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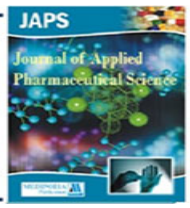

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Diet and Functional Foods in Treatment and Maintenance Therapy of Colon Disorders

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ABSTRACT

Dietary habits have been associated with variations in the risk of colon disorders, either its increase or decrease. Colon-specific approaches showed their potential to target and treat colon cancers and inflammatory diseases, but they vary in success rates for local recurrence, disease-free survival, and overall survival. Also, chemotherapies and radiotherapies have been applied as the surgical adjuvant treatments. The significant role of exogenously administered Lactobacilli in reducing toxin-producing bacteria in the gut and increases the longevity of the host, led to the coining of the term 'probiotics'. The evidence on the effects of inulin and oligofructose on colonization, translocation of pathogens and the prevention of intestinal diseases make them suitable candidates to treat colon disorders. Among potentially protective foods, growing attention has been dedicated to functional foods comprising probiotics, such as Lactobacilli or Bifidobacteria, and prebiotics such as fructo-oligosaccharides or fructans, as their consumption may treat inflammatory bowel diseases, like ulcerative colitis, crohn's disease as well as experimentally induced colon cancer in mammals. The readily apparent synergy of concomitantly using beneficial microorganisms and nutritive materials that support their growth led to the term "synbiotics" to describe foods or supplements that combine both probiotics and prebiotics. Various potential mechanisms are addressed in the present paper. This article discusses the real value of dietary components, which offers practical information to help patients as well as health professionals. Furthermore, article has focused on the possible value of probiotics, prebiotics and synbiotics in treatment and maintenance therapy of colonic ailments.

Keywords: Diet, Probiotics, Prebiotics, Synbiotics, Colon diseases

INTRODUCTION

Foods and dietary lifestyles are two important factors, associated with risk for colon diseases. Certain foods or diet-related items are seen to be important to either avoid or encourage so as to prevent disease (Young, 2000). A variety of colonic disorders were reported by different researchers including Inflammatory bowel diseases, like ulcerative colitis (Kornbluth and Sachar, 2010), crohn's disease (Roy *et al.*, 1997), pouchitis (Mahadevan and Sandborn, 2003); cancer, like colon cancer (Cappell, 2005), and diverticular disease (Stollman and Raskin, 1999). Severity and causative agents differentiate these inflammatory diseases into variety of colitis.

The differential diagnosis of various colitis with their clinical as well as histological characteristics is shown in the Table 1 (Kefalides and Hanauer, 2002). Various pharmaceutical approaches are used for targeting these colonic ailments. These colon targeted drug delivery systems are summarized with their features in Table 2 (Chourasia and Jain, 2003 & Kumar *et al*., 2011 & Philip and Philip, 2010). Multiparticulate technologies, tumor vaccines and immunotherapy are attractive alternative, or addition, to conventional cancer treatments, and their study has increased significantly (Krishnamachari *et al.*, 2011). Different approaches have certain advantages and disadvantages, selection of any approach depend on different physiological factors and also, the colonic microflora was found as an important constituent in the intestine's defence barrier and identified as being capable of influencing gastrointestinal diseases and disorders (Harish and Varghese, 2006). There exists a potential role for foods that contain probiotics and/or prebiotics to change the colonic microbiota in a way that might prevent colon diseases (Le Leu *et al*., 2010). Probiotics, prebiotics and synbiotics are considered in unique edible product category, which is known as Functional foods, because these comprise some bacterial strains and products of plant and animal origin containing physiologically active compounds beneficial for human health and reducing the risk of chronic diseases (Grajek *et al*., 2005).

Table. 1: Differential diagnosis of Ulcerative Colitis.

S.NO.	TYPE OF DISEASE	CLINICAL CHARACTERISTICS	HISTOLOGICAL CHARACTERISTICS
1	Ulcerative colitis	Bloody diarrhea	Distortion of crypts; acute and chronic diffuse inflammatory infiltrate; goblet cell depletion; crypt abscesses; lymphoid aggregates.
2	Crohn's colitis	Perianal lesions common; frank bleeding less frequent than in ulcerative colitis	Focal inflammation; submucosal involvement; granulomas; goblet cell preservation; transmural inflammation; fissuring.
3	Ischemic colitis	Older age groups; vascular disease; sudden onset, often painful	Mucosal necrosis; ballooning of capillaries; red blood cell congestion; hemosiderin and fibrosis (chronic disease).
4	Collagenous colitis	Watery diarrhea; rectal bleeding rare	> 10 µm–thick subepithelial collagen band; chronic inflammatory infiltrate.
5	Microscopic (lymphocytic) colitis	Watery diarrhea; often seen in older women; macroscopically normal colonic mucosa	Chronic inflammatory infiltrate; increased intraepithelial lymphocytes; crypt distortion Unusual.
6	Infective colitis	Sudden onset usual; identifiable source with other cases (eg, Salmonella); pain may predominate (eg, Campylobacter); pathogens present in stool	Crypt architecture usually normal; edema; superficial neutrophil infiltrate; crypt abscesses.
7	Pseudomembranous colitis	May be a history of antibiotics; "membrane" may be seen on sigmoidoscopy; Clostridium difficile toxin detectable in stools	Similar to acute ischemic colitis but may show "summit" lesions of fibrinopurulent exudate.
8	Amebic colitis	Travel in endemic area; amebae in fresh Stool	Similar to ulcerative colitis; amebae in lamina propria or in flask-shaped ulcers; identified by periodic acid–Schiff stain.
9	Gonococcal colitis	Rectal pain; pus	Intense neutrophil infiltration; purulent exudate; gram-positive cocci.

Table. 2: Various pharmaceutical approaches to colon targeted drug delivery systems.

APPROACH	FEATURES
1. Prodrug Formation	Covalent linkage of a drug with a carrier .
1.1. Azo conjugates	Drug is conjugated via an azo bond.
1.2. Cyclodextrin conjugates	Drug is conjugated with cyclodextrin.
1.3. Glycoside conjugates	Drug is conjugated with glycoside.
1.4. Glucuronate conjugates	Drug is conjugated with glucuronate.
1.5. Dextran conjugates	Drug is conjugated with dextran.
1.6. Polypeptide conjugates	Drug is conjugated with poly(aspartic acid) .
1.7. Polymeric prodrugs	Drug is conjugated with polymer.
2. Intact Molecule Delivery Approach	Delivery of intact molecule to the colon.
2.1. Coating with polymers	
2.1.1. Coating with pH-sensitive polymers	Formulation coated with enteric polymer release drug when pH moves towards alkaline range.
2.1.2. Coating with biodegradable polymers	Colonic bacteria degrade polymer, followed by release of drug.
2.2. Embedding in matrices	
2.2.1. Embedding in biodegradable matrix	Drug release by degradation of polymer.
2.2.2. Embedding in pH-sensitive matrix	Degradation of polymer in alkaline pH.
2.2.3. Hydrogels	Drug release by swelling.
2.3. Timed release systems	Multicoated formulation passes the stomach, the drug is released after a lag time of 3-5 h that is equivalent to small intestine transit time
2.4. Redox sensitive polymers	Drug formulated with azo polymer and disulfide polymer that selectively respond to the redox potential of colon provide colonic delivery.
2.5. Bioadhesive systems	Drug coated with bioadhesive polymer that selectively provide adhesion to colonic mucosa may release drug in the colon.
2.6. Coating with microparticles	Drug is linked with microparticles.
2.7. Osmotic controlled delivery systems	Drug released through semi permeable membrane due to osmotic pressure.
2.8. Pressure controlled drug delivery systems	Drug release occurs following the disintegration of a water-insoluble polymer capsule because of pressure in lumen of colon.
2.9. CODES™	Combined approach of pH dependent and microbially triggered CDDS.
2.10. Pulsatile drug delivery system	The drug is released rapidly after a well defined lag-time in Pulsed drug release manner.

SCIENTIFIC BASIS OF FUNCTIONALITY

Hundreds of microbial species live in association with humans-on skin and in visceral tracts. The human gastrointestinal tract contains about 10^{14} bacteria, with small numbers in the stomach ($<10^3$/mL) rising with descent of the tract to 10^{11}–10^{12}/mL in the colon. Here the anaerobes outnumber the aerobes 100-1000-fold (Jonkers and Stockbrügger, 2003). Bacterial populations have been estimated to reach 10^{14} cells at all sites of the human body (Tannock, 1994), a number that is more amazing when considered in the context of exceeding by 10-fold the number of human cells associated with the human body. Studies with germ-free (gnotobiotic) animals prove that microbial colonization is not required for survival. In fact, microbial colonization can have negative effects as a result of the toxic, genotoxic, mutagenic, or carcinogenic potential of microbial metabolites (Hill *et al.*, 1971). Germ-free animals, however, are more susceptible to infection than conventional counterparts (Hentges, 1992). The increased susceptibility to infection is attributed, at least in part, to poor immune function and the absence of "colonization resistance" (competition of normal microflora with invading microorganisms) (Vollaard and Clasener, 1994). Differences between conventional and germ-free animals provide a basis for the belief that microbial colonization has important health implications for host organisms.

It is a big jump, however, from the assertion that colonizing microflora has a profound effect on normal human health to the probiotic hypothesis that the addition of certain exogenous microorganisms to the intestinal ecosystem will have a positive effect. The intestinal tract is a fairly stable microbial ecosystem in the adult (Tannock, 1990). Acute perturbations as might result from antibiotic use, disease, or certain dietary changes seem to be self-correcting (Tannock, 1983). Probiotic bacteria consumed even in high numbers do not become permanent colonizers and are rarely detectable in fecal or intestinal samples beyond a couple of weeks after ingestion. This residence time likely coincides with washout kinetics, extended perhaps by some *in situ* replication of probiotics suited to the environment. Therefore, it is necessary to consider that probiotic effects may, in fact, be mediated by associations and mechanisms less intimate and more transient than those of native microflora.

Specific probiotic bacterium will have beneficial, detrimental, or no effect on health presumed strictly through determination of its genus or species. The tempting speculation that the members of one genus or species will consistently mediate specific effects is not supported by research. Strain-specific effects are frequently reported in a diversity of assays. Conversely, for targets including immune function, anti-cancer effects, and anti-diarrheal effects, similar positive effects have been demonstrated for different strains of different genera, e.g., lactobacilli, bifidobacteria and enterococci. Although direct comparisons of different strains are rarely done, it appears that generalizations about the probiotic performance of genera and species are difficult to make. Until mechanisms are better understood and controlled studies comparing isogenic strains differing in a well-defined manner are completed, it is prudent to assume that probiotic effects are strain-specific. In addition, physiological conditions of the host are likely to be as important to probiotic efficacy as the microbial strain (Sanders, 1999).

FOODS AND DIETARY MANAGEMENT

According to the definition, functional food is a part of an everyday diet and is demonstrated to offer health benefits and to reduce the risk of chronic disease beyond the widely accepted nutritional effects. In mid 1980s, the term 'functional foods' was introduced in Japan. This type of foods is known on the Japanese market as "Foods for Specified Health Use" (FOSHU). The functional foods comprise:

- Conventional foods containing naturally occurring bioactive substances (e.g., dietary fiber).
- Foods enriched with bioactive substances (e.g., probiotics, antioxidants).
- Synthesized food ingredients introduced to traditional foods (e.g., prebiotics),

Among the functional components, probiotics and prebiotics, soluble fiber, omega-3-polyunsaturated fatty acids, conjugated linoleic acid, plant antioxidants, vitamins and minerals, some proteins, peptides and amino acids, as well as phospholipids are frequently mentioned. These active substances constitute a focus of contemporary science of human nutrition (Grajek *et al.*, 2005).

The wide variation in incidence of colon diseases is largely attributed to national differences in diet and other environmental factors (Tamura *et al.*, 1996). In contrast to native Japanese, descendants of Japanese immigrants to America have, like other Americans, a high incidence of colon cancer attributed to dietary and other environmental adaptations (Haenszel and Kurihara, 1968). Indeed, the incidence of colon cancer has recently increased in native Japanese attributed to their adopting a Westernized diet and other environmental changes with industrialization (Tamura *et al.*, 1996). Also, the evidence for dietary modulation of cancer risk is greatest for colorectal cancer, which is one of the major causes of death from malignant disease in Europe and North America (Rowland, 2004). Some of colon diseases were thought to be causally related to diet (as shown in Table 3) (Young, 2000). The various diet-related risk factors are summarized in Table 4 (Cappell, 2005). Additionally, Irritable Bowel Syndrome patients have gastrointestinal symptoms (diarrhea, cramps, abdominal pain, nausea, gas, bloating, heartburn, etc.) caused by many of the following foods. Food induced gastrointestinal symptoms can begin within 5–15 minutes after eating, or up to twelve to forty eight hours later (due to fermentation). Foods and beverages are additive within and between meals. Foods and beverages eaten out at restaurants will cause problems due to sauces, spices, and hidden ingredients. Listed below are examples of some of the foods and beverages that IBS patients have found to aggravate their GI symptoms in Table 5 (MacDermott, 2007).

Table. 3: Colorectal disorders and diseases thought to be causally related to 'diet'.

S.NO.	TYPE OF DISEASE
1	Colorectal adenomas and cancer
2	Inflammatory bowel disease
3	Constipation and defecation difficulties
4	Anorectal disorders such as fissures and haemorrhoids
5	Certain food-induced diarrheal disorders and 'allergies'
6	Traveller's diarrhea
7	Diverticular disease
8	Irritable bowel syndrome

Table. 4: Diet-related risk factors for colon cancer.

S.NO.	DIET	PROPOSED MECHANISM	REFERENCES
1	High fat?	Various theories (e.g., increased bile secretion)	Willett *et al.*,1990
2	Low fruit and vegetable consumption	Anticarcinogenic substances in fruits and vegetables (e.g., folic acid)	Thun *et al.*,1992 & Kampman *et al.*, 1996
3	Low calcium?	Calcium binds to bile acids that are otherwise potentially colonotoxic	Bergsma-Kadijk *et al.*,1996
4	High red meat?	Animal fat in red meat or carcinogens (e.g., nitrosamines) in cooked meat	Fuchs *et al.*,1999
5	Low selenium?	Selenium can help neutralize toxic free radicals because of antioxidant effects	Ghadirian *et al.*, 2000
6	Low folate?	Folate needed for DNA synthesis and repair	Baron *et al.*,1998
7	Low carotenoid diet?	Carotenoids can help neutralize free radicals because of antioxidant effects	Modan *et al.*,1981
8	Low-fiber diet?	Dilution of carcinogens in stool cause by increased stool bulk and stool water with a high-fiber diet	Willett *et al.*,1990 & Howe *et al*., 1992

Table. 5: Foods and Beverages that induce and aggravate Irritable Bowel Syndrome Symptoms.

S.NO.	FOODS AND BEVERAGES
1	Milk and milk containing products: such as ice cream, cream cheese, cheese, cottage cheese, yogurt, ice milk, cream soups, butter, pudding, whipped cream, cream, cheesecake, chocolate, pastries, crackers, pretzels, cookies, etc.
2	Caffeine containing products such as coffee, tea, colas, sodas, chocolate, etc.
3	Alcohol products: beer, wine, coolers, foods containing or cooked in alcohol.
4	Fruits and fruit juices, particularly apples, apple juice or cider, citrus fruits, orange juice, tomatoes, tomato juice, etc.
5	Spices and seasonings; hot sauce; barbecue sauce; chili sauce; salsa.
6	Diet beverages, diet foods, diet candies, diet gum, sugar free products, "lite or light" products look good and taste good, but to not put on weight, go right through you, causing diarrhea, or stay in the GI tract and cause symptoms.
7	Fast foods and Chinese food: contain spices, sauces, and hidden ingredients.
8	Condiments: ketchup; mustard; mayonnaise; relish.
9	Fried foods and fatty foods.
10	Whole grain or multigrain breads; sourdough breads and bagels.
11	Salads: usually not the lettuce, but rather added ingredients such as bacon bits, croutons, onions, peppers, etc.
12	Salad dressings, particularly those containing mayonnaise, cheese and spices.
13	Vegetables, particularly cabbage, broccoli, cauliflower and corn.
14	Legumes: beans, lentils, chili, etc. Popcorn. Foods with high fiber content.
15	Red meats, i.e., steak, hamburger, sausage, bacon, prime rib. Spicy marinades or gravies tend to cause even greater problems.
16	Gravies, spaghetti sauce, cream sauces, cheese sauces, soups, stews, and stuffing.
17	Artificial flavorings, preservatives, and sweeteners.
18	Foods containing large amounts of fructose or high fructose corn syrup (honey, grapes, resins, nuts, etc).
19	Cookies, crackers, pretzels, cakes and pies.

Numerous epidemiological surveys link dietary substances to protection against colon disorders. Diet management has consistently been associated with protection in population studies. Various perceived mechanisms for beneficial actions of diet will be discussed. In brief, these include:

- Components of a healthy luminal environment such as high butyrate levels and lowered pH.
- Predominance of 'healthy' over 'unhealthy' bacteria.
- Rapid intestinal transit and high fecal bulk.
- Non-leaky epithelial barrier.
- Adsorption of carcinogens by fiber.
- Low bile salt concentrations and generation of toxic bile salts or protein derivatives (Young, 2000).

A variety of food substances and beverages are listed in the Table 6, that are well tolerated by patients with Irritable Bowel Syndrome. Many of food ingredients possess disease preventive properties. Epidemiological studies and randomized clinical trials carried out in different countries have demonstrated or at least suggested numerous health effects related to functional food consumption, such as reduction of cancer risk (Liong, 2008), decline in the development of colonic diverticulosis (Stollman and Raskin, 1999). At the moment, the most important and the most frequently used functional food compounds are probiotics, prebiotics and synbiotics.

Table. 6: Foods and Beverages that are well tolerated by patients with Irritable Bowel Syndrome.

S.NO.	FOODS AND BEVERAGES
1	Water. Flavored, noncarbonated water, ginger ale, Sprite. Gatorade.
2	Rice: cooked white, without sauces or additives.
3	Plain pasta noodles—(avoid tomato, spicy, or cream sauces).
4	Potato—boiled or baked without sour cream; Sweet potatoes. No French Fries.
5	Breads—French, Italian, whole white; English muffins; white rolls; cornbread.
6	Plain fish—broiled, without sauces. Tuna fish without mayonnaise.
7	Chicken or turkey—broiled or baked without spices or sauces.
8	Ham—plain, not smoked.
9	Eggs—soft boiled, poached, and scrambled (use water, not milk).
10	Cereals—dry or with soymilk or rice milk. Plain Cornflakes, Rice Krispies, Corn or Rice Checks, Cheerios. Avoid artificial colorings, flavorings, and sweeteners.
11	Soy or rice milk. Soy or rice based products.
12	Salads—lettuce, tomatoes, hard-boiled egg slices, oil and vinegar dressing.
13	Peas, carrots, cooked (avoid raw vegetables).
14	Crackers—Oyster, saltines, or animal crackers.
15	Applesauce, in small amounts.
16	Cantaloupe, watermelon, honeydew melon, in small amounts.
17	Fruit cocktail, peaches—nondietetic, canned or frozen.
18	Margarine, jams, jellies, peanut butter.

PROBIOTICS

The term probiotic, meaning "for life," is derived from the Greek language. The concept of probiotics evolved at the turn of the 20th century from a hypothesis first proposed by Nobel Prize winning Russian scientist Elie Metchnikoff, who suggested that the long, healthy life of Bulgarian peasants resulted from their consumption of fermented milk products. He believed that when consumed, the fermenting bacillus (Lactobacillus) positively

influenced the microflora of the colon, decreasing toxic microbial activities (Sanders, 1999). Different investigators defined probiotics according to their knowledge and work experiences, but the probiotic concept was found to be confined and unsatisfactory. Considering the various arguments, researchers found that there was need of revision in the concept of defining 'probiotic' term. Then, Havenaar and Huis In't Veld provided, one of the closest to, the definition of the term probiotic: "*A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host*" (Schrezenmeir and Vrese, 2001).

Reasons for the revision of 'probiotic' definition

Havenaar and Huis In't Veld definition tried to cover all aspects related to beneficial microorganisms. They came with definition which satisfy all and for justification, the reasons are as follows:

- The need to include products in addition to microorganisms, or preparations of microorganisms.
- The requirement of sufficient microbial numbers to exert health effects.
- Preference for the phrase "alteration of the microflora" over "improving the properties of the…microflora," because the optimal properties of the indigenous microflora were not defined until now and the evidence of benefit can be shown only by health effects.
- Definition of the term indigenous microflora refers to "the usually complex mixture of bacterial population that colonizes a given area in the host that has not been affected by medical or experimental intervention, or by disease" and use of to colonize to describe a bacterial population that establishes in size over time without the need for periodic reintroduction of the bacteria by repeated oral doses or other means (Schrezenmeir and Vrese, 2001).

The colon is a reservoir of large quantities of different bacterial species, some beneficial and others detrimental to health (as shown in the Table 7) (Sekhon and Jairath, 2010 & Fisher and Denison, 1996 & Phalipon and Sansonetti, 2007 & Novak and Juneja, 2002). Beneficial candidates of gut microflora perform three major tasks: colonization resistance to pathogens, modulation of gastrointestinal and systemic immune responses, and nutritional support (Isabel *et al.*, 2006 & Farthing, 2004 & Crittenden and Playne, 2006 & Guarner and Malagelada, 2003). Certain strains of bacteria have been discovered over the years to have probiotic properties, mainly consisting of lactic acid producing bacteria (lactobacilli, streptococci, enterococci, lactococci, bifidobacteria), and fungi such as Saccharomyces and Aspergillus (Liong, 2008). In other words, these are the microbial food supplement with nearly 20 known species, which beneficially affect the host by improving its intestinal microbial balance. Various types of probiotic bacteria include: Lactobacillus species (*L. acidophilus, L. reuteri, L. plantarum, L. casei, L. salivarius, L. bulgaricus, L. fermentum, L. gasseri, L. johnsonii, L. lactis, L. paracasei*), Bifidobacterium species (*B. bifidum, B. infantis, B. lactis, B. longum, B. breve, B. adolescentis*), Saccharomyces species (*S. boulardii*), Streptococcus species (*S. thermophilus*), other bacterium (*Propionibacterium freudenreichii, Enterococcus, Escherichia coli*). Lactobacilli and Bifidobacteria found in foods such as yogurt, cottage cheese, buttermilk or other cultured dairy products are the most familiar probiotics (Sekhon and Jairath, 2010).

Lactic acid bacteria constitute a diverse group of organisms providing considerable benefits to humankind. Attributes of lactic acid bacteria such as anti-microbial agent production and competition with potential pathogens in the gut provided the impetus for investigating a role for probiotics in colon diseases (Suvarna and Boby, 2005).

Probiotics and Inflammatory Bowel Disease

Several investigations have shown an influence of the intake of lactic acid bacteria and fermented-milk products on gut flora enzyme activities associated with colon disorders, especially Inflammatory Bowel Disease. Various studies conducted in laboratory animals, which encompass a wide range of tumor models and shorter-term, predictive assays conducted under diverse conditions, have provided extensive and quite compelling evidence for anti-cancer effects of specific probiotic bacteria. According to different researchers, positive, negative as well as neutral effects of probiotics on inflammatory bowel disease are compiled in Table 8 (Jonkers and Stockbrügger, 2003 & Mahadevan and Sandborn, 2003).

Table. 7: Beneficial and detrimental bacterial species.

SPECIES	BENEFICIAL	DETRIMENTAL
Lactobacillus	+	
Bifidobacterium	+	
Saccharomyces	+	
Streptococcus	+	
Bacillus	+	
Escherichia	+	
Propionibacterium	+	
Veillonella		+
Clostridium		+
Shigella		+

(+) means Effective

Table. 8: Effects of probiotics in patients with inflammatory bowel disease.

INFLAMMATORY BOWEL DISEASE	TREATMENT	NUMBER OF INDIVIDUAL	DURATION	EFFECT
Ulcerative colitis	Antibiotic + faecal enema	1 adult	Once	Induced remission
	L. plantarum (9 active /10 inactive)	19 adults	-	6 of 9 patients treated with active form: in remission
	Multispecies	20 adults	12 months	75% still in remission and changed fecal flora
	E. coli vs mesalazine	108 adults	12 weeks	Similar relapse rate
	E. coli vs mesalazine	116 adults	12 months	Similar remission and relapse rates
	E. coli vs mesalazine	327 adults	12 months	Similar relapse rate
Crohn's disease	*L. casei* GG	4 children	6 months	Decreased intestinal permeability, clinical disease activity
	S. boulardii + mesalazine	10 adults	6 months	Only 1 relapse
	L. casei GG	14 children	10 days	Increased mucosal IgA
	L. salivarius UCC118	20 adults	10 days	No remission, Increased quality of life
	Prednisolone + '*E. coli* vs placebo'	28 adults	12 months	Reduced relapse rate
	'*S. boulardii* + 5ASA' vs 5-ASA	32 adults	6 months	Reduced relapse rate
	L. casei GG vs placebo	37 adults	12 months	Similar endoscopic recurrence
Pouchitis	Antibiotics received, either placebo vs 6g daily oral dose of VSL-3	40 adults	9 months	Seventeen of 20 patients (85%) who were treated with VSL-3 maintained remission, compared to none of 20 patients who were treated with placebo.

VSL-3 contains 5×10^{11}/g of viable lyophilized bacteria consisting of 4 strains of lactobacilli (*L. acidophilus, L. delbrueckii* subsp. *bulgaricus, L. plantarum, L. casei*), three strains of bifidobacteria (*B. infantis, B. longum, B. breve)* and one strain of *Streptococcus salivarius* subsp. *thermophilus.*

Probiotics and Colon cancer

Many studies confirmed the involvement of the endogenous microflora in the onset of colon cancer. This makes it reasonable to think that changing the intestinal microflora could influence tumor development.

During the last two decades, several animal studies have demonstrated the protective effect of probiotics on colon cancer. For example, 25–50% inhibition of carcinogen-induced pre-cancerous colon lesions (aberrant crypt foci) has been reported in rats fed strains of *Bifidobacterium longum* in the diet. Furthermore, administration of dietary *B. longum*(1×10^{10} live bacterial cells/d) completely suppressed colon tumors induced by the compound 2-amino-3-methyl-3H-imidazo(4,5-f)quinoline, which is a carcinogen found in the human diet. *L. acidophilus* markedly reduced both the number and size of colon tumors induced by another carcinogen, 1,2-dimethylhydrazine (DMH). Interestingly, there was also a difference in the type of tumors: in the rats given *L. acidophilus*, in contrast to the ones given DMH alone, only benign tumors were seen, suggesting that probiotics may inhibit the development of malignant disease (Rowland, 2004).

Mechanisms by which lactic acid bacteria may be inhibiting colon cancer

The precise mechanisms by which lactic acid bacteria may inhibit colon cancer are presently unknown. However, mechanisms may include:

- Alteration of the metabolic activities of intestinal microflora
- Alteration of physico-chemical conditions in the colon
- Binding and degrading potential carcinogens
- Quantitative and/or qualitative alterations in the intestinal microflora incriminated in producing putative carcinogen(s) and promoters (e.g. bile acid-metabolizing bacteria)
- Production of antitumourigenic or antimutagenic compounds
- Enhancing the host's immune response and effects on physiology of the host (Rafter, 2002)

Probiotic and Infants

The infant GI tract is essentially sterile prior to birth, but is immediately exposed to maternal and environmental bacteria during delivery. The subsequent colonization process is influenced by the genetics of the host and several environmental factors, including mode of delivery, gestational age, hospitalization, antibiotic use by the mother or infant, and type of infant feeding. Studies using standard microbiological methods have shown that during the first few days of life, infants' intestinal microbiota are comprised almost entirely of enterobacteria and Gram-positive cocci, obtained predominantly during the birth process. Studies suggest that these facultative anaerobic bacteria generate conditions that favor the subsequent establishment of strict anaerobes such as bacteroides, bifidobacteria, and clostridia. These microorganisms perform numerous metabolic, growth-promoting, and protective roles (as shown in the Fig. 1) (Donovan *et al.*, 2008).

Dosage of probiotics

One to two billion colony forming units (CFU) per day of a mixed strain supplement probiotic are considered to be the minimum amount for the healthy maintenance of intestinal microflora. To get adequate amount of health benefits, a dose of $5x10^9$ CFU/d has been recommended for at least five days. According to Earl Mindell, an expert on nutrition, healthy persons should take 2 to 5 billion CFU of probiotics per day and those with problems in the GIT can take up to 10 billion CFU per day. The current daily intake recommended by the Natural Health Products Directorate of Canada, for prescription probiotics, is 5-10 billion CFU (Chakraborti, 2011).

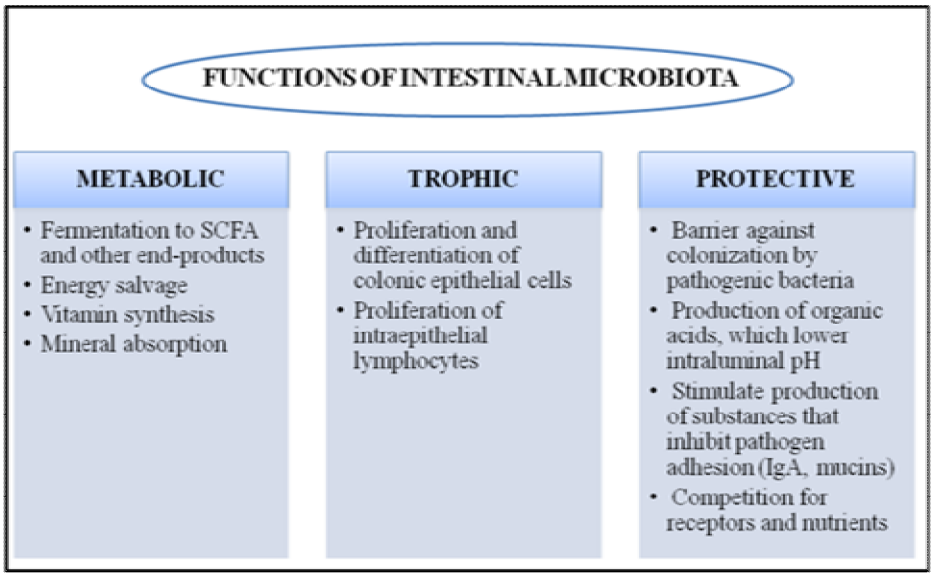

Fig. 1: Beneficial functions of intestinal microbiota in infants.

PREBIOTICS

Prebiotics are a concept that you may not have heard about, although again used in the 1950s (Lactulose was used 50 years ago as a prebiotic formula supplement to increase the numbers of Lactobacillus in infants' intestines) (Macgillivray *et al*., 1959). Generally, a prebiotic is a fiber found in some plants that reaches the colon undigested. The term prebiotic was introduced by Gibson and Roberfroid who exchanged "pro" for "pre," which means "before" or "for." They defined prebiotics as "*a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon*." This definition more or less overlaps with the definition of dietary fiber, with the exception of its selectivity for certain species (Schrezenmeir and Vrese, 2001). Different researchers worked on variety of naturally occurring or chemically synthesized oligosaccharides. These include inulin-type fructans, galactooligosaccharides, lactulose, isomaltooligosaccharides, xylooligosaccharides, soy-oligosaccharides, gentiooligosaccharides, and nigeroligosaccharides (Tuohy *et al*., 2005 & Roberfroid, 2007 & Swennen *et al*., 2006). Out of all above, only inulin-type fructans, galactooligosaccharides, resistant starch and lactulose fully meet the criteria established for classification as prebiotics (Tuohy *et al*., 2005 & Kolida *et al*., 2002 & Younes *et al*., 2001). They reach the colon largely intact where they are fermented by specific colonic microbial strains possessing a wide assortment of carbohydrolytic enzymes (Swennen *et al*., 2006 & Cummings *et al*., 2001). The main end products of carbohydrate metabolism are short chain fatty acids, namely acetate, butyrate and propionate, which are further used by the organism as an energy source (Sauer *et al*., 2007 & Gallaher and Khil, 1999).

Criteria to being classified as a prebiotic

For a food ingredient to be classified as a prebiotic it must fulfil the following criteria:

- Neither be hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract.
- Be selectively fermented by one or a limited number of potentially beneficial bacteria commensal to the colon, e.g. bifidobacteria and lactobacilli, which are stimulated to grow and/or become metabolically activated.
- Prebiotics must be able to alter the colonic microflora towards a healthier composition, for example by increasing numbers of saccharolytic species while reducing putrefactive microorganisms (Kolida *et al*., 2002)

Prebiotic effect of fructo-oligosaccharides

A variety of products containing inulin and/or oligofructose formulations, claiming to have beneficial effects on gut health and general well-being, are starting to become prevalent in the European market. These fructo-oligosaccharides have evidences to increase the level of various probiotics. The prebiotic effects of different oligosaccharides were summarized in Table 9. Bran and chicory are popularly fructans producing plant sources, used in the treatment therapy of diseases like diverticulitis and colon cancer, respectively (Painter, 1974 & Hughes and Rowland, 2001).

Table 9: Summary of studies designed to determine the prebiotic effect of fructo-oligosaccharides .

OLIGOSACCHARIDE	STUDY	MODEL	PREBIOTIC EFFECT	REFERENCES
Inulin	*In vivo*	Rats having colitis	Decrease in luminal pH between left and right colon	Videla, 1999
	In vivo	Eight healthy humans	Significant increase in bifidobacteria established by FISH	Kruse et al., 1999
	In vivo	Six healthy humans	Significant increase in stool frequency and fecal bulk	Den Hond et al., 2000
	In vivo	Rats having colitis	Increase in lactobacilli	Videla et al., 1998
	In vitro	Human fecal batch cultures	Highest decrease in pH with inulin and highest increase in butyrate very fast fermentation and high gas production	Karppinen et al., 2000
	In vivo	Mature Fisher Rats	Significantly reduced the total number of ACF	Verghese et al., 2002
Oligofructose	*In vivo*	Thirty healthy humans	Significant increase in bifidobacteria established via FISH at 7 g/d, no change in total bacterial levels	Tuohy et al., 2001
	In vitro	Human fecal flora	Increases in bifidobacteria. Lactobacilli outcompeted bifidobacteria at pH 5·2–5·4	Sghir et al., 1998
	In vivo	Rats	Increase in lactic acid bacteria after 2 weeks, but in the long-term any effect was lost	Le Blay et al., 1999
	In vitro	Human fecal flora	Significant bifidogenic effect compared to sucrose and inulin	Gibson and Wang, 1994
	In vivo	Twelve healthy humans	Significant increase in bifidobacteria, no change in total bacteria levels	Buddington et al., 1996
	In vivo	Ten healthy humans	Significant increase in bifidobacteria, some increase in lactobacilli	Williams et al., 1994
	In vivo	Forty healthy humans	Significant increase in bifidobacteria levels without excessive gas production at 10 g/d	Bouhnik et al., 1999
Inulin and oligofructose	*In vivo*	Eight healthy humans	15 g/d inulin or oligofructose led to bifidobacteria becoming predominant in feces	Gibson and Roberfroid, 1995
	In vitro	Human fecal flora	Significant increase in bifidobacteria, suppression of *E. coli* and clostridia	Wang and Gibson, 1993
Inulin and lactose	*In vivo*	Twenty five constipated humans	Significant increase in bifidobacteria decreases in enterococci and fuso-bacteria. Better laxative effect than lactose	Kleessen et al., 1997

FISH (Fluorescent *in situ* hybridization).
ACF (Aberrant crypt foci).

Prebiotics and Infants

In recent years, however, there has been an increase in the number of foods available for children and adults containing prebiotics, including yogurt and cereals. Prebiotic carbohydrates have been added to infant formulas in Japan for over 20 years, and 90% of infant formulas in Japan are purported to contain prebiotics. *In vitro* studies measuring carbohydrate utilization patterns and the production of short-chain fatty acids and gas by infant fecal bacteria have shown that larger, more complex carbohydrates, such as polydextrose and inulin, are fermented more slowly and less completely than short-chain materials such as galactooligosaccharides, fructooligosaccharides.

Thus, a combination of specific long-chain and short-chain carbohydrates may allow for slower fermentation by fecal bacteria, a process that may translate into a more sustained effect during gastrointestinal transit. This is a desirable trait as the distal (left) side of the large intestine has low saccharolytic activity compared to more proximal areas and is also more frequently affected by intestinal disorders. Further, *in vitro* data suggest that blends of prebiotic carbohydrates would be more likely to stimulate fermentation by a broader array of gastrointestinal bacteria, resulting in greater SCFA production and reduced pH-both conditions that are considered unfavorable for pathogens (Donovan *et al.*, 2008).

Dosage of prebiotics

The recommended dose has been found to be 10g daily of fructo-oligosaccharides. Oligofructose and inulin are available in nutritional supplements and in functional foods where their dose ranges from 4 to 10 g/d (Chakraborti, 2011).

SYNBIOTICS

In the 1970s and ’80s, Japanese investigators pioneered the use of non-digestible saccharides to favorably modify the intestinal microbiota using fructo-oligosaccharides, galacto-oligosaccharides, and lactulose (Yazawa *et al.*, 1978 & Minami *et al.*, 1983 & Hidaka *et al.*, 1986).

The 1980s and ’90s saw a marked increase in the use of probiotics to favorably modify the intestinal microbiota and a concomitant growth in interest in using prebiotics to achieve the same goal. In contrast to the probiotic strategy for microflora modification by providing living microorganisms, the prebiotic strategy seeks to stimulate the growth and/or enhance the metabolic activity of the healthful bacteria already colonizing the intestines. Prebiotics offer the ability to enhance the healthful strains in a person’s unique community of bacteria including beneficial strains not available as probiotics, such as Eubacterium species (Louis *et al.*, 2007).

The use of probiotics and prebiotics to prevent colon cancer has gained much attention due to positive outcomes of both *in-vivo* and molecular studies, individually and in combination. Various mechanisms have been proposed including its anticarcinogenic effects, antimutagenic properties, modification of differentiation processes in tumor cells, production of short chain fatty acids, modification of the composition of colonic bacterial ecosystem, and alteration of tumor gene-expressions (Rafter, 2002 & Wollowski *et al.*, 2001). Synbiotics refer to nutritional supplements combining probiotics and prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound (Schrezenmeir and Vrese, 2001 & Gibson and Roberfroid, 1995). Combinational therapy stimulates growth implantation as well as increase survival and activity of probiotic compound in the presence of selective prebiotic compound. Synbiotics show their potential either by improving the viability of probiotics or by delivering specific health benefits (Sekhon and Jairath, 2010).

Synbiotic intervention of probiotic and prebiotic in cancer

It has been suggested that a combination of a probiotic and a prebiotic, termed synbiotics, might be more active than either a probiotic or prebiotic alone (Le Leu *et al.*, 2010 & Roberfroid, 1998), in preventing colon rectal cancer. In a human intervention study, several colon rectal cancer biomarkers were shown to be altered favorably by a synbiotic intervention (Rafter *et al.*, 2007). There are also several reports in experimental animals whereby a synbiotic combination showed biological and anticancer effects beyond those of the individual components (Rowland *et al.*, 1998 & Femia *et al*., 2002 & Gallaher and Khil, 1999).

Microflora assistance

Strojný *et al*. (2011) observed synergistic effect of synbiotics on intestinal lactobacilli in 1,2-dimethylhydrazine exposed rats. Experimentation was done on Wistar rats (n = 60 [5 groups of 12 subjects], mixed sex) with age of 6 months. After feeding with high fat diet (10%) for 8 weeks, cancer induction was done with application of 1, 2-dimethylhydrazine (DMH) twice a week in a dose of 20 mg/kg subcutaneously. Different groups were treated with different functional food therapies. Significantly higher ($P < 0.05$) counts of lactobacilli were determined after the application of *Lactobacillus plantarum* along with *Lini oleum virginale* and *Lactobacillus plantarum* along with inulin enriched with oligofructose (2%). The study revealed the protective and assistive nature of synbiotic combination.

Impact on colonic aberrant crypt foci in rats

Challa *et al.* (1997) performed experiment on synergism of *Bifidobacterium longum* and lactulose, for suppression of azoxymethane-induced colonic aberrant crypt foci in male Fisher 344 weanling rats. Four groups of 15 rats were fed on four different types of diets including control, probiotic only, prebiotic only and both. All animals received a s.c. injection of azoxymethane at 16 mg/kg body wt at 7 and 8 weeks of age. Colons of 10 rats from each dietary group were analyzed for aberrant crypt foci (ACF), which are preneoplastic markers. Results indicate that feeding of lactulose and *B. longum* singly and in combination reduces the number of ACF ($P = 0.0001$) and the total number of aberrant crypts significantly ($P = 0.0005$). Synergistic effect was evolved and found that was a positive correlation between higher cecal pH and number of ACF.

Rowland *et al.* (1998) studied anticancer potential of synbiotic mixture on colonic aberrant crypt foci in male Sprague-Dawley rats. They used azoxymethane as carcinogen, *Bifidobacterium longum* as probiotic and inulin as prebiotic. They performed the experiment in four groups and found that consumption of either *B. longum* or inulin was associated with a decrease (26 and 41%, respectively) in AOM-induced small ACF (i.e. those comprising 1–3 aberrant crypts per focus). Combinational approach of the bifidobacterium and inulin resulted in more potent inhibition of ACF than administration of the two separately, achieving 80% inhibition of small ACF.

Femia *et al.* (2002) performed synbiotic activity on azoxymethane-induced colon carcinogenesis in rats, using probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* and prebiotic inulin enriched with oligofructose. These studies indicate that synbiotics exert an additive antitumorigenic effect in rat colon.

Alteration of cancerous biomarkers

Rafter *et al.* (2007) upgraded their research work by encouraging cancer risk factors reduction in cancer patients and studied cancer-related biomarkers. They conducted their study on human subjects (37 colon cancer patients and 43 polypectomized patients). They obtained fecal and blood samples before, during, and after the intervention, and colorectal biopsy samples were obtained before and after the intervention. They found significant changes in fecal flora of the subjects: Bifidobacterium and Lactobacillus increased and *Clostridium perfringens* decreased. The intervention significantly reduced colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells and improve epithelial barrier function in polypectomized patients. Also, found that synbiotic prevented an increased secretion of interleukin-2 by peripheral blood mononuclear cells in the polypectomized patients and increased the production of interferon γ in the cancer patients.

Synbiotics in colitis

Synbiotics has gained success in reducing the severity of enterocolitis. Mao *et al.* proved this synergistic effect in rats. Exogenous administration of *Lactobacillus plantarum* along with oat fibres showed decreased body weight loss, intestinal permeability and increased bowel mucosal mass in enterocolitic rats. They proved that effects of synbiotic were greater with fermentation than without fermentation or prebiotic alone (Mao *et al.*, 1996).

CONCLUSION

Probiotics have the potential to be exciting ingredients for foods with a “healthy” image. Support for the health of intestinal flora can take the form of supplementation with living probiotic organisms or prebiotic substances that nourish beneficial endogenous species. Probiotics and prebiotics, alone and in combination, act as a functional barrier against colonization by pathogens, promote normal gastrointestinal function, contribute to energy production, and exert colon protective activities. The health effects attributed to the use of synbiotics are numerous. Synergistic intervention of both probiotic and prebiotic showed various remarkable health concerns, which set the stage for expanded marketing of these functional foods. In the meantime, whole change in dietary lifestyle like acquiring a balanced approach to diet with some extra emphasis on insoluble fiber intake and avoidance of heavily burnt meat is prudent for preventing colorectal diseases. Dietary management along with incorporation of functional foods in a balanced and varied diet maximizes good health.

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