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MICROECOSYSTEM OF THE LARGE INTESTINE AS A TARGET-PLACE FOR PROBIOTICS AND PREBIOTICS USED AS FUNCTIONAL COMPOUNDS OF DIET – A REVIEW

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The conditions occurring in the large intestine, including benign environment, regular supply of substrates, regular emptying, and long transit time, create the most friendly habitat for microbial growth found within the whole gastrointestinal tract. The normal adult colon contains ~200 g of digesta, of which ~60% (dry weight basis) are microorganisms. Colonic microflora comprises ~10 dominating genera reaching the mean population numbers of 10^8–10^{10} cells per gram of contents. However, they were determined in a wide range of several log cycles in individuals, significantly influenced by host diet providing colonic food as substrates for bacterial growth and development. The activity of microflora makes large intestine the organ with the greatest metabolic activity in the whole body. Close contact of a variety and multiplicity of microbes as well as microbes and host, *via* enterocytes and gut-associated lymphoid tissue, produces interaction with epithelium resulting in systemic effects. Therefore, it seems reasonable to address the colon as target-place of probiotics and prebiotics exerting microflora-mediated effects. Their implementation to more and more western-type diet may result in the improvement of the health status of the population. Moreover, they also seem to be promising in clinical cases, however that statement needs confirmation in the controlled clinical trials. The review passes around scientific knowledge on the microecosystem of colon being the basis for use of probiotics and prebiotics as functional food compounds.

INTRODUCTION

The competition amongst microorganisms and their influence and interaction with host has been evolving for millions of years. The fact that a human is able to survive in the environment captured by microorganisms is, in a significant part, owing to the ability of commensal bacteria to protect the host from microbial-induced disease processes [Reid *et al.*, 2001]. Even though up-to-now knowledge on health impact of specific gut bacteria is fairly incomplete, two genera of intestinal bacteria, *Lactobacillus* and *Bifidobacterium*, are considered as entirely beneficial for host's health and important for normal development and maturation of normal bowel functions and the systemic body functions of the host. The human gastrointestinal tract evolved to adapt to a daily supply of live lactic acid bacteria associated with plant material as a source of bacterial substrates (oligo- and polysaccharides), presumably before they were consumed with milk. Archaeological studies enable placing the invention of lactic acid fermentation since the time of ~1.5 million years ago and to associate it with the activity of *Homo erectus* already before leaving Africa [Molin, 2001]. Even though plant-derived lactic acid bacteria and non-digested oligosaccharides (NDO) have accompanied the humankind from the beginning of its development, up-to-date ideas on their healthy activities are based on observations and premises rather than reliable scientifically proved results. The critical point seemed to be a change in the perception of the large intestine as the organ destined merely for conservation of water and mineral compounds, and faeces formation. As the polysaccharides of the plant cell wall (fibre) are no longer thought of as inert material passing through the gut, the colon started to be perceived as the important fermentation reservoir supplied with nutritive substrates for intensively growing microflora which *via* fermentation and *per se* affects the intestinal ecosystem, and moreover, influences comprehensive systems of the body, *i.e.* the human health [Stephen & Cummings, 1980].

In recent years, an increased activity of food industry and food science has been observed in the concept of functional food, especially in the field of development of food supplements which are able to effectively act in the gastrointestinal tract by influencing the composition and activity of intestinal microflora. Modulation of intestinal microflora seems to be one of the most interesting and currently one of the most extensively studied direction of functional food development [Gibson & Roberfroid, 1999]. The colon and its microflora, including a variety of species – pathogenic, opportunistic, and beneficial for host's health – functions as the most metabolically active organ in the body. Therefore, the modification of the composition and some metabolic activities of the colonic microflora by probiotics or the stimulation of the growth of endogenous benign bacteria by prebiotics seems to be reasonable and relevant. Beneficial action of probiotics for host's health may cover multiply effects. Bacterial lactase improves the absorption of lactose, the transient passage of lactic acid bacteria in the digestive tract may represent a microbial barrier against the development of pathogenic bacteria; probiotics reinforce the non-specific immune defence but also specific immu-

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nity; particularly the secretory immune system mediated by secretory IgA or IgM in response to the specific infectious antigens and perhaps to soluble food antigens; other possible mechanisms include the trophic effect on the intestinal layer, and a down-regulatory activity in cow's milk allergy as well as anti-inflammatory effects [Heyman, 2000].

Unfortunately, bowel digesta do not constitute the optimal medium for faecal flora considering both pH and substrates available [Ballongue, 1997]. Carbon source constitutes the main limiting factor. The lack of a carbon source directly usable by the bacteria induces competition between the genera and the species. Incorporation of prebiotics (for example fructooligosaccharides) to the diet provides additional pool of non-digestible food compounds, moreover, substrates selectively fermented in the colon, able to modify the composition of intestinal microflora in favour of bifidobacteria [Ziemer & Gibson, 1998]. The advantage of prebiotics is the lack of the significant quantitative losses as compared to the problems with probiotic survivability during passage through the gastrointestinal tract. However, the advantage of probiotics is their use for special target purposes. Perhaps, the combined action of probiotics and prebiotics in the form of synbiotics (synergistic pairs) may bring additive effects, but it needs scientific confirmation. In the future, along with further development of genetic techniques for qualitative and quantitative determination of the variety of gut microflora, monitoring of changes influenced by the active factor will certainly facilitate the comprehensive characterisation of the health-promoting role of probiotics, prebiotics and synbiotics as functional compounds of the diet.

MICROECOSYSTEM OF HUMAN LARGE INTESTINE, PROBIOTICS, PREBIOTICS

The human gastrointestinal tract is an inhomogeneous environment containing more and less friendly niches for bacterial colonisation. The major factors determining the final composition and distribution of microflora throughout the gastrointestinal tract are: (1) host mediated factors (pH, secretions such as immunoglobulins, bile, salts, enzymes; motility *e.g.* speed, peristalsis; physiology *e.g.* compartmentalisation; exfoliated cells, mucins, tissue exudate); (2) microbial factors (adhesion; motility; nutritional flexibility; spores, capsules, enzymes, antimicrobial components; generation time); (3) microbial interactions - synergy/antagonism (metabolic co-operation/competition; growth factors and vitamin excretion; short-chain fatty acids, amines; alteration to E_h, pH, O_2 tension; antimicrobial components, siderophores; nutritional requirements); (4) diet (composition, non-digestible fibre, drugs *etc.*) [Huis in't Veld *et al.*, 1997]. On the one hand, they constitute a powerful tool for efficient controlling of microbial growth and survival, on the other hand – promote their growth, colonisation and biochemical activity.

pH. One of the most significant factors influencing the composition of microflora is the pH level of digesta, different throughout the gastrointestinal tract. The environment in the stomach is strongly acidified due to the secretion of gastric juice ~2.5 L/day, a water solution of hydrochloric acid and enzymes. In the duodenum, pancreatic juice (2.5 L/day), alkaline in nature because of a high concentration of bicarbonate ions, neutralises the digesta to the value ~6, and at the end of the small intestine the pH value is ~7.4. Further on, as a consequence of the increased bacterial activity, the pH decreases to ~5.7 in the caecum, and it is running at ~6.7 in the colon [Fallingborg, 1999]. Considering the modulating effect of diet on gastrointestinal pH, it primarily affects the colonic pH. It should not be surprising when transit time at least is taken into consideration, a few dozen longer in the colon than in the small intestine.

Colonic transit time. Small intestine is a ~4–5 m long organ of 2.5–5 cm diameter with short transit time of contents (4–5 h from mouth to the caecum). In the colon, relatively short (130–150 cm) and with 8 cm lumen, the digesta is slowly moved. Transit through different regions of the colon has been measured using radiopaque markers visible on abdominal X-ray. Reported times were 7–24 h for the caecum and right colon, 9–30 h for the left colon and 12–44 h for the recto-sigmoid region [Cummings, 1997]. The normal values of total colonic transit time reported by Bouchoucha and Thomas [2000] were 44.3±29.3 h for males, and 68.2±54.4 h for females. The values of the mean colonic transit time for humans are difficult to generalise, as they depend the most on the diet and lifestyle, and vary a lot in different regions and populations. For example, in some countries, particularly in Africa, the normal gut transit time was estimated at 24–48 h, whereas in the United Kingdom, the reported values of mean transit time (MTT) and median MTT were 70 h and 60 h, respectively, with men 55 h and women 72 h, with a wide range (95%) of 30–168 h [Cummings, 1997]. However, Antoine *et al.* [2000], studying the transit time of elderly (60–75 years old), considered total transit time 40–55 h as slow, and above 55 h as very slow.

Total colonic transit time was significantly shorter ($p<0.05$) in the non-constipated than in the constipated adolescents, 30.2±13.1 *versus* 58.3±17.4 h, respectively [Zaslavsky *et al.*, 1998]. The similar values of total colonic transit time, 37.8±6.2 h *vs.* 59.9±5.4 h, were observed in the healthy and constipated children, respectively [Bautista Casasnovas *et al.*, 1991]. When the influence of age, gender, hormonal status and smoking habits on colonic transit time in 164 asymptomatic subjects was studied, Meier *et al.* [1995] noticed that it was significantly shorter in men than in women (30±2 *vs.* 42±3 h, $p<0.05$), shorter in non-smoking males in comparison with smoking males (26±2 *vs.* 40±5 h, $p<0.05$), and in females influenced only by height and menstrual cycle.

Both diet and bacterial fermentation influence the intensity of gut transit. In the native East Africans, shorter transit time was correlated with consuming a diet high in unrefined cereals, containing fibre, especially resistant starch [Topping & Clifton, 2001]. In study on 44 volunteers, 30 g barley bran flour accelerated gastrointestinal transit and increased faecal weight [Lupton *et al.*, 1993]. The gastrointestinal transit time also tended to shorten in 5 healthy subjects given different levels of dietary fibre [Saito *et al.*, 1991]. The strains of probiotic bacteria may affect the colonic transit time in opposite directions. *B. animalis* DN-173 010 was reported to shorten the colonic transit time in healthy women

[Marteau *et al.*, 2002], whereas propionibacteria, ingested in the amount of 5×10^{10} cfu/day for 2 weeks, significantly slowed the left colon transit by av. 5 h [Bougle *et al.*, 1999].

Microflora of the gastrointestinal tract. The physiological, environmental and structural differences within the gastrointestinal tract affect the composition and numbers of microflora in the segments. In the stomach, the numbers of microflora are generally low, 10^5–10^6 /g in rats (relatively high pH), 10^3–10^4 /g in humans [Danone, 1998]. Human stomach microflora comprises mostly facultative anaerobic G(+) bacteria, like streptococci. Due to a strong bactericidal effect of stomach secretions and bile, microflora of the first segments of the small intestine is rather poor (10^4/g). Closer to distal parts, the numbers of bacteria increase to 10^6–10^7/g, along with their variability. Next to Gram-positive bacteria, some Gram-negative facultative anaerobic bacteria start to appear, like enterobacteria. In the large intestine, anaerobic environment is reflected in the oxidation-reduction (redox) potential of -200 mV [Simon & Gorbach, 1984], and strictly anaerobic bacteria are plentiful, covering over 400 species, appearing in the concentration of up to 10^{11} per g of contents (or up to 10^{12} per g of dry weight). In spite of high diversity, the populations of a dozen or so species, belonging to *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Ruminococcus*, *Peptostreptococcus*, *Peptococcus*, *Lactobacillus*, *Clostridium*, *Streptococcus* (anaerobic), *Fusobacterium*, *Methanobrevibacter* and *Desulfovibrio*, are able to colonise the gut in the highest numbers [Macfarlane & Gibson, 1994; Cummings, 1997; Salminen *et al.*, 1998; Naidu *et al.*, 1999]. Facultative anaerobic bacteria are several log cycles less numerous, being a sub-dominant microflora, mostly opportunistic. Considering anaerobic metabolism of the vast majority of colonic bacteria which do not use oxygen as a terminal electron acceptor in respiratory processes, energy generation occurs primarily through substrate phosphorylation or fermentation. Colonic bacteria are also unusually rich in protein, which comprises 55% of their dry matter, with RNA and lipid accounting for 20 and 9%, respectively [Cummings & Macfarlane, 1997]. Temperate environmental conditions, availability of nutrients, adequate morphological and physiological parameters, and slow transit of digesta enable considering the colon as a fermenter of abundant microflora living in symbiosis with a host.

The role of intestinal microflora. As the effect of reciprocal adaptation, human large intestine and microflora colonising therein constitute a complex balanced ecosystem which enables the normal functioning of the host, unless it is dominated by harmful or pathogenic bacteria. Normal intestinal microflora is responsible for proper working of mechanisms providing resistance of the host to pathogen colonisation. This important first line of defence against pathogens was described in the bibliography as *bacterial antagonism*, *barrier effect*, or *colonisation resistance* [Tancrede, 1992]. The mechanism of creating the colonisation resistance covers several activities, including competition for the limited nutrients metabolised under the condition of low redox potential and ability to utilise variable available oligosaccharides, producing the inhibitory substances by dominating bacterial populations, and bacterial adhesion to the intestinal mucosa. Therefore, under *in vivo* conditions, interactions among bacteria occur and possible results are mutual stimulation, partial or total inhibition, or exchange of genetic material. The second line of defence is mediated *via* normal intestinal microflora and enables the activation of the secretory immune system associated with gut mucosa, resulting in the secretion of antibodies of IgA class (sIgA). The dual impact of the intestinal microflora on the immune system should be emphasised. Bacteria are considered either as a source of antigens or as non-specific immunomodulators. During the first months of life, intestinal microflora, dominated by bifidobacteria, plays an important immunomodulative role regarding maturation and moulding of intestinal and systemic immune mechanisms [Koletzko *et al.*, 1998]. The normal colonisation of the mammalian intestine with commensal microbes is hypothesised to drive the development of the humoral and cellular mucosal immune systems during neonatal life and to maintain the physiologically normal steady state of inflammation (non-inflammatory immune stimulators) in the gut throughout the subsequent life [Cebra, 1999]. As the intestinal microflora drives the maturation of the immune system, changes in its composition may play a role for the higher prevalence of allergy [Björkstén, 1999]. There is accumulating evidence that host microbial populations may significantly contribute to the pathogenesis of autoimmune diseases.

Despite the fact that the complex microflora contains potentially pathogenic bacteria, in the state of health, both host and microflora exist in harmony [Raibaud, 1992]. However, the outer factors, like invasive bacteria, antibiotic treatment, radiotherapy, marked change of diet, or stress, may disturb homeostasis of the normal microflora, which facilitates multiplying and even overgrowth of potentially pathogenic bacteria, expression of toxicity, and may cause infection or even acute or chronic diseases [Van Laere *et al.*, 1997]. *Clostridium difficile*, an indigenous colonic bacteria, was linked to pseudomembranous colitis as the primary causative agent, however, infection occurs due to the disruption of normal gut flora homeostasis arising from antibiotic treatment [Barbut & Petit, 2001]. Acute inflammatory reactions may cause diarrhoea and can be manifested by a number of bacterial genera, including *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, *Clostridium* and *Escherichia* [Macfarlane & Gibson, 1995]. Due to the complexity of intestinal microflora, the correlation between concrete bacterium and disorder is sometimes difficult to prove. As a result of various epidemiological studies, bacterial aetiology of inflammatory bowel disease, including ulcerative colitis and Crohn's disease, was ascribed generally to the functionally altered resident flora, and particularly to clinical isolates of *Mycobacterium paratuberculosis* and *Listeria monocytogenes*, or increased antibody responses to *Peptostreptococcus*, *Eubacterium*, *Coprococcus*, *Bacteroides* and *E. coli* in the case of Crohn's disease, and *Desulfovibrio* (sulphate-reducing bacteria), *Bacteroides*, *Streptococcus*, *Escherichia*, *Fusobacterium*, or *Shigella* in the case of ulcerative colitis, however their influence remains unproven [Sartor, 1997; Ziemer & Gibson, 1998; Farrell & Pepercorn, 2002; Loubinoux *et al.*, 2002]. Probiotic attempt to modify disease by favourable altering of bacterial composition, immune status, and inflammation may provide a simple and

attractive way of treatment [Gionchetti *et al.*, 2002], however clinicians urge for well-designed, randomised clinical studies to define the role of probiotics as therapeutic agents in inflammatory bowel disease [Tamboli *et al.*, 2003].

Probiotics. The term *probiotic* is derived from Greek and means *for health*. The definition of probiotics has been developed within years reflecting our understanding of mechanisms of affecting the human health and well-being. The most widely used one has been proposed by Fuller [1989] who called probiotic *a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance*. Yet, for the purposes of human nutrition, Salminen *et al.* [1998] suggested that probiotic is *a live microbial food ingredient that is beneficial to health*. However, when the mechanisms of probiotic action were focused on the role of concrete cellular substances, the definition covering live and not-live probiotics arose as follows: *probiotic is a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract* [Naidu *et al.*, 1999]. Recently, Schrezenmeir and de Vrese [2001] revised the existing definitions, describing probiotic as a *preparation of or a product containing* viable*, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonisation) in a compartment of the host and by that exert beneficial health effects in the host*.

Probiotic bacteria are commercially available mainly in fermented foods, especially dairy products. They belong mainly to the *Lactobacillus* and *Bifidobacterium* genera, considered to be safe [Adams & Marteau, 1995]. The current evidence suggests that probiotic effects are strain specific, therefore probiotics are identified at the strain level, and therefore beneficial results obtained for one strain cannot be extrapolated to other strains, even closely related ones. In the selection of probiotic strains several aspects should be taken into consideration, including: (1) safety – origin from healthy host, non-pathogenicity, antibiotic resistance; (2) functionality – viability and persistence in the GI tract, immunomodulation, antagonistic and antimutagenic properties; and (3) technological characteristics – ability to be manufactured under industrial conditions, necessity to survive and retain their functionality during storage, not producing off-flavours [Saarela *et al.*, 2000]. The guidelines for the evaluation of probiotics for food use were prepared by a Joint FAO/WHO Working Group [Report, 2002]. The protocol covers: strain identification by phenotypic and genotypic methods (genus/species/strain classification, strain should be deposited in the international culture collection); safety assessment *in vitro* and/or on animals and next in Phase 1 (safety) human study, along with functional characterisation using *in vitro* tests and in animal studies; and double blind, randomised, placebo-controlled (DBPC) Phase 2 (efficacy) human trial or other appropriate design with sample size and primary outcome appropriate to determine if strain/product is efficacious, with results preferably confirmed in the second independent DBPC study. The resulting probiotic food should be labelled detailing contents – genus, species, strain designation, minimum numbers of viable bacteria at the end of shelf-life, proper storage conditions and corporate contact details for consumer information.

Ingestion of probiotics resulted in several beneficial effects, both demonstrated and proposed, as: increased nutritional value (better digestibility, increased absorption of minerals and vitamins), alle*via*tion of lactose intolerance, positive influence on intestinal flora, prevention of intestinal tract infections, enhancement of the immune system, reduction of inflammatory reactions, prevention of cancer, anti-allergic activity, regulation of gut motility, reduction of serum cholesterol, prevention of osteoporosis, and improved well-being [Vaughan *et al.*, 1999]. Among numerous health-promoting effects attributed to the use of probiotics, Schrezenmeir and de Vrese [2001] distinguished the following well documented ones: (1) lower frequency and duration of diarrhoea associated with antibiotics (*Clostridium difficile*), rotavirus infection, chemotherapy, and to a lesser extent, traveller's diarrhoea; (2) stimulation of humoral and cellular immunity; and (3) decrease in unfavourable metabolites, *e.g.* ammonium and procancerogenic enzymes in the colon.

The most widely reported probiotic strains are *Lactobacillus rhamnosus* GG (Valio); *L. johnsonii* LJ-1, previously *L. acidophilus* LC1 or La1 (Nestle); *L. acidophilus* NCFB1748 (Rhodia); *L. casei* Shirota (Yakult), *L. plantarum* DSM9843 (299v) (Probi), *L. reuteri* MM53 (BioGaia Biologics), *L. casei* CRL431 (Chr. Hansen), *L. casei* DN114 (Danone), *L. gasseri* ADH, *Bifidobacterium bifidum* H1, *B. lactis* Bb-12 (previously *B. bifidum* Bb-12), *B. longum* BB536, *Saccharomyces boulardii* (*Saccharomyces cerevisiae* Hansen CBS 5926).

The minimum effective dose of probiotics is not precisely known. The oral administration in excess of 10^9 cfu per day is recommended to show a health effect, although dose response studies are often not included in clinical evaluations of probiotics [Sanders & Huis in't Veld, 1999]. A comparison of survival rate of properly selected probiotic strains, between 10–40%, and their counts in faecal samples suggests that they may be capable for growth in the intestinal tract, however the further research is essential to clarify this issue.

Substrates available for colonic microflora. The colonic microecosystem is characterised by a high fermenting capacity. Almost all bacteria dominating in the colon show saccharolytic potential (except for *Fusobacterium*), and some of them are amino acid fermenters. However, only two genera, *Bifidobacterium* and *Ruminococcus*, are able to degrade mucins, *i.e.* produce α-glycosidases specific for oligosaccharide chains of gut mucin glycoproteins [Falk *et al.*, 1998]. However reciprocal action exists, as acidification of large intestinal contents and production of short-chain fatty acids from carbohydrate fermentation were reported to stimulate mucus synthesis and mucus secretion [Meslin *et al.*, 1999]. The total amount of substrates reaching the colon per day was estimated at 20–60 g of carbohydrates and 5–20 g of protein. Daily amounts of substrates potentially available for colonic microflora were estimated at 5–35 g of resistant starch, 10–25 g of non-starch polysaccharides, 2–8 g of oligosaccharides, 2–5 g of sugars and sugar alcohols, unpredictable amount of synthetic carbohydrates (lactulose, polydextrose, pyrodextrins, modified celluloses), 1–12 g of dietary protein (Nx6.25), 4–8 g endogenous protein from pancreatic enzymes and other secretions, 0.5 g of

urea and nitrate, and unknown amounts of others, derived with mucus (3–5 g ?), bacterial recycling, sloughed epithelial cells and organic acids [Cummings, 1997]. The above estimations may constitute the basis for establishing the level of *e.g.* prebiotic supplementation/modification of diet. The above saccharide concentrations are not minimal, compared to the composition of bacterial nutrient media. The crucial difference is in type, as in contrast to easily and rapidly utilised mono- or disaccharides in media, mainly polysaccharides are carbohydrates able to avoid digestion and absorption in the small intestine. Their utilisation as substrates for colonic microflora depends on chemical structure, composition of monomer units, degree of polymerisation, possible linear or branched structure, and water solubility [Van Laere *et al.*, 1997]. Generally, saccharides with short and unbranched chains, and soluble in water are better fermented.

Dietary fibre. Colonic fermentation of different substrates *via* produced short-chain fatty acids affects the bowel structure and physiology. Moreover, it may bring some beneficial effects to the host. Some groups of colonic saccharides, like dietary fibre or prebiotics, were specified due to their effects. The principal acknowledged beneficial effects of dietary fibre were included in the recently proposed definition, read as follows: *dietary fibre consists of: carbohydrate polymers (DP≥3) of plant origin, which may or may not be associated in the plant with lignin or other non-carbohydrate components (polyphenols, waxes, saponins, cutin, phytates, phytosterols, etc.) OR carbohydrate polymers (DP≥3), processed (by physical, enzymatic or chemical means) or synthetic, included in the attached list whose contents may change on the basis of AFSSA recommendations. IN ADDITION dietary fibre is neither digested nor absorbed in the small intestine. It has at least one of the following properties: increase stools production, stimulate colonic fermentation, reduce prepprandial cholesterol levels, reduce post-prandial blood sugar and/or insulin levels* [Champ, 2002]. In parallel, the claims 'source of fibre' corresponding to a quantity of 3 g/100 g or 1.5 g/100 kcal, and 'rich in fibre' corresponding to a quantity of 6 g/100 g or 3 g/100 kcal were proposed for foods. Some foods, like the wholemeal bread, muesli, fresh not peeled apple, fresh orange pulp, strawberry, cooked lentils, cooked carrots, raw lettuce, cooked zucchini, are able to fulfil these criteria and can be recognised as rich in fibre.

The group of compounds covered by dietary fibre definition is not homogeneous due to functionality. Non-digestible oligosaccharides, just since 1980, were recognised as components of 'functional foods', as they reach the colon undegraded and provide a carbohydrate substrate particularly suited to the growth of bifidobacteria, which was regarded as beneficial to health [Playne & Crittenden, 1996]. Low calorie, prevention of tooth decay, intestinal control by enhancement of bifidobacteria, and dietary fibre-like effects are considered to be the main physiologically functional effects of NDO. The most intensive research on functional food properties of NDO has been focused on their prebiotic effect in human subjects, impact on bowel habit, increased calcium absorption, functioning of lipid metabolism and prevention against colon cancer [Van Loo *et al.*, 1999]. Another constituent, resistant starch (RS), especially that retrograded, may be distinguished by butyrogenic and bifidogenic properties. Brouns *et al.* [2002] suggested that increasing the RS content in the daily diet will be of benefit to the maintenance of gut health and to the reduction of risk factors associated with the development of intestinal inflammation and colorectal cancer. The proposed mechanism of RS action may include: stimulation of the local immune system (GALT), modulation of blood immune parameters, modulation of DNA synthesis and repair by butyrate, inhibition of abnormal cell growth and development by butyrate, and promotion of recovery from epithelial inflammation.

Prebiotics. Some non-digestible oligosaccharides and non-starch polysaccharides of dietary fibre recognised as capable to modify the composition of endogenous gut microflora and characterised with specific bifidogenic attributes were named prebiotics [Delzenne & Roberfroid, 1994]. According to the definition, a prebiotic is *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, and thus improves host health* [Gibson & Roberfroid, 1995]. The targeted bacterial genera are indigenous *Bifidobacterium* and *Lactobacillus*, as markers of healthy microflora [Van Loo *et al.*, 1999]. Prebiotics of proven efficacy that are commercially available are fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose. They all preferentially promote bifidobacterial growth in the large intestine. Many other prebiotic candidates are currently studied, including soybean oligosaccharides, glucooligosaccharides, maltooligosaccharides, and sugar alcohols (lactitol, xylitol, maltitol). Some authors consider also one type of resistant starch, namely high amylose maize starch, as a prebiotic able to promote the survival of lactic acid bacteria [Topping & Clifton, 2001]. However, the interaction of RS with the microflora in general remains to be elucidated.

The established effects of prebiotics include non-digestibility and low energy value, a stool bulking effect and modulation of the gut microflora, promoting bifidobacteria and repressing clostridia [Rastall & Gibson, 2002]. Postulated areas to be developed in the future might include prevention of intestinal disorders (*e.g.* ulcerative colitis, irritable bowel syndrome) and gastrointestinal infections including diarrhoea, modulation of the immune response, prevention of colon carcinogenesis, reduction in serum triacylglycerols and cholesterol and the improvement of bioavailability of minerals such as calcium and magnesium. Up-to-date, the greatest scientific interest has been focused on the nutritional and health benefits of oligofructose and inulin [Kleessen *et al.*, 1997; Roberfroid & Milner, 1999; Rao, 2001; Biedrzycka & Bielecka, 2004].

In populations consuming a Western-type diet, the intake of fructans has been estimated in the range of 1–4 g/day [Van Loo *et al.*, 1995]. Although no recommendation for daily dose of prebiotics exists, values from 3 to 20 g/day were shown to be effective. Non-digestible oligosaccharides and sugar alcohols may retain water and thus increase stool weight, whereas high amounts of these compounds, especially of short chain length, may cause diarrhoea, due to the increased osmotic pressure. However, as constipation is one of the most prevalent gastrointestinal complaints in the Western world, mildly laxative effect may be appreciated.

Hata and Nakajima [1985] determined the maximum effective dose without diarrhoea at ~21–24 g/day of short chain FOS, and dose with diarrhoea experienced by 50% of the tested subjects at ~50–55 g/day of short chain FOS. They also demonstrated that sorbitol caused diarrhoea at much lower doses. However, comparing lactulose and lactitol, possible side-effects of using lactulose, like nausea, vomiting, abdominal cramps, and diarrhoea, were not associated with lactitol [O'Sullivan, 1996].

When higher doses of low polymerised fructooligosaccharides (DP 2–4) were consumed, undesired side effects, like excessive flatus (>30 g FOS/day), borborygmi and bloating (>40 g/day), lastly, abdominal cramps and diarrhoea (50 g/day), were observed [Briet *et al.*, 1995]. When inulin with high chain length (DP 25) was administered to healthy volunteers in the amount of 15 g/day, the incidence (number of days per week) of abdominal cramps or flatulence observed in inulin *vs.* placebo groups was 1.2 *vs.* 0.0 or 0.8 *vs.* 0.0, respectively, however, the differences did not reach significance [Den Hond *et al.*, 2000].

Rao [2001] showed that even a dose of oligofructose, close to the minimum effect level deduced by means of a meta-analysis [Roberfroid *et al.*, 1998], 5 g/day, still has a significant bifidogenic effect. He also confirmed a hypothesis that the initial count of bifidobacteria, and not just the dose of oligofructose, is the influential factor in determining the relative increase in bifidobacteria, as it was adversely related to their initial count [Rao, 2001]. Therefore, elderly people and formula-fed infants, with significantly decreased colonic populations of bifidobacteria, may constitute the promising target groups for efficient prebiotic action. Kleessen *et al.* [1997] observed that inulin given to 10 elderly persons suffering from constipation in a dose of 20–40 g/day for 19 days significantly increased bifidobacteria by 1.3 log cycle and decreased enterococci in the number and enterobacteria in frequency, showed a better laxative effect than lactose and reduced functional constipation with only mild discomfort. As to prebiotics for infants, on the basis of scientific data on tolerance and effectiveness of fructooligosaccharides (FOS, oligofructose) and galactooligosaccharides (GOS), the Scientific Committee on Food at the European Commission [2001a, 2001b] accepted both oligosaccharides for use in follow-on formulae intended for older infants, in a concentration of up to 0.8 g/dL in the product ready for consumption (September 27, 2001), and next (December 14, 2001), extended the acceptance for a combination of 90% GOS and 10% oligofructose in infant formulae (aged between 0 and 6 months) and follow-on formulae (age >6 months) in the concentration as above.

Synbiotics. The consumption of appropriately selected probiotics as well as prebiotics may enhance the beneficial effect of each of them. The synbiotics, as a combination of probiotics and prebiotics, have not been extensively studied so far (reviewed recently by Rastall and Maitin [2002]). They stimulate indigenous bifidobacteria but also can improve the survival of the bacteria crossing the upper part of the gastrointestinal tract, thereby enhancing their effects in the large bowel [Fooks *et al.*, 1999; Roberfroid, 2000]. Perhaps individual advantages of probiotic and prebiotic component might be additive or even synergistic but this statement needs to be qualified. Generally, there are sparse studies on the development of synbiotics from properly selected probiotics and prebiotics, followed by the examination of their *in vivo* effectiveness.

CONCLUDING REMARKS

Due to species and metabolic diversity and complexity, the human colonic microecosystem plays a crucial role in host health. Probiotics and prebiotics, as functional food compounds, are used to control infections, protect against disease and maintain gut functions. They both optimise bodily function of the host influencing the composition of the colonic microflora and through microflora-mediated effects. The effects of probiotics in some of these conditions have been directly observed, in others they have been only suggested on the basis of *in vitro* studies and from experimental animal models. The official demands for probiotics are crystallising, including the confirmation of efficiency of probiotic strains with fully characterised properties in properly controlled, randomised clinical trials. Emphasis is placed on measurable effects. Special attention should be paid to the probiotic specialisation due to different age populations (especially children, aged people), immunoaltered patients and particular diseases. The same populations with altered microflora are promising target groups for prebiotics. The mechanisms of prebiotic action against colorectal cancer and irritable bowel disease, in human lipid metabolism and enhancement of mineral absorption still remains to be elucidated. However, the ground for functionality studies is a thorough knowledge of the colonic microecosystem. New advances may bring development and application of molecular methods for qualitative and quantitative determination of intestinal microflora and its functionality.

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