e., TNF-α, IL-6, and IL-1β (Hong et al. 2015). Interestingly, adenosine-monophosphate-activated protein kinase (AMPK) has also been reported to regulate

lipid metabolism through a variety of transcription factors that include PPAR-α (peroxisome proliferator-activated receptor-α), SREBP-1 (sterol regulatory element-binding protein 1), both of which play a significant role in the oxidation of fatty acids and lipogenesis. Studies have demonstrated that increased AMPK phosphorylation is beneficial in alcoholic fatty liver disease (Zhang et al. 2015). One of the chief transcription factors that plays a significant role in the gut wall functions maintenance, i.e., hypoxia-inducible factor (HIF) is also adversely affected by alcohol consumption. Intake of alcohol reduces the expression of HIF to a great extent. HIF significantly increases the intestinal trefoil factor (ITF) expression, xenobiotic clearance through P-glycoprotein (P-gp) as well as through other nucleotide signaling pathways (Wang et al. 2011).

In this chapter, the beneficial outcomes of probiotics in clinical and preclinical studies regarding the improvements in alcoholic liver disease along with different mechanisms are discussed.

9.2 Probiotic Products

There are a variety of probiotics, which are widely used in different conditions. *Saccharomyces cerevisiae* (boulardii) is the commonly given yeast strain. The bacterial probiotic species chiefly consist of *Lactobacillus* and *Bifidobacterium*, both saccharolytic species and are capable of fermenting carbohydrates to lactic acid (LA). Further, the role of LA in inhibiting bacterial growth of pathogenic strains is well established. The species of *Lactobacillus* genera include *L. rhamnosus, L. plantarum, L. sporogens, L. reuteri, L. bulgaricus, L. casei, L. delbrueckii, L. salivarius, L. johnsonii, and L. acidophilus*. The common species of *Bifidobacterium* genera that are used as probiotics include *B. bifidum, B. lactis, B. bifidus, B. breve (Yakult), B. infantis, and B. longum*. Other species of probiotics available on commercial scale include *Streptococcus acidophilus*, *Streptococcus thermophilus*, *Enterococcus SF68*, *Lactococcus lactis*, and *Escherichia coli* Nissle 1917 (Table 9.1) (Fijan 2014).

9.2.1 Pieces of Evidence in Support of Probiotics in Hepatic Diseases

Sizeable numbers of preclinical trials were conducted employing different models of alcoholic liver diseases including acute alcohol exposure, chronic exposure of alcohol, multiple-dose exposure of alcohol, and alcohol exposure plus lipopolysaccharide to study the effects of probiotics (Table 9.2). The different types of probiotic species like *L. rhamnosus* Gorbach Goldin (LRGG), thermally killed *Lactobacillus brevis* SBC8803, *L. rhamnosus* GG supernatant, *L. acidophilus*, *L. helveticus*, *De Simone Formulation*, *Bifidobacterium* have been evaluated for their effectiveness in the experimental models of alcohol-induced liver injury.

Table 9.1 Commonly used probiotic bacterial genera and species in alcohol-induced liver injury

Serial No.	Commonly used probiotic bacterial genera	Species
1.	Lactobacillus	*L. rhamnosus* *L. brevis* *L. plantarum* *L. sporogens* *L. reuteri* *L. casei* *L. bulgaricus* *L. delbruecki* *L. salivarius* *L. johnsonii* *L. helveticus* *L. acidophilus*
2.	Bifidobacterium	*B. bifidum* *B. lactis* *B. bifidus* *B. breve (Yakult)* *B. infantis* *B. longum*
3.	Miscellaneous	*Streptococcus thermophilus* *Streptococcus acidophilus* *Lactococcus lactis* *Enterococcus SF68* *Escherichia coli* Nissle 1917 *Akkermansia muciniphila*

Endotoxin derived from gut is considered an important mediator of ALD development. Beneficial outcomes of thermally killed *L. brevis* SBC8803 were observed on gut-derived endotoxin in an animal model of ethanol-induced alcoholic hepatic injury. Oral administration of *L. brevis* SBC8803 (500 mg/kg OD for 35 days) significantly upregulated the expressions of heat shock protein, Hsp25 mRNA (which are cytoprotective), and suppressed overexpression of TNF- α induced by alcohol and hepatic levels of SREBP-1, and SREBP-2. Also, *L. brevis* SBC8803 inhibited ethanol diet fed-induced increase in serum SGOT, SGPT levels with concomitant inhibition of elevated levels of hepatic cholesterol and triglycerides. It suggests that *Lactobacillus brevis* SBC8803 significantly ameliorates hepatic damage and fatty liver by inhibiting the upregulation of inflammatory cytokine (TNF-α) and transcription factors (SREBPs). The observed protective effects may be attributed to the cytoprotective heat shock proteins-induced appreciation of intestinal barrier function and inhibition of gut-derived endotoxin migration to hepatic tissues (Segawa et al. 2008). Furthermore, live LRGG treatment (once daily gavage of 2.5×10^7) in an animal model of alcoholic steatohepatitis significantly preserved the gut barrier functioning by attenuating oxidative stress in the intestine precipitated by alcohol intake, gut leakiness/hyperpermeability, inflammation, and severity of liver damage (Forsyth et al. 2009).

Table 9.2 Preclinical studies showing useful outcomes of probiotics in different models

Serial No.	Model	Probiotics treatment	Pharmacological Effects	Reference
1.	Mouse model of ethanol-induced alcoholic liver injury.	Thermally killed *L. brevis* SBC8803 (500 mg/kg once a day for 35 days)	Upregulated the expressions of heat shock protein, Hsp25 mRNA in the small intestine. Overexpression of certain cytokines like TNF- α and transcription factors like SREBP-1 and SREBP-2 which are induced by alcohol intake is suppressed.	Segawa et al. (2008)
2.	Rat model of alcoholic steatohepatitis	Live *Lactobacillus rhamnosus* Gorbach Goldin (GG) treatment (OD gavage of either 2.5×10^7)	Attenuated ethanol-induced intestinal oxidative stress, gut leakiness or hyperpermeability, the severity of inflamed intestinal and hepatic tissues	Forsyth et al. (2009)
3.	Mouse model of liver injury	*Lactobacillus rhamnosus* GG supplementation in Lieber-De Carli alcohol diet for 2 weeks	Reduced TNF-α production and decreased inflammation via inhibition of hepatic mRNA expression of TLR4- and TLR5-mediated endotoxin activation Decreased phosphorylation of p38 MAP kinase	Wang et al. (2013)
4.	Lieber-DeCarli alcohol diet-induced endotoxemia, hepatic steatosis, and injury	*Lactobacillus rhamnosus* GG treatment	Normalized HIF-2α levels that are reduced following alcohol intake and HIF responsive proteins, i.e., ITF levels	Wang et al. (2011)
5.	Lieber-DeCarli alcohol diet-induced endotoxemia, hepatic steatosis, and injury	*Lactobacillus rhamnosus* GG culture supernatant treatment for 5 days	Normalized ethanol-induced decrease in ileum mRNA levels of claudin-1, P-gp, and CRAMP Increased levels of these three named factors	Wang et al. (2012)

(continued)

Table 9.2 (continued)

Serial No.	Model	Probiotics treatment	Pharmacological Effects	Reference
6.	Hepatic steatosis and injury induced by acute and chronic exposure to alcohol	*Lactobacillus rhamnosus* GG	Enhanced hepatic AMPK phosphorylation and modulation of apoptosis mediated by Bax/Bcl-2	Zhang et al. (2015)
7.	Chronic and binge alcohol-fed animals.	*Lactobacillus rhamnosus* GG supernatant	Significantly improved intestinal function and hepatic injury as observed by a decrease in Escherichia coli protein levels in the liver, enhanced mRNA expression of tight junction proteins, and villus-crypt histology in the ileum in chronic and binge alcohol-fed animals. Lactobacillus rhamnosus GG supernatant potentially improved the functioning of the intestinal barrier, balanced Treg, and TH17 cells, and effectively attenuated liver damage in chronic and binge alcohol-fed mice	Chen et al. (2016)
8.	Rat model of chronic alcoholic liver injury	*Lactobacillus plantarum*	Suppressing inflammatory markers, i.e., TNF-α, NF-kB, and interleukin (IL)-12/p40 subunit and restore the histological changes of liver	Arora et al. (2014)
9.	Rat model of alcohol exposure-induced acute as well as chronic liver damage	*Lactobacillus plantarum C88*	Showed protection against the gut leakiness via upregulation of the tight junction proteins expressions and suppression of the inflammation mediated by endotoxins by diminishing P38 phosphorylation and through down-regulation of NF-κB	Zhao et al. (2017)

(continued)

Table 9.2 (continued)

Serial No.	Model	Probiotics treatment	Pharmacological Effects	Reference
			expression in alcohol-fed animals	
10.	Ethanol-induced steatosis in mouse model	*Lactobacillus plantarum LC27, Bifidobacterium longum LC67, their mixture (LM)*	Suppression of NF-κB activation induced by alcohol and decreased expression of α-smooth muscle actin in hepatic tissues and also enhanced the AMPK activation otherwise suppressed by alcohol intake. Similarly, the levels of alcohol dehydrogenase and acetaldehyde dehydrogenase, and tight junction protein expression were also elevated. There was an improvement of gut microflora composition *Vis-à-Vis* the estimated levels of proteobacteria, inhibition of fecal and blood lipopolysaccharide levels	Kim et al. (2018)
11.	Chronic-ethanol feeding-induced liver damage	*Lactobacillus plantarum*	Significantly attenuated the gut barrier dysfunction induced by alcohol, reduced inflammation, and hepatic injury mediated by endotoxins *Lactobacillus plantarum* abolished alcohol-induced effects via EGFR-dependent mechanism	Shukla et al. (2018)
12.	Alcohol-induced liver injury	De Simone Formulation, a probiotic mixture of live lyophilized *Bifidobacterium* species	Maintained the ecological balance of gut microbiota by down-regulating the expression of TNF-α and increasing tight junction proteins	Chang et al. (2013)

(continued)

Table 9.2 (continued)

Serial No.	Model	Probiotics treatment	Pharmacological Effects	Reference
			expression to decrease epithelial permeability	
13.	Ethanol-fed rats	*Streptococcus thermophilus, L. bulgaricus, B. longum*, *B. bifidum,* and *L. acidophilus,*	Inhibited the increase in plasma levels of endotoxin levels through restoration of permeability of the intestine and microbiological composition of fecal matter	Chiu et al. (2015)
14.	Mouse model of alcoholic liver disorder	Probiotics mixture comprising *Lactobacillus rhamnosus R0011* and *Lactobacillus acidophilus* R0052 (Lacidofil®)	Inflammatory cytokine levels were reduced following treatment with a probiotic mixture. A decrease in TLR4 levels	Bang et al. (2014)
15.	Mouse model of alcoholic liver disorder	*Akkermansia muciniphila*	*A. muciniphila* promoted intestinal barrier integrity via enhancing the expression of tight junction (TJ) proteins claudin-3 and occludin	Grander et al. (2018)
16.	Chronic-binge ethanol-fed mice	*Lactobacillus reuteri*	*Lactobacillus reuteri* producing IL-22 in intestine induces expression of REG3G to reduce ethanol-induced liver disease chronic-binge ethanol-fed mice	Hendrikx et al. (2019)
17.	Chronic-ethanol-fed rat	A Probiotic mixture, Golden Bifido (live *Lactobacillus bulgaricus*, *Bifidobacterium*, and *Streptococcus thermophilus*, LBS)	Golden Bifido ameliorates alcoholic liver disease in rats via the inflammation suppression and the regulation of the intestinal microbiota	Huang et al. (2019)

Another study has investigated the outcomes of probiotic LRGG therapy on gut-derived endotoxin and associated hepatic inflammation. Fourteen days of treatment with LRGG in Lieber-DeCarli alcoholic injury model significantly attenuated hepatic inflammation via reducing TNF-α production, hepatic mRNA expression of TLR4, and TLR5-mediated endotoxin activation. Additionally, a significant decrease in ethanol-induced p38 MAPK phosphorylation was also observed

following this treatment (Wang et al. 2013). Another set of experimentation by the same team of scientists also demonstrated that LRGG treatment significantly decreased Lieber-DeCarli alcohol diet-induced endotoxemia, hepatic steatosis, and injury. Lieber-DeCarli alcohol diet reduced the adaptive response-induced HIF and HIF responsive proteins, i.e., ITF levels, suggesting the key role of reduced supply of oxygen to the intestine and decreased HIF responsive proteins in precipitating alcoholic liver injury. Further, LRGG treatment normalized the levels of HIF-2α and ITF otherwise reduced by intake of alcohol, suggesting that HIF signaling plays an important role in the useful effects of probiotics therapy in ALD (Wang et al. 2011). The same group of co-workers also found that treatment with *Lactobacillus rhamnosus* GG culture supernatant for 5 days significantly restores the decreased ileum mRNA levels of claudin-1, P-gp, and CRAMP, all of which have significant important roles in maintaining intestinal barrier integrity. Interestingly, increased ileum RNA expression of HIF-2α, P-gP, and CRAMP was observed following LRGG culture supernatant treatment, confirming that administration of culture supernatant attenuates damage to hepatic tissues induced by alcohol through the promotion of HIF signaling and subsequently, suppressing the permeability of intestine and levels of endotoxins otherwise increase by intake of alcohol (Wang et al. 2012).

Zhang et al. investigated the possible role of AMPK in the protective effects of LRGG against acute alcohol exposure-induced hepatic steatosis and hepatic damage. It was observed that LRGG significantly ameliorates acute alcohol exposure-induced rise in lipogenic genes expression, SREBP, and stearoyl-CoA desaturase-1 along with a significant reduction in increased expression of PPAR-α, PPAR-γ coactivator protein-1α, and carnitine palmitoyltransferase-1, which leads to enhanced β-oxidation of fatty acids. Interestingly, chronic alcohol exposure reduced AMPK phosphorylation and enhanced acetyl-CoA carboxylase activity, which was shown to be ameliorated in the presence of LRGG treatment. Further, this treatment considerably increased Bcl-2 expression and reduced expression of Bax, which potentially inhibited acute alcohol exposure-induced hepatic apoptosis process. Therefore, it may be possible to suggest that LRGG treatment shows its useful outcomes in preventing acute alcohol exposure-induced hepatic steatosis and damage to hepatic tissues through enhanced hepatic AMPK phosphorylation and modulation of apoptosis mediated by Bax/Bcl-2- (Zhang et al. 2015).

Treatment with LRGG supernatant significantly improved the function of the intestine and attenuated hepatic injury as observed by an evident fall in *Escherichia coli* protein levels in the liver, rise in expression of mRNA related to tight junction proteins, and villus-crypt histology in the ileum in chronic and binge alcohol-fed animals. Interestingly, the analysis by flow cytometry showed that chronic-binge alcohol exposure significantly increased TH17 cell count, IL-17 secretion, and reduced Treg cell count, which were significantly attenuated following LRGG supernatant treatment. It indicates that LRGG supernatant promotes the functioning of the intestinal barrier, brings about a balance of Treg and TH17 cells to effectively attenuate liver damage in chronic and binge alcohol-fed mice (Chen et al. 2016).

L. plantarum C88 has also been investigated for its various useful properties such as reducing oxidative stress response, restoring gut microbiota, inhibiting endotoxemia-induced inflammation in the intestine, improving intestinal barrier dysfunction, and diminishing alcohol exposure-induced acute as well as chronic liver damage. In a study, *L. plantarum* treatment significantly ameliorated the liver injury by suppressing inflammatory markers, i.e., TNF-α, NF-kB, and interleukin (IL)-12/p40 subunit and restored the tissue levels changes in the liver thereby establishing the utility of probiotic species as a good pharmacological intervention for effective management of alcohol-induced liver damage (Arora et al. 2014). In separate experiments by Zhao et al., the ability of *L. plantarum* C88 to offer protection was studied against mice. *L. plantarum* C88 treatment was shown to exhibit protection against gut leakiness via the upregulation of the expressions of tight junction proteins and suppression of endotoxin-mediated inflammation by diminishing p38 phosphorylation and down-regulating NF-κB expression in alcohol-fed animals. The observed findings suggest that *L. plantarum* C88 may be used to effectively manage alcoholic chronic liver injury (Zhao et al. 2017).

Another study has observed the beneficial outcomes of *L. plantarum* LC27, *B. longum* LC67, and their mixture (LM) in ethanol-induced steatosis in a mouse model. LC27, LC67, or LM treatment largely restored alcohol-induced alteration in the hepatic and blood levels of ALT, AST, TG, and TC. The treatment also led to the suppression of alcohol-induced activation of NF-κB a, expression of α-smooth muscle actin in the hepatic tissues, and upregulation of alcohol-induced decrease in AMPK activation. Probiotics also increased the levels of enzymes, namely alcohol dehydrogenase and acetaldehyde dehydrogenase the levels of which are otherwise suppressed by alcohol exposure along with the tight junction protein expression in the colon and liver. Also, this probiotic therapy was found to improve the gut microbiota composition, assessed in terms of the increased count of proteobacteria, inhibition of levels of lipopolysaccharides in blood and fecal matter. The above finding strongly advocates that LC27, LC67, and LM may significantly attenuate alcoholic hepatic steatosis through inhibition of NF-κB activation and augmenting AMPK activation (Kim et al. 2018). Experiments by Shukla et al. unraveled the role of the EGFR-dependent mechanism in the observed beneficial outcomes of *L. plantarum* against chronic alcohol feeding-induced hepatic damage. *L. plantarum* supplementation significantly attenuated ethanol-induced gut barrier dysfunction, endotoxemia-mediated inflammation, and hepatic damage. The in vitro study depicted the key role of EGF receptor signaling in *Lactobacillus plantarum* induced shielding of tight junctions and intestinal barrier function from the harmful effects of acetaldehyde- a byproduct of alcohol metabolism in Caco-2 cell monolayers. In EGFR knockout mice, *L. plantarum* failed to prevent alcohol-induced tight junction protein disruption, oxidative stress, mucosal barrier dysfunction, inflammatory cytokines, endotoxemia, and hepatic injury, suggesting that *L. plantarum* abolishes alcohol-induced deleterious effects via EGF receptor-dependent mechanism (Shukla et al. 2018). A recent study has documented that alcohol exposure significantly diminished intestinal *Akkermansia muciniphila* in humans and mice. *Akkermansia muciniphila* is a Gram-negative anaerobic bacteria

that produce short-chain fatty acids (SCFA) such as propionates. SCFA propionates significantly inhibit histone deacetylase and epigenetically influence host gene expression. Further, *Akkermansia muciniphila* supplementation has been shown to reverse alcohol-mediated liver injury and gut microbiota dysbiosis. It has also been suggested that the probiotic *Akkermansia muciniphila* promoted intestinal barrier integrity via enhancing the expression of tight junction (TJ) proteins claudin-3 and occludin, thereby significantly ameliorated alcoholic liver injury (Grander et al. 2018). Ethanol-induced dysbiosis reduces levels of indole-3-acetic acid (IAA), a microbiota-derived ligand of the aryl hydrocarbon receptor (AHR), which regulates expression of IL-22. Interleukin 22 (IL-22) regulates the expression of regenerating islet-derived 3 gamma REG3G. It has been observed that *Lactobacillus reuteri* produces IL-22 in the intestine inducing expression of REG3G to reduce ethanol-induced liver disease in chronic-binge ethanol-fed mice (Hendrikx et al. 2019).

Interestingly it has been observed that De Simone Formulation, a probiotic mixture significantly prevents the entry of endotoxin and other bacterial products from the intestine into the systemic circulation. De Simone Formulation comprised of live freeze dried *B. longum*, *B. breve*, *B. infantis*, *L. plantarum*, *L. acidophilus, L. bulgaricus, L. paracasei*, and *Streptococcus thermophilus*. Treatment with De Simone Formulation was reported to maintain the ecological balance of gut microbiota by down-regulating the expression of TNF-α and through enhanced expression of tight junction proteins leading to decreased epithelial permeability in an animal model of alcoholic liver injury (Chang et al. 2013). Another study in an animal model of ethanolic liver damage concluded that when animal diets were supplemented with probiotic species such as *L. bulgaricus*, *L. acidophilus, B. longum*, *B. bifidum,* and *Streptococcus thermophilus* significantly inhibited the increase in levels of plasma endotoxin by restoring permeability of the intestinal membrane and microbiological contents of fecal matter (Chiu et al. 2015). Another set of experimentation that involved supplementation with a probiotic mixture comprising *L. rhamnosus* R0011 and *L. acidophilus* R0052 (Lacidofil®) was found to be efficacious in two different mouse models of alcoholic liver disorder. Also, a significant decrease in the toll-like receptors-4, TLR4 levels was observed in probiotics-treated animals (Bang et al. 2014). The harmful effects of altered ecology of gut microbiota such as inflammatory responses and liver injury are presumably exerted through TLR4 pathway. Treatment with probiotics mixture also significantly decreased the content of harmful cytokines like TNF-α and IL-1β (Hong et al. 2015), thus confirming the inhibitory effects of probiotics on the TLR4 pathway, often associated with liver damage and the release of TNF-α and IL-1β. Another recent study has also demonstrated that probiotic mixture, Golden Bifido (live *Lactobacillus bulgaricus*, *Bifidobacterium*, and *Streptococcus thermophilus*, LBS) ameliorates alcoholic liver disease in rats via the inflammation suppression and the regulation of intestinal microbiota (Huang et al. 2019) (Fig. 9.1).

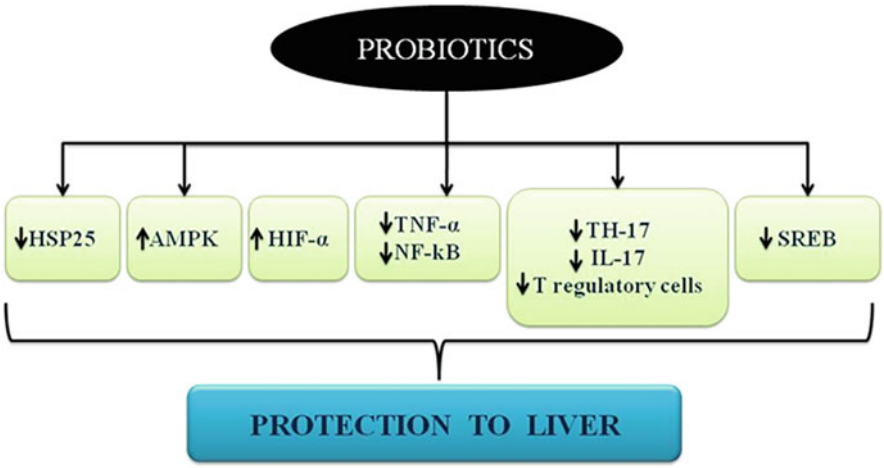

Fig. 9.1 Different mechanisms that may be involved in probiotics-mediated liver-protective actions; *HSP* Heat shock proteins, *TNF* Tumor necrosis factor-α, *HIF* Hypoxia-inducible factors, *SREB* Sterol regulatory element-binding proteins, *AMPK* Adenosine-monophosphate-activated protein kinase phosphorylation, *IL* Interleukin, *TH-17* T helper 17 cells

9.2.2 Clinical Studies Showing the Useful Effects of Probiotics

A large number of clinical trials have been conducted to investigate the efficacy of probiotic species in subjects with alcohol-induced hepatic damage. The subjects with alcohol-induced cirrhosis are more susceptible to infections and have high hospital mortality rates, which may be due to defects in the innate immune response with an inappropriate inflammatory response. Furthermore, several studies have described the impaired phagocytosis, bacterial killing, bowel flora, and neutrophil accumulation in alcoholic hepatitis subjects (Rajkovic and Williams 1986; Fiuza et al. 2000). A clinical study determined the effects of *L. casei* Shirota administration on the neutrophil function in subjects with alcoholic cirrhosis. This species is given three times daily in the prescribed manner (6.5×10^9 CFU, for 4 week time period, with abstinence state) significantly normalized the neutrophil phagocytic capacity in the selected patients. Furthermore, significant alterations in the neutrophil function were also linked with a rise in the surface receptor expression of TLR 4, which were found to be attenuated following *Lactobacillus casei* Shirota treatment. The study advocated that 4 weeks of probiotic treatment significantly decreased IL-10 production. Therefore, it can be suggested that the observed improvement in the neutrophil phagocytosis capacity may be mediated through modulation of secretion of IL-10 secretion and expression of TLR4 (Stadlbauer et al. 2008).

In another clinical study involving liver cirrhosis patients, probiotic treatment was shown to decrease hepatic encephalopathy. The study examined that liver cirrhosis subjects with minimal hepatic encephalopathy (MHE) had a significant change in the intestine microflora along with substantial fecal overgrowth of pathogens like Gram-

negative *E. coli* and Gram-positive *Staphylococcal* species. Further, synbiotic treatment for 30 days containing four lyophilized, non-urease-producing bacteria, namely *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *L. paracasei* subspecies paracasei, and *L. plantarum* along with bioactive fermentable fiber significantly modulated the gut flora, reduced blood ammonia levels, raised the fecal content of non-urease-producing *L.* species, and attenuated the endotoxemia associated with reversal of minimal hepatic encephalopathy in cirrhotic patients (Liu et al. 2004). In a clinical trial on alcoholic liver cirrhotic subjects, Yakult 400 treatment comprising *L. casei* twice a day for 4 weeks significantly increased the blood concentration of liver-specific transthyretin protein and decreased the levels of hypersensitive C reactive protein along with a significant improvement in gut flora (Koga et al. 2013). In a similar study, scientists explored the effects of probiotic treatment on bacterial overgrowth in the small intestine and permeability in subjects with chronic liver disease. A 4 week probiotics treatment (Duolac Gold probiotics containing six bacteria, namely *Streptococcus thermophilus, L. rhamnosus, L. acidophilus, B. longum*, *B. bifidum*, *B. lactis*) in chronic liver disease patients significantly alleviated the small intestinal bacterial overgrowth along with slight improvement in intestinal permeability. It suggested that the short-term probiotics treatment may be effective in attenuating small intestinal bacterial overgrowth but found to be useless when evaluated for its capacity to improve the permeability of the intestine and liver functions (Kwak et al. 2014). In a randomized controlled clinical study, the effects of probiotic treatment (Balgilac and Balgibif containing *L. plantarum* and *B. bifidum*) were studied in alcoholic hepatitis subjects. The study concluded that five-day oral supplementation with *B. bifidum* and *L. plantarum* 8PA3 led to normalization of altered bowel microbiota and improved ethanol-induced hepatic damage (Kirpich et al. 2008). A study by Dhiman et al. 2014 has reported that probiotic De Simone Formulation treatment significantly reduced liver disease severity in patients with alcoholic cirrhosis (Dhiman et al. 2014).

9.3 Conclusion

Evidence suggests that probiotics show protection against ethanol-induced liver damage through their antioxidant action, improvement in lipid metabolism, suppressing inflammatory cytokine expression in the intestine and liver, improvement in intestinal barrier function, and gut flora and modulation of the mucosal immune system. Probiotics have shown these protective effects through modulation of AMPK phosphorylation, hypoxia-inducible factor, toll-like receptor, epidermal growth factor, nuclear factor NF-kB, Bcl-2 expression, Bax expression, tight junction, and heat shock protein.

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Effect of Probiotic Supplementation on Modulation of Serum Lipids

10

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Abstract

An alteration in the intestinal microbiota due to a high-fat diet inherited disorder modulates lipid metabolism leading to cardiovascular diseases. Recently, to improve the lipid profiles, several pharmacological and non-pharmacological therapeutic strategies have been developed which involve the use of probiotics. Researchers noted mechanism wherein probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, causing decline in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or cholesterol redistribution from plasma to the liver. In certain bacteria, interference with cholesterol absorption from the gut by deconjugating bile salts through bile salt hydrolase affects the cholesterol metabolism or through direct cholesterol assimilation to stabilize their cell membrane and binding to the cell walls of probiotics in the intestine to convert cholesterol into coprostanol. The animal and human experimental model suggests significant reduction in serum triglycerides, total and LDL-cholesterol along with increased HDL-cholesterol using appropriate strains of lactic acid bacteria and bifidobacteria. At present, few clinical trials yielded conclusive results; therefore, need to have a precise understanding of the underlying mechanism, dosage, efficacy, safety concerns before probiotic consumption and when used in combination with drug therapy which could only be achieved through well-designed clinical trials.

Keywords

Intestinal microbiota · Cardiovascular diseases · Serum lipid profile · Probiotics · Lipid metabolism

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10.1 Overview

The urbanization and change in lifestyle patterns have led to an exorbitant increase in the rate of chronic diseases, particularly in cardiovascular diseases. According to the statistical reports, WHO accounts that approx. 17 million people (~31% worldwide) died from cardiovascular diseases (CVDs) mainly due to acute myocardial infarction (AMI) and stroke (WHO 2017). The WHO has already predicted that by the year-end of 2030, cardiovascular disorders (CVDs) are considered as the major contributing factor in the cause of human death affecting approximately 23.6 million people worldwide with the majority of cases in the South Asia region (Enas et al. 2007). This could lead to considerable economic losses and the requirement of a significant amount of expenditure for the healthcare sector (Gadelha and Bezerra 2019).

10.1.1 Contributing Factors for Cardiovascular Diseases (CVDs)

The risk factor which is contributing significantly is the buildup of visceral fat which is due to complex interactions between genetics and environmental factors and is associated with subclinical systemic inflammation (Luo and Liu 2016). The other contributing factors for high lipid levels that could finally lead to cardiovascular diseases (CVDs) are certain medical conditions such as diabetes, hypothyroidism, alcoholism, kidney disease, liver disease, and stress (Park et al. 2020). The prominent CVDs are hypercholesterolemia and hypertriglyceridemia, which led to an increased level of low-density lipoproteins (LDL) and reduction in high-density lipoproteins (HDL), and are also important targets for attempts to prevent heart-related disorders (Grundy et al. 2005; Sherbet et al. 2013).

To predict cardiovascular risk, lipid profile analysis has almost become a routine test. The major challenges faced to control the risk of CVDs are high epidemic proportions; it develops quietly and is associated with other contributing factors such as certain medical conditions which led to increased risk for cardiovascular diseases (Ezzati et al. 2002; Al-Hamad and Raman 2017; Fortes et al. 2018).

10.1.2 Pharmacological Approach Versus Non-pharmacological Approach

Although several studies indicate that drugs have shown convincing results in lowering cholesterol levels. But the pharmacological approach is associated with certain drawbacks. Certain treatments involved in CVDs have unwanted side effects when used for the long term; involve expensive drug therapy, use of statins (3-hydroxy-3-methylglutaryl coenzyme inhibitors), fibrates, niacin, cholesterol absorption inhibitors, and bile acid sequestrants (Bliznakov 2002). Therefore, the need of an hour is to work on the dietary management of serum cholesterol and triglyceride (TG) levels as the large population is affected by cardiovascular

diseases. In this context, realizing the drawbacks associated with the pharmacological approach, researchers and pharmacologists are working on finding a novel alternative method through non-pharmacological approaches which include lifestyle modification, adherence to low-fat/low saturated fat diet (Taylor and Williams 1998), involving the potential cholesterol- lowering plant-based products such as plant stanols, soy, cinnamon, and use of soluble fibers (WHO 2003). Still, the situation has become very difficult due to its multifactorial origin (Bilen et al. 2016). In this context, the role of intestinal microbiota has been realized and studied.

10.1.3 Role of the Intestinal Microbiota

Scientific evidence has clearly shown that the intestinal microbiota whether intestinal or systemic play a vital role in maintaining good health. Any type of change in the microflora of the intestine leading to gut instability or dysbiosis affects the emergence of many different diseases, especially non-transmissible chronic diseases. It has been reported that individuals with lower bacterial diversity in their intestinal microbiota have hypercholesterolemia as compared to the controls (Rebolledo et al. 2017). Therefore, the virtue of probiotics has already been recognized in terms of general gut health and immunity. Probiotics are defined as "living microbial supplements that beneficially affect the host animals by improving its intestinal microbial balances" or as live microorganisms that when administered in adequate amounts, confer a health benefit on the host (WHO 2003).

A different mechanism of lipid metabolism has been reported in the literature which has been discussed in the later section of this chapter. Several strains of lactic acid bacteria and bifidobacterium are reported to be involved in lipid metabolism wherein (1) bile salt hydrolases are produced which causes deconjugation of bile salts with low absorption and increased excretion. (2) In order to overcome the losses, this led to an increased demand for cholesterol to synthesize new molecules. (3) Inhibition of cholesterol transmembrane transporter expression in enterocytes; (4) The production of short-chain fatty acids led to the inhibition of hepatic synthesis of cholesterol and fatty acids; (5) The cholesterol is incorporated into the cell membrane of microorganism during microbial growth (Ooi and Liong 2010; Reis et al. 2017).

10.2 Hypocholesterolemic Potential of Probiotics: Earlier Research Evidences

Over the years, the use of animal and human models to evaluate the effect of probiotics on serum cholesterol levels has shown certain promising outcomes. To study the effect of new probiotic strains on lipid metabolism in humans, certain animals such as rats, mice, hamsters, guinea pigs, and pigs have been used. These animals have shown similarities with humans in terms of digestive anatomy and

physiology, nutrient requirements, bioavailability and absorption, and metabolic processes. The metabolic processes which are shared with humans are cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of hepatic cholesterol enzymes (Fernandez et al. 2000). Due to these aforesaid metabolic properties, the animals are therefore useful as experimental models for research applications (Patterson et al. 2008). The reliability of results thus obtained through the animal model has been confirmed through the human trial results.

The initial scientific reports of the use of probiotics in lipid metabolism date back more than 40 years and came from the tribes of Samburu and Maasai warriors in Africa wherein reduced serum cholesterol was noted after consumption of large amounts of milk fermented with a wild *Lactobacillus* strain (Shaper et al. 1963; Mann 1974). This led to the realization of the potential hypocholesterolemic effect in fermented milk products containing lactobacilli and/or bifidobacteria through animal and human studies.

10.3 Probiotic Market for Cholesterol Reduction

In the past two decades, the importance of prebiotics and probiotics in lowering blood cholesterol level has been recognized on a global level. In this direction, the European market has taken an initiative to penetrate through products claiming to lower the blood cholesterol level and thereby contributing towards a healthier heart (Young 1998). Danone launched Actimel Cholesterol Control® in Belgium, containing the suggested cholesterol-lowering probiotic *Lactobacillus acidophilus* and the branded prebiotic ACTILIGHT® (Beghin-Say), while, "Mona," a Danish company introduced a cultured dairy-based drink under the brand name Fysiq®. It contains the probiotic *Lactobacillus acidophilus* and the branded bifidogenic dietary fiber, RAFTILINE® (Orafti). On similar lines, MD Foods, a Danish company introduced a yogurt-style product "Gaio." The UK-based life sciences firm OptiBiotix Health has developed a naturally occurring bacterial strain, LP-LDL (*Lactobacillus plantarum* ECGC 13110402), known to lower LDL-cholesterol along with lowering systolic blood pressure. The CEO of OptiBiotix Health, Steve Prescott has preliminary talks with food and beverage brands in the USA for developing yogurt or other functional food having the claimed probiotic. The selected strain has high bile salt hydrolase activity, cholesterol removal potential, resistance to gastric, pancreatic, and bile acids, and a higher surviving rate on freeze-drying. The LP-LDL is shelf-stable at room temperature while it needs to be refrigerated in presence of moisture. UAS labs have developed *Lactobacillus reuteri* NCIMB 30242 (LRC); Kaneka claimed a product Floradapt Cardio containing three strains of *Lactobacillus plantarum* that could reduce LDL along with reduction in blood pressure (Watson 2020).

Still, it is difficult to understand the beneficial effects of these products in terms of blood lipid levels in humans (Pereira and Gibson 2002a, b).

10.3.1 Commercially Available Probiotic Products: Role and Research Evidence in Cholesterol Reduction

One of the fermented milk products launched by the company Gaio® having bacterial cultures, *Enterococcus faecium* and two strains of *Streptococcus thermophilus* (CAUSIDO® culture) were able to reduce plasma cholesterol levels on the intake of appropriate levels. These bacterial strains were isolated from the intestinal flora of inhabitants of Abkhasia (Caucasus), a region known for the longevity of its people and where fermented milk is a major part of their diet (Agerbaek et al. 1995). In one of the study, carried out by Agerbaek and coworkers, the consumption of 200 mL/ day of this fermented milk product for over 6 weeks by Danish middle-aged men (44 years old) with a subject group of 58 male volunteers led the reduction up to 6% of total plasma cholesterol and 10% reduction of LDL-cholesterol (Agerbaek et al. 1995). In this study, no change in serum HDL-cholesterol or plasma triacylglycerol levels was observed. The study was random, double-blind, and controlled. In another study, Richelsen and coworkers noted that there was a marked difference in serum LDL-cholesterol after the consumption of up to 200 mL/day of the Gaio® product for 1 month (Richelsen et al. 1996). However, it was also noted that long-term consumption up to 6 months did not show any marked difference in terms of reduction of LDL-cholesterol with that of placebo product.

Certain researchers concluded that although low-fat milk or fermented milk products may have some hypocholesterolemic effects but are not superior to the placebo milk product since a reduction in cholesterol levels was also noted in the placebo group (Taylor and Williams 1998). A similar trend has also been noted by Agerbaek and coworkers in both test and placebo experimental set (Agerbaek et al. 1995). During in vitro studies, Agerbaek and coworkers noted that the cultures present in the fermented milk play a vital role in determining the reduction in cholesterol levels. It was also inferred that though *Streptococcus thermophilus* is acid sensitive and does not, to any significant degree, survive passage through the small intestine during in vivo studies. So, it is the *Enterococcus faecium* which plays a significant role in possessing the beneficial effect of fermented milk product. It was also observed that reduction in the concentration of *Enterococcus faecium* in the test product could have contributed majorly in lowering of product efficacy and thereby decline in the effectiveness of cholesterol reduction (Agerbaek et al. 1995).

Similarly, Rossi and coworkers tested the ability of certain microorganisms which could reduce in vitro cholesterol levels and can tolerate bile salts. The test microorganisms were *E. faecium*, *L. acidophilus*, *L. jugurti*, *S. thermophilus,* and *L. delbrueckii.* The reduction in the cholesterol levels in the medium containing *E. faecium only* and in the mixture of *E. faecium* plus *L. acidophilus was observed* after 24 h of anaerobic incubation and a significant decline in vitro cholesterol level up to 53 and 65%, respectively, was noted (Rossi et al. 1999). Similar observations were made by Larsen and coworkers, wherein, a significant decline in LDL-cholesterol was reported in the Gaio® product group only (8.4%, $P < 0.05$) after 8 weeks. This decline would correspond to a reduction in the risk factor for

CHD up to 20–30%, which is of clinical relevance (Larsen et al. 2000). The prominent reason for the hypocholesterolemic effect of Gaio® product is related to the CAUSIDO® bacterial culture, particularly *E. faecium.*

In another study, conducted by Schaasfsma and coworkers, the effect on serum total cholesterol level was noted when "Danone" yogurt containing *Lactobacillus acidophilus* and 2.5% (w/v) fructo-oligosaccharides was fed for two treatment periods of 3 weeks separated by a washout period of 1 week. During the treatment periods, 30 male subjects consumed 125 mL of either test or reference product thrice daily. The study was randomized, placebo-controlled, double-blind with a two-way crossover trial. A significant reduction in serum total cholesterol (4.4%) and LDL-cholesterol (5.4%) was noted in test product (*Lactobacillus acidophilus* and 2.5% (w/v) fructo-oligosaccharides, 0.5% (w/v) vegetable oil and 0.5% (w/v) milk fat) against reference (traditional yogurt, containing 1% (w/v) milk fat). It further needs to be evaluated to know whether the hypocholesterolemic effect of the test product is either due to *Lactobacillus acidophilus* strain, or fructo-oligosaccharides (FOS), or both. The dosage and time factor also need to be taken into consideration to determine the effect on serum cholesterol levels (Schaafsma et al. 1998).

OptiBiotix Health conducted clinical studies and claimed that the use of probiotic *Lactobacillus plantarum* ECGC 13110402 led to 1% reduction in serum cholesterol along with lessening of artery disease risk by 2–3%. The clinical study was carried out with subjects having normal blood pressure which led to a significant reduction in systolic blood pressure up to 5.1% after 6–12 weeks. Further, studies need to be conducted using hypertension patients in a larger group for a clear representation of significant effects. Though certain studies were conducted for a third human trial by the University of Roehampton, UK, but it got delayed due to COVID-19. The 2017 study was conducted with a double-blind, placebo-controlled, randomized human intervention for over 12 weeks with 49 normal to mildly hypercholesterolemic adults aged 30–65. The results showed that twice daily ingestion of 2×10 CFU (Colony-Forming Unit) encapsulated LP-LDL led to a statistically significant (13.9%) reduction in LDL-cholesterol in volunteers with baseline total cholesterol of <5 mM during the 0–12 week period and a 13.1% decrease in the group with more elevated LDL. There was a 4.5% increase in HDL ("good") cholesterol for those taking the probiotic in the 6–12-week period, with a more meaningful (14.7%) increase for the over 60s (Watson 2020).

The main objective of this study is to critically review the scientific evidence available regarding the effect of probiotic supplementation in the prevention and treatment of lipid profile abnormalities and to decipher the mechanism lying behind the lowering of cholesterol through probiotics to develop a better formulation for human consumption.

10.4 Animal Studies on Probiotic Supplementation in Lowering Cholesterol Levels

Probiotic supplementation reduces cholesterol concentration in the serum of chickens (Mohan et al. 1995). Fukushima et al. reported that hypercholesterolemic male Fischer rats fed with 30 g/kg of *L. acidophilus*-fermented rice bran significantly showed an improved lipid profile in a four-week study. A reduction in serum total cholesterol and liver cholesterol of 21.3% and 22.9%, respectively, was noted against control (Fukushima et al. 1999). In one of the research studies, it was also reported that the supplementation of probiotics (*Lactobacillus acidophilus*, *Bifidobacterium bifidum,* and *Aspergillus oryzae*) at 100 mg/ kg in the diet of broiler chickens significantly reduced the serum cholesterol concentration. The dietary supplementation of a mixed culture of 12 strains of *Lactobacillus* at 1% in the basal diet of broilers resulted in higher body weight gain and lowered serum cholesterol concentration (Kalavathy et al. 2003). It was noted that administration of *Lactobacillus reuteri* led to the prevention of hypercholesterolemia in mice, there was a significant reduction in total cholesterol (22%) and triglycerides (33%), along with significant increase in the ratio of HDL to LDL up to 17% (Taranto et al. 2000). Arun and coworkers reported that the dietary supplementation of *Lactobacillus sporogenes* (6×10 spore per g) at 100 mg kg diet significantly lowered total cholesterol, VLDL cholesterol, and triglycerides concentrations in the serum of broiler chickens (Arun et al. 2006).

Ichim and coworkers evaluated the effect of DBR on the activity of gut microbiota through in vitro studies in terms of cholesterol metabolism. The Daily Body Restore (DBR) is a proprietary blend composed of 9 probiotic organisms of the genera *Lactobacillus* and *Bifidobacterium*, and 10 digestive enzymes. The Shime® system consisting of sequential colon reactors was supplemented with DBR for analysis of short-chain fatty acid production. After 8 weeks of DBR treatment, LDL concentrations were dramatically reduced by 78%, and HDL was increased by 52% relative to control mice. The addition of DBR to the Shime® system led to significantly increased production of propionate in colon reactors, indicative of microbial production of short-chain fatty acids known to inhibit cholesterol synthesis (Ichim et al. 2016). The cholesterol-lowering potential of *L. fermentum* MTCC 5898 was noted in rats fed with high-fat diet. It was also noted that besides the decline in total cholesterol, a significant reduction in triglycerides, VLDL, and LDL was also observed. An increment in HDL level was also noted in the probiotic treated group (Yadav et al. 2018).

Fazeli et al. noted that the consumption of *L. plantarum* A7 (10^8 CFU mL^{-1}) for 14 days is effective in lowering serum lipid levels in rats (Fazeli et al. 2010). On similar lines, Salaj and coworkers while working with *Lactobacillus plantarum* strains, i.e., *Lactobacillus plantarum* LS/07 and *Lactobacillus plantarum* Biocenol LP96, examined its effects on lipid metabolism and body weight in rats fed with high-fat diet. It was noted that *Lactobacillus plantarum* LS/07 reduced serum cholesterol and LDL-cholesterol, while *Lactobacillus plantarum* Biocenol LP96 decreased triglycerides and VLDL. In both the strains, no change in serum HDL

and liver lipids was noted. Findings also showed that the effect of lactobacilli on lipid metabolism may differ among strains and both the strains involved in the study, could improve lipid profile (Salaj et al. 2013). Aminlari and coworkers also reported that on evaluating two probiotic bacteria, *L. plantarum*, and *Bacillus coagulans,* on lipid panel parameters. It was observed that there was a decline in serum concentrations of total cholesterol, triglycerides, LDL, and VLDL in comparison with the enriched-cholesterol diet group (Aminlari et al. 2018). In support of an earlier study, El-shafie and coworkers also noted the hypocholesterolemic effect of *Lactobacillus plantarum* NRRL B-4524 used as a single or as mixed culture with *Lactobacillus paracasei* in rat diets (El-Shafie et al. 2009). Introduction of *L. paracasei* TD3 in rat's diet could significantly reduce serum cholesterol levels (~9.2%), whereas there was no significant difference between experimental groups for triglycerides, LDL, and HDL levels. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes were significantly decreased in the probiotic group (Dehkohneh et al. 2019). Recently, Chen and coworkers tried to investigate the role of *L. plantarum* FZU3013, a probiotic isolated from *Hongqu* rice wine via a traditional brewing process in improving the nonalcoholic fatty liver (NAFL) associated with hyperlipidemia in mice fed with a high-fat diet. The results have shown a significant reduction in the HFD-induced body weight gain which inhibits the excessive accumulation of liver lipids, promotes excretion of bile acids from feces. These findings clearly show the potential of *L. plantarum* FZU3013 in improving lipid metabolism disorders by modulating specific intestinal microbial phylotypes and regulate hepatic lipid metabolism related genes, thereby preventing NAFL and hyperlipidemia (Chen et al. 2020). In another study, Yamasaki and coworkers, while working with *Lactobacillus plantarum* 06CC2 (LP06CC2), an isolate from Mongolian dairy product noted the suppression of an increase in liver cholesterol and hepatic damage indices. The mice fed with LP06CC2 have also shown an increase in cecal content and fecal butyrate. A bile acid deconjugation using glycocholate leading to a decrease in bile acid absorption is indicated in presence of LP06CC2. It is evident from results that LP06CC2 is a promising microorganism for the reduction of the cholesterol pool via modulation of bile acid deconjugation (Yamasaki et al. 2020).

Table 10.1 clearly shows the evidence of animal clinical trials conducted to evaluate the effects of probiotics on the lipid profile and other variables.

In the aforesaid studies, the inclusion criteria were limited only to the use of probiotics and not on synbiotics in lowering cholesterol levels. It has also been noted and reported that the cholesterol-lowering effect of probiotics varies from strain to strain.

10.4.1 Limitations of Using Probiotics as Cholesterol-Lowering Adjunct

The evaluation of probiotics as a cholesterol-lowering adjunct has its limitations since the use of high dosage and a large amount was reported on a regular basis

Table 10.1 Research evidences showing human clinical trials conducted to evaluate the effects of probiotics on the lipid profile and other variables

Probiotic strain/s	Animal/subjects	Dose/ duration of the study	Study outcome	Reference
Bifidobacterium longum Bb-46 (fortified in buffalo milk-yogurt)	48 male hypercholesterolemic albino rats	50 g regular basis 35 days	A significant reduction in total cholesterol (TC) level by 50.33%, LDL-cholesterol by 56.3% and triglycerides by 51.2% compared to the control	El-Gawada et al. (2005)
Lactobacillus reuteri (containing bile salt hydrolase) (BSH)	20 pigs	Twice daily 13 weeks	A decline in total, as well as LDL-cholesterol was observed to be 11% and 26%, respectively	De Smet et al. (1998)
L. plantarum CK 102 (healthy human isolate)	32 Sprague-Dawley (SD) male rats; 5 weeks old; induced hypercholesterolemic; mean BW of 129 ± 1 g	5.0×10^7 CFU/mL daily, 6 weeks	A significant decrease in total cholesterol (27.9%), 28.7% decline in LDL-cholesterol, and 61.6% decrease in triglycerides ($P < 0.05$)	Ha et al. (2006)
L. plantarum KCTC3928 (Cellbiotech Co. Ltd., Korea)	21 six-week-old C57BL/6 male mice; induced hypercholesterolemic	1×10^9 CFU/mL of *L. plantarum* KCTC3928, 4 weeks	A significant decrease in total cholesterol up to 33%, 42% decline in LDL-cholesterol, and 32% reduction in triglycerides ($P < 0.05$) An increase in HDL-cholesterol up to 35% was noted ($P < 0.05$)	Jeun et al. (2010)
L. acidophilus (wild chickens & human isolates)	30 Awassi weaning lambs; hypercholesterolemic	1×10^9 CFU/ capsule 2 capsules daily, 120 days	A significant decline in total cholesterol up to 22.6% ($P < 0.05$)	Lubbadeh et al. (1999)
L. plantarum PH04 (isolated from infant feces)	12 male hypercholesterolemic mice	14 days	A significant reduction in total serum cholesterol (~7% reduction) and in triglycerides	Nguyen et al. (2007)

(continued)

Table 10.1 (continued)

Probiotic strain/s	Animal/subjects	Dose/ duration of the study	Study outcome	Reference
			(~10% reduction) against control	
L. gasseri	Rats	Fed milk with probiotic strain	Lowering of total and LDL-cholesterol levels up to 42 and 64%, respectively. Decline in triglyceride levels was also noted.	Usman and Hosono (2000)

(Pereira and Gibson 2002a, b). A dosage level of 10^9 to 112×10^9 CFU/day was reported and it was noted that individuals fed with larger doses are safe in terms of beneficial effects along with no adverse clinical effects. The effect of probiotics is strain-dependent and a combination of different strains gives better results (Rajkumar et al. 2014). Therefore, as per the research carried out by Rajkumar and coworkers, there is a need to test small dosage amounts over a long period. In the current scenario, the thrust area of study must be on a *priority basis*, to examine the proven probiotic strains in lipid metabolism on human subjects and *secondly*, to ensure that the probiotic food additive use should reach the colon alive and have recommended viable numbers (1×10^7 cfu/g) (Ranadheera et al. 2010). The variation in the culture viability depends on the handling of the product (generally kept under cold temperature conditions), *thirdly,* the mechanism suggested so far for cholesterol assimilation in growing cells, bile salt hydrolase enzyme, and the incorporation in the cellular membrane through probiotic bacteria only a few probiotic strains can do so with small effect compared to that of the cholesterol-lowering drugs (Guo et al. 2012; Liong and Shah 2006).

Several studies were conducted wherein divergent results were obtained due to the specificity and combination of the strains employed, the doses administered, the duration of the studies, and other extraneous variables. It is recommended that further studies be conducted, designed to identify the long-term effects and the influence of probiotics when used in combination with drug-based treatment (Gadelha and Bezerra 2019). Research studies indicate that supplementation with probiotics, as investigated in well-controlled studies can be used as an adjuvant to traditional treatments for dyslipidemia.

A synergistic effect was observed when probiotic supplementation was combined with other treatments. In one of the studies, soy isoflavones when combined with probiotics have an additive effect as compared to groups given supplementation only. The combination of physical exercise with probiotic administration stimulates an increase in HDLc. The soy products containing isoflavones exhibited significant reductions in electronegative LDL in hypercholesterolemic individuals (Cavallini et al. 2016). The probiotic supplementation led to the improvement in inflammatory

profile, glycemic control, body mass, and immunological markers, which are generally been considered risk factors for the development of CVDS (Gadelha and Bezerra 2019). On the contrary, there are few research evidences and clinical reports wherein it was noted that all the patients do not respond equally well when probiotic was fed as an adjunct. Therefore, it is advisable to keep these patients on cholesterol-lowering drugs such as statins with slight modifications in lifestyle.

10.5 Studies on Probiotic Supplementation Lowering Cholesterol Levels in Humans

The hypocholesterolemic potential of probiotics has also been evaluated in human subjects. In one of the studies conducted using 48 hypercholesterolemic volunteers for 10 weeks, wherein a daily consumption of 200 g of yogurt containing *L. acidophilus* L1 contributed to a significant reduction in serum cholesterol concentration compared to control (Anderson and Gilliland 1999). Xiao and coworkers examined the effect of yogurt consumption fermented with *L. acidophilus* cultures on 30 Dutch healthy men involved as subjects for several weeks, it was noted that there was a significant reduction in total as well as LDL-cholesterol levels by 4.4 and 5.4%, respectively, when compared with controls (Xiao et al. 2003).

Several interesting data were also obtained wherein a decline in serum cholesterol levels was noted in bottle-fed babies as the number of *Lactobacillus acidophilus* in their stools get increased (Harrison and Peat 1975). In one of the research reports, it has been noted that the consumption of 2 L of whole fat milk and skim milk led to the reduction of serum cholesterol by 5 and 15%, respectively. On the other hand, the same literature report also suggested that if an equivalent amount of milk fat is replaced with butter there was an increase in serum cholesterol by 7% (Howard and Marks 1977). Yogurt has also been reported to cause a decline in serum cholesterol levels in humans (Hepner et al. 1979) and the effect is transient (Rossouw et al. 1981). Ashar and Prajapati (2001) reported the hypocholesterolemic effect of probiotic diet in humans and showed total cholesterol reduction to an extent of 12–21% by feeding on acidophilus milk.

Schaarmann and coworkers also tried to decipher the relationship between the intake of probiotic yogurt and the concentration of cholesterol fractions. The group conducted an experiment using 29 healthy women as subjects. The groups were divided into normal cholesterolemic group (total cholesterol <250 mg/dL) and a hypercholesterolemic group (total cholesterol >250 mg/dL. The experiment consists of three periods (placebo, standard yogurt, and probiotic yogurt), and each lasting for 51 days, the product consumed was having a concentration of 300 g/day. The probiotic yogurt composed of *Lactobacillus acidophilus* and *Bifidobacterium* longum strains, whereas standard yogurt has *Streptococcus thermophilus* and *Lactobacillus lactis* as microbial strains. Results showed a decline in LDL-cholesterol and triacylglycerides after consumption of standard and probiotic yogurts. Though a large decline was noted after intake of probiotic yogurt along with an increase in HDL cholesterol compared to standard yogurt but the difference was not significant

in the hypercholesterolemic group. Therefore, no significant change was observed in the normocholesterolemic and the hypercholesterolemic groups when probiotic yogurt was fed (Schaarmann et al. 2001). In one of the study carried out by Schaafsma et al. wherein 54 volunteers were used as subjects, it was noted that there was a 5–10% decline in serum cholesterol levels after several weeks of moderate consumption of yogurt fermented with *Lactobacillus bulgaricus* and *S. thermophilus* (Schaafsma et al. 1998).

Fuentes and coworkers noted that daily intake of *L. plantarum* in the form of a capsule containing 1.2×10^9 CFU led to lowering of TC and LDL-C concentrations in hypercholesterolemic subjects after 12 weeks of study (Fuentes et al. 2013). In agreement with reports of Fuentes and coworkers, Wu et al. noted that the consumption of lactobacilli strains such as *L. reuteri and L. plantarum* has shown a significant reduction in TC and LDL-C. The researchers also suggested that the consumption of synbiotic food, containing *L. sporogenes* and inulin has a beneficial effect on TG and HDL-C (Wu et al. 2017).

Several scientific reports suggest a significant reduction in the total cholesterol (TC), LDL-cholesterol (LDLc), and triglycerides along with an incremental increase in the levels of HDL-cholesterol (HDLc) in the system when supplemented with probiotics (Ahn et al. 2015; Fuentes et al. 2016). The results are highly heterogenous; therefore, a subset was analyzed and noted that TC more than 200 mg/dL had shown better response to treatment with probiotics.

Cho and Kim conducted 30 random trials; subjects treated with probiotics demonstrated reduced total cholesterol and LDL-cholesterol compared to control while there was no significant effect of probiotics on HDL-cholesterol as well as triglycerides. The major factors determining the significant effect of probiotics on total cholesterol and LDL-cholesterol were greater for higher baseline total cholesterol levels, longer treatment durations, and certain probiotic strains (Cho and Kim 2015). Similar observations have also been reported during studies conducted by Yan and coworkers (Yan et al. 2019).

Sharma et al. reported that meta-analysis of 14 randomized clinical trials with normal, borderline, and borderline high baseline cholesterol levels were noted when fed with probiotics, a significant decline in serum TC (−8.40 mg/dL) and LDL-cholesterol levels (−6.63 mg/dL) observed compared to control (devoid of probiotics). The probiotic strains studied in this meta-analysis were *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium animalis,* and *Enterococcus faecium*. Out of these 14 trials, 13 trials showed a trend in the reduction of both serum TC and LDL-cholesterol levels. Out of these 13 trials, only 6 trials showed statistically a significant decline in serum TC levels along with 4 trials wherein a significant decline in serum LDL-cholesterol levels was noted. On the other hand, a significant change in HDL-cholesterol and TG levels was not observed with the use of probiotics (Sharma et al. 2016).

Park and coworkers conducted a double-blind, randomized, placebo-controlled study which includes 70 participants (both sexes), age 20+ having blood triacylglyceride (TG) levels below 200 mg/dL (normal value) in order to investigate

the effect of *Lactobacillus plantarum* Q180 (LPQ180) on postprandial lipid metabolism and the intestinal microbiome environment. It was noted that there was a significant decline in LDL-cholesterol ($p = 0.042$) and apolipoprotein (Apo) B-100 ($p = 0.003$) levels, after 12 week of treatment with LPQ180. Besides this, there was a significant decrease in total indole and phenol levels ($p = 0.019$). Healthy postprandial lipid metabolisms in subjects and a healthy intestinal environment with a higher level of enteric bacteria such as *R. bromii*, *K. alysoides*, *B. intestinihominis*, and *F. plautii* were observed due to ingestion of LPQ180. A higher level of these enteric bacteria led to a higher SCFA content after LPQ180 supplementation for 12 weeks. It could be due to large deviations from small number of subjects in each group and healthy TG levels (Park et al. 2020). Table 10.2 indicates research evidences showing human clinical trial conducted to evaluate the effects of probiotics on the lipid profile and other variables.

The main limitations faced during clinical trial studies were that these were of short duration varying from 15 days to 12 weeks, small sample size, and no analysis of the intestinal microbiota (IM), and the majority of subjects involved were having dyslipidemia.

10.6 Contradictory Results on Probiotic Usage in Both Animal and Human Studies

Several research reports showed a lowering in cholesterol levels in several fermented milk products, while some researchers failed to do so even when supplemented with dietary supplements in both animals and humans, which have led to doubtful results. In the majority of the studies, volunteers were fed with yogurt fermented with *L. acidophilus* as probiotic to study its influence on serum lipids, but no significant change in plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides was noted (De Roo et al. 1998; Lewis and Burmeister 2005; Fabian and Elmadfa 2006; Pawan and Bhatia 2007).

Kikuchi-Hayakawa et al. (2000) while working with *L. casei* strain Shirota (Yakult®) in hamsters clearly states that cholesterol metabolism is strain specific. Although this strain could grow well in presence of mixed lipid micelles containing bile acids and under anaerobic conditions but still could not significantly remove cholesterol (only 11%) from culture broth even after 24 h of incubation. Contrary to the other strains tested such as *L. acidophilus, L. crispatus, L. gasseri* which has shown a significant removal of cholesterol (~80%).

Lewis and Burmeister conducted an experimental study wherein 80 volunteers (20–65 years) consumed two capsules containing freeze-dried *L. acidophilus* (3×10^{10} CFU/2 capsules) three times daily for 6 weeks, in order to determine the effect of *Lactobacillus acidophilus* on human lipid profiles. It was found that *L. acidophilus* capsules did not significantly change plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides of the subjects (Lewis and Burmeister 2005). Fabian and Elmadfa (2006) also observed that the mean concentrations of total, HDL- and LDL-cholesterol in 33 female volunteers showed

Table 10.2 Research evidences showing human clinical trials conducted to evaluate the effects of probiotics on the lipid profile and other variables

Probiotic strain/s	Subjects Age (years)	Dose/duration of the study	Study outcome	Reference
Lactobacillus curvatus HY7601 and *L. plantarum* KY1052	121 non-diabetic people with hypertriglyceridemia	2 grams of powder containing *Lactobacillus sp.* 0.5 × 1010 CFU of each 12 weeks	18.3% reduction in TG and a 15.6% reduction in LDL along with 21.1% increase in apo A-V. TG and apo A-V values were inversely correlated.	Ahn et al. (2015)
Bifidobacterium lactis HN019	51 people with metabolic syndrome (18–60 years)	26 subjects, consumed milk fermented with 2.72×10^{10} CFU of *Bifidobacterium lactis HN019;* 45 days	Significant reductions were observed in TC ($p = 0.009$) and LDL-c ($p = 0.008$).	Bernini et al. (2016)
Lactobacillus casei shirota	30 healthy volunteers (55–74 years)	*Lactobacillus casei shirota* with 1.3×10^{10} CFU/day. 4 weeks	No significant reduction in TC or TG	Dong et al. (2013)
Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12	60 people with type-2 diabetes	6 week	A significant reduction of 4.54% in total cholesterol and 7.45% reduction in LDL-cholesterol. No change in HDL-cholesterol and triglycerides	Ejtahed et al. (2011)
E. faecium M-74	43 volunteers	56 weeks	Reduction of serum cholesterol concentration by 12%	Hivak et al. (2005)
Lactobacillus acidophilus La5 and *Bifidobacterium animalis*, subspecies *lactis* Bb12	156 people with metabolic syndrome Mean age: 67 years G1: Yogurt, plus placebo	Subjects consuming probiotic ingested at least 3×10^9 CFU/day.	No differences in lipid profile markers between groups ($p < 0.05$)	Ivey et al. (2015)

	capsule; G2: Probiotic capsule plus milk; G3 and G4: Placebos			
L. fermentum ME-3	45 healthy volunteers (50–75 years), with BMI from 24–30 kg/m^2 and borderline risk factors for cardiovascular disease	2 capsules per day containing the probiotic *L. fermentum* ME-3 (6×10^9 CFU/day), plus other compounds. 4 week	Significant reductions in LDL-c, TC, TG, and OX-LDL ($p < 0.05$) and a tendency to improvements in HDL.	Kullisaar et al. (2016)
L. plantarum 299v (ProViva)	36 healthy volunteers with moderately elevated fibrinogen concentrations (>3.0 g/L); 35–45 years old	400 mL of rose-hip drink containing 5.0×10^7 CFU/mL daily, 6 weeks.	A decrease in total cholesterol level up to 2.5% and LDL-cholesterol level up to 7.9%.	Naruszewicz et al. (2002)
Saccharomyces boulardii	11 hypercholesterolemic men (21–69 years)	5.6×10^{10} CFU/day of *Saccharomyces boulardii* 8 weeks	Only remnant lipoprotein (RLP) exhibited a significant reduction ($p < 0.03$)	Ryan et al. (2015)
Lactobacillus acidophilus La-5 and *Bifidobacterium animalis lactis* BB	45 people, 35–60 years with type 2 Diabetes mellitus	120 g/day of milk fermented with probiotics (*Lactobacillus acidophilus* La-5, *Bifidobacterium animalis lactis* BB-12; 10^9 CFU of each). 6 weeks	Reduced LDL-c ($p = 0.03$) and TC ($p = 0.04$)	Tonucci et al. (2017)
B. longum BL1 (fortified low fat yogurt)	32 subjects (aged 28–60 years old)	4 weeks	A significant decline in serum total cholesterol, LDL-cholesterol and triglycerides along with 14.5% increase in HDL-cholesterol	Xiao et al. (2003)

no relevant differences between the two groups, one enriched with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and the other served as control (devoid of probiotics). On the similar lines, Simons and coworkers while working with *Lactobacillus fermentum* (2×10^9 CFU per capsule; four capsules daily) in 46 volunteers (aged 30–75 years) noted that there was no change in the lipid profile even after 10 weeks of the study period on the consumption of probiotics (Simons et al. 2006). Hatakka and coworkers studied the hypocholesterolemic effect of probiotics and noted that the administration of *L. rhamnosus* LC705 (10^{10} CFU/g per capsule; two capsules daily) in 38 men with a treatment period of 4 weeks did not show any influence on blood lipid levels (Hatakka et al. 2008).

10.6.1 Attributing Factors Leading to Contradictory Results

The aforesaid results thus obtained could be attributed to several factors, though in vivo trials utilize real-life models with real pathological systems, but these trials are affected by external factors such as different strains of probiotics, administration dosage, analytical accuracy of lipid analyses, clinical characteristic of subjects, duration of the treatment period, inadequate sample sizes, failure to control the nutrient intake and energy expenditure during the experiments variations in the baseline levels of blood lipids, and lack of suitable controls or placebo groups (Liong 2007; Greany et al. 2008).

Literature reports are available on probiotic strains to be developed and tested for serum cholesterol reduction (Shaper et al. 1963; Pereira and Gibson 2002a, b). In case of animal model, a large number of strains have demonstrated the effect of probiotics on serum lipid level with consistent results (Pereira and Gibson 2002a, b). While, on the other hand, when this effect was examined in human studies, inconsistent results were noted wherein some showing positive significant effects while others with no effects (Taylor and Williams 1998; Huang et al. 2013; Wang et al. 2012; Wu et al. 2017). The data variation could be due to differences in the experimental design based on the following factors such as type and quantity of the fermented milk product; age and sex distribution and starting plasma cholesterol levels of the subjects studied; and length of the study period). Therefore, direct comparisons are not possible (Pereira and Gibson 2002a, b).

To overcome the conflicting results, meta-analysis can be conducted wherein in the randomized controlled trials the effect of probiotics on serum lipid levels could be examined. In meta-analysis, changes in the mean and standard deviation of lipid parameters (TC, LDL-cholesterol, HDL-cholesterol, and TGs) were studied (Sharma et al. 2016).

10.6.2 Role of Minimal Effective Probiotic Dosage for Hypocholesterolemic Potential

At present, there is much research evidence that reports the hypocholesterolemic potential of probiotics but the "*minimal effective dosage"* of probiotics needs to be examined to reduce blood cholesterol levels. There are no regulatory standards for probiotic product to produce cholesterol-lowering effect and a tremendous variation is noted in the concentration of probiotics in food products (FAO 2002). It has been noted from the previous research studies that the effective administrative dosage of probiotics varies and is dependent on the strains used, along with the clinical characteristics of subjects, such as lipid profiles. Thus, more studies are needed, to determine the effective dosage of probiotics to exhibit hypocholesterolemic effects. The prescribed dosage should also work for in vivo studies involving the lipid profiles.

10.6.3 Role of Analyzing Lipid Profile

The effect on lipid profile during consumption of fermented milk product was noted in patients suffering from mild to moderate primary hypercholesterolemia by Bertolami et al. (1999). The study conducted was prospective, randomized, double-blinded, and placebo- controlled with a crossover design. In this study, 32 subjects between the age group (36–65 years) were included in the trial for a period (8 weeks). It was observed that fermented milk (Gaio®) caused a statistically significant decline in total serum cholesterol. However, there was some discrepancy in the results, not all the subjects respond to the product, among these three subjects have shown an elevated cholesterol level.

The manipulation of intestinal microbiota with probiotic supplementation aids in several benefits to the host (Lin et al. 2014). The use of probiotics has already been there in human healthcare, for the prevention and treatment of diseases through modulation of intestinal microbiota (Coppola and Gil-Turnes 2014; Kechagia et al. 2013). The probiotics must have the capacity to adhere to the intestinal mucosa, overcoming the barriers imposed by the gastrointestinal tract, primarily the gastric pH, bile salts, and pancreatic enzymes (Soccol et al. 2010).

The existing research evidence from animal and human studies indicates that use of the fermented dairy products has shown moderate lowering action of cholesterol. However, the potential mechanism behind the lowering is still unclear. Herein, we have examined the various mechanisms of action of probiotics reported in the literature for lowering cholesterol levels.

10.7 Mechanism of Action of Probiotics for Cholesterol Metabolism

Several researchers reported through in vitro studies that some of the strains of Lactobacilli (Gilliland et al. 1985; Rasic et al. 1992; Noh et al. 1997) and Bifidobacterium (Tahri et al. 1995, 1996) can assimilate cholesterol in presence of bile acids.

(a) *Role of physiochemical conditions for cholesterol removal:* Gilliland and coworkers while working with *Lactobacillus acidophilus* strains noted that the removal of cholesterol from growth medium could only be possible in presence of bile and under anaerobic conditions (Gilliland et al. 1985). While in vivo cholesterol assimilation by cells or attachment of cholesterol to the surface has been explained by Meei YN Lin based on the ability of six *L. acidophilus* strains noted during in vitro studies. It was noted that when *L. acidophilus* ATCC4356 was grown anaerobically in a medium supplemented with bile acids for 24 h, the maximum uptake of 57% was reported (Lin and Chen 2000). Generally, these are the conditions that occur in the intestine and help in part of the cholesterol ingestion in diet thereby making it impossible for cholesterol to be absorbed in the blood. Among the *L. acidophilus* strains, a considerable variation was found in terms of their ability to grow in presence of bile and to remove cholesterol from laboratory medium (Gilliland et al. 1985; Gilliland and Walker 1990; Walker and Gilliland 1993).

No metabolic degradation via. *Alteration of cell wall/membrane*: The binding ability of intact cells to cholesterol varied widely among strains which could be due to differences in chemical and structural properties of the bacterial cell wall peptidoglycans. Still, it remains unclear whether the variation in cholesterol uptake in different strains is due to differences in their cell membrane or some other cell components. The studies of De Rodas et al. (1996) were supported with research evidence given by Noh et al. (1997) wherein authors reported that assimilation of cholesterol by *L. acidophilus* ATCC43121 was not metabolically degraded. These researchers noted that the cells grown in presence of cholesterol micelles and bile salts were resistant to lysis by sonication and are more resistant to sonic disruption due to the possibility of the alteration of the cell wall or membrane by cholesterol. Kimoto and coworkers also noted a difference in the fatty acid distribution pattern for cells grown in the presence and absence of cholesterol (Kimoto et al. 2002). Lye and coworkers noted that an increased concentration of saturated and unsaturated fatty acids was noted in the cells incorporated with cholesterol. This altered composition in presence of cholesterol also led to an increased membrane strength and subsequently higher cellular resistance toward lysis (Lye et al. 2010). Further evaluation by the same group was conducted to determine the possible location of the incorporated cholesterol within the membrane phospholipid bilayer of probiotic cells. The researchers incorporated fluorescence probes into the membrane bilayer of probiotic cells that were grown in the absence and presence of

cholesterol. It was noted that when probiotic cells were grown in the presence of cholesterol, the incorporation of cholesterol was noted in regions of the phospholipid tails, upper phospholipids, and polar heads of the cellular membrane phospholipid bilayer.

However, certain in vitro studies such as growth performance in bile containing medium as well as the ability to bind to cholesterol were conducted using 28 different strains of *L. gasseri*, by Usman and Hosono (1999). During studies, it was noted that there was greater variation in bile tolerance contrary to the earlier studies (Gilliland et al. 1985; Klaver and van der Meer 1993). The variation in bile tolerance among 28 different strains could be due to differences in growth performance. Later on, the hypothesis of cholesterol removal by probiotic cells during different growth conditions was supported by Kimoto and coworkers. The authors observed that live and growing cells could remove more cholesterol than those which are non-growing (live but suspended in phosphate buffer) and dead (heat-killed). The cholesterol removal from media by non-growing and dead cells indicates that some cholesterol is bound to the cell surface (Kimoto et al. 2002). It has been suggested that in order to assimilate cholesterol in the intestinal tract, the organism must be bile tolerant. However, no correlation between the two has still been noted.

The in vivo studies were conducted using young pigs as experimental models to test the cholesterol assimilation in the intestine (Gilliland et al. 1985). Pigs are considered as an animal model for in vivo studies of cholesterol assimilation in the intestine, since their digestive system, the distribution of coronary arteries, and the atherosclerotic tendencies resemble those of humans (Ratcliffe and Luginbuhl 1971). The in vivo experimental studies when conducted using *L. acidophilus* RP32L significantly inhibit an increase in serum cholesterol when fed with high lipid diet. This is due to cholesterol assimilation by the *L. acidophilus* strain (Gilliland et al. 1985; Rasic et al. 1992). Agerbaek and coworkers noted diverse variation in the hypocholesterolemic effect of yogurt and other fermented milk products which could be due to different bacterial strains used in fermentation in different human studies (Agerbaek et al. 1995). The other important reason for this effect could be the viability of ingested and their ability to colonize in the small intestine, wherein the cholesterol absorption takes place.

(b) *Co-precipitation with deconjugation of bile salts:* Certain bacteria interfere with cholesterol absorption from the gut through enzymatic deconjugation of bile acids by bile salt hydrolase (BSH) and affecting cholesterol metabolism. The bacteria reported hydrolyzing conjugated bile acids are *Bacteroides* spp., Bifidobacteria fusobacteria, Clostridia, Lactobacilli, and Streptococci (Hylemon and Glass 1983). The other mechanism for cholesterol assimilation in the intestine was studied using *Lactobacillus acidophilus* and *Bifidobacterium bifidum* by Klaver and van der Meer (1993), wherein they proposed the hypothesis that cholesterol removal from the culture medium by *L. acidophilus* RP32 and other species could be related to co-precipitation with deconjugated bile salts in an acidic environment and not due to bacterial uptake of cholesterol.

Reports suggest that gut flora not only hydrogenates, dehydrogenates, and oxidizes bile acids, but also cleaves side chains to yield steroids. It was reported that the deconjugated bile acids are less soluble and less likely to get absorbed from the intestinal lumen compared to conjugated bile salts. Therefore, greater excretion of free bile acids was noted from the intestinal tract compared to their conjugated forms. Increased excretion of bile acids results in lowering in serum cholesterol concentration and therefore there will be a decline in the amount of bile acids reaching liver and a further decline in the secretion back into the intestine through enterohepatic circulation. In order to replace the excreted bile acids, more bile acids need to be synthesized from cholesterol in the liver or to reduce the absorption of cholesterol through the intestinal lumen in lipid fed subjects. This hypothesis was supported by the study conducted by Lye and coworkers (Lye et al. 2009). *L. gasseri* SBT0270 has shown the ability to suppress the reabsorption of bile acids into the enterohepatic circulation (by deconjugation) and therefore more cholesterol utilization for de novo bile acid synthesis in homeostatic response enhances the excretion of acidic steroids in feces in vitro (Usman and Hosono 1999) resulting in lowering of serum cholesterol (Begley et al. 2006; Ooi and Liong 2010).

It is still unclear in terms of microbial free bile acid properties whether it could be related to the hypocholesterolemic effects observed in vivo (Gilliland et al. 1985) since pH in the intestinal tract of humans is usually neutral to alkaline. Though it has shown promising results during in vitro studies.

(c) *pH-dependent phenomenon for cholesterol assimilation:* The aforesaid hypothesis mentioned was studied in *Bifidobacterium* species and an intense binding between the cell surface and cholesterol was observed which indicates cholesterol uptake into cells. The pre-requisites to remove cholesterol were dependent on cell growth and the presence of bile salts. The authors also concluded that cholesterol removal from the broth is not only attributed to the co-precipitation of cholesterol with deconjugated bile salts, but rather to conjugation of both effects (Tahri et al. 1995). Later on, Tahri and his team did further research to support this mechanism and studied the effect of pH on cholesterol assimilation in absence of microbial cells. At pH below 5.5, the cholesterol was partially removed when deconjugated bile salts were added. While, as soon as the pH level increased to pH 7, the precipitated cholesterol gets re-dissolved so it indicates that it is a transient phenomenon and is dependent on pH. It was also noted that resting cells of Bifidobacterium did not interact with cholesterol and it is only the growing cells that could assimilate cholesterol in their cell membrane (Tahri et al. 1996). Similar has been noted and reported for probiotics in the intestine to take up and assimilate cholesterol for stabilization of their cell membrane and binding cholesterol to cell walls of probiotics bacteria (Razin 1975; Noh et al. 1997; Tanaka et al. 1999; Lepercq et al. 2004).

(d) *Role of excessive dietary calcium in cholesterol metabolism:* There are certain research hypotheses related to the effect of the presence of dietary calcium. It is clearly stated that excessive dietary calcium binds with bile acids and suppresses

reabsorption into the enterohepatic circulation and lowers down the LDL-cholesterol level.

(e) *Enzymatic conversion of cholesterol to coprostanol:* In one of the proposed mechanisms for cholesterol metabolism, it has been reported that cholesterol is converted in the intestine to coprostanol and excreted in feces. This way there is a decline in the concentration of cholesterol being absorbed. In one of the research study, carried out by Chiang and coworkers, it was noted that *Sterolibacterium denitrificans* produces cholesterol dehydrogenase/isomerase which catalyzes the transformation of cholesterol to cholest-4-en-3-one, an intermediate cofactor in the conversion of cholesterol to coprostanol (Chiang et al. 2008). Thereafter, several researchers worked on this mechanism using strains of probiotic bacteria. The lactobacilli probiotic bacteria such as *Lactobacillus acidophilus, L. bulgaricus,* and *L. casei* ATCC 393 were evaluated for their conversion via fluorometric assays by Lye and coworkers during in vitro studies (Lye et al. 2010). It was also determined that cholesterol reductase is present both intracellular and extracellular in most of the probiotic strains thereby leading to the conversion of cholesterol to coprostanol. A decline in the cholesterol concentration along with an increment of coprostanol was also noted. Further studies need to be undertaken regarding this enzyme cholesterol reductase as it is already been administered to humans to lower blood cholesterol levels.

(f) *Generation of short-chain fatty acids:* As per another set of theory for the mechanism behind the reduction in the systemic levels of blood lipids through probiotics was explained through research evidences. The researchers suggested that the probiotics can ferment the food-derived indigestible carbohydrates and yield short-chain fatty acids (SCFA). The generation of short-chain fatty acids causes a decline in plasma cholesterol concentration either by inhibiting hepatic cholesterol or redistribution of cholesterol from plasma to the liver (Fuller and Gibson 1998). St-Onge and coworkers reported SCFA production in the large intestine at a concentration range of 100–450 mmol/day. The SCFA comprises acetate, propionate, and butyrate with a ratio of 60:20:15. The ratio depends upon the substrate used (St-Onge et al. 2000). It has been noted that generally the presence of acetate in serum increases total cholesterol, while propionate increases blood glucose and lowers hypocholesterolemic response caused by acetate. The SCFA, propionate reduces utilization by the liver for fatty acid and cholesterol synthesis. In one of the studies conducted by Wolever and coworkers, it was reported that sufficient propionate must be generated to overcome the effect of acetate generation which is a precursor for lipid synthesis. The plasma cholesterol concentration is influenced by the proportion of each fatty acid produced during fermentation (Wolever et al. 1996). Figure 10.1 depicts the schematic representation of different proposed mechanisms reported for cholesterol reduction.

Our research and development team, Department of probiotics, Synbiome, while working with *Lactobacillus plantarum* spp. strains MSD1 and MSD2 during in vitro

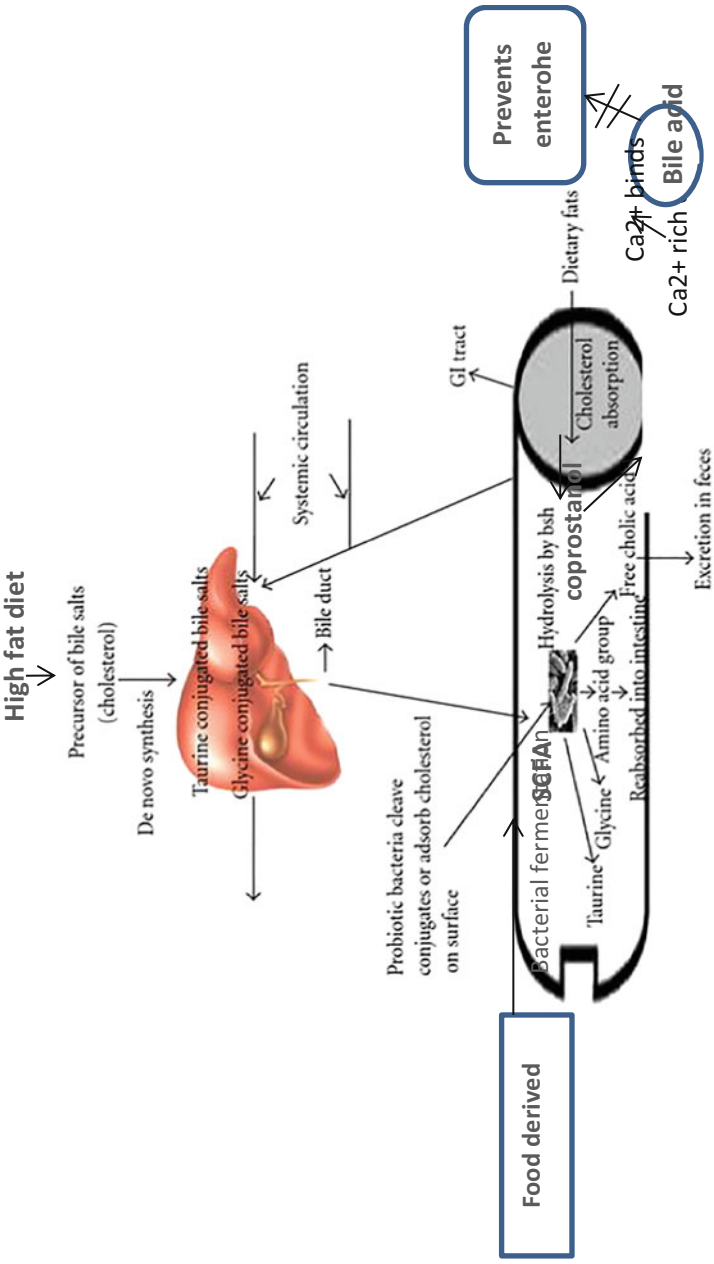

Fig. 10.1 Schematic representation of different proposed mechanisms reported for cholesterol reduction

studies experimented with three different sets for the cholesterol assimilation, wherein, in the first set, MRS medium containing cholesterol, in the second set, cholesterol supplemented with 6 mM taurocholate, and in the third set, cholesterol supplemented with 6 M sodium tauroglycocholate was used. Our findings have shown that both the strains of *L. plantarum* had significant results in terms of the amount of cholesterol assimilation. However, strain MSD1 has a greater capacity to assimilate the amount of cholesterol in all three sets along with a higher percent cholesterol removal compared to other strain MSD2. The amount of cholesterol assimilation by *Lactobacillus* spp. MSD1 in MRS medium, containing cholesterol supplemented with 6 mM sodium tauroglycocholate has led to significant cholesterol assimilation (46.72 μg/mL) with a percent cholesterol removal (66.74%) compared to the other two sets. The strain, *L. plantarum* spp. MSD1 has 2.8 times more cholesterol removal capacity in medium containing cholesterol supplemented with 6 mM sodium tauroglycocholate compared to the medium containing solely cholesterol. The amount of cholic acid released by the action of bile salt hydrolase (BSH) was assayed through the plate assay technique showing precipitation of cholic acid and through TLC technique. It was also noted that *L. plantarum* spp. MSD1 occurred in long chains in presence of bile salts and could grow even at higher concentrations of 14% bile salt till 48 h of incubation(*data not published*).

To date, most of the studies have been conducted in vitro and very few attempts have been made through in vivo trials to evaluate the possible hypocholesterolemic mechanism involved.

10.8 Conclusions

The dairy products fermented with appropriate bacterial probiotic strains could aid in lowering of circulating cholesterol concentration and thereby diminishing the risk of CHD. The fermented dairy products can be considered as functional foods which are involved in lowering of high cholesterol concentration. If the bacterial strains fulfilled certain criteria such as being bile tolerant, can deconjugate bile acids, and bind cholesterol it could lower down blood cholesterol levels. Several mechanisms have been suggested for lowering of cholesterol levels through probiotics. These include the end products of SCFA fermentation, cholesterol assimilation, binding of cholesterol to the bacterial cell wall, and enzymatic deconjugation of bile acids. The major concern was raised with the mechanism of deconjugation of bile acids wherein it was suggested the potential increased risk for colon cancer due to the carcinogenic properties of deconjugated bile acids (Sanders 2000).

However, still, the exact mechanism of action of probiotic bacteria on lowering serum cholesterol is not clear. It could be concluded that there are several mechanisms that have been studied and reported for mediating hypocholesterolemic effect by probiotics. However, all these mechanisms have been reported via in vitro studies and the mechanism is not firmly established for in vivo studies. To date, the products containing live bacteria (yogurt, acidophilus milk, and Kefir) did not retain in the human intestinal tract and are eliminated in feces. It is necessary to consume

probiotic products daily for the long-term effect on metabolism but there are certain reports on animal as well as human dietary studies wherein this concept of daily probiotic consumption has given conflicting results. Further, research studies through proper-designing of in vivo trials may disclose additional understanding and knowledge on defining the exact mechanism for lowering of cholesterol using probiotics, better safety assessment prior to consumption, to improve the strain stability characteristics, and to solve the problem of survivability in large bowl to eliminate the existing controversies on the use of probiotics for regulating lipid metabolism. Besides this, the in vitro cholesterol reduction needs to be confirmed in mixed culture and mixed substrate environment.

The underlying mechanism of cholesterol-lowering effects by probiotics needs to be explored in order to have a better understanding of the mechanisms and better formulations for human consumption.

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Use of Prebiotics for Addressing Gut Dysbiosis and Achieving Healthy Gut–Brain Axis

11

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Abstract

The bacteria in the gastrointestinal tract which forms the gut microbiome plays a vital role in maintaining body homeostasis and health of the host. Any change in the normal gut microbiome composition and function imposes gut dysbiosis, defined as an imbalance of the bacteria in the gut. The central nervous system (CNS) and the gut microbiome are in constant bidirectional communication involving endocrine, neuronal, and immunological mechanisms forming the gut–brain axis (GBA). Emerging preclinical studies suggest that gut dysbiosis may result in GBA dysfunction leading to neurodegenerative and neurodevelopmental diseases, as well as age-related cognitive decline. Therefore, modulation of gut microbiota composition and functionality offers a promising tool for treating or managing gut dysbiosis and in turn achieving a healthy gut–brain axis. Use of prebiotics is gaining attention as the most robust and safe method of achieving such modulation. Prebiotics refer to non-digestible food ingredients predominately some fermentable carbohydrates that can selectively modulate the composition and/or activity of the microbiota of the gut, thus conferring beneficial physiological effects on the host. The metabolism of prebiotics by the gut microbiome induces changes in the gut barrier integrity and promotes the release of metabolites (mainly SCFAs) contributing to the improvement of host health, particularly in the context of GBA. In this chapter, we discuss the concept of prebiotics, microbiota modulation by prebiotics, and the impact prebiotics on GBA.

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Keywords

Prebiotics · Gut dysbiosis · Gut–brain axis (GBA) · Gut microbiome · Central nervous system (CNS)

11.1 Introduction

The gut-brain crosstalk has garnered the attention of researchers in the past few decades as studies very clearly indicate the overpowering role of the gut microbiome not only in the modulation and regulation of metabolism and immunity, but also in the functioning of the central nervous system (CNS). The gut microbiome plays a key role in influencing the development and function of the nervous system through its constant bidirectional communication with the CNS (Carabotti et al. 2015; Liu et al. 2019). Microbial metabolites are also known to transmit signals to the brain directly or through the autonomic neurons establishing the gut–brain axis (GBA) (Clapp et al. 2017). Given the enormous sharing of function between host and microbiome, the concept of the holobiont has emerged, which regard eukaryotes as a composite structure in which neither the host nor the microbiome can be considered as functioning independently (Zilber-Rosenberg and Rosenberg 2008). Dysbiosis typically occurs when the microbes that are resident in our gastrointestinal tract (GIT) are disrupted triggering an imbalance in the gut microbiome and disruption in the microbiome–gut–brain axis (Carding et al. 2015; Noble et al. 2017). Conventionally, gut dysbiosis has been implicated in several chronic gastrointestinal tract related diseases and disorders, such as irritable bowel syndrome (IBS) (Menees and Chey 2018), colorectal cancer (Sobhani et al. 2013), celiac disease (Marasco et al. 2016), and also in metabolic disorders such as type 2 diabetes (T2D) and obesity (Belizário et al. 2018). However, gut microbiome dysbiosis has also been observed to impact the GBA, ultimately affecting the CNS and functions related to behaviour and cognition (Carding et al. 2015), clearly suggesting the microbial control of the GBA.

11.2 Microbial Control of the Gut–Brain Axis (GBA)

The gut–brain axis is inclusive of the central nervous system (CNS), autonomic nervous system (ANS), enteric nervous system (ENS), the gut microbiota, and the endocrine and immune systems (Carabotti et al. 2015). This bidirectional interaction also includes the modulation of gut physiology by the CNS and its influence on functions of the gut such as motility, nociception, and immune function (Fung et al. 2017). The vagus nerve connects the ENS to the CNS, thus providing a direct communication pathway between the gut microbiome and the CNS facilitating the modulation of the CNS by neurotransmitters or other metabolites produced by the bacteria of the gut (Forsythe et al. 2014). Neurotransmitters are endogenous chemical messengers which diffuse signals across a chemical synapse from one neuron to

another neuron, gland cell, or muscle cell (Lodish et al. 2000). The ability of specific bacterial species of the gut microbiota to produce and modulate neurotransmitters and related receptors has been demonstrated by a number of studies (Strandwitz 2018; Wu et al. 2020). In a study with normal mice, ingestion of *Lactobacillus rhamnosus* JB-1 induced region-dependent changes in the expression of gamma aminobutyric acid (*GABA*) receptor levels in the brain and reduced anxiety and depression related behaviour, with the vagus nerve identified as the major communication pathway between the gut and the brain (Bravo et al. 2011). Studies in gem free mice have shown increased activity related to transcriptional pathways in the amygdala (Stilling et al. 2015), increase in levels of noradrenaline and dopamine and 5-hydroxytryptamine (5-HT) in the striatum (Diaz Heijtz et al. 2011), and decrease in levels of 5-HT and 5-HT1A receptor expression in the hippocampus and amygdala (Neufeld et al. 2011; Diaz Heijtz et al. 2011). Studies have also thrown light on the ability of gut bacteria to produce several neuroactive compounds, for example, serotonin by *Candida, Streptococcus, Escherichia* and *Enterococcus* species, norepinephrine by *Escherichia*,
Saccharomyces, and *Bacillus* species, acetylcholine by *Lactobacillus* species, GABA by *Bacillus* and *Bifidobacterium*, and dopamine by *Bacillus* and *Serratia* species (Lyte 2011). The neurotransmitters that are unable to cross the blood–brain barrier exert their action by stimulating the vagus nerve, consequently affecting brain functions (Barrett et al. 2012).

One of the systems known for its close interaction with the gut microbiota is the hypothalamic–pituitary–adrenal (HPA) axis, the major neuroendocrine system of the body. The communication between the HPA axis and gut microbiota is closely associated with the immune system, gut hormones, as well as the autonomic nervous systems (Mayer 2000). In response to stress, paraventricular neurons of the hypothalamus release corticosterone-releasing factor (CRF), which then induces the anterior pituitary gland to release of adrenocorticotropic hormone (ACTH) (Foster et al. 2017). The release of ACTH will induce the release of catecholamines, glucocorticoids, or mineralocorticoids which can influence both behaviour and intestinal microenvironment (Farzi et al. 2018). Various studies have been published that have demonstrated the influence of stress on gut microbiome composition (Bailey and Coe 1999; Bailey et al. 2011). In addition, gut microbiota can modulate the expression of CRF in the hypothalamus (Crumeyrolle-Arias et al. 2014) and the expression of 2A subtype Of N-methyl-D-aspartic acid (NMDA) receptor, brain-derived neurotrophic factor (BDNF), and 5-HT1a receptors in the cortex and hippocampus (Ka et al. 2016), thus influencing the function of the HPA axis.

The host gut microbiome can modulate the maturation and function of microglia (Erny et al. 2015) and influence the activation of peripheral immune cells (Fung et al. 2017). Pathogen-associated molecular patterns (PAMPs), for example, lipopolysaccharides (LPS), can stimulate host immune cells to produce various peripheral various proinflammatory cytokines such as TNF-α, IL-1α, IL-1β, and IL-6 (Dantzer 2009). PAMPs and peripheral cytokines stimulate the macrophage like cells in the circumventricular organs (CVOs) and choroid plexus, to produce brain proinflammatory cytokines that diffuse by volume propagation into the brain

parenchyma (Sherry et al. 2010). The peripheral cytokines can also activate the vagal afferents providing a rapid signalling pathway. In both these events, the activity of brain proinflammatory cytokines can be mediated by either prostaglandins that diffuse to brain targets or by activation of neural pathways within the brain bringing about alteration in neurological functions (Dantzer et al. 2000; Dantzer 2009)

Microbial fermentation of complex polysaccharides/prebiotics in the intestine may increase the production of short-chain fatty acids (SCFAs), such as butyric, acetic, and propionic acid which are capable of crossing the blood–brain barrier (BBB) and able to elicit neurological response (Silva et al. 2020). SCFAs interact with their receptors on enteroendocrine cells and indirectly signal the brain via either the systemic circulation or through vagal pathways by stimulating the secretion of neurotransmitters such as GABA and 5-HT (Sherwin et al. 2018) and gastrointestinal tract (GIT) hormones such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) (Cherbut et al. 1998). SCFAs can cross the BBB and upregulate the expression of tight junction proteins, ultimately influencing integrity of the BBB (Silva et al. 2020). In the CNS, SCFAs contribute to the biosynthesis of serotonin (Reigstad et al. 2015), increase neurogenesis, (Kim et al. 2009) and impact neuroinflammation by influencing glial cell morphology and function and moderating the levels of neurotrophic factors (Savignac et al. 2013). Thus, interaction of SCFAs can indirectly or directly influence the pathophysiology of brain disorders as well as emotion and cognition.

11.3 Dysbiosis and Disorders Related to Gut–Brain Axis

Gut microbiota can affect neurological functions via many complex pathways evident by the fact that many neurological disorders are associated with dysbiosis in the gut. In addition, the rate of psychiatric disorders (especially depressive and anxiety disorders) has been found to be considerably high among patients with functional gastrointestinal disorders (Lydiard and Falsetti 1999). There a number of studies that support the hypothesis that gut dysbiosis can result in altered gut–brain axis resulting in neurobiological disorders (Griffiths and Mazmanian 2018), neurodevelopmental disorders (Stilling et al. 2015; Lacorte et al. 2019), and impaired cognitive function (Novotný et al. 2019). Increased gut permeability is also speculated to be strongly correlated with behavioural changes. In fact, many of the functional GI disorders such as IBS, functional dyspepsia are associated with increased gut permeability, chronic inflammation, and anxiety and depressive disorders (Barry and Dinan 2006; Jones et al. 2017). Further, a clinical study of patients with alcohol addiction reinforced the link between increased gut permeability and depression and anxiety (Leclercq et al. 2014). A few representative studies related to the involvement of gut dysbiosis in various neurological and psychiatric disorders are discussed below.

Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by a progressive decline in behaviour, cognitive function, and social skills. AD is associated with the formation of amyloid beta (Aβ) plaques and neurofibrillary

tangles (DeTure and Dickson 2019). The bacteria of the GIT are source of a significant amount of amyloids. In the gut, the exposure to bacterial amyloid may result in the activation of immune cells which subsequently enhances formation of neuronal amyloid in the brain (Kowalski and Mulak 2019). The role of bacterial amyloid in triggering AD is evident from a number of studies. Rats exposed to *E. coli* that produced curli (bacterial amyloid) displayed increase in deposition of neuronal alpha-synuclein (α-syn) in both the brain and gut, and augmented astrogliosis and microgliosis compared to rats exposed to mutant bacteria lacking the ability to produce curli (Chen et al. 2016). Similarly, α-syn expressing *C. elegans* fed on curli-producing bacteria displayed greater α-syn aggregation (Chen et al. 2016). The prevalence of bacterial components such as *E. coli* pili protein (Zhan et al. 2016) or nucleic acids (Emery et al. 2017) is greater in the brain of AD patients. *H. pylori* infection is also linked with AD. It has been reported that AD patients with *H. pylori* infection have low Mini-Mental State Examination scores corresponding with serious cognitive dysfunction (Kountouras et al. 2009). Vogt et al. (2017) reported gut microbiota alterations in AD patients characterized by lower microbial diversity, decreased abundance of *Bifidobacterium and Firmicutes*, and increased abundance of *Bacteroidetes*.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by neuroinflammation

and loss of midbrain dopaminergic neurons and manifested by motor symptoms such as rigidity, tremors, and bradykinesia (Poirier et al. 2016). Growing evidence suggests that motor impairments are usually preceded by nonmotor symptoms mainly constipation, depression, sleep behaviour disorder, and olfactory deficit, sometimes by up to a decade (Chaudhuri and Schapira 2009). A recent study reported significant decrease in the abundance of *Prevotellaceae* in PD patients and a positive association between the abundance of *Enterobacteriaceae* and the severity of instability of posture and gait difficulty, strongly implying the role of the bacteria of the gut in the PD phenotype (Scheperjans et al. 2015). Interestingly, abnormally aggregated Lewy bodies (α-synuclein) which are the pathohistological hallmark of PD are reported to be observed in the ENS before it appears in the CNS (Braak et al. 2006). In addition, experiments have demonstrated the spread of α-synuclein from the intestinal wall to the vagus nerve and hence the CNS (Goehring et al. 2014). Another study reported significant decrease in the concentration of SCFA in the faeces of PD patients compared to controls. This was accompanied by reduction in abundance of bacterial phylum *Bacteroidetes* and the bacterial family *Prevotellaceae* and increase in abundance of *Enterobacteriaceae* (Unger et al. 2016). These studies provide direct evidence of the spread of PD pathology from GIT to CNS via the gut–brain axis.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which includes repetitive patterns of behaviour that influences how a person perceives and socializes with others, causing problems in communication and social interaction (Faras et al. 2010). ASD has been reported to be associated with GIT problems, such as overgrowth of intestinal pathogenic bacteria, abnormal gastrointestinal fistula, indigestion, and poor absorption in children (Fond et al. 2015). Alterations

in the composition of the gut microbiota and its metabolites have been demonstrated both in ASD children and animal models of ASD (De Angelis et al. 2015; Kushak et al. 2016). Fine gold and colleagues reported higher levels of *Desulfovibrio* species and *Bacteroides vulgatus* in faeces of severely autistic children compared to control (Finegold et al. 2010). *Bacteroidetes* produce propionic acid which may influence CNS and autism behaviour. Kang et al. (2013) reported lower levels of carbohydrate-degrading and/or metabolizing bacteria of the genera *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* in autistic children. Gastrointestinal *Candida albicans*, a fungus which is known to release autistic behaviour inducing ammonia and other toxins, was reported to be two times more abundant in toddlers with ASD compared to normal individuals (Iovene et al. 2017).

Multiple sclerosis (MS) is a common neurological disease typified by an autoimmune inflammatory response in which immune cells affect brain and spinal cord cells resulting in demyelination and damage to the axon (Tremlett et al. 2016a). Studies have shown that MS patients have lower proportion of *Faecalibacterium* (Cantarel et al. 2015). This dysbiosis is significant because reduction in the population of *Faecalibacterium* spp. results in decrease in levels of its metabolite butyrate leading to decrease of Treg cells and proinflammatory cytokines (Sokol et al. 2008). In a study with 18 relapsing-remitting MS cases and 17 controls, it was noted that MS subjects had a significant augmentation in relative abundance of members of the *Desulfovibrionaceae* (*Bilophila*, *Desulfovibrio*, and *Christensenellaceae*) and reduction in *Lachnospiraceae* and *Ruminococcaceae*. In addition various other studies provide evidence on the prominence of the changes in composition of gut microbiota in MS (Tremlett et al. 2016a; Adamczyk-Sowa et al. 2017).

Major Depressive Disorder (MDD) also referred to as clinical depression is a psychological state characterized by persistent feeling of sadness and loss of interest, accompanied by several psychophysiological changes, such as loss of appetite, or sexual desire, disturbances in sleep pattern and constipation (Verduijn et al. 2015). Over the past decade, increasing number of studies have reported altered gut microbiota constitution in major depressive disorder (MDD) patients. Aizawa et al. (2016) reported reduction in *Bifidobacterium* and/or *Lactobacillus* counts in patients with MDD compared with normal individuals. Remarkably, attenuation of depression related behaviours could be achieved by intervention using probiotic *Bifidobacterium* (Desbonnet et al. 2008; Savignac et al. 2014) *and Lactobacillus* (Messaoudi et al. 2011; Bravo et al. 2011) *and prebiotic fructooligosaccharides and galactooligosaccharides* (Burokas et al. 2017). Furthermore, the counts of bacteria such as *Prevotella*, *Klebsiella*, *Streptococcus*, and *Clostridium* XI were found to be higher in MDD patients (Lin et al. 2016).

The gut microbiota has also emerged as a vital influencer of cognitive health (Desbonnet et al. 2008; Noble et al. 2017). Fröhlich et al. (2016) reported that recognition of novel objects was compromised in mice with antibiotic treatment induced dysbiosis. This cognitive deficit was correlated with alteration in the expression of cognition-relevant signalling molecules of the brain such as serotonin transporter, neuropeptide Y system, brain-derived neurotrophic factor, and N-methyl-D-aspartate receptor subunit. Additionally, Lee et al. (2019) reported that

suppression of gut dysbiosis by *Bifidobacterium longum* can alleviate cognitive decline in mouse model. Studies investigating the link between gut dysbiosis and neurological and psychiatric disorders are summarized in Table 11.1.

In addition to the studies mentioned above one can find myriad studies relating gut dysbiosis and neurological and psychiatric disorders (Douglas-Escobar et al. 2013; Rogers et al. 2016; Clapp et al. 2017). While the mechanistic details still remain to be determined, these recent advances suggest that modulating the composition of the microbiota appears to be a viable therapeutic option for modulating neurological and psychiatric disorders and may improve quality of life. This can be achieved in part by effective prebiotic intervention. The following section highlights different types of prebiotics that can be effectively used for addressing gut dysbiosis and consequently achieving healthy gut–brain axis.

11.4 The Concept of Prebiotics

Several therapeutic strategies have been employed to re-establish and/or to maintain the equilibrium in the microbial ecosystem of the intestine. These include the consumption of probiotics, prebiotics, and synbiotics (Gagliardi et al. 2018), phage therapy (Scarpellini et al. 2015), bacterial consortium transplantation (BCT), and faecal microbiota transplantation (FMT) (Li et al. 2015). In the recent years, use of prebiotics is becoming increasingly popular as a safe dietary approach for overcoming gut dysbiosis. Prebiotics and the metabolites formed by their fermentation in the gut play a vital role in management of gut dysbiosis and hence modulate the gut–brain axis (Franco-Robles et al. 2019).

The concept of prebiotics was introduced in 1995 by Glenn Gibson and Marcel who first defined prebiotics as 'nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health' (Gibson and Roberfroid 1995). Accordingly to classify a compound as prebiotic it should be resistant to the acidic pH of stomach; should not be digested/hydrolysed by mammalian enzymes nor be absorbed in the gastrointestinal tract; should be fermented by intestinal microbiota and should selectively stimulate the growth and/or activity of the intestinal bacteria that confer health benefits on the host (Gibson et al. 2010). Most of the first prebiotics evaluated in humans and used commercially were shown to enrich *Lactobacillus* and/or *Bifidobacterium* specifically (Didari et al. 2014). Over the last two decades, prebiotics and the concept around it have constantly been debated and the definition of prebiotics has seen an evolution to include all perspectives. In December 2016, a panel of experts in nutrition, biochemistry, microbiology, and clinical research convened by International Scientific Association of Probiotics and Prebiotics (ISAPP) updated the definition of a prebiotic to 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' thus expanding the beneficiary role of prebiotics to body sites other than the GIT, and provide inclusion of diverse classes of food and non-food categories. It recognizes the health benefits derived from prebiotic stimulation of not only *Lactobacillus and*

Table 11.1 Studies investigating the link between gut dysbiosis and neurological and psychiatric disorders

Neurological disease/ disorder	Study design and sample	Gut dysbiosis-Altered gut microbiota and / or metabolites (As compared to control)	Reference
Alzheimer's disease	*AD patients with dementia and non-demented control participants.* Sample: Faecal sample	*Bifidobacterium and Firmicutes* ↓ *Bacteroidetes* ↑	Vogt et al. (2017)
Parkinson's disease	*PD patients and age-matched control* Sample: Faecal sample	*Enterobacteriaceae* ↑ Bacteroidetes ↓ *Prevotellaceae* ↓ SCFA↓	Unger et al. (2016)
Parkinson's disease	*PD patients and healthy control* Sample: Mucosa and Faecal sample	Faecal Sample Putative 'anti-inflammatory' butyrate producing bacteria from the genera *Blautia*, *Coprococcus*, and *Roseburia* ↓ Mucosa Sample *Faecalibacterium* ↓ Putative, 'proinflammatory' proteobacteria of the genus *Ralstonia* ↑	Keshavarzian et al. (2015)
Autism	Neurotypical and autistic children Sample: Faecal sample	*Prevotella, Coprococcus,* and unclassified *Veillonellaceae* ↓	Kang et al. (2013)
Autism	Autistic subjects and non-autistic control (sibling and non-sibling) Sample: Faecal sample	Bacteroidetes ↑ *Desulfovibrio* species and *Bacteroides vulgatus*↑ Firmicutes ↓	Finegold et al. (2010)
Major depressive disorder	MDD patients and Control Sample: Faecal sample	*Bifidobacterium* and *Lactobacillus* ↓	Aizawa et al. (2016)
Major depressive disorder	MDD patients and Control Sample: Faecal sample	*Bacteroidetes, Proteobacteria,* and *Actinobacteria* ↑ *Firmicutes* ↓	Jiang et al. (2015)
Major depressive disorder	MDD patients and Control Sample: Faecal sample	*Prevotella*, *Klebsiella*, *Streptococcus*, and *Clostridium* XI ↑	Lin et al. (2016)
Multiple sclerosis	MS patients and Control Sample: Faecal sample	*Ruminococcus* ↑ *Faecalibacterium* and *Bacteroidaceae* ↓	Cantarel et al. (2015)
Multiple sclerosis	MS patients and Control Sample: Faecal sample	*Desulfovibrionaceae (Bilophila, Desulfovibrio, and Christensenellaceae)* ↑ *Lachnospiraceae and Ruminococcaceae* ↓	Tremlett et al. (2016b)

Abbreviations: *AD* Alzheimer's Disease, *PD* Parkinson's Disease, *MDD* Major Depressive Disorder; *MS* Multiple Sclerosis, *SCFA* Short Chain Fatty acids

Bifidobacterium but also of other beneficial taxa which include (but not limited to) *Eubacterium*, *Roseburia*, or *Faecalibacterium* spp. (Gibson et al. 2017). Substrates that influence gut microbiota composition through mechanisms different from selective utilization by host microorganisms are excluded from the prebiotic bracket, for example, antibiotics, minerals, vitamins, and bacteriophages (Gibson et al. 2017). Another term of interest in the context of prebiotics is *Dietary fibre*. Codex Alimentarius Commission in 2009, defined Dietary fibre as 'carbohydrate polymers with 10 or more monomeric units, which are neither digested nor absorbed in the human small intestine'. They include naturally occurring edible carbohydrate polymers in food; edible carbohydrate polymers extracted (physically, enzymatically, or chemically) from food raw material, and edible synthetic carbohydrate polymers with beneficial physiological effect (Codex Alimentarius Committee 2010). The flexibility in the definition of dietary fibre is evident by the fact that many countries include non-digestible carbohydrates with greater than three monomeric units under the bracket of dietary fibre (Jones 2014).

To date, prebiotic properties have been ascribed primarily to carbohydrates, especially non-digestible oligosaccharides (NDO) and a few complex carbohydrates; however some compounds that are not carbohydrates are also recommended to be classified as prebiotics, for example, cocoa-derived flavanols (Tzounis et al. 2011); whey derived protein, glycomacropeptide (GMP) (Sawin et al. 2015), and polyunsaturated fatty acid (PUFA) (Gibson et al. 2017). Since majority of the substrates studied for their prebiotic potency are dietary carbohydrates, in the present chapter we will focus only on the impact of dietary carbohydrates as prebiotics.

11.4.1 Prebiotic Dietary Carbohydrates

Prebiotic dietary carbohydrates are **carbohydrates** present in food that are speculated to be able to

11.4.1.1 Oligosaccharides as Prebiotics

Over the past few decades, different types of oligosaccharides have been reported to possess prebiotic potency, among them inulin-type fructans [inulin, oligofructose, and fructooligosaccharides (FOS)], lactulose, and galactooligosaccharides (GOS) are the only dietary carbohydrates that are reported to fulfil all the criteria for classification as prebiotics (Davani-Davari et al. 2019). An interesting *class* of oligosaccharides included in the prebiotic category are the *human milk oligosaccharides* (*HMOs*). HMOs play a very important and crucial role in shaping infant gut microbiome (Pannaraj et al. 2017). Xylooligosaccharides (XOS), isomalto-oligosaccharides (IMO), raffinose family oligosaccharides (RFO), lactitol and a range of other oligosaccharides are included under emerging prebiotics.

Inulin-Type Fructans

Inulin-type fructans (ITF) are polymers of fructose with β-(2←1) fructosyl-fructose linkages with degree of polymerization (DP) varying from 1–60. Both $F_{py}F_n$

[fructopyranosyl-(fructofuranosyl)$_n$-fructose] and $G_{py}F_n$ [glucopyranosyl-(fructofuransoyl)$_n$-fructose] are included under this nomenclature. ITF include native inulin (DP, 2–60), inulin HP (DP, 10–60) oligofructose (OF), and fructo-oligosaccharides (FOS) (DP, 2–8) (Roberfroid 2007). ITF can be extracted from plants (native chicory inulin), produced from enzymatic hydrolysis of inulin (e.g. oligofructose), or enzymatically synthesized from sucrose (e.g. FOS) (Roberfroid 2007). In addition to these, two ITF products; (i) 'Synergy' containing long-chain inulin and short-chain oligofructose and (ii) 'scFOS' containing a mixture of three oligosaccharides of DP3–5 are also available commercially (Hidaka et al. 1986). Owing to β-configuration of the anomeric C_2 in its fructose monomers, ITF can resist digestion/hydrolysis by mammalian digestive enzymes which are known to be specific for α-glycosidic bonds, making ITF excellent prebiotic substrates (Roberfroid 2007).

Galactooligosaccharides (GOS)

Galactooligosaccharides (GOS), also known as oligolactose, or oligogalactosyllactose, are oligosaccharides of β-D-galactopyranosyl units (2–8) with a terminal (reducing end) D-glucose. Conventionally, GOS are prepared from lactose by transglycosylation reaction using the enzyme β-galactosidase, which adds D-galactopyranosyl monomers to the nonreducing end of lactose, forming a family of oligosaccharides of varying chain length comprising a mixture of (1 → 4) and (1 → 6) linkages (BeMiller 2019). GOS produced from transglycosylation reaction are termed as trans-galactooligosaccharides (TOS). β-galactosidases are derived from various fungal and bacterial sources such as *Aspergillus* (Vera et al. 2012), *Bifidobacteria* (Rabiu et al. 2001), and *Lactobacilli* (Iqbal et al. 2011). The yield, degree of polymerization, and glycosidic linkages differ based on the source from which β-galactosidase is derived (Zárate and López-Leiva 1990). Recently, a unique second-generation prebiotic GOS was produced using galactosidase enzymes obtained from *Bifidobacterium bifidum* NCIMB 41171 (Tzortzis et al. 2005). This GOS referred to as B-GOS (Bimuno® 52 % GOS content; Clasado Biosciences Ltd) contains GOS in β-and α-anomeric configuration (Tzortzis 2010).

Human Milk Oligosaccharides (HMOs)

Human milk oligosaccharides are a complex group of glycans found in human milk at a concentration of 20–25 g/L in colostrum and 10–15 g/L in mature milk (Coppa et al. 1999). More than 200 different oligosaccharides have been reported in human milk with their carbohydrate chain containing lactose (Galβ1-4Glc) at the reducing end, which may be extended by the addition of β1-3- or β1-6-linked lacto-*N*-biose (type 1 chain) or *N*-acetyllactosamine (type 2 chain) (Bode 2012). The principle monosaccharides of HMOs are D-galactose, D-glucose, L-Fucose, N-acetylglucosamine, and sialic acid. Based on their structure and substitution, HMOs are classified as sialylated acidic HMOs, fucosylated neutral HMOs, and non-fucosylated neutral HMO (Vandenplas et al. 2018). Among the huge repertoire of soluble glycan structures of HMO, 2′-fucosyllactose (2′-FL) is reported to be the most abundant (Erney et al. 2000).

11.4.1.2 Complex Polysaccharides

Complex polysaccharides which are abundant in plant-based diet reach the intestine unaltered. Many complex polysaccharides are soluble and are easily fermented by the intestinal microbiota and thus serve as prebiotics (Flint et al. 2012). Fermentable complex polysaccharides which are known for their prebiotic potency include arabinoxylans, beta-glucans, resistant starch, glucomannans, and fucoidan.

i. Arabinoxylans are non-digestible polysaccharides mainly found in the bran tissues of most cereals (Hopkins et al. 2003). Arabinoxylans consist of unsubstituted β-(1-4) linked xylose backbone with α-(1-3) arabinofuranosyl or α-(1-2) L-arabinofuranosyl or a double α-(1-2) and α-(1-3) arabinofuranosyl linked to the xylose backbone with or without uronic acid (galacturonic acid, glucuronic acid, and mannuronic acid) and phenolic acid (mainly ferulic acid and p-coumaric acid) substitution (Bajpai 2014).
ii. Resistant starch is a portion of dietary starch that cannot be digested by amylases of the GIT and reaches the colon to be fermented by microbiota (Englyst and Cummings 1985). Currently, 5 types of resistant starch have been identified: RSI-Physically inaccessible starch, RSII-Granular starch with the B- or C-polymorph, RSIII-Retrograded starch, RSIV-Chemically modified starches, and RSV-Amylose-lipid complex (Birt et al. 2013).
iii. Beta-glucans are non-starch polysaccharides consisting of repeating glucose residues forming either linear chains or branched structures (Lam and Chi-Keung Cheung 2013). The primary structure, branching pattern and degree of branching, molecular weight (MW), and solubility are involved in the biological activity exhibited by beta-glucan and vary according to the source (Zeković et al. 2005).
iv. Glucomannans are neutral polysaccharides produced by many plants, especially the *Amorphophallus* family (e.g. Konjac). These polysaccharides predominately comprise mannose units with glucose as the second most abundant sugar, and may contain some acetylated residues and galactose side chains (Al-Ghazzewi et al. 2007). Konjac glucomannans typically have high molecular weight ($>1 \times 10^6$ Da), and are commonly used in the food industry as a gelling and thickening agent owing to their exceptionally high swelling characteristics when hydrated (Akesowan 2002).
v. Fucoidan is a fucose-enriched, sulphated polysaccharide that is primarily extracted from brown algae. Along with L-fucose and sulphate groups, fucoidan consists of one or more units of mannose, galactose, xylose, glucose, arabinose, rhamnose, glucuronic acid, and acetyl groups (Luthuli et al. 2019).

11.5 Prebiotics in Management of Dysbiosis

Based on the emerging appreciation of the link between the brain and the gut microbiota, it is evident that management of gut dysbiosis has direct beneficial impact on the gut–brain axis, hence restoration of dysregulated microbiota has

therapeutic consequences. This can be achieved by boosting bacterial growth using *prebiotics. Additionally, the metabolites produced by the intestinal bacteria in the process of prebiotic fermentation have a positive impact on host physiology* (Tsai et al. 2019). *Research over the years has identified specific strains of bacteria whose abundance in the gut would bring about a positive impact on the microbiome quality and on health condition and is termed as beneficial bacteria. Some examples of beneficial bacteria include Lactobacillus reuteri* (Gao et al. 2015)*, Lactobacillus rhamnosus*JB-1 (Bravo et al. 2011), *Lactobacillus acidophilus, Bifidobacterium animalis* subsp. *lactis, Prevotella* (Ou et al. 2013)*, Faecalibacterium prausnitzii* (Scott et al. 2015)*, Bacillus subtilis* HU58 (Tam et al. 2006), etc. In fact, a host of bacterial species belonging to the lactobacilli and bifidobacterial genera are considered beneficial/ probiotic (Fijan 2014). *Currently the dysbiosis management strategies are focussed towards increasing the population of these beneficial bacteria.*

Different approaches are employed to study the ability of dietary carbohydrates to selectively propagate the growth of beneficial bacteria and induce the production of specific SCFAs, and thus modulate the gut microbiome. The simplest and the most widely applied approach is the in vitro studies. There are numerous in vitro studies on the ability of dietary carbohydrates to enhance the growth of specific strains of bacteria (Su et al. 2007; Ward et al. 2007; Pastell et al. 2009; Kunová et al. 2012; Ramnani et al. 2012; Sims et al. 2014; Liu et al. 2016). *Additionally*, in vitro fermenters have been employed in an attempt to mimic intestinal conditions using colon simulators (Gibson and Wang 1994; Macfarlane et al. 1998; Mäkeläinen et al. 2010). *In addition to evaluating their prebiotic potency, researchers have also analysed the ability of dietary carbohydrates to* inhibit the growth of selected human intestinal pathogens (Fooks and Gibson 2002). *In vitro studies are helpful in proposing the prebiotic potency of dietary fibre; however, these studies need to be validated by in vivo experiments.* In vivo studies generally use animal models such as rats or mice or human clinical trials, to determine the effect of prebiotic supplementation on host faecal microflora. Rats or mice provide limited representation of the situation in the human colon, hence the results may not indicate true effect (Shanks et al. 2009). Therefore, the most efficient approach is the human volunteer Randomized Controlled Trial (RCT) study. Human trials are usually done by faecal sampling after diet supplementation with dietary carbohydrates for a fixed experimental period. Various in vivo studies with human volunteers have reported the ability of dietary carbohydrates to selectively stimulate the growth of bifidobacterial, lactobacilli, and other genera, inhibit the growth of pathogenic bacteria, and increase the concentration of specific SCFA (Table 11.2). However, the major drawback of human trials is that analysis of the different regions of the gut is not possible and only faecal matter is readily available.

Table 11.2 Examples of *in vivo* studies (Human volunteers) designed to determine the ability of prebiotic dietary fibre to modulate gut microbiome and impact gut ecology

Prebiotic	Volunteers	Study Design	Dosage	Changes in gut-microbiome ecology	Reference
Bi2muno Galactooligosaccharide (B-GOS)	45 overweight adults with risk factors for metabolic syndrome.	12 weeks, randomized, double-blind, cross-over (4-week wash-out period), placebo-controlled design.	5.5 /day	*Bifidobacterium*↑ *Bacteroides* spp. ↓ *C. histolyticum* ↓ *Desulfovibrio* spp↓	Vulevic et al. (2013)
Trans-galactooligosaccharide (T-GOS)	44 patients with Rome II positive IBS. Age: 18 and 80 years	12-week, single centre, parallel, patient blinded, randomized cross over controlled design.	3.5 or 7 g/day	At 3 g and 7 g *Bifidobacterium* spp. ↑ At 7 g *C. perfringens* subgroup *histolyticum* ↓ *Bacteroides/Prevotella* spp. ↓	Silk et al. (2009)
Inulin type fructans (Synergy 1)	30 overweight females Age: 18–65 years BMI: >30 kg/m^2	12 weeks, randomized, double-blind, parallel, placebo-controlled design.	16 g/day	*Bifidobacterium longum* ↑ *Bifidobacterium pseudocatenulatum* ↑ *Bifidobacterium adolescentis*↑ Total SCFA↓ Acetate↓ Propionate↓	Salazar et al. (2015)
Agave Inulin	29 Healthy adults (15F, 14M) Age: 20–40 years BMI: 18.5–29.5 kg/m^2	3-week, randomized, double-blind, placebo-controlled, cross-over design.	5.0 or 7.5 g/day	*Bifidobacterium* ↑ *Ruminococcus*↓ *Lachnobacterium*↓ *Desulfovibrio*↓	Holscher et al. (2015)
Inulin-oligofructose	12 healthy volunteers	21-days, controlled, randomized, cross-over design.	10 g/day	*Faecalibacterium prausnitzii* ↑ *Bifidobacterium adolescentis* ↑ *Bifidobacterium bifidum* ↑	Ramirez-Farias et al. (2009)

(continued)

Table 11.2 (continued)

Prebiotic	Volunteers	Study Design	Dosage	Changes in gut-microbiome ecology	Reference
				Bifidobacterium longum ↑ SCFA-NC Lactate-NC pH-NC	
FOS	10 patients with active ileocolonic Crohn's disease Age: 18–84 years	3-week dietary intervention.	15 g/day	*Bifidobacterium* ↑	Lindsay et al. (2006)
XOS and Inulin + XOS (INU-XOS)	60 healthy adults (34F, 36M) Age: 18–24 years BMI: 18·5–27 kg/m^2	4-week, randomized, parallel, placebo-controlled, double-blind design.	5 g XOS (XOS group) 3 g inulin + 1 g XOS (INU–XOS group).	XOS *Bifidobacterium* ↑ Butyrate ↑ Acetate↓ p-cresol↓ pH↓ INU-XOS *Bifidobacterium* ↑ Total SCFA↑ Propionate↑	Lecerf et al. (2012)
Resistant starch	14 obese males Age: 27–73 years BMI: 27.9–51.3 kg/m^2	3-week, randomized cross-over design.	22–29 g/d	*Oscillospira guillermondii* ↑ *Ruminococcus bromii* ↑ *Sporobacter termitidis*↑ *Clostridium leptum* ↑ *Clostridium cellulosi* ↑ *Papillibactercinnamivorans* ↓ *Alistipes* spp. ↓ Acetate ↓	Salonen et al. (2014)

				Propionate ↓ Butyrate↓	
Lactulose	16 healthy volunteers (5M, 11F) Age: 19–42 years	6-week, controlled, randomized, double-blind, parallel group design.	5 g/day	*Bifidobacterium* ↑ Total anaerobes-NC *Lactobacillus* -NC pH-NC	Bouhnik et al. (2004)
Arabinoxylan-oligosaccharides (AXOS)	63 healthy subjects (30F, 33M) Mean Age: 42 ± 17 years Mean BMI: 23.3 ± 3.2 kg/m^2	1-week run-in period, followed by three 3-week treatment periods with 2-week wash-out periods. Double-blind, randomized, placebo-controlled, cross-over deign.	3 or 10 g/day	*Bifidobacterium*↑ Acetate↑ Propionate↑ Butyrate↑ pH↓	François et al. (2012)

Abbreviations: M Male; *F* Female; *NC* No change, *BMI* Body Mass Index

11.6 Influence of Prebiotics on Gut–Brain Axis

The selective stimulation of beneficial bacteria by prebiotics not only helps in the management of dysbiosis but may also has a positive impact on the gut–brain axis. Though the mechanism of action of prebiotics on the gut–brain axis is still not conclusively determined, SCFAs are speculated to play a vital role. SCFAs are organic acids (saturated fatty acids) with a chain length ranging from one to six carbon atoms (Miller and Wolin 1996). The principle SCFAs released by the microbial fermentation of prebiotic substrates include butyrate, acetate, and propionate with the amalgamated concentration greater than 100 mM in the lumen of the intestine (Boets et al. 2017). Other SCFAs such as formate, caproate, and valerate are produced in lesser amounts (Macfarlane and Macfarlane 2003). SCFAs improve the gut health by exerting a number of local effects which include maintaining intestinal barrier integrity (Peng et al. 2009), provide protection from intestinal inflammation, affect mucous production in the gastrointestinal tract (Barcelo et al. 2000), influence gastrointestinal motility (Cherbut et al. 1998), and reduce the risk of colorectal cancer (Encarnação et al. 2015). In addition to the local effects, SCFAs are speculated to play a vital role in the crosstalk along the microbiome gut–brain axis owing to their effects directly on the CNS or indirectly via the immune and endocrine signalling pathways (Stilling et al. 2016; Dalile et al. 2019).

Studies conducted in rodent models and a few human trials have contributed immensely towards our understanding of the effect of prebiotics on neurobiological processes and consequently on the affective and cognitive functions. Sprague–Dawley rats administered with FOS, GOS, or water, over 5 weeks, showed increased expression of hippocampal brain-derived neurotrophic factor (BDNF) and NR1 subunit of *N*-methyl-D-aspartate receptor (NMDAR), with B-GOS additionally enhancing hippocampal NR2A subunits, and frontal cortex NR1 and D-serine (Savignac et al. 2013). The authors noted that GOS displayed superior neurostimulatory activity in comparison to FOS owing to the greater bifidogenic capacity of the former. The effect of supplementation of Bimuno formulation of galactooligosaccharide (B-GOS) has been studied in neonatal male and female Sprague–Dawley rat pups (Williams et al. 2016). Animals fed with B-GOS showed increased expression of hippocampal NMDAR subunit GluN2A, synaptophysin, BDNF, but not MAP2, suggesting that in neonates B-GOS feeding modifies neurotransmission rather than synaptic architecture. Based on the suggestion that BDNF may confer anxiolytic state, and NMDAR subunits may regulate cognitive functions it proposed that prebiotics can alter mood and cognitive abilities, via the modulation of microbiota (Savignac et al. 2013; Williams et al. 2016).

Salivary cortisol awakening response (CAR) and a validated test battery of emotional processing were used to assess neuroendocrine and affective effects (brain functions concerned with emotions) of prebiotics in healthy male and female participants ($n = 45$) who consumed either FOS, B-GOS, or a placebo (Schmidt et al. 2015). Amplified waking cortisol is a biomarker of psychological stress and emotional disturbances (Mannie et al. 2007; Shibuya et al. 2014). Results showed that the intake of B-GOS was linked with reduced waking salivary cortisol reactivity

and altered attentional bias in comparison with intake of FOS and placebo. Furthermore, B-GOS intake showed attenuated attentional vigilance to negative versus positive information in a dot-probe task in participants, suggestive of anxiolytic and antidepressive role of B-GOS. These studies strongly support the key role of gut microbiota modulating prebiotics in the regulation of affective function. Dietary intervention with scFOS (5 g/day) vs placebo for 4 weeks to treat IBS in patients is reported to increase faecal *Bifidobacterium* count and reduce anxiety scores (Azpiroz et al. 2017). Silk et al. (2009) evaluated the ability of a novel prebiotic trans-galactooligosaccharide (T-GOS, at doses 3.5 and 7.5 g/day) in managing colonic microbiota, improving IBS symptoms including managing anxiety and depression in patients suffering from IBS. Results indicated that T-GOS significantly improved anxiety/depression and subjective global assessment (SBA) scores. T-GOS treatment, at doses 3.5 and 7 g/day, resulted in significant increase in relative population of *Bifidobacterium* spp. The higher dose (7 g/day) of T-GOS resulted in lower proportion of *Bacteroides-Prevotella* spp. and *Clostridium perfringens* subgroup histolyticum, whereas lower T-GOS dose (3.5 g/day) resulted in higher proportion of *Eubacterium rectale*/*Clostridium coccoides* spp. Gronier et al. (2018) reported that rats ingesting B-GOS showed increase in the plasma acetate, and acetyl Co-A carboxylase mRNA, and cortical GluN2B subunits levels. Additionally, increase in neuronal responses to iontophoretically applied N-methyl-d-aspartate (NMDA) and improvement in intra-dimensional to an extradimensional set shifting in B-GOS fed rats were observed, thereby indicating heightened cognitive flexibility. Overall, the data demonstrated the association between pro-cognitive effect of B-GOS intake with an escalation in cortical NMDAR function, however the role of circulating acetate produced the B-GOS metabolism by the gut bacteria was not addressed. In another study, co-administration of B-GOS (0.5 g/kg/day) with olanzapine (antipsychotic drug) in adult female Sprague–Dawley rats significantly attenuated olanzapine-induced weight gain and had a positive effect on cognitive function (Kao et al. 2018). It was shown that in humans, FOS may modulate appetite by regulation of hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (Cani et al. 2009). Studies suggest that fructooligosaccharides from *Morinda officinalis* (OMO) exert effectual memory improvements in Alzheimer disease (AD)-like animals, and are effective in alleviating AD by affecting the gut–brain axis (Chen et al. 2013; Chen et al. 2017)

HMOs are reported to be crucial nutrients for neurological development in infants and essential for optimal *development* of cognitive abilities (Jacobi et al. 2016). A study in male rodents (both C57BL/6 mice and Sprague–Dawley rats) showed heightened associative learning and working memory associated with HMO glycan 2′-FL. Chronic administration of 2′-FL augmented the expression of phosphorylated calcium/calmodulin-dependent kinase II (pCaMKII), postsynaptic density protein 95 (PSD-95), and brain-derived neurotrophic factor (BDNF) in cortical and subcortical structures. These molecules are reported to be important in the storage of newly acquired memories, suggesting that dietary 2′-FL can affect cognitive domains and improve learning and memory in rodents (Vázquez et al. 2015). Another study revealed that oral supplementation of 2′-FL during lactation improved cognitive

abilities, both in childhood and adulthood (Oliveros et al. 2016). Two possible and nonexclusive mechanisms of action have been proposed to explain the neurobeneficial effects of HMO, a central mechanism according to which 2′-FL reaches the brain via systemic circulation (Goehring et al. 2014) and a local mechanism involving stimulation of the vagus nerve by 2′-FL (Murrey and Hsieh-Wilson 2008). All in all, whether the mode of action is via direct stimulation of the nervous system or indirectly via the microbiota, the molecular integrity of 2′FL is critical for induction of its effects.

Complex polysaccharides and polysaccharide-rich extracts modulate cognition, behaviour, and provide neuroprotective effects. Dietary intervention (14 days) with breakfast high in wheat bran fibre (3.5 g of wheat bran) in healthy, habitual low-fibre consumers significantly improved subjective perception of bowel function, digestive feelings, and general wellbeing (Lawton et al. 2013). Ambrotose Complex (a proprietary mixture of NSP) was observed to induce significant improvement in recognition and working memory performance, in healthy middle-aged adults (Best et al. 2010; Best et al. 2015). Similarly another study demonstrated that intervention with complex carbohydrates (6.5 g of fibre) is favourable in comparison to a simple carbohydrate breakfast, because of the higher degree of satiety and lower perception of fatigue associated with complex carbohydrate consumption (Pasman et al. 2003). Pectic polysaccharides have been reported to have anti-fatigue activity and improve the antioxidant status in the hippocampus of treated animals (Klosterhoff et al. 2018). Pectic polysaccharide consumption is also associated with improved intestinal barrier function resulting in prevention of lipopolysaccharide (LPS) entry into the circulation and reduction of influence of systemic inflammation on the brain. Supplementation with a Beta 1,3/1,6 glucan (250 mg, commercially available as Wellmune WGP®) for 4 weeks improved overall health, increased vigour, and reduced fatigue, tension, anger, and confusion, compared to 250 mg of rice flour placebo (Talbott and Talbott 2009).

Oral administration of isolichenan (*Cetraria islandica* derived alpha-glucan) to ethanol-fed mice reversed the ethanol-induced impairment (Smriga et al. 1999). Oral or intravenous injection of a new (1–3) (1–4) (3:2) α-glucan, isolated from the lichen *Flavoparmelia caperata*, resulted in potent, dose-dependent enhancement in tetanically evoked synaptic short-term potentiation (STP) in the hippocampus of rats (Smriga et al. 1996). Sherry et al. (2010) noted a basal up-regulation of IL-4 mRNA accompanied by doubling of endotoxin-induced IL-1RA expression in the brain of mice fed soluble fibre (pectin rich diet) in comparison with the mice fed insoluble fibre, indicating that the impact of soluble fibre is not limited to the gut and peripheral immune system but goes beyond and affects the neuroimmune system. In a neurotoxin (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)) induced animal model of Parkinson, fucoidan derived from the brown alga *Laminaria japonica* resulted in evident increase in tyrosine hydroxylase expression, increased levels of striatal dopamine and its metabolites, reduced behavioural deficits, and lowered cell death. In the same study, extended to *in vitro* model of PD, fucoidan shielded mouse dopaminergic MN9D cells from MPTP toxicity (Luo et al. 2009). In another study, intraperitoneal administration of Bladderwrack fucoidan reduced the

extent of hypoxia-ischemia induced neural damage in the cortex, hippocampus, and striatum of rat (Uhm et al. 2004). Arabinoxylan from *Triticum aestivum* (wheat) and beta-glucan from barely have been reported to have ameliorating effect against vascular dementia (Han et al. 2010). A uncharacterized polysaccharide fraction of *Panax ginseng* has been reported to promote learning and memory (Lyubimov et al. 1997). The impact of consumption of prebiotics on neurological disorders, cognition, and behaviour is summarized in Table 11.3.

11.7 Conclusion

The relationship demonstrated between the gut microbiome and the brain and the ability of prebiotics to modulate gut microbiome and thus impact gut–brain axis has garnered interest of researchers working towards developing diet-based therapies to manage neurological and psychiatric disorders. Although research in this direction has been initiated, there is limited understanding on the detailed mechanism of action of prebiotics. Developing therapeutic formulations using prebiotics requires deeper research into investigating the right dose, appropriate inclusion of probiotics, duration of treatment, and knowledge of associated side effects. It is also worthwhile to note that many dietary carbohydrates not bracketed under prebiotics have an impact on the CNS via routes that do not involve modulation of gut microbiome. Further, the involvement of food and pharmaceutical companies is required in terms of investment for large scale human trials. Prebiotic containing diet-based therapy in managing neurological and psychiatric disorders has a long road ahead.

Table 11.3 Studies investigating the impact of prebiotics on neurological disorders, cognition, and behaviour

Prebiotic	Study Model	Main finding	Inference	Reference
Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS)	Adult male Sprague–Dawley rats	FOS and GOS increased hippocampal BDNF, NR1 subunit and *N* methyl-D-aspartate receptor (NMDAR) subunits expression Increased BDNF mRNA expression in dentate gyrus, GOS increased hippocampal NR2A subunits, and frontal cortex NR1 and D-serine and elevated plasma D-alanine and peptide YY (PYY).	FOS and GOS *could potentially* be used to treat *neuropsychiatric disorders*	Savignac et al. (2013)
Fructooligosaccharides from *Morinda officinalis* (OMO)	Adult male Sprague–Dawley AD-like symptoms rats.	OMO administration can ameliorate learning and memory disabilities in AD-like animals significantly.	FOS may have therapeutic effect in Alzheimer's disease	Chen et al. (2017)
Bimunogalactooligosaccharide (B-GOS) or Fructooligosaccharides (FOS)	Human: Healthy volunteers	B-GOS intake was associated with decreased waking salivary cortisol reactivity and altered attentional bias compared to FOS and placebo. Participants showed reduced attentional vigilance to negative versus positive information.	Anxiolytic and antidepressive role of B-GOS	Schmidt et al. (2015)
Short-chain fructooligosaccharides (scFOS)	Human: IBS patients	scFOS significantly reduced anxiety scores and increased faecal *Bifidobacteria*.	Anxiolytic effect of short-chain fructooligosaccharides (scFOS)	Azpiroz et al. (2017)
Bimuno™ galacto-oligosaccharide (B-GOS®)	Adult male Sprague–Dawley rats	B-GOS® administration augmented cortical neuronal responses to NMDA iontophoresis, partially hindered the reduction of NMDA responses HA-966 (glycine site antagonist) and resulted in	Pro-cognitive effect of B-GOS®	(Gronier et al. 2018)

		improvement in intra-dimensional to an extradimensional set shifting.		
Human milk oligosaccharides (HMO)-2′-fucosyllactose (2′-FL)	C57BL/6 mice and Sprague–Dawley rats	2′-FL enhanced associative learning and working memory and increased the expression of phosphorylated calcium/calmodulin-dependent kinase II (pCaMKII), postsynaptic density protein 95 (PSD-95), and brain-derived neurotrophic factor (BDNF) in cortical and subcortical structures-molecules reported to be important in the storage of newly acquired memories.	Positive impact of HMO-2′-FL on cognition.	Vázquez et al. (2015)
Beta 1,3/1,6 glucan (commercial name Wellmune WGP®)	Human trial: marathon runners	Beta-Glucan treated group reported significantly fewer upper-respiratory tract symptoms (URTI), decreased confusion, fatigue, anger, and tension, increased vigour, and better overall health.	Beta-glucan improves overall health and mood	Talbott and Talbott (2009)
Proprietary mixture of non-starch polysaccharides (NSPs) (Ambrotose® complex)	Human trial: middle-aged adults	Significant improvement in recognition and working memory performance was observed in the group that consumed NSP.	NSP may enhance memory performance	Best et al. (2015)
Oligofructose-enriched inulin	Human trial: Healthy adults	Inulin consumption was associated with greater accuracy on a recognition memory task, and improved immediate and delayed recall performance.	Positive effect of inulin on mood and memory	Smith et al. (2015)
Pectin	C56BL/6J mice injected i.p. with LPS	Pectin diet resulted in quicker recovery from LPS induced social withdrawal compared with cellulose diet.	Protective effect of pectin against endotoxin-induced sickness behaviour	Sherry et al. (2010)

(continued)

Table 11.3 (continued)

Prebiotic	Study Model	Main finding	Inference	Reference
Fucoidan	Neurotoxin induced animal model (C57/BL mice) of Parkinson	Fucoidan led to evident increase in tyrosine hydroxylase expression, increased levels of striatal dopamine and its metabolites, reduced behavioural deficits, and lowered cell death.	Protective effect of fucoidan against neurotoxin-induced neurodegeneration.	Luo et al. (2009)

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Designer Probiotics in Metabolic Disorders 12

Rishi Gupta and Shailendra Raghuwanshi

Abstract

Microbes play several vital physiological and metabolic functions in human body. It has been observed that alteration in human gut microbiota has resulted in various chronic and acute metabolic diseases such as obesity, hypertension, neurogenic diseases (Parkinson's and Alzheimer), diabetes, etc. Hence, re-establishment of microbial population, with the help of commensal probiotic bacteria, to improve the gut dysbiosis, has always been the topic of interest. Currently, with the growing knowledge of synthetic biology, genetic engineering, metabolic engineering, and other advanced tools, researchers are attempting to design recombinant probiotic strains, which are capable of carrying therapeutic molecules to the target site. These designer probiotics will enhance the efficacy of the carried molecule without showing any side effects. However, currently, the consumer acceptance of such "Designer Probiotics" is very low. The current chapter envisages a brief introduction about designer probiotics, their developmental strategies, applications of designer probiotics in regulating metabolic diseases, and the challenges in the path of their development discussing examples of few designer probiotic strains. Overall, this chapter intends to provide insight towards the development of designer probiotics to improve the human health.

Keywords

Phenylketonuria · Patho-biotechnology · Receptor mimicking · Synthetic oligosaccharides · Anti-microbial peptide

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12.1 Introduction

In human body ecosystem, microbes are found most abundantly in the gastrointestinal (GI) tract, and these gut microbiota plays a significant beneficial role in human life by participating in various physiological functions advantageous to the host (Tlaskalova-Hogenova et al. 2004; Raghuwanshi et al. 2015; Kristensen et al. 2016). Here, the body works as a host, which provides a suitable condition for growth, while the commensal microbes perform their counterparts by preventing pathogens (Hand 2016), increasing host-immunity (Round and Mazmanian 2009; Patel and DuPont 2015; Macpherson et al. 2017; Raghuwanshi et al. 2018), enhancing the stimulus for GI-hormones (Saulnier et al. 2013), and controlling brain behavior (De Palma et al. 2014; Steenbergen et al. 2015; Kristensen et al. 2016; De Palma et al. 2017). The uniqueness of these gut microbiota is their capability evolve naturally and inhabiting every potential tissue.

Though the normal gut microbiota are essential for several vital processes, however, occasionally they fails, which can be the result of gut microbiota manipulation through hygiene, lifestyle changes, and diet, for example, diet can cause an impact to promote phylogenetic variations in the microbiota (Graf et al. 2015). Moreover, physical activity is also known to affect the gut-microbiome diversity as it is evident that athletes have a more diverse gut microbiome than non-athletes (Clarke et al. 2014). Besides these passive factors, the active manipulators of gut microbiota are antibiotics. The use of antibiotics has been linked to dysbiosis (Langdon et al. 2016), leading to low diversity and evenness among gut microbes (Dethlefsen and Relman 2010; Francino 2016). Moreover, presence and expression of microbial genes are altered following antibiotic therapy, which also lead to detrimental functions of microbiota (Reijnders et al. 2016).

All these factors for gut dysbiosis including overly use of antibiotics, passive lifestyle, use of pesticides in farms, etc., may lead to antibiotic resistance against pathogens, increase obesity epidemic, inflammation, resistance to insulin, diabetic condition, heart diseases (CVDs), brain-related disorders, dyslipidemia, pathophysiological conditions such as allergy, intestinal inflammatory diseases, and even cancers (Amaral et al. 2012; Benbouziane et al. 2013; Andreu and Torrent 2015; Aydin et al. 2015). However, enhancing the functional repertoire of probiotics, the commensal organisms that can be harnessed for therapeutic benefit, is a promising approach to combat these issues. Probiotics can affect the host either directly or through their products, or even can influence the activity of resident bacteria in the host (Scott et al. 2015). The first health benefit report of probiotics was discussed by Russian scientist, Eli Metchnikoff (1907), according to whom "*the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes with useful microbes*." Later, WHO and FAO defined the probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO 2006; Hill et al. 2014). There are several reports of beneficial impacts of probiotic organisms in metabolic disorders, inflammatory disorders, CNS related disorders, pathogen infections, dyslipidemia, and regulating

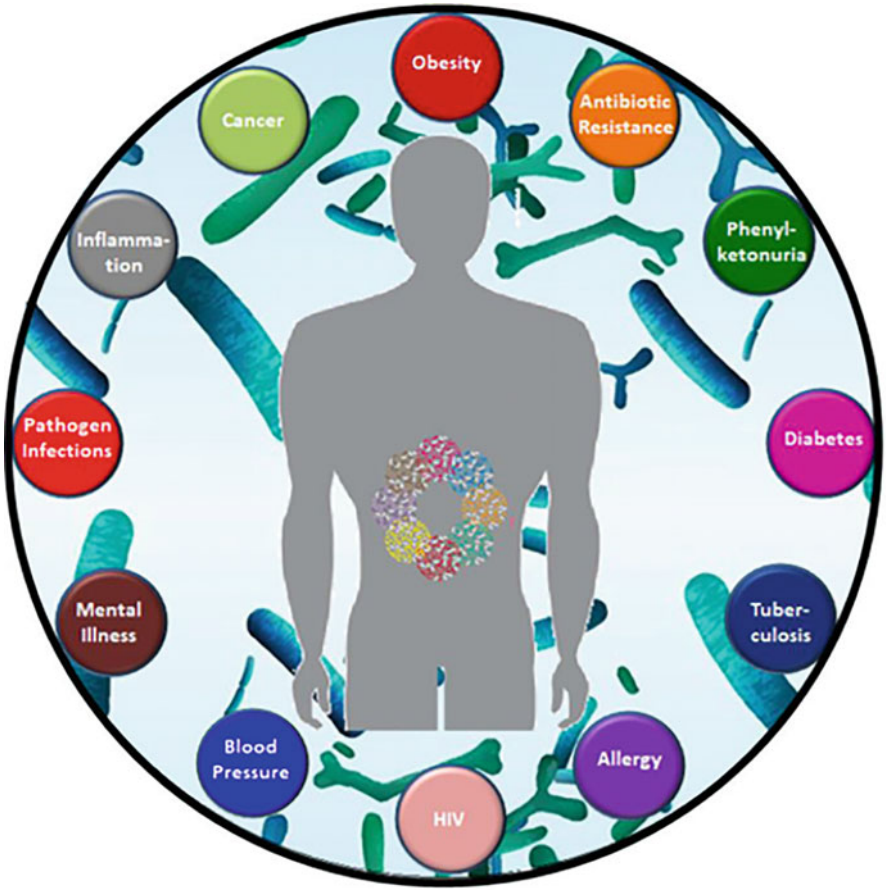

Fig. 12.1 Schematic illustration of health benefits of probiotics

mucosal immune response (Fig. 12.1) (Miettinen et al. 1996; Kwon et al. 2010; Yin 2010; Chen et al. 2011; Yan and Polk 2011; Klaenhammer et al. 2012; Asemi et al. 2013; Kim et al. 2013; Plaza-Diaz 2014; Reichold et al. 2014; Savcheniuk et al. 2014; Wang et al. 2014; Kasińska and Drzewoski 2015; Di Cerbo et al. 2016; Kobyliak et al. 2016; Nazemian et al. 2016; Wallace and Milev 2017).

12.2 Why Designer Probiotics?

The probiotics are categorized into mono-strain or multi-strain probiotics and it has been well documented that multi-strain probiotics poses significant positive effects due to symbiosis among the strains used in formulation (Timmerman et al. 2004). A list of few multispecies probiotic consortium and their positive impacts are shown in Table 12.1. However, recognition of the importance of microorganism–receptor

Table 12.1 List of probiotic consortium and their role in improving human diseases

Generic Name	Microorganisms	Role	Reference
De Simone Formulation	*Streptococcus thermophilus, Eubacterium faecium, Bifidobacterium breve, B. infantis, B. longum, Lactobacillus acidophilus, L. plantarum, L. casei, and L. delbrueckii subspecies bulgaricus*	Ulcerative colitis, gestational diabetes mellitus (GDM)	Venturi et al. (1999), Timmerman et al. (2004), Jafarnejad et al. (2016)
EcologicR tolerance/ SyngutTM	*B. lactis, L. acidophilus, L. plantarum, L. lactis*	To strengthen the gut barrier function, have beneficial effects on post-immunological induced stress, inhibit Th2, and stimulate IL-10 levels, thus providing beneficial effects in patients with food intolerance	Besseling-van der Vaart et al. (2016)
Ecologic AAD	*B. bifidum, B. lactis, B. longum, E. faecium, L. acidophilus, L. paracasei, L. plantarum, L. rhamnosus, L. salivarius*	Reduced diarrhea-like bowel movements when administered in healthy volunteers taking amoxicillin	Koning et al. (2008)
Multispecies probiotic consortium	*L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. breve, B. longum, S. thermophilus*	Prevented rise in fasting plasma glucose (FPG), to decrease high sensitivity C-reactive protein (hs-CRP), and to increase plasma glutathione (GSH) in diabetic patients	Asemi et al. (2013)
Ecologic® 641	*L. acidophilus, L. casei, L. salivarius, L. lactis, B. bifidum and B. infantis*	Bacterial translocation, morbidity and mortality in a rat model of acute pancreatitis	van Minnen et al. (2007)
Fermented milk	*L. rhamnosus, Propionibacterium freudenreichii, B. lactis*	Effect of a multi-strain probiotic on IBS: abdominal symptoms, quality of life, gut microbiota, inflammatory markers	Kajander et al. (2008)
Multistrain probiotic consortium	*B. longum, B. bifidum, B. lactis, L. acidophilus, L. rhamnosus, and S. thermophilus*	Inhibiting pathogen growth and atopic dermatitis, suggesting further application on other diseases like IBD	Yoon et al. (2013)

interactions in the pathogenesis of disease and limited probiotic strain diversity was the main drawback (Marotz and Zarrinpar 2016). To combat such issues, researchers used synthetic biology and genetic engineering approaches to develop recombinant probiotic strains, commonly called as "Designer Probiotics." Moreover, it was also postulated that genetic engineering of target specific probiotic strains or development of probiotics as a vehicle to carry vaccine and drug molecules is a promising approach (Braat 2006; Paton 2012; Kumar 2016; Maxmen 2017). Such designer probiotics as vaccine vehicle also offer an advantage of no possibility of reversion to a virulent phenotype, which always remains a threat with the attenuated pathogenic strains (Seegers 2002).

Moreover, according to researchers (Paton et al. 2006; Sleator and Hill 2008) this approach has several other advantages as well.

(i) Oral administration of probiotic,
(ii) Well characterized receptors recognized by enteric pathogens/toxins,
(iii) Inhibition of pathogen adherence leading to lower infection,
(iv) Sequestration of a toxin by the improved host immune system which prevent clinical symptoms,
(v) It does not apply a selective pressure on the pathogen, so development of resistance against it is very unlikely.

12.3 Strategies of Developing Designer Probiotics

Among various strategies to develop designer probiotics, few main approaches are as below:

12.3.1 Patho-Biotechnology Based Designer Probiotics

Patho-biotechnology describes the concept of using a pathogenic organism for beneficial use in the biotechnological application. This stands for the use of their ability to adapt against stress, their strong host invasion system and virulence related abilities to other different areas. Recently, it has been suggested to use them in the field of development of probiotics as food supplements (Sleator and Hill 2006). In addition, patho-biotechnology can also improve strain's resistance during manufacturing process and storage period for improved probiotic response. The most common example is the use of *Listeria monocytogenes, L. salivarius*, and *Bifidobacterium breve* for the development of targeted designer probiotics. For instance, the betaine transporter gene (betL) from L. monocytogene resulted in reducing the stress and improved the survival rate of probiotics (Hoffmann et al. 2013; Sleator et al. 2003a).

12.3.2 Receptor-Mimicking Based Designer Probiotics

In this approach, the probiotics are developed by engineering the expression of host-receptor-mimics on the surface of a commensal bacterium. Paton and group developed a designer probiotics for the prevention of gastrointestinal infections using a strategy involving the expression of host cell receptor-mimics on the surface of probiotic strains (Paton et al. 2006; Sleator and Hill 2008). Expression of two galactosyl-transferase genes (*lgtC* and *lgtE*) from *Neisseria gonorrhoeae* into *Escherichia coli* strain generated a lipopolysaccharide terminating in Gal(α1, 4) Gal(β1, 4)Glc, which mimics Shiga-toxin (*stx*) receptor and was found effective against shigatoxigenic *E. coli* (STEC) (Paton et al. 2000). Using a similar strategy to that mentioned above, an *E. coli* strain was engineered to produce a chimeric *LPS* receptor mimic capable of binding a heat-labile enterotoxin (Paton et al. 2005). Similarly, a probiotic strain with an altered *LPS* was designed by Focareta et al. (2006). This altered *LPS* receptor terminates in a structure that mimics the GM1 ganglioside terminus, which is the binding receptor for cholera toxin (Focareta et al. 2006). Similar approaches have also been used to develop probiotics for enterotoxigenic *E. coli* (ETEC) (Paton et al. 2005).

12.3.3 Synthetic Oligosaccharide-Based Designer Probiotics

In this approach, oligosaccharides in specific conformation or in multivalent interaction correspond to a given receptor epitope to inhibit the ligand binding (Zopf and Roth 1996; Mulvey et al. 2001). Using this strategy, a probiotic ("Synsorb-pk") comprising silica particles linked to synthetic Gal(α1,4)Gal(β1,4)Glc oligosaccharide was developed for severe gastroenteritis, which can progress to hemolytic uremic syndrome (HUS) (Paton and Paton 1998). Similarly, probiotic (Synsorb 90) displaying a Gal(α1,3)Gal(β1,4)GlcNAc epitope was developed against *Clostridium difficile* (Heerze et al. 1994). Merritt et al. (2002) developed a probiotic for cholera toxin consisting of a pentacyclic core, each displaying m-nitrophenyl-α-d-galactoside, which showed enhanced binding to the toxin. "SUPERTWIGS" having dendrimers with multiple tri-saccharides was also developed using similar strategy against an O157:H7 STEC (Nishikawa et al. 2002; Nishikawa et al. 2005; Watanabe et al. 2004).

12.3.4 Anti-Microbial Peptides Based Designer Probiotics

Probiotics produced from commensal bacteria are capable of expressing antagonism against pathogenic microorganisms. Expression of anti-microbial peptides with potential to overcome the antibiotic resistance among pathogens resulted in combined benefits of anti-microbial peptides as well as of probiotics (Proctor 2011; Reid et al. 2015). This can be achieved by cloning and expression of anti-microbial peptide specific genes in the probiotic bacteria.

12.4 Designer Probiotic Strains

Most of the probiotics as well as probiotic consortium are developed from *Bifidobacterium*, *Lactobacillus* species, and other lactic acid bacteria (LAB) or specific yeast strains (*Saccharomyces cerevisiae, S. boulardii, Kluyveromyces lactis,* and *Pichia pastoris*) (Govender et al. 2013). While on the other hand, when we consider designer probiotics, there are some specific promising bacterial species that are currently under consideration.

12.4.1 Faecalibacterium Prausnitzii

F. prausnitzii is a bacterium of Clostridium cluster IV (Martín et al. 2017), and its anti-inflammatory response proposed its usage as targeted anti-inflammatory drugs for Crohn's disease (Quévrain et al. 2016). In addition, it can induce the Clostridium-specific IL-10-secreting regulatory T cell subset, and reduce IL-12 and IFNg production, to maintain the gut barrier immune function (Quévrain et al. 2016). Also it is reported to reduce the severity of trinitrobenzene sulfonic acid (TNBS) colitis and correct the associated dysbiosis (Sokol et al. 2008). These studies suggest that *F. prausnitzii* can be regarded as a potential probiotic candidate for chronic gut inflammation and Crohn's disease (Sokol et al. 2008; Martín et al. 2017).

12.4.2 Akkermansia Muciniphila

Schneeberger et al. (2015) reported the impact of *A. muciniphila* in lipid metabolism, inflammatory markers in adipose tissue, regulation of glucose level, resistance to insulin, and occurrence of plasma. This led to investigate the role of this bacterium in adipose tissue homeostasis and metabolism. A study conducted by Dao et al. (2016) suggested *the A. muciniphila* is associated with body fat distribution and glucose homeostasis. While the effect of *A. muciniphila* in reversing the atherosclerotic lesson and improving the metabolic endotoxemia-induced inflammation and controlling the dysbiosis has also been reported (Li et al. 2016). All these prospects put *A. muciniphila* in the category of potential designer probiotics (Dao et al. 2016).

12.4.3 Bacteroides Fragilis

Bacteroides species are gram −ve, obligate anaerobe, and non-spore forming commensal bacteria, which constitutes approximately 25% of our gut microbiota (Wexler 2007). They passed from mother to infant during vaginal delivery and thus are considered as gut's primary colonizers. Among various Bacteroides species, *B. fragilis* is the most common and this produces an immunomodulatory molecule, polysaccharide A (PSA), which play a vital role in homeostasis and development of the host immune system and preserves the balance between T cell types (Troy and Kasper 2010; Round et al. 2011).

12.4.4 Bacteroides Uniformis

Oral administration of *B. uniformis* has shown considerable improvement in lipid profile, glucose insulin and leptin levels, TNF-a production, and phagocytosis in mice studies (Gauffin Cano et al. 2012). Moreover, it has also been reported that their administration can improve immunological dysfunction and metabolic disorder related to gut dysbiosis (Gauffin Cano et al. 2012; Yang et al. 2016).

12.4.5 Eubacterium Hallii

E. hallii is an anaerobic bacterium that resides in our gut and affects the gut metabolism (Engels et al. 2016). It is a natural butyrate producer and is supposed to lower mucosal inflammation and oxidative status, enhance the host–gut microbiota homeostasis, strengthen the gut barrier, increase energy metabolism, improve insulin sensitivity, and act as energy (Canani et al. 2011; Engels et al. 2016). Moreover, it has also been regarded safe as its high dose did not cause any negative impact (Udayappan et al. 2016).

12.4.6 Clostridium Cluster Members

Patients suffering from inflammatory bowel disease (IBD) have reduced number of bacterium related to Clostridium spp. clusters IV and XIVa, which are supposed to be exceptional Tregs (Regulated T cells) inducer in the colon (Atarashi et al. 2011). These Tregs are also considered as potential therapeutic agents for allergies and IBD (Atarashi et al. 2011). Later, Atarashi et al. (2013) again reported that these Clostridia clusters XIVa, IV, and XVIII were also playing role in Treg cell differentiation and accumulation (Atarashi et al. 2013). Moreover, these Clostridium clusters were also reported to produce short chain fatty acids (SCFAs) to improve the gut dysbiosis conditions (Atarashi et al. 2013).

12.4.7 Listeria Monocytogenes

It is a pathogenic bacterium, which as auxotrophic mutant or after selective elimination of virulence genes can be used as novel vaccine and drug delivery vehicles (Zhao et al. 2005). Also these bacterium can be used to incorporate stress tolerant genes into non-pathogenic probiotic strains (Sleator and Hill 2006). Such genes can support the survival of probiotic strain in stressful conditions such as gastric juice, bile juice, low pH, etc. (Mattila-Sandholm et al. 2002; Sleator and Hill 2007a). *L. monocytogenes* serves as an ideal candidate for this concept as its genome has been fully sequenced and can be manipulated to resisting numerous stresses and also eliciting a strong host immune response in probiotics (Glaser et al. 2001; Hamon et al. 2006; Gray et al. 2006; Lecuit 2005). *L. monocytogenes* has three solute uptake

genes *betL*, *gbu,* and *opuC* (Wemekamp-Kamphuis et al. 2002). Among them, *betL* is reported to be a betaine transporter gene (Sleator et al. 1999; Sleator et al. 2000; Hoffmann et al. 2013), whose cloning has contributed to probiotic survival under a variety of stresses (Sheehan et al. 2006; Sleator et al. 2003a).

12.4.8 Bifidobacterium Breve

B. breve UCC2003 strains expressing *betL* were shown to exhibit significantly increased tolerance to simulated gastric juice (pH 2.5) as well as osmotic stress. Moreover, the heterologous expression of the *BilE* system from *L. monocytogenes* would increase bile tolerance and subsequent gastrointestinal persistence of the probiotic strains (Sheehan et al. 2007). Interestingly, *B. breve* UCC2003 expressing *betL* gene has improved survival rate even in gastric juice (Sheehan et al. 2007), which may be attributed to the fact that improving compatible solutes accumulation leads to more physiologically robust probiotic strains (Sleator et al. 2003b).

12.5 Applications of Designer Probiotics in Metabolic Disorders

Enzymes are the crucial factors in cellular metabolism and their deficiency can result in metabolic disorder. Manipulating the gut ecosystem by designer probiotics expressing therapeutic biomolecules such as enzymes might serve as alternative approaches against metabolic disorders (Singh et al. 2017; Isabella et al. 2018; Kurtz et al. 2019).

12.5.1 Diabetes

Diabetes is a condition of hypo-secretion of insulin resulted in higher blood glucose levels in the body, which may subsequently create several acute health concerns such as cardiovascular disease and Alzheimer, etc. (Li et al. 2015). Diabetes are of 2 types, type 1 is related to the impaired cells in the pancreas and type 2 occurs due to insulin resistance (Klöppel et al. 1985). Compared to conventional insulin injection, use of probiotics capable of secreting pro-insulin or cytokines would be a better alternative because of less pain and negligible side effects (Vinay et al. 2005; Van Belle et al. 2011; Bluestone et al. 2010). Recently, several attempts have been made to engineer probiotics carrying interleukins (ILs), pro-insulin, glucagon like proteins (GLPs), and other therapeutic protein, which have shown proven records in combating diabetes in mouse models. Liu (2016) developed a probiotic strain of *Lactococcus lactis* expressing HSP65-6IA2P2 protein (pro-insulin autoantigen), which showed significant improvement in diabetes mellitus conditions among diabetic mice (Liu 2016). Oral administration of IL-10, and this designer probiotics with anti-CD3 showed stability against diabetes in 59% of tested mice (Liu 2016). Similarly, engineered *L. lactis* NZ9000 expressing fusion protein HSP65-6P277

was found effective against diabetes (Ma et al. 2014). Moreover, *L. lactis* harboring auto-antigen GAD65370-575 and IL-10 brought about stabilization in pancreatic inflammation (Robert et al. 2014). While designer probiotic strain of *Lactobacillus casei* induces SP (Usp45)-INS-specific antibodies, which improve the levels of IL-4 and protect them from pancreas injury (Schwenger et al. 2015). GLP-1 (1–37) fused with USP45-LEISS secretion marker and polyhistidine tag, expressed in *Lactobacillus gasseri* ATCC 33323 showed improved insulin secretion by converting rat cells into insulin-secreting cells (Duan et al. 2015). In another report, *Lactobacillus paracasei* expressing exendin-4 peptide also enhanced insulin secretion (Zheng et al. 2017).

12.5.2 Phenylketonuria (PKU)

Phenylketonuria (PKU) another metabolic disorder is caused by defect in the phenylalanine hydroxylase (PAH) that prevents the action of phenylalanine ammonia lyase (PAL), which breakdown the phenylalanine into ammonia and cinnamic acid (Wang et al. 2005). The rise in level of phenylalanine results in acute health concerns resulting in reduced intellectual ability, seizures, etc. (Mitchell et al. 2011). Researchers are working for developing probiotic strains expressing PAL gene to be a potential solution for PKU. Overexpressing the PAL gene from *Rhodosporidium toruloides* in *E. coli* significantly reduced the phenylalanine level in mouse model (Sarkissian et al. 1999). Similar results were obtained when *Lactobacillus reuteri* having PAL gene from *Anabaena variabilis* was administered (Durrer et al. 2017). Recently, *E. coli* Nissle 1917 was genetically engineered to overexpress PAL and L-amino acid deaminase to convert phenylalanine into phenylpyruvate, which substantially lowered the level of phenylalanine in blood (Isabella et al. 2018).

12.5.3 Hyperammonemia

Hyperammonemia is caused due to an enzymatic defect in metabolizing free ammonia to urea resulting in increased ammonia level in blood (Leonard and Morris 2002). This condition can be reversed using lactulose or antibiotics; however, the use of antibiotics may have many side effects (Auron and Brophy 2012). Recently probiotic strain SYNB1020 was developed from *E. coli* Nissle 1917 by deleting *thyA* and *argR* genes (−ve regulators for arginine translocation) and incorporating an *argA215* gene (N-acetyl glutamate) to convert ammonia into L-arginine. This designer probiotics on administration resulted in 50% improvement in the survival rate of hyperammonemia suffering mice model (Kurtz et al. 2019).

12.5.4 Parkinson's Disease

Parkinson's disease is linked with the low level of dopamine, a neurotransmitter, in the brain cells. The medicine, which is used for the treatment of Parkinson's disease,

is Levodopa. This levodopa is converted to dopamine on decarboxylation mediated by an enzyme acid decarboxylase (AADC) (Bergmann et al. 1974). However, besides brain, this levodopa can also be decarboxylated in gut causing side effects and reduced its bioavailability. Recently, pyridoxal-5-phosphate dependent tyrosine decarboxylase from *Enterococcus faecalis* was reported to decarboxylate the L-dopa, while *Eggerthella lenta* was found to have dopamine dehydroxylase (*Dadh*) gene for conversion of L-Dopa to m-tyramine in gut (Rekdal et al. 2019). They also observed that in dopamine dehydrogenase enzyme the variants have arginine at 506th position could metabolize L-Dopa while the variants with Serine at 506th position could not. This indicated that an SNP mutation in enzyme can predict the L-Dopa metabolism in complex gut microbiota (Rekdal et al. 2019).

12.5.5 Alzheimer's Disease

Alzheimer's disease is a chronic neurodegenerative condition caused by abnormal accumulation of amyloid and tau protein in and around the brain cell, which decreased the level of acetylcholine and resulted in memory loss and cognitive loss (Selkoe and Hardy 2016). Recently, its progression was found to be linked with dysbiosis in gut microbiota, which caused accumulation of amino acids. This amino acid accumulation further activates the M1 microglia that is responsible for cognitive loss (Wang et al. 2019). For the reversal of this condition, a drug molecule (GV-971), a mixture of acidic linear oligosaccharides with varied degree of polymerization, was used to improve cognition by re-establishing the gut flora (Wang et al. 2019). This suggests that designer probiotic strains linked to these oligosaccharide molecule will be a potential candidate to improve this metabolic disease.

12.5.6 Obesity

Obesity is now considered a worldwide pandemic, which is associated with alteration in gut flora (Robert et al. 2014). Researchers have attempted to engineer probiotics having potential to deliver therapeutic molecules such as leptins and N-acylphosphatidyl-ethanolamines (NAPEs) to reduce the obesity (Steidler et al. 2000). Stritzker and Szalay (2013) reported reduction in the level of obesity in mice administered with *E. coli* expressing NAPEs. Similarly, *E. coli* Nissle 1917 expressing NAPEs was found to reduce obesity in mice (Chen et al. 2014), while those expressing pyrroloquinoline quinone and fructose dehydrogenase facilitate in treating fructose induced hepatic steatosis in mice (Somabhai et al. 2016).

12.5.7 Angiotensin Level Linked Hypertension

Angiotensin is a hormone, which functions as vaso-contractor, i.e., it narrows the blood vessels, which resulted in hypertension (high blood pressure conditions),

however if the receptors for angiotensin are blocked, this condition can be reversed. In this aspect, angiotensin-converting enzyme inhibitory peptides (ACEIPs) are reported to reduce the blood pressure by relaxing the blood vessels. Yang et al. (2015) engineered a probiotic strain *Lactobacillus plantarum* NC8 expressing ACEIPs, which on administration to mice was observed to decrease the angiotensin levels and subsequently reduced the blood pressure.

12.6 Challenges in Development of Designer Probiotics

The use of designer probiotics has the potential to significantly impact the mortality/morbidity rates. However, being genetically engineered, these designer probiotics contain additional genetic elements for inducing antigenicity, immunomodulation, and effect on normal metabolic pathways, and hence safety of bioengineered probiotics is an important issue (Singh et al. 2016). Besides the issue of biological containment, the stress due to change in water activity and temperature are other challenges for probiotics (Sleator and Hill 2007b), Also, the viability percentage of the bacterial strain during product manufacturing and storage processes is also a matter of concern (El Hage et al. 2017).

In addition, the probiotics have also to face the regulatory issues, as worldwide they are also classified into different categories depending on their use in a particular condition. For instance, in the USA, as a dietary supplement, probiotics are considered as "food" and should be regulated by the Dietary Supplement Health and Education Act (DSHEA); and in case of therapeutic purpose, the probiotic comes under drug category and hence it must be approved by the FDA. On the other hand, in Japan probiotics are categorized as foods as well as drugs. Since, the classification and definition of probiotics by different regulatory bodies vary across world; the regulatory status of these probiotics is still unknown (El Hage et al. 2017).

12.7 Conclusions

Development of antibiotic resistance in pathogenic bacteria and increase in metabolism related diseases have led an urge to search for alternative cost-effective approaches to conventional antibiotic prescription. In this aspect, probiotics have shown proven records for health benefits by preventing illness, increasing immunity and maintaining the gut microbiome. Moreover, with the growing understanding about the synthetic biology and genetic engineering of microbes, researchers are now inclining towards development of recombinant "designer probiotics" to be used as drug delivery system, gene therapy vectors, invaders to pathogenic microbes and also career of therapeutic proteins in a more target specific manner. These designer probiotics comprising an anti-microbial, anti-inflammatory, and immunomodulatory repertoire not only help the host against infectious diseases but also their target specific nature reduce the production level and subsequently the cost. Few designer probiotics strains and their role in prevention and treatment of human diseases are listed in Table 12.2.

Table 12.2 List of designer probiotic organisms depicting their engineered element/proposed hypothesis in reducing the metabolic disorders

Metabolic disorders	Probiotic organism	Genetically modified element/ Proposed hypothesis	Reference
Hypertension	*Lactobacillus plantarum NC8*	Expressing ACEIP coding sequences from TFP and YFP joined by an arginine linker	Yang et al. (2015)
Obesity	*Escherichia coli*	N-acylphosphatidylethanolamines (NAPEs)	Chen et al. (2014)
	E. coli	N-acylphosphatidylethanolamines (NAPEs)	Stritzker and Szalay (2013)
	E. coli	Genes for pyrroloquinoline quinone and fructose dehydrogenase	Somabhai et al. (2016)
Hyperammonemia	*E.coli*	Deletion of thyA and argR genes and integration of argA215 gene	Kurtz et al. (2019)
PKU	*E. coli*	PAL gene	Sarkissian et al. (1999)
	Lactobacillus reuteri	PAL gene	Durrer et al. (2017)
	E. coli	PAL gene and L-amino acid deaminase	Isabella et al. (2018)
Diabetes	*Lactobacillus gasseri*	Glucagon like protein GLP-1 (1–37)	Duan et al. (2015)
	Lactococcus lactis	HSP65-6IA2P2 protein	Liu (2016)
	Lactococcus lactis	Fusion protein HSP65-6P277	Ma et al. (2014)
	L. lactis	Auto-antigen GAD65370-575	Robert et al. (2014)
	Lactobacillus casei	SP (Usp45)- INS-specific antibodies	Schwenger et al. (2015)
	Lactobacillus paracasei	Exendin-4 peptide	Zheng et al. (2017)
Parkinson's disease	Gut microbiota	Integration of tyrosine decarboxylase and dopamine dehydroxylase (Dadh)	Rekdal et al. (2019)
	Inactive DadH producing gut microbiota	SNP mutation (Arg506 in place of Ser506) of inactive dopamine dehydrogenase present in gut microorganisms for conversion of L-Dopa to m-tyrosine	Rekdal et al. (2019)
Alzheimer disease	Probiotic strains	Synthetic oligosaccharides used in GV-971	Wang et al. (2019)

Though this concept is gaining interest and popularity in view of long-term protection against chronic diseases, however, the issue of biological containment and consumer acceptance of recombinant probiotics is still a significant roadblock. In conclusion, we are optimistic that utilizing the advancement in technologies such

as synthetic biology, genetic engineering, and patho-biotechnology, research in this area will continue to generate stable recombinant designer probiotics with strongly regulated gene expression and clearly demonstrable medical benefits. Moreover, with the use of rigorous biological containment strategies, detailed risk analysis, scientific evidences, and consumer education, these designer probiotics will attain broader acceptance in the near future.

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Animal Models Used for Studying the Benefits of Probiotics in Metabolic Disorders

13

Aakriti Garg, Anoop Kumar, Faheem Hyder Pottoo, and Pooja A. Chawla

Abstract

The GIT flora is disturbed due to various reasons such as metabolic disorders, immunosuppressive therapy, administration of antibiotics, radiations, etc. The introduction of beneficial bacterial species can therefore be an attractive choice for restoring microbial equilibrium in GIT and preventing disease. Probiotics are live microorganisms which appear to provide medical benefits when ingested usually by enhancing the intestinal microbiota. In literature, various studies have indicated the benefits of probiotics in metabolic disorders. Thus, in the first part of chapter, we will summarize available animal models to study benefits of probiotics in metabolic disorders. The advantages and limitations of individual animal model will be discussed in second part. Finally, this chapter will highlight current challenges and future perspectives.

Keywords

Animal models · Probiotics · Metabolic disorders · Intestinal microbiota · Microbial equilibrium

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
BBDP rat	BioBreeding diabetes-prone rat
BW	Body weight
FAO	Food and Agriculture Organization
GM	Genetically modified
HBA1c	Hemoglobin A1c
HFD	High-fat diet
HFSD	High-fat-sugar diet
HIV	Human immunodeficiency virus
HSD	High sucrose diet
IDDM	Insulin-dependent diabetes mellitus
IL-6	Interleukin-6
IM	Intramuscular
IP	Intraperitoneal
IV	Intravenous
MSG	monosodium glutamate
NIDDM	non-insulin-dependent diabetes mellitus
NOD mice	Non-obese mice for type 1 diabetes
ob gene	Obese gene
SC	Subcutaneous
STZ	Streptozotocin
TNFα	Tumor necrosis factor
WHO	World Health Organization

13.1 Introduction

According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), probiotics are "live microorganisms which, when administered in adequate amount, confer a health benefit on the host" (Kumar et al. 2015). Probiotics help to maintain the body health in combination with the gut microflora. It has been reported that gut microflora is an important determinant of metabolic diseases like diabetes and obesity. The obesogenic diet can bring about a change in the bacterial population in intestine which may further lead to metabolic disorders. Thus, to prevent such deleterious effects, probiotics can be used to reshape the microflora in intestine and improve gut health. However, before administration of probiotics in human beings, there is need to evaluate their efficacy in metabolic disorders in animal models to understand their stability, safety, and mechanism.

Animal models are living, non-human organisms which are used scientifically to investigate the biological phenomenon and physiological function between the animal model and target species. Animal models are most widely used method to determine the pharmacological and toxicological profile of a drug before administration in human. Mice and rats are the most commonly used animals used to check the efficacy of probiotics in metabolic disorders. The animal model should mimic the anatomy, physiology, and pathogenesis so as to extrapolate the data for human. Thus, a sound knowledge of genetics, anatomy, and physiology will be of great help in choosing an animal model. Phylogenetic closeness, however, cannot guarantee the similar results, for example, chimpanzee does not acquire human immunodeficiency virus (HIV), thus cannot be used to study acquired immunodeficiency syndrome (AIDS) (King 1986). New animal model are constantly being developed to understand the mechanism of action, pharmacokinetics, metabolic diseases, diagnosis, therapeutic procedures, safety, and efficacy of chemical substances for human use. Different types of animal models can be used, the efficacy of probiotics is as follows:

1. In induced models, the disease condition to be investigated in induced using biological, chemical, and physical methods in healthy animals. However, there is difference between the etiology and categorization of disease in induced animal models (Hau 2008).
2. Spontaneous models are the animals that develop the disease under natural conditions. The manifestations of the disease in such models are similar to those of target species. It is a common practice to compare the disease and response between the animal model and the target species. For example, athymic nude mice (Pantelouris 1968).
3. Genetically modified (GM) animal models are developed by alteration in the genome to produce a desired disease. GM are further of two types, namely transgenic animals in which DNA is inserted into genome and knock-outs in which a specific gene is removed from the genome to produce a particular genotype. However, the development of GM animal models may lead to unpredictable and undesired results.
4. Negative models designed in such a manner that a specific disease does not develop but the animals exhibit absence of response to certain stimuli. These models are commonly used to understand the physiology and mechanism of resistance to a disease.
5. In orphan animal model, the disease occurs naturally which is not yet described in human, e.g., bovine spongiform encephalopathy in cows. These are useful to investigate similar diseases found in target species.

Various factors such as breed, species, strain, genotypes, etc. are known to affect the suitability and selection of particular model. During the investigation of the efficacy of probiotics in metabolic disorders, the microbial factor plays an important role as they may alter the outcome and inferences from the animal model. Infections in experimental animals may be attributed to various microbes such as bacteria,

virus, fungi, etc. Thus, it should be taken in account that the selected model is devoid of undesired microbial species in order to get accurate, valid, and reproducible results. However, there may be variations due to chemical substance, genotype, environmental conditions, or microbial flora, thus the efficacy of probiotics should be investigated carefully in animal models.

In the current book chapter, various animal models used to check the efficacy of probiotics in metabolic disorders such as diabetes and obesity are summarized. Further, the advantages and limitations of each model are also discussed. The chapter concludes with the current challenges and future perspectives.

13.1.1 Diabetes

Diabetes is a metabolic disorder characterized by high blood sugar level. Diabetes mainly develops due to inability of beta cell to produce sufficient insulin and insulin resistance, which leads to decreased consumption of glucose by tissues, thus raising blood glucose level. Thus, animal models used for diabetes tend to have beta cell failure and/or insulin resistance. Some of the frequently employed animal models to test the efficacy of probiotics for diabetes are outlined below:

13.1.1.1 High-Fat Diet Induced Diabetes

High-fat diet (HFD) fed model is the commonly used animal model to induce type 2 diabetes. This model was first introduced by Surwit et al. (1988). As compared to other strains, HFD fed model is found most effective model in C57BL/6J mice. HFD consists of 35.8% fat, 20.5% protein, 3.6% ash, 0.4% fiber, 36.8% carbohydrate, and 3.1% moisture administered for 1 week. HFD leads to increase in weight, stable hyperglycemia, followed by hyperinsulinemia (Winzell and Ahren 2004), indicating the continuous worsening of insulin resistance (Fig. 13.1). Moreover, after 1 week of HFD administration, there is elevation of baseline glucose and insulin, impaired insulin secretion, and reduced glucose elimination. Thus, the characteristic feature of type 2 diabetes, i.e., beta cell dysfunction and insulin resistance are induced in this mode.

The diabetes and obesity in human are induced majorly due to environmental manipulations, thus this model is of advantage as it mimics the human situation more

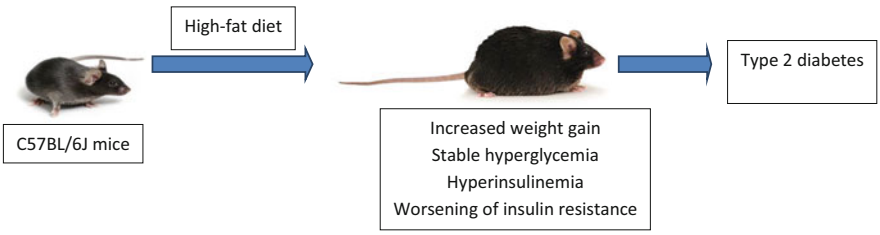

Fig. 13.1 Representation of type 2 diabetes induced by high-fat diet

accurately. However, the percentage of diet in the given diet exceeds the common dietary intake among the developed nations (Harika et al. 2013). Moreover, there may be some differences in the studies on HFD-induced diabetes model due to difference in age, strain and gender of mice, diet composition, fat content, and duration of feeding (King and Bowe 2016).

Yadav et al. (2013) used the HFD-induced type 2 diabetes model to show the antidiabetic activity of probiotic, De Simone Formulation. Also, it was shown that Lactobacillus rhamnosus enhances adiponectin and improved sensitivity to insulin in HFD fed mice (Kim et al. 2013).

13.1.1.2 High-Fat Diet and Streptozotocin Induced Type 2 Diabetes

Among the available models for type 2 diabetes, the streptozotocin (STZ) combined with HFD has been widely used by researchers to investigate the antidiabetic activity of chemical molecules. Also, this model has been employed to check the efficacy of probiotics against type 2 diabetes by researchers (Chen et al. 2018; Yan et al. 2019). In this model, rats are fed with HFD consisting of 48% carbohydrate, 22% fat, and 20% protein with a calorific value of 44.3 kJ/kg for a period of 4 weeks followed by IP administration of STZ (25–30 mg/kg) (Zhang et al. 2008) (Fig. 13.2). HFD helps to initiate the insulin resistance, an important feature of type 2 diabetes. Following insulin resistance by HFD, STZ causes alkylation of DNA, causing β cell death, thus inducing insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). In this model, a lower dose of STZ is used to induce mild insulin secretion impairment as its high dose impairs insulin secretion, thus mimicking type 1 diabetes.

HFD in combination with ST induced type 2 diabetes animal model is cheap, practical, and easily accessible for the investigation of antidiabetic property of probiotics. Moreover, stable hyperglycemia is achieved using this model. Further, this model can be customized as per need to resemble the slow pathogenesis of type 2 diabetes which cannot be found in most humans (Fang et al. 2019).

The major disadvantage of HFD in combination with STZ induced type 2 diabetes is that it is time-consuming, thus increases the cost of overall investigational protocol (Srinivasan et al. 2005). Moreover, the difference in fatty acid compositions in HFD may lead to considerable difference in the results and outcomes, affecting the

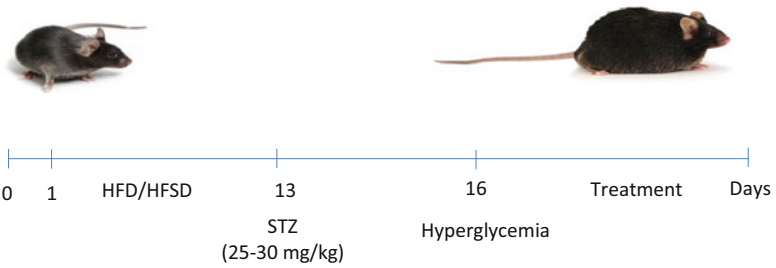

Fig. 13.2 HFD/HFSD and STZ induced diabetes

reproducibility of the result (Buettner et al. 2007). Further, the effects of HFD are difficult to prevent or reverse, thus the effect of antidiabetic drugs on obesity cannot be studied using this model, hence, model where low percentage of fat is administered might be useful to design such studies (Gheibi et al. 2017). Despite the mentioned limitations, HFD/STZ is a practical and reasonable animal model for type 2 diabetes to represent the later stage of ailment.

Chen et al. (2018) described the beneficial effects of *Lactobacillus* in HFD/STZ induced diabetic mice.

13.1.1.3 High-Fat-Sugar Diet and Low Dose of Streptozotocin Induced Type 2 Diabetes

In this model, high-fat-sugar diet (HFSD) consisting of 20% sucrose, 5% milk powder, 12% lard oil, 2% egg, and 61% normal fodder is administered orally for 6 weeks followed by once intramuscular (IM) or intraperitoneal (IP) injection of STZ (30–35 mg/kg) (Zhuo et al. 2018) (Fig. 13.2). The excess accumulation lipids due to HFSD trigger the mitogen-activated protein kinase (MAPK) pathway (Savary et al. 2012), which in turn increases the secretion of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNFα). These further attack islets cells of pancreas and interfere with the insulin signaling mechanism and hence lessen glucose uptake efficiency. Also, as discussed earlier, the low dose of STZ helps to induce destruction of small part of beta cell, reducing the production of insulin rather than the complete destruction.

The main advantage of HFSD/STZ induced type 2 diabetes is that it helps in achieving the characteristic pathogenesis of type 2 diabetes. It brings out dysfunction of and insulin resistance without the genetic manipulation or multiple congenic breeding techniques which may lead to undesired results like β cell driven metabolic disorders or massive obesity. Thus, the development of this model is cheap, easy to breed, and widely available (Barrière et al. 2018).

Manaer et al. (2015) and Dang et al. (2018) reported the antidiabetic effect of shubat, a probiotic and *L. paracasei*, respectively, in high-glucose-fat induced type 2 diabetes.

13.1.1.4 Alloxan Induced Diabetes

Use of alloxan to induce insulin-dependent diabetes mellitus is very well documented (Dunn and McLetchie 1943; Gomori and Goldner 1945) for a variety of species like mice, rabbits, monkeys, dogs, and cats (Goldner and Gomori 1944; Cruz Jr et al. 1961). Alloxan can be administered through different routes such as intravenous (IV), subcutaneous (SC), and IP either in single dose or multiple doses. The species of animals, their status of nutrition, and route of administration also play a significant role in the determination of the dose of alloxan to induce diabetes (Federiuk et al. 2004). However, single IP dose of 170–200 mg/kg of body weight (BW) of alloxan is most preferably used and is effective to induce diabetes. The administration of alloxan causes blockage of secretion of insulin stimulated by glucose and leads to reactive oxygen species formation, thus promoting selective necrosis of pancreatic β cells (Fig. 13.3).

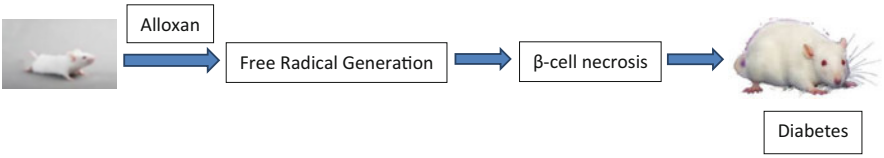

Fig. 13.3 Alloxan induced diabetes

There are some disadvantages of alloxan-induced diabetes like auto-reversal of hyperglycemia induced by alloxan and poor diabetogenicity after IP administration of 150 mg/kg and below. Moreover, very young animals are less susceptible and offer high resistance to the diabetogenic effects of alloxan, thus other animals should be used to induce diabetes using alloxan (Ighodaro et al. 2017).

Al-Salami et al. (2008) investigated the antidiabetic activity of probiotics in alloxan-induced diabetic animal model. They reported that probiotics have no effect on blood glucose level in healthy animals, however, it significantly decreases the blood glucose level in diabetic animals.

13.1.1.5 Db/db Mouse

Db/db mouse are genetically modified experimental animals for diabetes. They express autosomal recessive mutations in leptin receptor leading to obesity (Bogdanov et al. 2014), decreased insulin receptor sensitivity, decreased β cell function, and elevated Hemoglobin A1c (HBA1c) levels. This leads to the progressive development of hyperglycemia with age which provides clinical relevance. At 6 weeks of age, db/db mouse have near normal or slightly increased fasting plasma glucose level and comparatively normal β cell function. The level of fasting plasma glucose elevates gradually over several weeks. After 16 weeks of age, there is entire degeneration of β cell function and fasting plasma glucose becomes very high, i.e., >400 mg/dL. (Fajardo et al. 2014).

However, genetically modified db/db mouse are homogenous and development of hyperglycemia is genetically determined which differs from heterogeneity observed in humans. Moreover, db/db mouse have limited availability, are expensive, and require high maintenance (Srinivasan and Ramarao 2007).

Yun et al. (2009) demonstrated that *Lact. gasseri* BNR17 (derived from human breast milk) decreased blood glucose level and ameliorated the symptoms associated with diabetes after oral feeding in db/db mice. Wang et al. (2020) explored the antidiabetic mechanism of 14 probiotics in db/db mouse. They found that probiotics remarkably enhanced blood lipid and blood glucose levels. Furthermore, they improved morphological changes in liver, kidney, and pancreas and protected pancreas from apoptosis.

13.1.1.6 Non-obese Mice for Type 1 Diabetes (NOD Mice)

Type 1 diabetes is an autoimmune disease caused by infiltration of pancreatic islets by immune cells which attack β cells, leading to β cell destruction and ultimately insulin deficiency. The NOD mouse is being widely used as an experimental model

for Type 1 diabetes as it shares similarities with Type 1 diabetic patients (Li et al. 2019). The pathogenic events in NOD mouse begins after 3 weeks of birth with the presentation of islets antigens in lymph nodes of pancreas (Hoglund et al. 1999). At this stage there is infiltration of islets with antigen presenting cell (APCs), i.e., dendritic cells and macrophages, and then with lymphocytes, resulting in insulitis which gradually progresses over 15 weeks (Rosmalen et al. 1997). After 18–20 weeks, there is development of Frank diabetes, i.e., blood glucose level above 250 mg/dL (Kachapati et al. 2012).

The advantage of NOD mice is that the type 1 diabetes pathogenesis is similar to that in human, i.e., due to destruction of β cell resulting I insulin deficiency and ultimately hyperglycemia. Furthermore, chronic hyperglycemia can lead to serious complications like neuropathy, cardiovascular diseases, retinopathy, nephropathy, etc. Many of these complications are depicted by NOD mice and hence it provides a good animal model to study type 1 diabetes and its complications (Aldrich et al. 2020).

However, these mice require high maintenance (Caquard et al. 2010) and the physiological variations between mice and human, for example, islet architecture, immune system components, metabolism, etc. should be kept in mind while employing them to study type 1 diabetes therapy (Roep et al. 2004). It has been shown that it is relatively easy to treat diabetes in young NOD mice, therefore, the point of intervention should also be considered while using this experimental model (Roep 2007). Dose conversion of drug from NOD mice to human also poses a limitation in using it as an experimental model (von Herrath and Nepom 2005).

Despite the above-mentioned limitations of the NOD mouse, it is still used widely as it represents various aspects of human disease and is helpful to identify genetic as well as signaling pathway leading to type 1 diabetes. Kim et al. (2020) showed that the incidence of diabetes reduced significantly after the administration of a probiotic combination consisting of *Lactobacillus acidophilus*, *Lactobacillus reuteri, Lactobacillus casei, Streptococcus thermophiles*, and Bifidobacterium bifidum 6 times a week for 36 weeks to 4 weeks NOD mice. This combination also ameliorated insulitis and β cell mass in NOD mice. In a study, it was shown that probiotics belonging to families *Lactobacillaceae* and *Bifidobacteriaceae* and genus *Streptococcus thermophilus* ameliorated type 1 diabetes in NOD mice (Dolpady et al. 2016). In another experiment it was observed that oral administration of *L. lactis* in NOD mice prevents type 1 diabetes progression (Takiishi 2012). Calcinaro et al. (2005) showed that feeding De Simone Formulation prevents type 1 diabetes and reduces insulitis in NOD mice.

13.1.1.7 BioBreeding Diabetes–Prone (BBDP) Rat

The BioBreeding diabetes–prone (BBDP) rat is an important model to understand the pathogenesis as well as investigate the therapeutic intervention of type 1 diabetes. In BBDP rat, type 1 diabetes spontaneously through T cell mediated autoimmune destruction of β cells presents in pancreatic islets. In BBDP rats, diabetes develops after puberty with similar prevalence in females and males (Mordes et al. 2004). About 90% of rats develop diabetes within 8–16 weeks of age. The diabetes is quite

severe and is characterized by hyperglycemia, weight loss, hyperinsulinemia, insulitis, and ketonuria which requires insulin administration for survival. Metabolic and clinical symptoms are followed by histological abnormalities in islets of pancreas. The advantage of this model is that it is genetically similar to that of human diabetes and also insulitis is morphologically similar to that of human insulitis. Moreover, the hyperglycemia occurs in BBDP rat under well controlled circumstances and within short duration, thus facilitating its utility for understanding the stages of type 1 diabetes development (Bortell and Yang 2012).

Valladares et al. (2010) demonstrated that the administration of *L. johnsonii* isolated from BioBreeding diabetes resistant rat delayed or inhibited the onset of type 1 diabetes in BioBreeding diabetes-prone rats.

13.1.2 Obesity

The disparity between the intake of energy and its expenditure is the most common cause for the appearance of obesity. Microflora of the gut also plays a significant role in obesity as it influences the metabolism of whole body by influencing the energy balance, gut barrier function, and integrating peripheral as well as central intake regulatory signals. Thus, probiotics can be of use in obesity as they contribute to enhance the gut microflora, affect appetite, food intake, metabolic functions, and body weight through modulation of bacterial species in intestine and gastrointestinal pathways. Following are the most prevalent animal models to investigate the anti-obesity activity of probiotics:

13.1.2.1 Diet Induced Obesity

Diet induced obesity is the frequently used animal model to test the anti-obesity activity of probiotics. HFD and/or high caloric diet can be used to induce obesity in animals. HFD leads to lipid assimilation in the body and thus is detrimental to health, leading to quick gain of weight (Roseno et al. 2015; Sampey et al. 2011; Kumar et al. 2014).

In HFD-induced obesity, mice are fed with HFD consisting of 45% fat, 16.4% protein, and 25.6% carbohydrate (5.252 Cal/g) for 8 weeks. The physiological mechanisms involved in this model are the overconsumption of HFD leading to low satiety which in turn cause storage of dietary fat in the body and alteration in hormones required for the energy balance (Fig. 13.4) (For example, suppression of

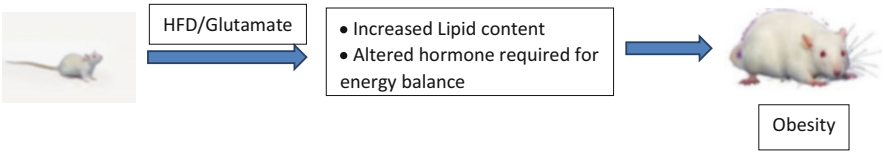

Fig. 13.4 High-fat diet/Glutamate induced obesity

ghrelin secretion after consumption of HFD; leptin and insulin resistance caused due to HFD-induced hyperinsulinemia and hyperleptinemia) (Hariri and Thibault 2010).

This model is simple to induce and closely mimic the time taken for the gradual development of obesity in human. However, there are some drawback of this models as the standardized, single, and defined diet is still lacking (Barrett 2016).

Another model used to induce obesity is high calorie diet induced obesity. In this model, the mice are ingested with high sucrose diet (HSD) consisting of 50.0% sucrose, 5.0% fat, 20.0% protein, and 15.0% cornstarch by weight (Kang et al. 2013). This model is commonly used by researchers to investigate the anti-obesity activity of testing compound as it takes longer time to induce obesity after hypercaloric diet as observed in humans (Rashmi et al. 2019).

However, HFD-induced obesity is more effective to induce obesity in animal as compared to HSD because of the low satiating effect of HFD and large storage capacity of adipose tissues as compared to low capacity of glycogen stores.

Various researchers have used diet induced obesity animal models to determine the anti-obesity activity of probiotics such as *L. plantarum* (Lee et al. 2007; Takemura and Sonoyama 2010; Park et al. 2013) and *L. curvatus* (Yoo et al. 2013; Kang and Cai 2018; Park et al. 2013); *Lactobacillus rhamnosus* (Lee et al. 2006; Liao et al. 2017; Kang and Cai 2018); *Bifidobacterium* (Stenman et al. 2014; Yin et al. 2010; An et al. 2011); *Lactobacillus gasseri* SBT2055 (Miyoshi et al. 2014); *Lactobacillus paracasei* ST11 (NCC2461) (Tanida et al. 2008); *L. casei* IMV B-7280 (Bubnov et al. 2017); *Pediococcus pentosaceus* LP28 (Zhao et al. 2012). Soundharrajan et al. (2020) recently checked the metabolic effect of selected probiotics in HFD fed mice. Their study showed that 29 potential probiotic strain may alleviate the obesity development and its associated metabolic disorders.

13.1.2.2 Glutamate Induced Obesity

Administration of glutamate leads to obesity by causing imbalance between the absorption and energy expenditure. In this model, 2–4 mg/g of BW of monosodium glutamate (MSG) can be administered SC or IP during the neonatal period for 4–10 doses, causing obesity (Von Diemen and Trindade 2006).

There is great increase in body lipid content and decrease in hormone-stimulated lipolysis in MSG-induced obese mice (Fig. 13.4), thus resembles the genetically induced mice. Moreover, it is found that the mortality rate in MSG-induced obese mice is low (Bunyan et al. 1976).

Savcheniuk et al. (2014) opined that multi-probiotic when administered from childhood stage prevents adiposity in glutamate-induced obesity in rats.

13.1.2.3 Other Models

Db/db Mouse

As discussed above can also be used as a genetically modified experimental animal for obesity. Everard et al. (2014) showed that the administration of *Saccharomyces boulardii* exhibits reduced body weight in db/db mouse.

Ob/ob Mouse

In these mice, there is a spontaneous mutation in obese (ob) gene leading to markedly obese phenotype. The obesity in ob/ob mouse is observed due to lack of leptin which further leads to hyperphagia, hypothermia, and decreased expenditure of energy. Further defects include hypercorticosteronemia, insulin resistance associated with hyperglycemia and hyperinsulinemia, growth hormone deficiency, and hypothyroidism leading to linear growth inhibition (Lutz and Woods 2012). The advantage of selecting spontaneous animal model is that there is no need to use labor-intensive feeding schemes to induce obesity. However, in humans, in most of the obese individuals, the obesity does not develop due to decrease in leptin production, therefore the physiology of ob/ob mouse does not entirely reflect to that of human. Moreover, ob/ob mice include high maintenance, expensive, and moreover they are infertile; therefore, limiting their use in routine study.

Yadav et al. (2013) demonstrated that administration of De Simone Formulation, a probiotic reversed obesity in ob/ob mice.

13.2 Current Challenges and Future Perspective

Mortality and morbidity are the risk factors often attached to disorders of metabolism like obesity and diabetes. Various studies have reported the beneficial activity of probiotics against obesity and diabetes by alteration in gut microflora, lowering of cholesterol, and regulation of insulin secretion. However, elucidation of the interaction between ingested probiotics and intestinal microflora possesses a great challenge to consider probiotics as a therapy against metabolic diseases. Thus, thorough and more specific in vivo studies can be of great help to understand the pharmacology and toxicology of probiotics when administered in the metabolic disorders. The in vivo studies will help researchers to gain insight regarding the basic mechanism and will enable researchers to conduct more optimal safety studies before such probiotics are approved for human consumption.

Moreover, there does not exist any standardized safety guidelines related to ingestion of probiotics in human. Thus, there is a need for the careful evaluation of individual probiotics in order to check the potential side effects. There is a need for advanced investigation to further improve the understanding of the relationships that exist between microflora of the intestine and the ingested probiotics.

13.3 Conclusion

Probiotics have shown to be beneficial in metabolic disorders as they help in improving lipid profile, insulin resistance, and glucose tolerance. A further exploration of the efficacy of probiotics in metabolic diseases may be beneficial in the management of metabolic diseases. Thus, further studies need to be designed in animal models to understand the exact mechanism, efficacy, and safety of probiotics in metabolic diseases.

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