Fructan rich diet to improve gut microbiota in disease and health

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1. Microbiota

Microbiota is a community of microorganisms that populate a certain environment and specially the population of microorganisms inhabiting in or on the human body. Human body forms a platform on which diverse microbial ecosystems are established. After the birth of a mammal, a life-long process of colonization by foreign microorganisms that inhabit most environmentally exposed surfaces such as the skin, mouth, gut and vagina is initiated [1, 2]. The human microbiota involves more than 10^{14} symbiotic microbial cells [3] and 10^{15} viruses [4] harbored by each person, predominantly bacteria in the gut; the human microbiome consists of the genes these cells harbor [5, 6]. In short, the microbial population that inhabits in and on the human body establish our microbiota, and the genes they encode are defined as our microbiome. This complex community involves bacteria, eukaryotes, viruses, and at least one archaeon that interact with one another and with the host, greatly impacting human health and physiology. Cultivation of these microorganisms are difficult and only small number of them can be cultured. However, culture-independent high-throughput sequencing provides a great information about these microorganisms [3, 7]. Many samples can be characterized and compared rapidly by the highly multiplexed studies [8, 9]. These studies enable the detection of spatial, temporal, and disease-associated patterns in human microbiota. In humans, the microbiota plays a significant role in health and disease and it can be occasionally referred to as our ‘‘forgotten organ’’ [10]. The microbiota takes a role in energy harvest and storage, besides its fundamental role in a variety of metabolic functions such as fermenting and absorbing undigested carbohydrates [11]. Most significantly, the gut microbiota interacts with the immune system and provides signals for the maturation of immune cells and the normal development of immune functions [12].

2. Gut Microbiota

The microbiota colonization occurs on every surface of the human body that is open to the external environment. Microorganisms live in our skin and in the genitourinary, gastrointestinal, and respiratory tracts [13-15]. The gastrointestinal tract (GIT) is the most colonized organ; only the colon contains more than 70% of all the microorganisms in the human body [1, 3]. The human gut has 200 m^2 surface [16] that represents a significant surface for microbial colonization. Moreover, the GIT is a favored surface for colonization since it provides a variety of nutrients for microbes. Strict anaerobes are dominant in gut microbiota with respect to the facultative anaerobes and aerobes [17-19]. The gut microbiota of healthy individuals is composed of six bacterial phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* [20]. However, only the *Bacteroidetes* and the *Firmicutes* dominate in the human gut microbiota [21]. Variety of bacterial species in the human gut reaches as much as 35,000 species [22].

The GIT has non-homogenous microbiota. The number of bacterial cells in stomach, duodenum, jejunum, ileum and column are approximately 101, 103, 104, 107 and 1011 bacteria per gram, respectively [10]. Furthermore, the composition of microbial community differs in different parts. Biopsy samples from colon and small intestine of healthy people were compared and enrichment of different bacterial communities were observed at these two sites [22]. Bacilli class of the *Firmicutes* and *Actinobacteria* were dominant in small intestine, however; *Bacteroidetes* and the *Lachnospiraceae* family of the *Firmicutes* were enriched from colonic samples. Furthermore, there is also difference in microbiota of intestinal lumen between attached and embedded in the mucus layer which separates intestinal epithelium from the lumen. For example, *Bacteroides*, *Bifidobacterium*, *Streptococcus*, members of *Enterobacteriacea*, *Enterococcus*, *Clostridium*, *Lactobacillus*, and *Ruminococcus* were all detected in feces, on the other hand, only *Clostridium*, *Lactobacillus*, and *Enterococcus* were found in the mucus layer and epithelial crypts of the small intestine [23].

Bacteria in the GIT can be divided into two main groups: useful bacteria (good bacteria) and harmful bacteria (bad bacteria) which must be kept in the intestine (Fig. 1). Numerous factors can affect gut flora and it has been linked to various diseases including obesity, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), non-alcoholic steatohepatitis (NASH), colon cancer, liver cancer, type II diabetes mellitus, allergic diseases and cardiovascular diseases [24, 25]

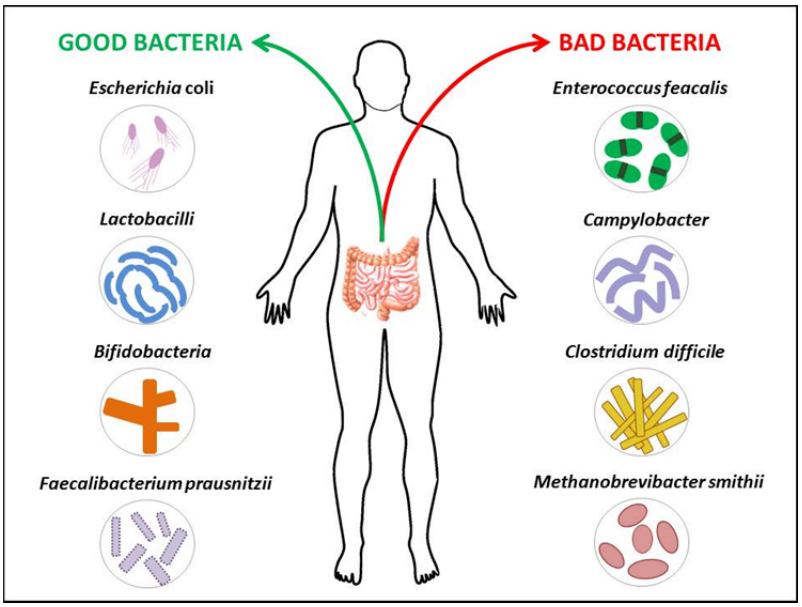

Fig. 1 Bacterial flora of gut.

Formation of microbiota in the human gut starts with the birth. During the canal of birth, infants are exposed to varying microbial groups [26] in the vaginal microbiota of their mothers, similarity between microbiota of infants intestine and that of mother vagina is an evidence for this colonization in the infant during the birth [27]. Moreover, different microbial populations were observed from the infants delivered through cesarean section [28]. Microbiota of intestine during the first year of baby is very simple, but it can change from individual to individual and from time to time. However after the first year, gut microbiota of baby becomes stable and similar to that of a young person [27, 29]. Microbial community of individual GIT was affected by many other factors such as host physiology, genetics, and environmental factors [30, 31]. Increasingly, diet is recognized as a key environmental factor that mediates the composition and metabolic function of the gastrointestinal microbiota [32]. Indeed, consumption of specific dietary ingredients, such as fiber and prebiotics, is an avenue by which the microbiota can be modulated.

3. Dietary fibers and prebiotics

Dietary fibers are defined as [33]: carbohydrate polymers with ten or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: (i) edible carbohydrate polymers naturally occurring in foods as consumed, (ii) edible carbohydrate polymers which have been obtained from food raw materials by physical, enzymatic, or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence, and (iii) edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence. Prebiotics are also classified as dietary fibers. Prebiotics are recently defined as according to International Scientific Association of Probiotics and Prebiotics (ISAPP) [34]: "selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health."

Target genera of prebiotics are dominantly *Lactobacilli* and *Bifidobacteria*. When two genera were compared, changes were observed more often in *Bifidobacteria* since more *Bifidobacteria* usually reside in the human colon than *Lactobacilli*, and they exhibit a preference for oligosaccharides [35]. To classify an ingredient as a prebiotic, it should resist gastric acidity, be hydrolyzed by mammalian enzymes, absorbed in the upper GIT, be fermented by the GIT flora, selectively stimulate the growth/activity of GIT bacteria potentially associated with health and well-being. The known health benefit for prebiotics intake is rather limited than for dietary fibers. However, it has been put forward that prebiotic intake may ; reduce inflammation and inflammatory bowel disease symptoms, duration and frequency of antibiotic-associated diarrhea, lower the risk of cardiovascular diseases, protect against colon cancer, enhance absorption and bioavailability of calcium, magnesium, iron minerals and prevent obesity while supporting weight lost and satiety [35].

New technologies in molecular and computational studies show the effect of diet on composition and function of GIT microbiota. For instance, metagenomics provide the information for gastrointestinal microbiome which is 150 fold more than genome of the human [11]. Most fibers and prebiotics cannot be digested by the human enzymes, less than 20 glycosidases involved in digestion of dietary polysaccharides have been recognized in the human genome [36]. From this perspective, metabolization of dietary polysaccharides by the gastrointestinal bacteria is an example of the symbiotic relationship between the host and the microbiota. Furthermore, this relationship provides an opportunity for dietary modulation of the microbiota since microbial growth and metabolism depend on substrate availability, i.e., the type of dietary fiber or prebiotic consumed by the host.

4. Fructans as prebiotics

Fructans are non-structural carbohydrates that occur in bacteria, algae, flowering plants and mosses [37]. Fructans are fructose-based oligo- and polysaccharides containing maximal one glucose unit [38]. They are the most common and known group among prebiotics and can be divided into five main groups based on their chemical structure. (i) inulin

with β-(1-2)linked fructose residues, (ii) neo-inulin with two β-(1-2)-linked fructose chains attached to the sucrose starter unit, (iii) β-(2-6) linked levan, (iv) neo-levan with internal glucose residue and (v) graminans (mixed fructans) consist of β-(2-6)-linked fructose residues with β-(1-2) branches [37, 39].

Fructans provide improvement of blood parameters, resistance against intestinal and extra-intestinal pathogens, modulation of immune system and reduced allergies [40]. The intestinal benefits of fructans as well as their symbiotic association with probiotic bacteria, encompass prevention and treatment of infectious diseases, including viral or bacterial diarrhea, and chronic inflammatory diseases such as ulcerative colitis [41]. It is known that their prebiotic activity changes with structural features like; composition of monomers, type of glycosidic linkage, degree of polymerization (DP) and degree of branching [42].

Prebiotic fructans cannot be digested in stomach and small intestine on the other hand, might be partially hydrolyzed due to acidic environment [37]. When they reach colon, glycoside hydrolase (GH32) secreting bacteria degrade them and they serve as bifidogenic factor on beneficial probiotic bacteria and can be used as carbon and energy source [43] At the end of this metabolic activity, short chain fatty acids (SCFAs) like butyrate, propionate, acetate, lactate and hydrogen, methane, and carbon dioxide are produced. SCFAs are used as carbon and energy source for bifidogenic bacteria and play important role in the enhancement of host health through enhancing immune system, facilitating mineral uptake, neuronal and hormonal feedbacks [40, 43]. Butyrate is known to increase growth of *Lactobacillus* and *Bifidobacteria* [44]. Moreover, butyrate plays important role on epithelial cell proliferation and differentiation [37]. Acetate join circulation and goes to liver and it is used as energy source while propionate is absorbed and metabolized aerobically and play key role in many mechanisms for instance appetite regulation. [45, 46]. It is reported that FOS play a key role in production of butyrate and propionate in high amounts [47]. Long term effects of FOS consumption is studied by Le Blay et al. [48], and rats fed with diet containing FOS (9-10 g/day) during 27 weeks and they reported transitory increase in lactate and constant increase in butyrate.

4.1 Inulin

Inulin is a β-(1-2) linked fructose polymer generally with terminal glucose and its DP varies from 2 to 60 units [49, 50]. Inulin can be synthesized enzymatically from sucrose or extracted mainly from plants. Inulin type fructans are generally obtained from chicory (*Cichorium intybus L*) or artichoke (*Helianthus tuberosus*) but recently Agave cactus and oats are also considered as rich soruce of inulin fructans [51, 52]. It is reported that inulin based dietary fiber should contain at least 3-6 g /100 g inulin while 3-8 g of inulin per portion should be consumed for bifidogenic activity [47].

4.2 Fructooliosaccharides

Fructose oligomers with low DP are called as fructooligosaccharides (FOS). FOSs originate from sucrose molecules and main monomeric units are fructose [47, 53]. Inulin type FOS and levan type FOS are two different types of FOS [54]. Partially hydrolysis of FOSs generate oligofructoses like glucopyranosyl-(fructofuranosyl)n-1fructose (GFn) and fructopyranosyl-(fructofuranosyl)n-1 fructose (Fn) with different DP degrees (1-4) [55]. 1-kestose (GF2), nystose (GF3), fructofuranosyl nystose (GF4) are also called FOS because of fructose monomers present in their composition [56]. Oligofructans are synthesized by hydrolysis of long fructan chains or sucrose based enzymatic synthesis. Inulin type oligofructans can be obtained from hydrolysis of inulin by endo-inulinase (EC 3.2.1.7) [57] or synthesized from sucrose by *Aspergillus aculeatus* derived fructosyltransferase (EC 2.4.1.9) [58] , *Aspergillus niger* derived β-fructofuranosidase (EC 3.2.1.26) enzymes (58)**.** FOSs can be found commonly in chicory, Jerusalem artichoke, asparagus and onion family members [59]. Daily consumption amount for bifidogenic activity is reported as 2-10 grams [60]. FOS can be used for prevention of intestinal infections and intestinal infections; inhibition of pathogens, ordering intestinal flora; regulation of intestinal immune system; enhancement of immune response; stimulation of probiotic growth of *Lactobacilli* and *Bifidobacteria* species; optimization of colonic function and metabolism; production of short chain fatty acids; increase of mineral absorption; reduction of food intake and obesity management and control of diabetes type 2 and prevention of cancer [41, 54, 61-72].

4.3 Levan

Levan is β-(2-6) linked fructose polymer commonly found in nature. Water soluble, nontoxic and strongly adhesive polymer have many application fields such as pharmacy, food, chemistry, medical and cosmeceutical industries [73]. It is produced extracellularly from sucrose based substrates by various microorganisms including first extremophilic levan producer *Halomonas smyrnensis AAD6T* [74]. Several studies with levan as antioxidant, anticancer, anticoagulant, biocompatible microcarrier for peptide and protein drugs, adhesive multilayer films, bioactive surfaces, anti-irritant, prebiotic activities, antidiabetic and lipid metabolism regulator are reported by various researchers [74, 75]. Levan is a non-toxic soluble dietary fiber and hydrolysates of levan improve function of gut. Linkage type, length of polysaccharide and branching are significant physiological parameters that affect fermentation of levan in GIT. *In vitro* studies indicate enrichment of *Bifidobacterium adolescentis*, *B. pseudocatenulatum*, *B. breve*, *B. longum*, *Lactobacillus plantarum* and *Pedicoccus pentosaceus* with levan oligosaccharides [66, 76].

5. Fructans rich diet in health

5.1 Studies on prebiotic activity of fructans

Prebiotics are reported as more effective and simpler modulators on gut microbiota compared to probiotics because they stimulate the life cycle of the resident gut microbiota [37]. Fructans show their prebiotic activities through toll like receptors (TLR) and their fermentation products show their activity through AMPK and/or nuclear factor kappa B (NF-κB) signaling pathways [40]. Effect of inulin type fructan rich Jerusalem artichoke tuber on 72 wistar rats for 12 weeks is investigated by Samal et al. [77]. Increase of beneficial bacteria in colon and rectal digesta (*Lactobacillus* spp. and *Bifidobacterium* spp.), acetic acid, propionic aicd and total SCFA concentration and improved fiber digestibility observed. Chung et al [78] studied prebiotic effect of inulin and apple pectin *in- vitro*. Increased *Bacteroides*, *Eubacterium eligens* is observed for inulin. Prebiotic effect of Agave inulin (BIOAGAVE) (0, 5, 7.5 g/day) on 29 healthy humans for 3 weeks is observed by Holscher et al. [79]. Concentration dependent increase in *Bifidobacterium adolescentis*, *B. breve, B. longum, and B. pseudolongum* is observed while *Desulfovibrio* and *Clostridium sp*. decreased. Majid et al. [80] studied prebiotic activity of oligofructose and inulin containing fiber-prebiotic enriched solutions (7 g/day) (Nutrison Protein Plus Multifibre, Nutricia UK) on 22 diarrhea enteral nutrition fed patients for 7 days. No bifidogenic effect is observed but *Faecalibacterium prautsnizii* and *Bacteroides*/*Prevotella* decreased in fecal samples. *Bacillus subtilis* natto CCT 7712 FOS is investigated by Silva et al. [81] for its prebiotic activity. FOS was recorded as a good energy source. Highest growth on FOS was observed for *Lactobacillus plantarum* ATCC 14917 followed by *Lactobacillus casei* (LC-1). Study concluded that FOS and probiotics (*Lactobacillus casei* and *Lactobacillus plantarum* ATCC 14917) could be used as symbiotic in foods. Martine-Gutierrez et al. [82] investigated prebiotic effect of *Agave salmina* fructooligosachharides and inulin (ORAFTI®). Growth of *Lactobacillus acidophilus* is observed at the highest level in presence of *Agave salmina* FOS with lowest pH and highest lactate production. Porras-Dominguez [83] investigated prebiotic activity of levan-type oligofructans hydrolyzed by endo-levanase (EC 3.2.1.65) and high molecular weight levan and reported that levan-type oligofructans improve growth of Bifidobacteria more in comparison to levan. Commercially available branched levan (from *Erwinia herbicola* and *Lactobacillus sanfranciscensis* LTH 2590) were investigated for their prebiotic activities and *L. sanfranciscensis* levan showed bifidogenic activity [84, 85]. Prebiotic activity of inulin, *Agave tequilana* fructan and *Halomonas* levan were investigated by Arrizon et al [86]. Growth of *Lactobacillus* species was observed in all fructans. On the other hand, inulin and levan increased also growth of *Bifidobacteria*. Moreover, growth of pathogenic bacteria (*Salmonella typhimurium*, *Listeria monocytogenes* and *Clostridium spp.*) decreased by inulin and *Halomonas* levan while *Agave tequilana* fructan had no effect. Adamberg et al. [87] studied effects of levan on gut microbiota and enriched *Bacterioides*, *Escherichia*, *Streptococus* and *Faecalibacterium* is observed. Mardo et al. [88] investigated biochemical properties of endolevanase BT1760 on six bacterial levan synthesized by levan sucrase Lsc3 of *Pseudomonas syringae pv*. Tomato, mutant Asp300Asn mutant type, levan from *Zymomonas mobilis*, *Ervinia herbicola* and *Halomonas smyrnensis* levan and timothy grass isolated levan degradation into fructooligosaccharides. BT1760 degraded levans into FOS with DP 2 to 13. All levans were hydrolyzed successfully by BT1760 at body temperature (37°C) and pH between 5-6 and study concluded that plant or bacteria derived levan serve as a prebiotic for *B. thetaiotaomicron* and promote SCFA synthesis by gut microbiota. Enzymatically derived low molecular weight oligosaccharide β-(2-6) (FOS) from levan is investigated as a new carbon source for bifidobacterial growth (*Bifidobacterium adolescentis*, *B. longum*, *B. breve*, and *B. pseudocatenulatum*) by Marx et al [66] and formation of SCFAs were different between species and highest SCFA production is observed in *B. adolescentis*. Zhao et al [89] investigated effect of levan on 96 pigs for 6 weeks and concluded that levan decreased *E. coli* count and increased growth of *Lactobacillus.*

5.2 Immunity and gut barrier function

Barrier function of gut is important for health of host and generally maintained by epithelial barrier. Corruption of epithelial barrier or failure in barrier function may result with several diseases like pathogen infection, obesity, necrotizing enterocolitis, irritable bowel syndrome, inflammatory bowel disease and diabetes [90]. Prebiotics effect host immunity via interaction with immune system cells. Various surface receptors of immune system cells like T and B lymphocytes recognize several carbohydrate structures and some of them bind to those structures of fibers. Neutrophils, macrophages, and dendritic cells have several carbohydrate receptors like Ca^{+} dependent mannose receptor and langerin and Ca^{+} independent Dectin 1 and Dectin 2. Most of those receptors belong to C type lectin (CLR) family that have conserved residues for recognition of carbohydrates [47]. β-glucan groups of carbohydrates have ability to bind directly to macrophage, monocyte, neutrophil and dendritic cell receptors [37] Dectin-1 receptor binds β-glucan structures of fibers and stimulate immune response via cytokine secretion from dendritic cells and stimulates secretion of IL-2, IL6, IL-10, IL-12 and TNF-α [37, 91, 92]. Dectin-1 also play role in TLR related inflammatory signal production via inducing inflammatory gene expression through protein kinase and phosphatase signaling [47]. Endocytosis and phagocytosis is initiated by activation of Dectin 1 [37]. Prebiotic and antipathogenic activities of levan was studied by Yang et al [93, 94] and they reported that enterotoxigenic *E.coli* adhesion to the mucosa is decreased while growth of

beneficial bacterial populations was increased. Fooks and Gibson [95] searched effect of several prebiotics on probiotic and pathogen bacteria and indicated that FOS, inulin, XOS and their mixture inhibited pathogens (*E.coli*, *C. jejuni* and *S. enteridis*) greater than lactulose, lactitol, starch and dextran. It is reported that β-(2-1)-fructans improve intestinal epithelial cell barrier function through Toll-like-2 (TLR-2) receptors. TLR activity and cytokine production is related with chain length of β-(2-1)-fructans and short chain β-(2-1)-fructans are reported to induce regulatory cytokine secretion (IL-10 / IL-12 ratio) more compared to long chain β-(2-1)-fructans [96]. Vogt et al. [96] studied effect of different chain length β-(2-1)-fructans on T84 human intestinal epithelial cell line *in-vitro* and found that time and chain length dependent cell barrier protective effect is maintained on T84 cells via TLR-2 and this showed that β-(2-)1-fructans might target mainly TLR-2 receptor and less TLR-4, 5, 6, 7, and 8 on epithelial cells that result in NF-κB/AP-1 activation to improve host health on TLR-2 dependent mechanisms. Fransen et al. [97] investigated effect of short and long chain β-(2-1) fructans (Frutalose® OFP and Frutafit® TEX) on conventional and germ free mice for 5 days. .Long and short chain fructans increased T-helper cells, enhanced 2-alpha-l-fucosyltransferase 2 expression and IL- 22 dependent genes in conventional mice ileum. Long chain fructans affect B cell response in germ free mice while short chain fuctans increased T regulatory and dendritic cells. Enhanced immunity is observed in germ free mice thus study concluded that immunity is partially dependent to microbiota in a chain length independent manner. Li and Kim [98] investigated effect of levan-type fructan (0, 0.05, 0.1 and 0.2%) on growth, blood profile and fecal microbiota on 80 growing pigs for 42 days. *Lactobacillus* is increased in tract while weight gain is also improved. *E. coli* LPS injection induced blood lymphocyte, serum cortisol level, IL-6, TNF-α increase and levan-type fructan fed pigs showed decrease in these levels at the time dependent interval. Study concluded that 0.1 % levan type fructan supplementation can enhance growth, digestibility, fecal *Lactobacillus* count and immune response in inflammation. Effect of inulin and short chain FOS on gut epithelial barrier function was investigated by Wu et al. [99] *in- vitro*. They concluded that barrier function enhancement can be maintained by activating host epithelial cell signaling through protein kinase C (PKC) δ-dependent mechanism and tight junction induction. Effect of inulin type fructan (DP 10-60, DP 25) on immunity was investigated by Voght et al [100] *in- vitro* and *in- vivo* with 40 heathy human subjects vaccinated against hepatitis B. Development of Anti-HbsAg-titer and lymphocyte are investigated. Th-1 cell increase and TLR-2 stimulation is observed for inulin type fructans (DP 10-60) better than DP 2-25 in *in- vitro* studies. DP 10- 60 increased anti- HBsAg titer, Th-1 cell and transitional B cells. Both fructans increased cytokine production and NK- cells time dependent. Results concluded immunity against pathogenic hepB epitopes was only supported by long chain fructans. Thick mucus layer of gut epithelium with digestive enzymes, antimicrobial peptides and immunoglobulins protect host from pathogens [37]. It was reported that gel- like chemical barriers formed by higher amount of mucin inhibit translocation or colonization of pathogens such *Salmonella* spp., *Shigella* spp. *Vibrio cholerae* and *E.coli* [37]. Acetate protects organism from lethal infections through epithelial cell defense by binding G protein couples receptors on immune cells and regulate immune response [37]. Butyrate is known to induce expression of antimicrobial peptide LL37 that prevent bacterial infections while supplying energy for colon epithelium and increasing proliferation of those cells during injury and reduce colonic inflammation [37, 101, 102]. Rodriguez et al. [103] investigated symbiotic effect of FOS (Beneo®-95) and resistant starch (RS) (Fibersol®-2) on healthy and trinitrobenzenesulphonic acid-(TNBS) colitic rats and found that symbiotic consumption increased *Lactobacilli* and *Bifidobacteria* furthermore increase expression of MUC2 in colon and TNBS models anti-inflammatory activity in the colon.

6. Fructans rich diet in disease

6.1 Colorectal Cancer

Consumption of prebiotics like β-glucans, dietary fibers, fructans and resistant starch play important role in prevention of colorectal cancer through production of SCFA (acetate, butyrate, and propionate), modulation of gene expression in tumor cells, decreasing activity of cancer triggering bacteria, action as diluting agent to reduce interaction of mutagens and carcinogens with epithelial cells. Production of SCFAs decrease pH in the colon which result in increased mineral solubility like calcium, magnesium, and iron. By the increase of calcium absorption pathogen growth can be prevented. Increased calcium absorption also reduces the risk of osteoporosis via bone calcium depletion [37]. Butyrate induces cell apoptosis, differentiation and reduces proliferation of malignant cells through initiation of cell cycle arrests at phase G_1. It also stimulates protein synthesis like alkaline phosphatase, hormone receptors and glycoproteins and act on colonocytes to reduce colorectal cancer [37, 45]. Bolognani et al. [104] investigated effects of oligosaccharides and lactic acid producing bacteria on early neoplasia precursor aberrant crypt foci (ACF) induction by carcinogens on ACF induced rats. *L. acidophilus* or inulin fed rats showed significant ACF decrease and protection from colorectal cancer. Taper and Roberfroid [105] investigated role of inulin and oligofructose (15%) on transplantable mouse tumor and showed that tumor growth is inhibited by supplementation of inulin and oligofructose. Synergy 1® (chicory inulin) and Metlin® (Mexican agave inulin) are tested against colon cancer and bone calcium metabolism in mice and rats with two different models by Riviera- Huerta et al. [106]. Results indicated that inulin inhibited dextran sulfate sodium induced colitis and colon cancer development in mice through reducing concentration of TNF-α (tumor necrosis factor alpha).

Formation of polyps and villous atrophy and lymphoid hyperplasia are prevented. Studies with rats showed increase of bone densitometry in femur and vertebra.

6.2 Irritable Bowel Syndrome and Inflammatory Bowel Disease

Irritable Bowel Syndrome (IBS) is a gastrointestinal disorder which results in abdominal pain, discomfort and change in stool characteristics and frequency. IBS is related with gut barrier function [90]. Butyrate is known to have positive effect on reducing symptoms of IBS [107] and improving gut barrier function via epithelial proliferation. Symbiotic approach to reduce symptoms and improve health of IBS patients via increasing production of lactic acid and butyrate is reported by various studies [90]. Beneficial effect of inulin-type fructans and FOS on IBS disease is reported [108]. Inflammatory Bowel Disease (IBD) is the general name of two inflammatory gut related diseases; Chron's disease (CD) and Ulcerative colitis (UC) and mucosal inflammation and impaired barrier function is observed in IBD patients [109] CD is a transmural mononuclear inflammation and generally effect colon and terminal ileum while UC only effect colon. Loss of goblet cells, epithelial cell damage and neutrophil infiltration occurs in UC [90]. Gut microbiota, barrier function of gut and mucus secretion play important roles in reducing symptoms of IBD thus prebiotic support to improve gut microbiota may be beneficial in reducing IBD symptoms while improving quality of life in those patients. *Lactobacillus* GG is reported beneficial in Children Chron's disease by improving gut barrier function [110]. Paineau et al [111] investigated effect of short chain fructooligosaccharides (sc-FOS) on patients with functional bowel disorders and improved daily activity, and life quality is reported thus, consumption of FOS may improve digestive comfort due to their prebiotic activities. Ulisse et al [112] investigated effect of probiotic mixture VSL#3 on biopsy specimens of pouchitis patients and cytokine secretion (IL-10) was increased. Cox et al investigated [113] effect of fermentable carbohydrates on functional gastrointestinal symptoms (FGS) in IBD. Patients with IBD and FGS get fructan (12g/day), Galactooligosachharide (GOS) (6 g/day), Sorbitol (6 g/day) and glucose placebo (12g/day). Fewer patients reported relief of FGS in fructan diet while GOS and sorbitol exacerbated symptoms of FGS in IBD.

6.3 Metabolic Syndrome, Diabetes, Obesity, and Cardiovascular Diseases

Diabetes, obesity, cardiovascular diseases, and metabolic syndrome are closely related and recently increasing disorders and one can trigger another. Side effects of drugs and increased popularity on prebiotic consumption as a treatment for such diseases made researchers investigate effects of prebiotics in disease and their interaction with host and microbiota. Glucose intolerance, insulin resistance, hyperinsulinemia, hypertension, dyslipidemia and impaired fasting glycemia together with obesity refers to metabolic syndrome [114]. It is reported that metabolic syndrome have risk for development of type 2 diabetes, atherosclerosis and hypertension [115]. Development of metabolic syndrome could be reduced by consumption of prebiotics (oligosaccharides) to improve gut microbiota, gut barrier function and reducing oxidative stress [114]. Diabetes mellitus (DM) is an insulin metabolism related disease where body cannot use or produce insulin effectively (type 1 and type 2) [116]. Diabetes may result in dementia, kidney and cardiovascular diseases, increased mortality and morbidity , hypertension and steatosis hepatis and cancer [117].

Risk of heart diseases can be increased with plasma LDL cholesterol levels and dietary content can reduce plasma cholesterol levels via production of SCFAs. Especially propionate inhibits cholesterol synthesizing enzymes, arrange distribution of cholesterol from plasma to liver, improve secretion of bile acids [37, 118]. Lowering blood lipid and cholesterol levels, reduction of obesity and diabetes and improving absorption of minerals could reduce the risk of hypertension and hearth diseases [108].

Obesity is an impaired lipid metabolism related disease and increase in serum triglycerides and cholesterol is observed [119]. It is known hat obesity can trigger hypertension [120], myocardial infarct [121] and diabetes [122]. Gut microbiota play crucial role on obesity and related metabolic disorders. Nutrition is important for establishment of this microbiota. Indirect effect of colonic fermentation is on the pancreas and adipose tissue hormones which have role energy metabolism thus, colonic fermentation, microbiota and prebiotics are associated with obesity [45, 123].

Effect of inulin type fructans (oligofructose and inulin) on metabolic syndrome is investigated by Rault-Nania et al [124] on fructose fed metabolic syndrome rat model for 4 weeks. Inulin type fructan supplemented diet prevented induction of high blood pressure, heart peroxidation susceptibility and renal damages and hypertriglyceridemia. Increased levels of oxidative stress and lowered AMPK activity is also observed which promote metabolic syndrome. Parnell et al [125] investigated effects of oligofructose supplementation on 37 obese subjects (BMI 30.4 kg/m^2) for 12 weeks. Study concluded that obesity related inflammation markers might be mitigated by oligofructose supplementation via reducing PAI-1, risk factor for thrombosis, and metabolic endotoxemia through reducing plasma lipopolysaccharide levels. Effect of oligofructose enriched inulin (8 g/day) on body composition, markers of inflammation, fecal bile acid levels and microbiota composition is investigated by Nicoloucci et al.[126] on obese children for 16 weeks. Decreased body weight, percent body fat, percent trunk fat, reduction in IL-6, serum triglycerides is observed. *Bifidobacterium* and *Bacteroides vulgatus* increased in fecal samples and bile acid concentration if fecal samples only in placebo group. Dalzenne et al [127] investigated metabolism on FOS (20% of body weight) and inulin (10% body weight) fed rats for 30 days. Decreased serum and liver triglyceride level is observed in FOS while no change on cholesterol levels. Only the ratio of HDL and LDL cholesterol is increased. Seric and hepatic lipid modifications observed both groups. Kok et

al. [128] investigated effect of fructooligosaccharides (Raftilose ®) (100g/kg) on rats for 30 days and found that FOSs reduced de-novo fatty acid synthesis in liver and lowered serum insulin levels. Propionate inhibits de-novo synthesis of fatty acids, hepatic gluconeogenesis in hepatocytes, suppress plasma triacylglycerol, control blood glucose level that play role in insulin resistance, and show anti-inflammatory effects [45, 129]. Patent application of Haber et al [130] claimed role of levan in treatment for hyperlipidemia and hypercholesterolemia and as an arteriosclerosis reducing agent. Yamamoto et al [131] studied 1-5% high molecular levan containing cholesterol free diet fed rats for 1 month and results showed increased fecal sterol and lipid excretion which may conclude sterol absorption preventing activity of levan while decreased serum cholesterol level is also observed. Regulation of appetite through hormones and diet play important role in body weight reduction, blood glucose level regulation and obesity. Appetite can be modulated with prebiotic supplementation by increasing plasma gut peptide concentrations and SCFAs in gut like acetate, butyrate, and propionate. Propionate is known to reduce body weight through stimulation of leptin. SCFAs can suppress appetite through hormonal stimulation like Glucagon like peptide- 1 (GLP-1) and Peptide YY in gut lumen secreted by L colonocytes [46]. Appetite regulation of acetate through parasympathetic nervous system and stimulated insulin, leptin and ghrelin secretion is reported by Perry et al [132]. A study about appetite, inulin and FOS relationship is reported by Cani et al [133]. 10 healthy adults received 16 grams of prebiotics for 2 weeks and results showed that lowered hunger and appetite is observed. Plasma GLP-1level increased while postprandial plasma glucose response is decreased. Cani et al [134] investigated effect of oligofructose (Orafti, Belgium) on obese mice and results showed that prebiotic modulation increased *bifidobacteria*, and increased gut barrier function via GLP-2. Effect of oligofructose (Orafti, Belgium) on high fat diet fed mice is also investigated by Cani et al [135]. It is concluded that oligofructose uptake reduces the development of diabetes and obesity via glucagon like 1 receptor. GLP-1 receptor play important role in decreasing blood glucose level and steatosis in obesity and diabetes [136]. Kang et al [137] studied effect of *Zymomonas* levan on obese and hyperlipidemia suppressed lab rats. Reduced daily weight gain even on high fat diet fed rats is observed. Hyperinsulinemia, hyperglycemia, levels of free fatty acids serum triglycerides, serum cholesterol, unilocular fat tissue improvement and adipocyte hypertrophy is reduced in dose depending manner. Parnel et al. [138] investigated effect of fructooligosaccharides (21g/day) for 22 weeks in obese adults and enhanced weight loss and improved blood glucose level is observed. Oh and Lee et al [139] searched anti-obesity activity of fermented red ginseng, levan and their combination for 11 weeks on high fat diet fed mice. Combination of levan and red fermented ginseng consuming group showed decreased body weight gain, white adipose tissue weight, insulin resistance, leptin, and fasting blood glucose level. Energy intake and appetite control in oligofructose enriched inulin supplementation (8 g/day) is investigated by Hume et al [140] in 42 overweight and obese children (7-12 y) for 16 weeks. Feeling of fullness, fasting adiponectin and ghrelin increased in prebiotic consumption while prospective food consumption at breakfast buffet, energy intake and BMI is decreased.

Kazak et al. [141] investigated antidiabetic activity of *Halomonas* levan on pancreatic INS-1E pancreatic cell line *in-vitro*. Decreased reactive oxygen species (ROS) generation and apoptosis is observed. *Bacillus* levan containing diet decreased plasma glucose and serum lipid levels and increased glycogen level on diabetic rats [142]. Gao et al [143] studied effect of butyric acid (5% wt/wt) on dietary-obese high fat diet fed mice and found that prevention of insulin resistance formation is prevented by butyrate while fatty acid oxidation and thermogenesis, mitochondrial function and biogenesis are enhanced and adiposity is reduced. Another study about HDL cholesterol increasing and LDL cholesterol lowering effect of *Bacillus* levan on cholesterol rich diet fed diabetic rats for 2 months is reported by Belghith et al [144]. Gobinath et al [145] investigated antidiabetic effect of xylooligosachharides and fructooligosaccharides (10% w/w) on diabetes induced rats for 6 weeks. Hyperglycemia and cholesterol levels are reduced and body weight is improved and *Bifidobacteria* and *Lactobacillus* is increased in both samples. Activity of antioxidant enzymes (catalase and glutathione reductase) increased in the blood of diabetic rats. Glycation end products, glycosuria, proteinuria, diabetic neuropathy and concentration of blood creatinine and urea reduced. Chen et al. [146] investigated effect of inulin type fructans; (DP 2-25 and DP 10-60) on gut barrier function related type 1 diabetes (TD1) in nonobese diabetic rats. Long chain fructans reduced the incidence of TD1and increased $CD25^+$, $FoxP3^+$, $CD4^+$ and decreased IL-17A, $CD4^+$, Th 17 cells, cytokine production in pancreas, spleen and colon is observed. Fructans also enhanced tight junction proteins ocludin, claudin-2, antimicrobial peptides defensin-1 and cathelicidin related peptide production. Result concluded that long chain fructans delay development of diabetes via modulation of gut and pancreatic immunity, barrier function and microbiota. Oligofurctose containing a yogurt drink (Orafti® P95) and inulin containing fruit jelly (Orafti® GR) is investigated on 40 -42 healthy adults by Lightowler et al. [147] and Inulin consumption lowered glycemic response in higher amount than Oligofructose consumption. In both studies insulin response is lowered. Study concluded that substitution of glycemic sugar by inulin or oligofructose reduce blood glucose response to foods.

7. Conclusions and future perspectives

The complex and diverse gut microbial communities play crucial roles in human health due to their metabolic, immunologic, and protective properties in a number of diseases. To improve bacterial community and their functions,

dietary approach play significant role. The most important dietary strategy is to modulate metabolic function of microbiota via consumption of dietary fibers and prebiotics [148]. Carbohydrate polymers are called dietary fibers or prebiotics if they cannot be digested by human alimentary enzymes and absorbed in colon. Therefore these carbohydrates are selectively fermented in colon by gut microbiota and generate short chain fatty acids (SCFAs) like butyrate, acetate and propionate [35, 149]. Among prebiotics most known group is fructans that are found commonly in nature. Recent studies put forth that use of fructans as prebiotic is beneficial to human health and may counteract the development of various diseases such as metabolic syndrome, diabetes, obesity, cardiovascular diseases, colorectal cancer, irritable bowel syndrome and inflammatory bowel disease. Although fructans are valuable functional food ingredients, they requires further research. In further studies, derivatives of fructans and their combinations both with each other and with natural fructans can be investigated for their healing effects in various diseases.

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References

[1] Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006;124(4):837-48.
[2] Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human–microbe mutualism and disease. Nature. 2007;449(7164):811-8.
[3] Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proceedings of the National Academy of Sciences. 1998;95(12):6578-83.
[4] Haynes M, Rohwer F. The human virome. Metagenomics of the human body: Springer; 2011. p. 63-77.
[5] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett C, Knight R, Gordon JI. The human microbiome project: exploring the microbial part of ourselves in a changing world. Nature. 2007;449(7164):804.
[6] Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutrition reviews. 2012;70(suppl 1):S38-S44.
[7] Shendure J, Ji H. Next-generation DNA sequencing. Nature biotechnology. 2008;26(10):1135-45.
[8] Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010;7(5):335-6.
[9] Hamady M, Walker JJ, Harris JK, Gold NJ, Knight R. Error-correcting barcoded primers allow hundreds of samples to be pyrosequenced in multiplex. Nature methods. 2008;5(3):235.
[10] O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO reports. 2006;7(7):688-93.
[11] Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. science. 2006;312(5778):1355-9.
[12] Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK. Host–bacterial symbiosis in health and disease. Advances in immunology. 2010;107:243.
[13] Hull MW, Chow AW. Indigenous microflora and innate immunity of the head and neck. Infectious disease clinics of North America. 2007;21(2):265-82.
[14] Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology. 2009;136(1):65-80.
[15] Verstraelen H. Cutting edge: the vaginal microflora and bacterial vaginosis. Verhandelingen-Koninklijke Academie voor Geneeskunde van Belgie. 2007;70(3):147-74.
[16] Gebbers J, Laissue J. Immunologic structures and functions of the gut. Schweizer Archiv fur Tierheilkunde. 1988;131(5):221-38.
[17] Gordon JH, Dubos R. The anaerobic bacterial flora of the mouse cecum. Journal of Experimental Medicine. 1970;132(2):251-60.
[18] Harris MA, Reddy CA, Carter GR. Anaerobic bacteria from the large intestine of mice. Applied and environmental microbiology. 1976;31(6):907-12.
[19] SAVAGE DC. Associations of indigenous microorganisms with gastrointestinal mucosal epithelia. The American journal of clinical nutrition. 1970;23(11):1495-501.
[20] Mirzaei MK, Maurice CF. Menage a trois in the human gut: interactions between host, bacteria and phages. Nature Reviews Microbiology. 2017;15(7):397-408.
[21] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. science. 2005;308(5728):1635-8.
[22] Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proceedings of the National Academy of Sciences. 2007;104(34):13780-5.
[23] Swidsinski A, Loening-Baucke V, Lochs H, Hale LP. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. World journal of gastroenterology: WJG. 2005;11(8):1131.
[24] Guarner F, Malagelada J-R. Gut flora in health and disease. The Lancet. 2003;361(9356):512-9.
[25] Fu J, Bonder MJ, Cenit MC, Tigchelaar E, Maatman A, Dekens JA, et al. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. Circulation research. 2015:CIRCRESAHA. 115.306807.
[26] Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Review of infectious Diseases. 1990;12(5):856-72.
[27] Mändar R, Mikelsaar M. Transmission of mother's microflora to the newborn at birth. Neonatology. 1996;69(1):30-5.
[28] Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of delivery–effects on gut microbiota and humoral immunity. Neonatology. 2008;93(4):236-40.

[29] Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. The American journal of clinical nutrition. 1999;69(5):1035s-45s.
[30] Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. The ISME journal. 2010;4(2):232-41.
[31] Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell host & microbe. 2008;3(4):213-23.
[32] Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. Nature. 2016;535(7610):56-64.
[33] Meeting JFWECoFA, Organization WH. Evaluation of Certain Food Additives: Seventy-first Report of the Joint FAO/WHO Expert Committee on Food Additives: World Health Organization; 2010.
[34] Blatchford P, Ansell J, de Godoy M, Fahey G, Garcia-Mazcorro J, Gibson G, et al. Prebiotic mechanisms, functions and applications-A review. International Journal of Probiotics & Prebiotics. 2013;8(4):109.
[35] Slavin J. Fiber and prebiotics: mechanisms and health benefits. Nutrients. 2013;5(4):1417-35.
[36] Cantarel BL, Lombard V, Henrissat B. Complex carbohydrate utilization by the healthy human microbiome. PloS one. 2012;7(6):e28742.
[37] Di Bartolomeo F, Startek J, Van den Ende W. Prebiotics to fight diseases: reality or fiction? Phytotherapy Research. 2013;27(10):1457-73.
[38] Di Bartolomeo F, Van den Ende W. Fructose and fructans: opposite effects on health? Plant foods for human nutrition. 2015;70(3):227-37.
[39] Van den Ende W, El-Esawe SK. Sucrose signaling pathways leading to fructan and anthocyanin accumulation: A dual function in abiotic and biotic stress responses? Environmental and Experimental Botany. 2014;108:4-13.
[40] Peshev D, Van den Ende W. Fructans: prebiotics and immunomodulators. Journal of Functional Foods. 2014;8:348-57.
[41] Saad N, Delattre C, Urdaci M, Schmitter J-M, Bressollier P. An overview of the last advances in probiotic and prebiotic field. LWT-Food Science and Technology. 2013;50(1):1-16.
[42] Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. Journal of molecular biology. 2014;426(23):3838-50.
[43] Sonnenburg ED, Zheng H, Joglekar P, Higginbottom SK, Firbank SJ, Bolam DN, et al. Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. Cell. 2010;141(7):1241-52.
[44] Roy CC, Kien CL, Bouthillier L, Levy E. Short-chain fatty acids: ready for prime time? Nutrition in clinical practice. 2006;21(4):351-66.
[45] Lovegrove A, Edwards C, De Noni I, Patel H, El S, Grassby T, et al. Role of polysaccharides in food, digestion, and health. Critical reviews in food science and nutrition. 2017;57(2):237-53.
[46] Kumari M, Kozyrskyj A. Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. Obesity Reviews. 2017;18(1):18-31.
[47] Venema K, Do Carmo AP. Probiotics and prebiotics: Caister Academic Press; 2015.
[48] Le Blay G, Michel C, Blottière HM, Cherbut C. Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in cecal butyrate in rats. The Journal of nutrition. 1999;129(12):2231-5.
[49] Roberfroid M, Slavin J. Nondigestible oligosaccharides. Critical Reviews in Food Science and Nutrition. 2000;40(6):461-80.
[50] Roberfroid M. Prebiotics: the concept revisited. The Journal of nutrition. 2007;137(3):830S-7S.
[51] Holscher HD, Doligale JL, Bauer LL, Gourineni V, Pelkman CL, Fahey GC, et al. Gastrointestinal tolerance and utilization of agave inulin by healthy adults. Food & function. 2014;5(6):1142-9.
[52] Paseephol T, Sherkat F. Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. Journal of Functional Foods. 2009;1(3):311-8.
[53] THAMER KG, PENNA AL. Efeito do teor de soro, açúcar e de frutooligossacarídeos sobre a população de bactérias lácticas probióticas em bebidas fermentadas. Revista Brasileira de Ciências Farmacêuticas, São Paulo. 2005;41(3).
[54] van den Heuvel EG, Muijs T, Brouns F, Hendriks HF. Short-chain fructo-oligosaccharides improve magnesium absorption in adolescent girls with a low calcium intake. Nutrition research. 2009;29(4):229-37.
[55] Roberfroid MB. Introducing inulin-type fructans. British Journal of Nutrition. 2005;93(S1):S13-S25.
[56] Yun JW. Fructooligosaccharides—occurrence, preparation, and application. Enzyme and microbial technology. 1996;19(2):107-17.
[57] Singh RS, Singh RP. Production of fructooligosaccharides from inulin by endoinulinases and their prebiotic potential. Food Technology and Biotechnology. 2010;48(4):435.
[58] Ghazi I, Fernandez-Arrojo L, Garcia-Arellano H, Ferrer M, Ballesteros A, Plou FJ. Purification and kinetic characterization of a fructosyltransferase from Aspergillus aculeatus. Journal of Biotechnology. 2007;128(1):204-11.
[59] Wack M, Blaschek W. Determination of the structure and degree of polymerisation of fructans from Echinacea purpurea roots. Carbohydrate research. 2006;341(9):1147-53.
[60] Rivero-Urgell M, Santamaria-Orleans A. Oligosaccharides: application in infant food. Early Human Development. 2001;65:S43-S52.
[61] Sabater-Molina M, Larqué E, Torrella F, Zamora S. Dietary fructooligosaccharides and potential benefits on health. Journal of physiology and biochemistry. 2009;65(3):315-28.
[62] Vega R, Zuniga-Hansen M. The effect of processing conditions on the stability of fructooligosaccharides in acidic food products. Food chemistry. 2015;173:784-9.
[63] Lorenzoni AS, Aydos LF, Klein MP, Rodrigues RC, Hertz PF. Fructooligosaccharides synthesis by highly stable immobilized β-fructofuranosidase from Aspergillus aculeatus. Carbohydrate polymers. 2014;103:193-7.
[64] Charalampopoulos D, Rastall RA. Prebiotics in foods. Current opinion in biotechnology. 2012;23(2):187-91.
[65] Selak M, Rivière A, Moens F, Van den Abbeele P, Geirnaert A, Rogelj I, et al. Inulin-type fructan fermentation by bifidobacteria depends on the strain rather than the species and region in the human intestine. Applied microbiology and biotechnology. 2016;100(9):4097-107.

[66] Marx SP, Winkler S, Hartmeier W. Metabolization of β-(2, 6)-linked fructose-oligosaccharides by different bifidobacteria. FEMS Microbiology Letters. 2000;182(1):163-9.
[67] Pinelo M, Jonsson G, Meyer AS. Membrane technology for purification of enzymatically produced oligosaccharides: molecular and operational features affecting performance. Separation and Purification Technology. 2009;70(1):1-11.
[68] Jakob F, Pfaff A, Novoa-Carballal R, Rübsam H, Becker T, Vogel RF. Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function. Carbohydrate polymers. 2013;92(2):1234-42.
[69] Yen C-H, Kuo Y-W, Tseng Y-H, Lee M-C, Chen H-L. Beneficial effects of fructo-oligosaccharides supplementation on fecal bifidobacteria and index of peroxidation status in constipated nursing-home residents—A placebo-controlled, diet-controlled trial. Nutrition. 2011;27(3):323-8.
[70] Jakobsdottir G, Nyman M, Fåk F. Designing future prebiotic fiber to target metabolic syndrome. Nutrition. 2014;30(5):497-502.
[71] Delgado GTC, Tamashiro WM, Pastore GM. Immunomodulatory effects of fructans. Food Research International. 2010;43(5):1231-6.
[72] Licht TR, Ebersbach T, Frøkiær H. Prebiotics for prevention of gut infections. Trends in food science & technology. 2012;23(2):70-82.
[73] Kang SA, Jang K-H, Seo J-W, Kim KH, Kim YH, Rairakhwada D, et al. Levan: applications and perspectives. Microbial production of biopolymers and polymer precursors Caister Academic Press, Norwich. 2009:145-61.
[74] Öner ET, Hernández L, Combie J. Review of levan polysaccharide: From a century of past experiences to future prospects. Biotechnology advances. 2016;34(5):827-44.
[75] Erginer M, Akcay A, Coskunkan B, Morova T, Rende D, Bucak S, et al. Sulfated levan from Halomonas smyrnensis as a bioactive, heparin-mimetic glycan for cardiac tissue engineering applications. Carbohydrate polymers. 2016;149:289-96.
[76] Kang T, Jung S, Kang S, Jang K, Jang E, Kim S, et al. Preparation of levan oligosaccharides by acid hydrolysis and its application in growth of lactic acid-producing bacteria. Korean J Biotechnol Bioeng. 2002;17:137-41.
[77] Samal L, Chaturvedi V, Pattanaik A. Effects of dietary supplementation with Jerusalem artichoke (Helianthus tuberosus L.) tubers on growth performance, nutrient digestibility as well as activity and composition of large intestinal microbiota in rats. J Anim Feed Sci. 2017;26(1):50-8.
[78] Chung WSF, Walker AW, Louis P, Parkhill J, Vermeiren J, Bosscher D, et al. Modulation of the human gut microbiota by dietary fibres occurs at the species level. BMC biology. 2016;14(1):3.
[79] Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GC, Swanson KS. Agave inulin supplementation affects the fecal microbiota of healthy adults participating in a randomized, double-blind, placebo-controlled, crossover trial. The Journal of nutrition. 2015;145(9):2025-32.
[80] Majid HA, Cole J, Emery PW, Whelan K. Additional oligofructose/inulin does not increase faecal bifidobacteria in critically ill patients receiving enteral nutrition: a randomised controlled trial. Clinical Nutrition. 2014;33(6):966-72.
[81] Silva P, Garcia S, Baldo C, Celligoi M. Prebiotic activity of fructooligosaccharides produced by Bacillus subtilis natto CCT 7712. Acta Alimentaria. 2017;46(2):145-51.
[82] Martinez-Gutierrez F, Ratering S, Juárez-Flores B, Godinez-Hernandez C, Geissler-Plaum R, Prell F, et al. Potential use of Agave salmiana as a prebiotic that stimulates the growth of probiotic bacteria. LWT-Food Science and Technology. 2017.
[83] Porras-Domínguez JR, Ávila-Fernández Á, Rodríguez-Alegría ME, Miranda-Molina A, Escalante A, González-Cervantes R, et al. Levan-type FOS production using a Bacillus licheniformis endolevanase. Process Biochemistry. 2014;49(5):783-90.
[84] Korakli M, Gänzle M, Vogel R. Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by Lactobacillus sanfranciscensis. Journal of applied microbiology. 2002;92(5):958-65.
[85] Dal Bello F, Walter J, Hertel C, Hammes WP. In vitro study of prebiotic properties of levan-type exopolysaccharides from lactobacilli and non-digestible carbohydrates using denaturing gradient gel electrophoresis. Systematic and Applied Microbiology. 2001;24(2):232-7.
[86] Arrizon J, Hernández-Moedano A, Oner ET, González-Avila M. In vitro prebiotic activity of fructans with different fructosyl linkage for symbiotics elaboration. International Journal of Probiotics & Prebiotics. 2014;9(3):69.
[87] Adamberg K, Tomson K, Talve T, Pudova K, Puurand M, Visnapuu T, et al. Levan enhances associated growth of Bacteroides, Escherichia, Streptococcus and Faecalibacterium in fecal microbiota. PloS one. 2015;10(12):e0144042.
[88] Mardo K, Visnapuu T, Vija H, Aasamets A, Viigand K, Alamäe T. A Highly Active Endo-Levanase BT1760 of a Dominant Mammalian Gut Commensal Bacteroides thetaiotaomicron Cleaves Not Only Various Bacterial Levans, but Also Levan of Timothy Grass. PloS one. 2017;12(1):e0169989.
[89] Zhao P, Wang J, Kim I. Evaluation of dietary fructan supplementation on growth performance, nutrient digestibility, meat quality, fecal microbial flora, and fecal noxious gas emission in finishing pigs. Journal of animal science. 2013;91(11):5280-6.
[90] Bron PA, Kleerebezem M, Brummer R-J, Cani PD, Mercenier A, MacDonald TT, et al. Can probiotics modulate human disease by impacting intestinal barrier function? British Journal of Nutrition. 2017;117(1):93-107.
[91] Wismar R, Brix S, Frøkiær H, Laerke HN. Dietary fibers as immunoregulatory compounds in health and disease. Annals of the New York Academy of Sciences. 2010;1190(1):70-85.
[92] Yamasaki S, Matsumoto M, Takeuchi O, Matsuzawa T, Ishikawa E, Sakuma M, et al. C-type lectin Mincle is an activating receptor for pathogenic fungus, Malassezia. Proceedings of the National Academy of Sciences. 2009;106(6):1897-902.
[93] Yang Y, Galle S, Le MHA, Zijlstra RT, Gänzle MG. Feed fermentation with reuteran-and levan-producing Lactobacillus reuteri reduces colonization of weanling pigs by enterotoxigenic Escherichia coli. Applied and environmental microbiology. 2015;81(17):5743-52.
[94] Yang Y, Zhao X, Le MH, Zijlstra RT, Gänzle MG. Reutericyclin producing Lactobacillus reuteri modulates development of fecal microbiota in weanling pigs. Frontiers in microbiology. 2015;6.
[95] Fooks LJ, Gibson GR. In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. FEMS Microbiology Ecology. 2002;39(1):67-75.

[96] Vogt L, Ramasamy U, Meyer D, Pullens G, Venema K, Faas MM, et al. Immune modulation by different types of β2→ 1-fructans is toll-like receptor dependent. PLoS One. 2013;8(7):e68367.
[97] Fransen F, Sahasrabudhe NM, Elderman M, Bosveld M, Aidy SE, Hugenholtz F, et al. β2→ 1-Fructans Modulate the Immune System In Vivo in a Microbiota-Dependent andn-Independent Fashion. 2017.
[98] Li J, Kim I. Effects of levan-type fructan supplementation on growth performance, digestibility, blood profile, fecal microbiota, and immune responses after lipopolysaccharide challenge in growing pigs. Journal of animal science. 2013;91(11):5336-43.
[99] Wu RY, Abdullah M, Määttänen P, Pilar AVC, Scruten E, Johnson-Henry KC, et al. Protein kinase C δ signaling is required for dietary prebiotic-induced strengthening of intestinal epithelial barrier function. Scientific reports. 2017;7:40820.
[100] Vogt LM, Elderman ME, Borghuis T, Haan BJ, Faas MM, Vos P. Chain length-dependent effects of inulin-type fructan dietary fiber on human systemic immune responses against hepatitis-B. Molecular Nutrition & Food Research. 2017.
[101] Ashida H, Ogawa M, Kim M, Mimuro H, Sasakawa C. Bacteria and host interactions in the gut epithelial barrier. Nature chemical biology. 2012;8(1):36-45.
[102] Guilloteau P, Martin L, Eeckhaut V, Ducatelle R, Zabielski R, Van Immerseel F. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutrition research reviews. 2010;23(02):366-84.
[103] Rodríguez-Cabezas ME, Camuesco D, Arribas B, Garrido-Mesa N, Comalada M, Bailón E, et al. The combination of fructooligosaccharides and resistant starch shows prebiotic additive effects in rats. Clinical nutrition. 2010;29(6):832-9.
[104] Bolognani F, Rumney CJ, Pool-Zobel BL, Rowland IR. Effect of lactobacilli, bifidobacteria and inulin on the formation of aberrant crypt foci in rats. European journal of nutrition. 2001;40(6):293-300.
[105] Taper H, Lemort C, Roberfroid M. Inhibition effect of dietary inulin and oligofructose on the growth of transplantable mouse tumor. Anticancer research. 1997;18(6A):4123-6.
[106] Rivera-Huerta M, Lizárraga-Grimes VL, Castro-Torres IG, Tinoco-Méndez M, Macías-Rosales L, Sánchez-Bartéz F, et al. Functional Effects of Prebiotic Fructans in Colon Cancer and Calcium Metabolism in Animal Models. BioMed Research International. 2017;2017.
[107] Vanhoutvin S, Troost F, Kilkens T, Lindsey P, Hamer H, Jonkers D, et al. The effects of butyrate enemas on visceral perception in healthy volunteers. Neurogastroenterology & Motility. 2009;21(9):952-e76.
[108] Yeo S-K, Ooi L-G, Lim T-J, Liong M-T. Antihypertensive properties of plant-based prebiotics. International journal of molecular sciences. 2009;10(8):3517-30.
[109] Pastorelli L, De Salvo C, Mercado JR, Vecchi M, Pizarro TT. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Frontiers in immunology. 2013;4:280.
[110] Gupta P, Andrew H, Kirschner BS, Guandalini S. Is Lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. Journal of pediatric gastroenterology and nutrition. 2000;31(4):453-7.
[111] Paineau D, Payen F, Panserieu S, Coulombier G, Sobaszek A, Lartigau I, et al. The effects of regular consumption of short-chain fructo-oligosaccharides on digestive comfort of subjects with minor functional bowel disorders. British Journal of Nutrition. 2008;99(02):311-8.
[112] Ulisse S, Gionchetti P, D'Alò S, Russo FP, Pesce I, Ricci G, et al. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. The American journal of gastroenterology. 2001;96(9):2691-9.
[113] Cox SR, Prince AC, Myers CE, Irving PM, Lindsay JO, Lomer MC, et al. Fermentable carbohydrates (FODMAPs) exacerbate functional gastrointestinal symptoms in patients with inflammatory bowel disease: a randomised, double-blind, placebo-controlled, cross-over, re-challenge trial. Journal of Crohn's and Colitis. 2017:jjx073.
[114] Van den Ende W, Peshev D, De Gara L. Disease prevention by natural antioxidants and prebiotics acting as ROS scavengers in the gastrointestinal tract. Trends in Food Science & Technology. 2011;22(12):689-97.
[115] Agirbasli M, Tanrikulu AM, Berenson GS. Metabolic syndrome: Bridging the gap from childhood to adulthood. Cardiovascular therapeutics. 2016;34(1):30-6.
[116] Wu J, Shi S, Wang H, Wang S. Mechanisms underlying the effect of polysaccharides in the treatment of type 2 diabetes: A review. Carbohydrate polymers. 2016;144:474-94.
[117] Dina C. New insights into the genetics of body weight. Current Opinion in Clinical Nutrition & Metabolic Care. 2008;11(4):378-84.
[118] Arora T, Sharma R, Frost G. Propionate. Anti-obesity and satiety enhancing factor? Appetite. 2011;56(2):511-5.
[119] Kang SA, Hong K, Jang K-H, Kim Y-Y, Choue R, Lim Y. Altered mRNA expression of hepatic lipogenic enzyme and PPARα in rats fed dietary levan from Zymomonas mobilis. The Journal of nutritional biochemistry. 2006;17(6):419-26.
[120] Matsuo T, Sairenchi T, Suzuki K, Tanaka K, Muto T. Long-term stable obesity increases risk of hypertension. International journal of obesity. 2011;35(8):1056-62.
[121] Yusuf S, Hawken S, Ôunpuu S, Bautista L, Franzosi MG, Commerford P, et al. Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. The Lancet. 2005;366(9497):1640-9.
[122] Bray G. Obesity increases risk for diabetes. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 1992;16:S13-7.
[123] Nilsson AC, Östman EM, Holst JJ, Björck IM. Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. The Journal of nutrition. 2008;138(4):732-9.
[124] Rault-Nania M-H, Demougeot C, Gueux E, Berthelot A, Dzimira S, Rayssiguier Y, et al. Inulin supplementation prevents high fructose diet-induced hypertension in rats. Clinical Nutrition. 2008;27(2):276-82.
[125] Parnell JA, Klancic T, Reimer RA. Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. Obesity. 2017;25(3):510-3.
[126] Nicolucci AC, Hume MP, Martínez I, Mayengbam S, Walter J, Reimer RA. Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity. Gastroenterology. 2017.

[127] Delzenne NM, Kok N, Fiordaliso M-F, Deboyser DM, Goethals FM, Roberfroid MB. Dietary fructooligosaccharides modify lipid metabolism in rats. The American Journal of Clinical Nutrition. 1993;57(5):820S-S.
[128] Kok N, Roberfroid M, Robert A, Delzenne N. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. British Journal of Nutrition. 1996;76(06):881-90.
[129] Sa'ad H, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2010;1801(11):1175-83.
[130] Haber B, Ter Meer H-U, Hausmanns S. Cholesterol-reducing agent made of dietary fibre and cholesterol-reducing substances. Google Patents; 2003.
[131] Yamamoto Y, Takahashi Y, Kawano M, Iizuka M, Matsumoto T, Saeki S, et al. In vitro digestibility and fermentability of levan and its hypocholesterolemic effects in rats. The Journal of nutritional biochemistry. 1999;10(1):13-8.
[132] Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al. Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome. Nature. 2016;534(7606):213-7.
[133] Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, et al. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. The American journal of clinical nutrition. 2009;90(5):1236-43.
[134] Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58(8):1091-103.
[135] Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R. Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. Diabetes. 2006;55(5):1484-90.
[136] Delzenne NM, Cani PD. Gut microbiota and the pathogenesis of insulin resistance. Current diabetes reports. 2011;11(3):154-9.
[137] Kang SA, HONG K, Jang K-H, KIM S, LEE KH, Chang B-I, et al. Anti-obesity and hypolipidemic effects of dietary levan in high fat diet-induced obese rats. Journal of microbiology and biotechnology. 2004;14(4):796-804.
[138] Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. The American journal of clinical nutrition. 2009;89(6):1751-9.
[139] Lee SR, Hwang KT, Ji GE. The Anti-Obesity Effects of the Dietary Combination of Fermented Red Ginseng with Levan in High Fat Diet Mouse Model. Phytotherapy research. 2014;28(4):617-22.
[140] Hume MP, Nicolucci AC, Reimer RA. Prebiotic supplementation improves appetite control in children with overweight and obesity: a randomized controlled trial. The American Journal of Clinical Nutrition. 2017;105(4):790-9.
[141] Kazak H, Barbosa A, Baregzay B, da Cunha MA, Oner ET, Dekker R, et al. Biological activities of bacterial levan and three fungal β-glucans, botryosphaeran and lasiodiplodan under high glucose condition in the pancreatic β-cell line INS-1E. Adaptation biology and medicine: new developments. 2014;7:105-15.
[142] Dahech I, Belghith KS, Hamden K, Feki A, Belghith H, Mejdoub H. Oral administration of levan polysaccharide reduces the alloxan-induced oxidative stress in rats. International journal of biological macromolecules. 2011;49(5):942-7.
[143] Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 2009;58(7):1509-17.
[144] Belghith KS, Dahech I, Hamden K, Feki A, Mejdoub H, Belghith H. Hypolipidemic effect of diet supplementation with bacterial levan in cholesterol-fed rats. International journal of biological macromolecules. 2012;50(4):1070-4.
[145] Gobinath D, Madhu AN, Prashant G, Srinivasan K, Prapulla SG. Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. British Journal of Nutrition. 2010;104(01):40-7.
[146] Chen K, Chen H, Faas MM, Haan BJ, Li J, Xiao P, et al. Specific inulin-type fructan fibers protect against autoimmune diabetes by modulating gut immunity, barrier function, and microbiota homeostasis. Molecular Nutrition & Food Research. 2017.
[147] Lightowler H, Thondre S, Holz A, Theis S. Replacement of glycaemic carbohydrates by inulin-type fructans from chicory (oligofructose, inulin) reduces the postprandial blood glucose and insulin response to foods: report of two double-blind, randomized, controlled trials. European Journal of Nutrition. 2017:1-10.
[148] Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017;8(2):172-84.
[149] De Preter V, Verbeke K. Functional Aspects of Prebiotics and the Impact on Human Health. 2014.