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Role of Lactic Acid Bacteria and Bifidobacteria in Synbiotic Effect

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Dedicated to the memory of Professor Vera Johanides

Summary

Some genera of lactic acid bacteria and bifidobacteria are the main subjects of this review because they are most commonly incorporated in probiotic products. Since these bacteria are also indigenous to the colon, a strategy for increasing their numbers and/or activity is the use of prebiotics, non-digestible oligosaccharides, that stimulate autochthonous and allochthonous (probiotic) bacteria. At the same time, the potential for using probiotics and prebiotics in combination was recognised and the term synbiotic was proposed for products containing both supplements.

Key words: lactic acid bacteria, bifidobacteria, probiotic, prebiotic, synbiotic

Introduction

In the last few years great attention was dedicated to probiotics and prebiotics or their combined use (synbiotics) in improvement of human health in a natural way. Some genera of lactic acid bacteria and genus *Bifidobacterium* make an extremely important group of probiotic bacteria. As members of the autochthonous microflora of the gastrointestinal tract of humans and animals, they offer considerable potential as probiotics because of their history of safe use and the general body of evidence that supports their positive roles (*1–4*). Namely, probiotics are microorganisms which had been included in food for many years without any adverse effects or which were present in the gastrointestinal tract of the healthy humans and animals (*5*).

Two separate approaches exist to increase the number of health-promoting organisms in the gastrointestinal tract. The first is the oral administration of live beneficial microorganisms. At present, these microorganisms, called probiotics, have been selected mostly from lactic acid bacteria and bifidobacteria that form a part of the normal intestinal microflora of humans, since these organisms are indigenous to the colon. Another strategy for increasing their number is to supply those already present in the intestine with selective carbon and energy source that provides them with competitive advantage over other bacteria in this ecosystem, thus selectively modifying the composition of the microflora using dietary supplements. These selective dietary components were named »prebiotics« by Gibson and Roberfroid in 1995 (*6*). They are specific, naturally occurring carbohydrates, mainly of plant origin, and fructo-oligosaccharides (FOS) are mostly known representatives.

The concept of »synbiotics« (a mixture of probiotic and prebiotic) has recently been proposed to characterise health-enhancing food and supplements used as functional food ingredients in humans (*6–8*). From the

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ongoing research more of promising potential health effects of synbiotics, are being observed (*9–13*).

Probiotic Concept

Definition of probiotics and selection criteria for their choice

The term »probiotics« was introduced by Lilly and Stillwell in 1965 (*14*) for growth promoting factors produced by microorganisms. The word »probiotic« is derived from Greek and means »pro life«. In 1974, Parker (*15*) used the term for »organisms and substances« which influenced the intestinal microflora and had beneficial effects on animals. The term »substances« is imprecise and would include even antibiotics. Therefore, in 1989 Fuller (*16*) defined »probiotics« as »a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance«. However, according to this definition probiotics were restricted to feed supplements, animals and the intestinal tract, and the term »probiotic« thus could not be used for living microorganisms administered in any other way than in food or feed, or for locations other than the gastrointestinal tract. Consequently, in 1992 Havenaar and Huis in't Veld (*17*) proposed to broaden Fuller's definition into »a probiotic is a mono- or mixed culture of live microorganisms which, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora«.

Probiotic strains can be used only, and are active only, on or in the body of the host if they fulfil a large number of criteria. The criteria for the selection and assessment of probiotic microorganisms were the result of the collaboration of research institutions and universities with food industries. The list of properties expected from potential probiotic strains of lactic acid bacteria, compiled by several authors (*1,18–22*) are:

- accurate taxonomic identification
- normal inhabitant of the species targeted: human origin for human probiotics
- nontoxic and nonpathogenic
- genetically stable
- capable of survival, proliferation, and metabolic activity at the target site
- adherance and colonization potential preferred
- stability of desired characteristics during culture preparation, storage, and delivery
- viability at high populations preferred at $10^6 - 10^8$
- production of antimicrobial substances, including bacteriocins, hydrogen peroxide, and organic acids
- antagonistic toward pathogenic/cariogenic bacteria
- able to compete with the normal microflora, including the same or closely related species; potentially resistant to bacteriocins, acid, and other antimicrobials produced by residing microflora
- resistant to bile
- resistant to acid
- immunostimulatory
- able to exert one or more clinically documented health benefits
- amenable to production processing: adequate growth, recovery, concentration, freezing, dehydration, storage, and distribution
- provision of desirable organoleptic qualities (or no undesirable qualities) when included in fermented products.

The intestinal microflora and its importance

The importance of an indigenous microflora in the gastrointestinal tract as a natural resistance factor against potential pathogenic microorganisms was already recognised in the 19th century by Metchnikoff during his research on cholera (*23*). Many decades later, the crucial work was done in the development of this concept after findings in laboratory where animals were orally treated with antibiotics (*24–26*). They showed that by administering antibiotics to experimental animals *per os*, they could render mice more susceptible to infection with *Salmonella typhimurium*, *Shigella flexneri* and *Vibrio cholerae*.

One of the most convincing proofs of the importance of the intestinal microflora in resistance to diseases was provided by Collins and Carter in 1978 (*27*). They showed that the germ-free guinea-pig was killed by 10 cells of *Salmonella enteritidis* but it required 10^9 cells of this bacterium to kill a conventional animal with complete intestinal microflora (*28*).

It is now recognised that the indigenous microflora of humans and animals provide protection against infections with pathogenic microorganisms. This phenomenon is often called »bacterial antagonism« (*26*) »barrier effect« (*29*), »competitive exclusion« (*30*) or widely used term »colonization resistance« (*31*).

Savage (*32*) has observed that about 90 % of 10^{14} cells associated with the human body are microorganisms, and that the vast majority of these are bacteria residing in the colon. Viable bacterial counts in excess of 10^{11}/g dry weight were found in colonic contents. More than 400 bacterial species have been identified in the faeces of a single individual (*33–35*). The vast majority of these microorganisms are anaerobes, but they exhibit varying degrees of tolerance towards oxygen, ranging from relatively oxygen tolerant bacteroides (*36*) and bifidobacteria, to very strictly anaerobic methanogenic bacteria (*37*). Anaerobic bacteria outnumber aerobic species approximately by the factor of 1000. Gram-negative rods belonging to the genus *Bacteroides* are the predominant bacteria in the colon (*38*). The other main groups consist of Gram-positive rods and cocci. Many of them are lactic acid bacteria (genera *Lactobacillus*, *Streptococcus*, *Peptostreptococcus*) and bifidobacteria (*39*). The intestinal microflora has significant influence on its host as it has been observed in experiments in which the germ-free (absence of a microflora) and conventional (presence of a microflora) animals were compared (*40–42*). These comparisons showed that many biochemical, physiological and imunological characteristics of an animal host are strongly influenced by the presence of the intestinal microflora (*42*). The ability to compete for limiting nutrients and possibly for adhesion sites on food particles or on the colonic mucosa is likely to be the most important factor that determines the composition of intestinal microflora. Species that are unable to compete successfully are rapidly eliminated from the intestinal ecosystem (*39*). This leads to a consideration of the substrates (prebiotics) that are available for the growth of beneficial autochthonous and allochthonous (probiotic) bacteria in the colon.

Disturbance of intestinal microflora

Although the composition of the intestinal microflora is rather stable in healthy individuals, it can be altered by many endogenous and exogenous factors (Table 1).

Table 1. Factors affecting disturbance of intestinal microflora

Exogenous	Endogenous
antibiotic therapy	nutrient availability
excessive hygiene	types of diet
emotional stress	pH value of intestinal lumen
ageing	redox potential
travelling	diarrhoea
peristaltic disorders	bacterial antagonism
surgical operations	bacterial co-operation
liver or kidney diseases	mucin
radiation therapy	lysozyme
chemotherapy	defensins
pernicious anemy	
disorders of immune system	

Disturbances in intestinal ecosystem are generally characterised by a remarkable increase in bacterial counts in the small intestine, by an increase of aerobes, mostly *Enterobacteriaceae* and streptococci, by the reduction or disappearance of bifidobacteria and/or often by the incidence of *Clostridium perfringens* (*43*).

These evidences would suggest that the loss of indigenous microorganisms implies deregulation of autogenic factors and vacated habitats. Consequently, commensal or transient microorganisms have chance to take possession of these vacant niches. If these microorganisms are potentially pathogenic, the outbreak of an opportunistic infectious disease is quite possible (*17*).

Whereas excessive hygiene prevents the acquisition of a protective microflora, oral antibiotics suppress its activity even after it has been acquired. Thus diarrhoea is a common side-effect of *per os* antibiotic treatment. The disease pseudomenbranous colitis is almost always associated with administration of antibiotics *per os*, and *Candida* infections are often an unpleasant consequence of antibiotic therapy (*5,16,28*).

Disturbance of intestinal microflora can also be due to stress (*44*). During stress conditions the number of lactobacilli decreases and the number of enterotoxigenic strains of *E. coli* increases. Stress can be produced by drastic changes in the physical or emotional environment from which hormonal changes ensue and can affect the production of mucus, which may in turn reduce some groups of microflora beneficial members associated with it. The inevitable stress that accompanies space flight and the preparation for it is associated with a change in the lactic acid bacteria present in intestinal environment (*45,46*). Salminen *et al.* (*47*) published the results confirming that some strains of lactic acid bacteria may provide protection against traveller's diarrhoea.

In addition, a layer of mucus covers the entire surface of digestive tract. It forms a barrier protecting the delicate underlying cells from damage by digestive enzymes, acid, abrasion and pathogenic bacteria. The major antimicrobial compounds of the mucus are mucins, lysozyme and defensins (*48,49*), a group of small antimicrobial peptides possessing broad-spectrum activity against bacteria, fungi, protozoa and viruses.

The mucins are huge glycoprotein molecules comprising a protein backbone covered in oligosaccharide chains. In the colon especially, many of these mucin oligosaccharides chains are normally sulphated and they have host-protective role. The Mucin and Anaerobic Microbiology Research Group (*50*) have discovered and purified a mucin-desulphating sulphatase that removes sulphate from the mucin oligosaccharide chains. The enzyme is produced by an anaerobic bacterium (*Prevotella* sp.) isolated from colonic mucosa. They have found that the activity of mucin-desulphating enzymes increases markedly in ulcerative colitis. All of these compounds (mucins, lysozyme, defensins) produced by small and large intestinal epithelial cells contribute substantially to host protection and in many cases are responsive to alterations of the intestinal microflora.

All these conditions, where the balance of the gut microflora was disturbed, are situations in which probiotic microorganisms can have significant effect on its re-establishment.

Lactic acid bacteria and bifidobacteria as probiotics

Physiological and taxonomical characteristics of lactic acid bacteria and bifidobacteria

Traditionally, the lactic acid bacteria are defined by formation of lactic acid as a sole or main end-product from carbohydrate metabolism. Lactic acid bacteria comprise a diverse group of Gram-positive, non-spore forming bacteria. They occur as cocci or rods and are generally lacking catalase, although pseudo-catalase can be found in rare cases. They are chemoorganotrophic and grow only in complex media. Fermentable carbohydrates are used as energy source. Hexoses are degraded mainly to lactate (homofermentatives) or to lactate and additional products such as acetate, ethanol, CO_2, formate or succinate (heterofermentatives). Lactic acid bacteria are found in foods (dairy products, fermented meat, sour dough, fermented vegetables, silage, beverages), on plants, in sewage, but also in the genital, intestinal and respiratory tracts of humans and animals (*51,52*).

Modern classification mainly based upon comparative sequence analysis of 16S ribosomal ribonucleic acid (16S rRNA), determined phylogenetic relationships of lactic acid bacteria and bifidobacteria (53). Based on 16S and 23S rRNA sequence data, Gram-positive bacteria form two lines of descent. One phylum consists of Gram-positive bacteria with a DNA base composition of less than 50 mol % guanine plus cytosine (G+C), the so-called *Clostridium* branch, whereas the other branch (*Actinomyces*) comprises organisms with a G+C content that is higher than 50 mol %. The typical lactic acid bacteria such as genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Streptococcus* and *Carnobacterium* have a G+C content of less than 50 mol % and belong to the *Clostridium* branch. Originally, the genus *Bifidobacterium* was considered to be a member of the lactic acid bacteria, but based on the high DNA G+C content (55 – 67 %) and from 16S rRNA data, it is now quite clear that bifidobacteria belong to the *Actinomyces* branch (*51*). Physiologically, bifidobacteria resemble genuine lactic acid bacteria since they are saccharoclastic organisms that produce lactic acid and acetic acid without generation of carbon dioxide, except during degradation of gluconate (*54*).

Progress in understanding of the conservative nature of specific macromolecules (16S rRNA) allows one to treat the lactic acid bacteria and bifidobacteria under the aspects of a systematic taxonomy. This enables us to combine the well-known physiological and biochemical data with phylogenetic ones in a polyphasic approach for arranging of the hitherto confusing multitude of the species of the lactic acid bacteria and bifidobacteria (*53,55,56*).

Probiotic activity mechanism of lactic acid bacteria and bifidobacteria

The scientific basis for the development of probiotics is in their protective role in the host (humans and animals) against colonisation of intestinal tract by non-indigenous microorganisms. The mechanism of probiotic action is still unknown but different approaches could be developed. According to Fuller (*16,57*) and Huis in't Veld and Havenaar (*58*) probiotic effect of lactic acid bacteria and bifidobacteria may be expressed by three main mechanisms of action:

1. Suppression of pathogenic microorganisms in intestinal tract by:
   a) production of antibacterial substances including primary metabolites, such as lactic acid, acetic acid, carbon dioxide, diacetyl, acetaldehyde, hydrogen peroxide (*59–61*) and bacteriocins which are proteinaceous compounds that manifest antimicrobial activities against other closely related bacteria (*62, 63*);
   b) competition for nutrients. In discussion about the large intestine Freter *et al.* (*64*) have stated that competition for limiting nutrients (specific carbohydrates) is one of the determining factors that has received the greatest scientific support;
   c) competition for adhesion receptors on the gut epithelium. Probiotic strains can adhere specifically or non-specifically. Specific adhesion occurs when an adhesin on the bacterial cell binds to a receptor on the epithelial cell, which is often defined as a lock and key function. Non-specific adhesion is a more general phenomenon mediated by hydrophobic or electrostatic interaction. Non-specific adhesion may not have any significance in the colonisation of epithelia *in vivo*, but may possibly be important in the colonisation of luminal contents. For example, non-specific adhesion may enhance substrate uptake and thus enforces growth (*65*).
2. Alteration of microbial metabolism in intestinal tract:
   a) increasing the activity of useful enzymes, *e.g.* β-galactosidase in the alleviation of lactose maldigestion in lactose-intolerant people (*66*);
   b) decreasing the activity of some colonic enzymes such as β-glucuronidase, β-glucosidase, nitroreductase, azoreductase and steroid-7α-dehydroxylase known to have carcinogenic effect (*67,68*).
3. Stimulation of immunity

Recent reports have shown that orally administered lactobacilli can improve immune status by increasing circulating and local antibody levels, gamma interferon concentration, macrophage activity and the number of natural killer cells (*69*). The entry of lactic acid bacteria as members of physiological indigenous microflora into the mucosa and subsequent translocation to other organs is currently regarded as a crucial step for the development of the normal mucosal and systemic immunity (*70,71*).

Categories 2 and 3 include such purported health benefits as reductions in large bowel (colon) carcinogens and mutagens (*72*), antitumor properties (*73,74*), cholesterol-lowering effects (*75*), increased lactose digestion (*66,76*), relief from constipation (*77*), stimulation of immunocompetent cells (*78*) and enhancement of phagocytosis (*79*).

On the basis of the above-mentioned probiotic activity mechanism of lactic acid bacteria and bifidobacteria there is some evidence for their beneficial effect on human health. According to Rowland (*80*), beneficial effects claimed for probiotics belong to five areas with varying degrees of experimental support and those are: alleviation of lactose intolerance, preventive and therapeutic effects against diarrhoea, effects on the immune system, plasma cholesterol lowering and prevention of cancer.

Prebiotic and Synbiotic Concept

Prebiotic and synbiotic definition

According to many authors (*68,81–83*), the low fibre diets of industrial societies are causative factors in the development of civilisation diseases like constipation, obesity, haemorrhoids, diverticulosis, cardiovascular disease, diabetes, colon cancer and others, whereas the better colonic activity achieved by high fibre diets protects against these diseases and can even cure some of them.

Taking into consideration that many potentially health-promoting microorganisms, such as lactic acid and bifidobacteria, are already resident in the human colon, Gibson and Roberfoid (*84*) have introduced the prebiotic concept. According to them 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, that can improve the host health«. Like probiotics, the prebiotics belong to a more general class of »colonic foods«, *i.e.* »foods entering the colon and serving as substrates for the endogenous colonic bacteria, thus indirectly providing the host with energy, metabolihc substrate and essential micronutrients« (*84*). However, the relationship between the change in the number per gram of faeces of a particular bacterial species or strain and the dose of the prebiotic substrates is not yet clear. Indeed the initial number of the bacteria in faeces before any intake of the prebiotics seems to be a key parameter determining the multiplication factor (inverse relationship with the dose of the prebiotic), as well as the absolute increase in the number of bacteria (direct relationship) (*85,86*). For a food ingredient to be classified as a prebiotic, it must:

1. neither be hydrolysed nor absorbed in the upper part of the gastrointestinal tract;
2. be a selective substrate for one or a limited number of potentially beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated;
3. consequently, be able to alter the colonic microflora towards a healthier composition, for example by increasing the number of saccharolytic species and reducing putrefactive microorganisms such as asaccharolytic clostridia and *Enterobacteriaceae* (*86,87*).

According to Crittenden (*88*), the prebiotic approach for increasing beneficial bacteria in the colon potentially provides some advantages over the probiotic strategy. Namely, consumed probiotic bacteria must survive transit through the hostile conditions in the stomach and then adapt quickly to their new environment (survivability and colonisation may be a problem). On the contrary, prebiotics offer not only the potential to increase the number of beneficial bacteria, but also their metabolic activity through the supply of fermentable substrate. The increase in metabolic activity of autochthonous or allochthonous (probiotic) microorganisms is fundamental to many of the currently proposed mechanisms of health promotion by prebiotics (Fig. 1).

Further step in the development is the use of synbiotics, where probiotics and prebiotics are combined. Thus, the living microbial additions would be used in conjuction with a specific substrate for growth. For example, the combination of a fructose-containing oligosaccharide with *Bifidobacterium* strain is a potentially effective synbiotic, as is the use of lactilol or lactulose in conjuction with lactobacilli (*68*).

The results of many researches (*8–13*) point to a synergistic effect of probiotic and prebiotic combination on faecal microflora of experimental animals. This effect was demonstrated by increased total anaerobes, aerobes, lactobacilli, and bifidobacteria counts as well as by decreased clostridia, *Enterobacteriaceae* and *E. coli* counts. The combination of probiotics and non-digestible carbohydrates may be a way of stabilisation and/or improvement of the probiotic effect. Such synbiotics indicate a realistic way of using biological preparations in the prevention of gastrointestinal diseases in humans and animals.

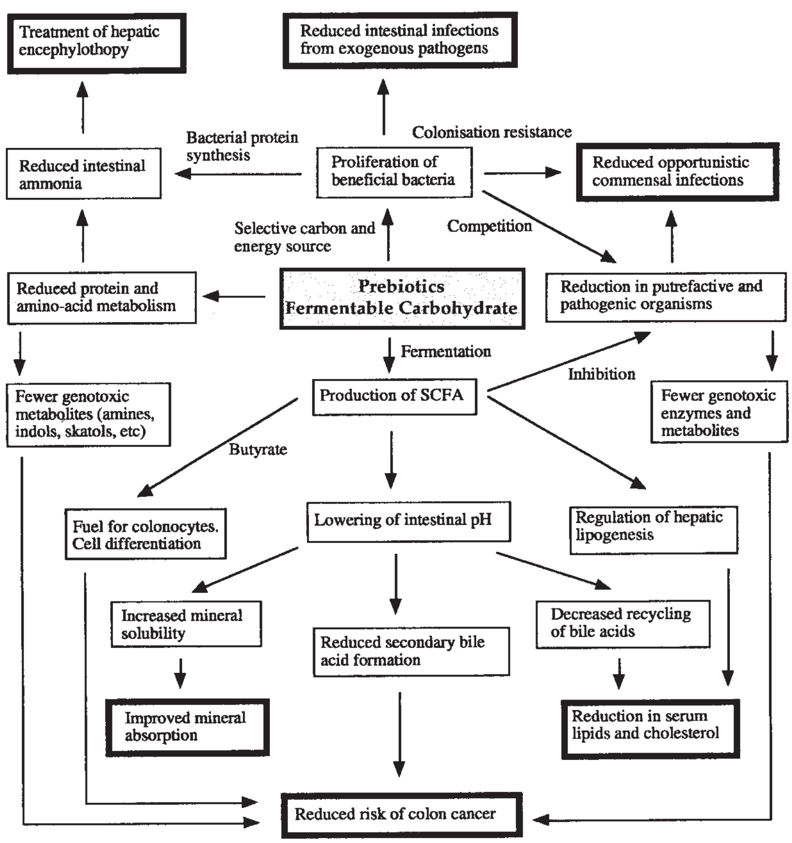

Fig. 1. Proposed mechanisms of prebiotic action to improve human health; figure by courtesy of Dr. R. G. Crittenden (*88*)

Table 2. Types of prebiotic substrates and their chemical composition

Type	Chemical composition
POLYOLS (sugar alcohols)	
Xylitol	$C_5H_{12}O_5$
Sorbitol	$C_6H_{14}O_6$
Mannitol	$C_6H_{14}O_6$ (stereoisomer of sorbitol)
DISACCHARIDES	Synthetic derivatives of lactose:
Lactulose	4-*O*-β-D-galactopyranosyl-D-fructose
Lactitol	4-*O*-β-D-galactopyranosyl-D-glucitol
OLIGOSACCHARIDES	
Raffinose	(α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru)
Soybean oligosaccharides (raffinose + stachyose)	raffinose: (α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru) + stachyose: (α-D-Gal-(1→6)-α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru)
Fructo-oligosaccharides (FOS)	(α-D-Glc-(1→2)-[(β-D-Fru-(1→2)-]^n n = 2-4
Oligofructose (mixture of oligosaccharides)	(α-D-Glc-(1→2)-[(β-D-Fru-(1→2)-]^n n = 2-6 and β-D-Fru-(1→2)-[(β-D-Fru-(1→2)-]^n n = 1–6
Galacto-oligosaccharides (TOS – transgalactosylated oligosaccharides)	(α-D-Glc-(1→4)–[(β-D-Gal-(1→6)-]^n n = 2–5
OTHER NON-DIGESTIBLE OLIGOSACCHARIDES	
Palatinose	
Isomalto-oligosaccharides	
Lactosucrose	
Galactosyl lactose	
POLYSACCHARIDES	
Inulin	α-D-Glc-(1→2)–[(β-D-Fru-(1→2)-]^n
Resistant starch (starch modifications that are resistant to the host endogenous glycolytic enzymes)	$(C_6H_{10}O_5)_n$

Types of prebiotic substrates and their effect on the composition of the intestinal microflora

Among the large number of bacterial species present in the colon, three groups can be distinguished. The first one is the beneficial group, consisting of bifidobacteria, lactobacilli and other genera of the lactic acid bacteria. These bacteria are thought to be health improving. The second group comprises *Enterobacteriaceae* and species from genus *Clostridium*, which are both considered negative for general health. The third group comprises all the other bacteria, and are considered neutral (*89*).

Any fermentable dietary component that arrives undigested in the colon has the potential to act as a prebiotic. To the present day almost all prebiotics described and all those produced commercially have been carbohydrates. These range from small sugar alcohols and disaccharides, to oligosaccharides and large polysaccharides (all with a variety of sugar compositions and glycosidic linkages) which are presented in Table 2. Such a diverse range of chemical structures would be expected to provide an equally diverse range of effects on the colonic microflora (*88*).

Data from *in vitro* studies with batch culture fermenters demonstrated that fructo-oligosaccharides were specifically fermented by bifidobacteria (*90*). This was subsequently confirmed in human volunteer trial using both oligofructose and inulin at a level of 15 g per day (*91*), where feeding fructo-oligosaccharides to healthy human volunteers caused bifidobacteria to become the numerically predominant bacterial genus in faeces (*87*). The ability to efficiently utilise such a variety of substrates indicates that bifidobacteria possess an array of glycosidases, making them nutritionally versatile and allowing them to adapt and compete in an environment with changing nutritional conditions (*88*). Most of the bifidobacteria grow more rapidly on prebiotic fructo-oligosaccharides than on glucose (*90,92*). Other non-digestible oligosaccharides that may have prebiotic potential include raffinose and stachyose, as well as those that contain xylose, galactose and maltose (*93*). In particular, feeding 5 % (w/v) galacto-oligosaccharides to human microflora associated rats has been shown to significantly increase populations of bifidobacteria and lactobacilli, while decreasing enterobacteria (*94*).

As important as the impact of prebiotics on selective proliferation of beneficial microbial populations is their influence on metabolic activity of the microflora. Prebiotics may stimulate autochthonous bacteria not only to grow, but also to produce compounds beneficial to the host. Their colonic fermentation produces short chain fatty acids (SCFAs) and lactic acid, which are important factors determining the pH of the colonic lumen. More than 300 mmol of SCFA are produced daily, representing the predominant luminal anion in the colon (*95*). The three main SCFA, acetate (60 %), propionate (20 %), and butyrate (18 %) are taken up by colonocytes and actively metabolised (*96*). The SCFA have a strong effect on the metabolism of the host (*97*). Acetate and propionate are gluconeogenic and influence cholesterol production (*98*). Butyrate is a major source of energy for colonic epithelial cells (*99*), and low concentrations cause differentiation of mammalian cells as well as colon carcinoma cells (*100,101*). At the present time, there is consequently great interest in the metabolic functions of butyrate, and it is the subject of considerable research activity in the prebiotic concept.

Prebiotics have generally been observed to increase lactate and acetate concentrations suggesting fermentation by lactic acid bacteria and bifidobacteria (*8,102–107*). However, propionate and butyrate are also produced during prebiotic fermentation indicating that other members of the microflora also utilise these substrates (*108,109*). Furthermore, it has recently been observed that the longer the chains of non-digestible carbohydrates, the slower they are fermented. The longer chains thus allow stimulation of bacterial metabolism in a more distal part of the colon, whereas the short chains are readily fermented in the proximal part of the colon. Typically, the distal part of the colon is very much energeticaly depleted. Bacteria are starving, and the proteolysis of dead cells and the subsequent strictly anaerobic fermentation of the released amino acids result in production of (cytotoxic) putrefactive metabolites (*9*). Therefore, the balance between »fermentation« (forming SCFAs) and »putrefaction« (forming phenolic compounds – skatol, indole and cresol) is very important for the prebiotic concept (*42,102*).

Conclusion

The use of both probiotics and prebiotics will probably increase dramatically worldwide because of strong commercial interests in providing these supplements to both humans and animals. Therefore, it is important to underline when considering the effectiveness and biological activity of probiotics, prebiotics or their combination (synbiotics) that they are food products and not drugs. Furthermore, in many cases, their effects are mainly prophylactic in nature, rather than therapeutic, *i.e.* preventive rather than curative.

Besides, modern concept of the intestinal microflora composition and microflora – host relationships, accurate identification of intestinal bacteria and potentially probiotic bacteria using 16S rRNA technology combined with the polymerase chain reaction will enlarge to possibilities for monitoring accuracy of probiotic and prebiotic efficacy.

With the advent of the »functional food« concept, it is clear that there is an important niche for the pro-, pre- and synbiotic approaches. The implantation of live bacteria into the human or selective increase of certain bacterial genera resident therein is a functional claim. However, more rigorous research is required before health claims gain improved scientific credibility.

At the end let us remember the great Metchnikoff who wrote in the beginning of the last century:

»A reader who has little knowledge of such matters may be surprised by my recommendation to absorb large quantities of microbes, as a general belief is that microbes are harmful. This belief is erroneous. There are many useful microbes, amongst which the lactic bacilli have an honourable place« (*110*).

This »honourable« place of lactic acid-producing bacteria in human health should not get lost because of high commercialisation of probiotic products with insufficient scientific credibility about presence and activity of these bacteria. Therefore, the scientific community must be responsible to the public whom it serves and should act as gatekeepers or watchdogs in their health protection.

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Uloga bakterija mliječne kiseline i bifidobakterija u sinbiotičkom učinku

Sažetak

Neki rodovi bakterija mliječne kiseline i bifidobakterije su glavna tema ovoga preglednog rada jer se one najčešće nalaze u probiotičkim proizvodima. Budući da su te bakterije i vrlo korisni sudionici autohtone mikroflore debeloga crijeva, korištenje prebiotika, tj. slaboprobavljivih oligosaharida koji neprobavljeni dospijevaju u debelo crijevo i tamo služe kao supstrat za te bakterije, učinkovit je način stimulacije rasta i metaboličke aktivnosti autohtono prisutnih ili unesenih, alohtonih (probiotičkih) bakterija. Istodobno, kombinirana primjena probiotika i prebiotika prepoznala se kao mogućnost sinergističkog učinka, te je za takve proizvode, koji sadržavaju oba dodatka, predložen naziv sinbiotik.