USING PREBIOTICS TO IMPROVE HUMAN HEALTH

UPOTREBA PREBIOTIKA ZA UNAPREĐENJE ZDRAVLJA LJUDI

MATIJEVIC, Bojan

Abstract: *Functional food is considered as acceptable way to improve health and prevent diseases, and includes products that contain probiotics and prebiotics with target action on gut microflora. Prebiotics are obtained from plants or they can be synthesized from starch and other carbohydrates using appropriate enzymes. Bacteria of Laktobacillus spp. and Bifidobacterium spp. in intestines ferment prebiotics and make various metabolites and biologically active compounds that favourably affect the condition of the colon, absorption of minerals, immune system, lipid metabolism, and they have anticancer activity. This paper shows different types of prebiotics and results of research on their impact on health which has been conducted last ten years.*

Key words*: prebiotics, probiotics, functional foods, therapeutic effects*

Sažetak: *Funkcionalna hrana je prihvatljiv način za poboljšanje zdravlja i sprečavanja bolesti, a uključuje proizvode koji sadrže probiotike i prebiotike s ciljanim djelovanjem na mikrofloru crijeva. Prebiotici su dobiveni iz biljaka ili se mogu sintetizirati iz škroba i drugih ugljikohidrata pomoću odgovarajućih enzima. Bakterije iz roda Laktobacillus i Bifidobacterium u crijevima fermentiraju prebiotika i proizvode različite metabolite i biološki aktivnih tvari koje povoljno utječu na stanje debelog crijeva, apsorpcija minerala, imunološki sustav, metabolizam lipida i imaju antikancerogeno djelovanje. Ovaj rad prikazuje različite vrste prebiotika i rezultate istraživanja o njihovom utjecaju na zdravlje u posljednjih deset godina.*

Ključne riječi: *prebiotici, probiotici, funkcionalna hrana, zdravstveni učinci*

Authors´ data: Bojan, **Matijevic**, Ph.D., Senior Lecturer, Karlovac University of Applied Sciences, Karlovac, Croatia, bojan.matijevic@vuka.hr

1. Introduction

During last twenty years, more and more attention has been paid to production of food which directly and/or indirectly influences human health. That fact is result of a number of different types of research which have enabled better understanding of biochemistry, molecular biology, physiology and pathology, and they also support the hypothesis that food controls and directs various functions in the body and thus affects health of entire organism [1]. This type of food is called functional food. By its definition, functional food, beside its basic nutritive value, also contains biologically active ingredients, which have positive effect on one or more target functions in the body. Biologically active ingredients can be a natural food ingredient, or food can be enriched with an ingredient during the process of production [2] [3]. Functional food also includes products which contain probiotics and prebiotics with target action on intestinal microflora [4] [5]. Aim of this paper is to show various types of prebiotics and to point out the most important results of research about their influence on health: improvement of colon condition and absorption of minerals, strengthening of immune system, influence on metabolism of lipids and anticancer effect, which have been defined during last ten years.

2. Importance of probiotics and prebiotics for intestinal microflora

Human colon is a specific ecosystem, where, in perfect natural balance, a few hundred, so far isolated and described, microbial species live in concentration higher than 10^{12} organisms per a gram of total solid [6]. This big microbial biomass and its metabolic activity are not without any influence, since they can have both positive and negative effects on healthy people. The latest research has relieved that lactobacilli and bifidobacteria are the most numerous anaerobic bacteria in colon microflora [7]. In healthy persons, intestinal microflora content is stable in most cases, and it depends on individual physiology. However, different endogenous and exogenous factors (getting old, curing, stress, nutrition and other factors form the environment) can cause imbalance. Changed intestinal microflora is characterized with higher number of aerobic bacteria, mainly enterobacteria and streptococci, and number of lactobacilli and bifidobacteria is becoming less [6]. Regardless the cause, the most common consequence of those disorders is diarrhea, of which almost 4 billion people in the world suffer every year [8]. It is possible to get higher number of useful microorganisms in intestine by oral intake of probiotics and/or prebiotics.
Chosen types of *Lactobacillus* spp., less types of *Bifidobacterium* spp. [9] [10] are mostly used as probiotics, but other lactic acid bacteria also can be used as probiotics. Although there are different opinions, it is supposed that presence of minimum 10^6 CFU/mL of each probiotic bacteria or g of product is necessary to obtain desired effect, but to obtain progress in health, above limits of normal nutrition [11], it is necessary to take at least 10^9 CFU [12] [13]. It is possible to obtain increased number of useful microorganisms by introducing selected sources of carbon and energy, which provide them competitive advantage to other bacteria in intestine. In that way intestinal microflora is modified by using food additives called prebiotics [14].

Prebiotics are more useful than probiotics for regulation of intestinal microflora as they can be added in different food products and they pass through upper part of digestive system unchanged [15]. Some prebiotics are isolated from natural sources such as chicory, onion, artichoke, asparagus, bananas, garlic and soya. However, thanks to industrial processes, production of prebiotics as fructo-, galacto- and xylooligosaccharides has been developed, and they are commercially available and being added into different food products. Bacteria in intestine such as *Lactobacillus* spp. and *Bifidobacterium* spp. have enzymes necessary to use prebiotic substrates by which their successful propagation is enabled as well as formation of metabolites useful for health [16]. That is why, importance of prebiotics has been recognized, both independently and together with probiotic bacteria as synbiotics, for human health improvement [17].

3. Criteria used to choose prebiotics

Prebiotic is defined as indigestible food component, which has favorable effect on the host by selective stimulation of growth and/or activity of one or limited number of bacteria in colon, which improves health of the host [5]. Similarly to probiotics, prebiotics belong to the class ”food of colon’’, that is, food that entered the colon and serves as substrate of colon endogenous bacteria which indirectly supplies the host with energy, metabolic substrates and essential micronutrients [15]. Prebiotic effect can be attributed to a lot of food components such as oligosaccharides and polysaccharides, including also diet fibers. However, not all indigestible food components are prebiotics. If a food component is categorized as prebiotic, it has to fulfill certain conditions [16], such as:

1. it must not hydrolyze, and it does not absorb in the upper part of digestive system
2. it selectively stimulates growth of potentially useful bacteria in colon
3. it prevents growth of pathogens and virulence, inducing systemic effects which can be useful for health.

It is often hard to fulfill and prove all mentioned conditions, and it is made by in vitro and in vivo tests. Much of the early literature describes tests performed on pure cultures. Typically, this involves the selection of a range of strains of *Bifidobacterium* spp., *Lactobacillus* spp. and other representative bacteria such as *Bacteroides* spp., *Clostridium* spp. and *Escherichia coli.* A carbohydrate is usually judged to be prebiotic if species of bifidobacteria (for example) metabolise the oligosaccharides more efficiently than other bacteria. The problem with this approach is, of course, that the strains selected cannot truly be considered as representative of the colonic microbiota. Such studies cannot establish that the test substrate is selectively metabolised and should be used for initial screening purposes only [17]. A more meaningful in vitro method for studying prebiotic oligosaccharides is the use of mixed culture. Study of the changes in populations of selected genera or species can then establish whether the fermentation is selective. The use of faecal inocula probably gives a representation of events in the distal colon. However, more proximal areas are likely to have a more saccharolytic nature and both the composition and activities of the microbiota indigenous to the colon is variable dependent upon the

region sampled. Tests which are carried out in vitro on animals provide only approximate data (such as formation of short-chain fatty acids). They cannot be representative at humans because of different morphology of digestive system comparing to experimental animals [16] [18].

4. Types of prebiotics

Chemically prebiotics are mostly of carbohydrate composition, which can be made of small sugar alcohols, disaccharides, oligosaccharides, and polysaccharides, with different composition of sugar and glycosidic bonds. Stability of prebiotics depends on presence of other sugars residues, ring shape (pentose and hexose), configuration and type of bond of monosaccharide units. Generally, β-bond is stronger than α-bond, and also hexoses are strongly linked than pentoses [19]. Different chemical structures of prebiotics do not necessarily mean different effect on colon microflora [20]. Literature states a number of different prebiotics which are commercially available and which selectively stimulate growth of bifidobacteria in colon, and they are called bifidogenic or bifidus factors [18] [21] [22] [23].

5. Therapeutic effects of prebiotics

5.1. Improvement of the colon

Direct therapeutic effect of prebiotics on health is improvement of the colon. Prebiotics as “food of colon” stimulate growth of bacteria in digestive system and increase of volume of feces. Prebiotics which completely ferment in intestine can increase feces for about 1.5 to 2 g/g of prebiotic, and degradation products stimulate peristalsis [16].

Positive effect of prebiotics is shown in people who suffer from chronic constipation. They come to the colon undigested and they affect metabolism. That causes changes in microflora and prevents infection. Research, done on people who travel to countries of high risk, also shown that intake of prebiotics can reduce the incidence of diarrhea by 50% [24]. In a randomized, controlled study, oligofructose and/or galactooligosaccharides were shown to have an effect on relapse of *Clostridium difficile* - associated diarrhoea [25]. Oligofructose administration to young childrens attending day care centre increased *Bifidobacteria* and decreased a potential pathogens, such as clostridia. These effects on colon microflora were accompanied by less flatulence, diarrhoea, and vomiting [26].

5.2. Improvement of minerals absorption

Research shows that prebiotics improve absorption of minerals from food. Most studies show that when prebiotics are taken, Ca, Mg, Fe and Zn are absorbed well, but not heavy metals such as Cu or Hg [16]. That directly affects density and structure of bones. It is interesting that all prebiotics do not stimulate absorption of minerals. Prebiotics which have big molecular mass and which consist of 12 to 65 monosaccharides units are more efficient. Better effect was obtained with β (2→1) fructosyl bond (Synergy 1) in which ratio of short (DP 2-8) and long (DP 12-65)

chains is 1:1 [24]. Research conducted on animals and humans shows different results in stimulation of absorption of minerals from food. Prebiotics with low DP equally well stimulate absorption of Mg at both humans and animals, but they do not stimulate absorption of Ca. However, when prebiotics with high DP were used, absorption of Ca was better [16]. Influence of prebiotics on absorption of minerals can be explained with presence of more acid which converts minerals into soluble form. Formation of butyrates, a selective source of energy for intestinal cells, stimulates absorption of mucosal cells. Some explains that it is because of increased activity of substances responsible for transfer of Ca (calbidin) in colon [16].

5.3. Anticancer activity

Carcinoma of the colon or rectum causes death in 50% of cases. There are a number of causes of this disease, but diet is of big importance. People who intake big amount of indigestible fibres with food, influence the reduction in risk of this disease. Prebiotics in intestine are decomposed by probiotic bacteria which make butyrate and with that they influence the ways of carcinoma development [28]. Butyric acid is used by the epithelial cells of the colon mucosa as energy source, being in addition a growth factor. Recent preclinical studies have reported that butyrate might be chemopreventive in carcinogenesis or protector agent against colon cancer by promoting cell differentiation. Research about influence of prebiotic chicory fructans on mice determined a reduced amount of nitrogen compounds which cause cancer [28]. In different animal models, inulin and/or oligofructan diet was found to suppresses azoxymethane-induced large intestine tumor at the promotion stage in young rats tumor and 5 to 15% supplementation of inulin or oligofructose lowered breast tumors incidence in rats and mice and metastasis in the lung. In addition, combination of probiotic *Lb. rhamnosus* and *Bifidobacterium animalis* subsp. *lactis* with inulin enriched with oligofructose was shown to display an antitumorigenic action on azoxymethane-induced colon carcinogenesis in rats.

5.4. Stimulation of immune system

The functional foods are reported to enhance the immunity of the consumers. Indeed, the dietary components and their fermentation metabolites are in closely contact with the gut associated lymphoid tissue (GALT) which is the part of the vast intestinal immune system. The presence of food in the small intestine may be necessary for adequate function and development of GALT. Although, no information is available on how host organisms recognize ingested prebiotics in the process of expressing the immunomodulating effects and subsequent events. Another mechanism on immunity system is assumed that innate defence responses can be activated through the interaction of sugar moieties with innate receptors on the plasma membrane of host cells, in particular in macrophages and dendritic cells.

Research shows that inulin increases phagocytic capacity of macrophages [22] and IgA is much more secreted in intestine [31]. Furthermore, inulin showed an anti-inflammatory effect on distal colitis induced in rats by dextran sodium sulphate and improves lesions of the intestinal mucosa. At mice infected with *Listeria* or

Salmonella bacteria, a significant difference was observed when they, in diet, used fructans which are degraded slowly [16].

5.5. Influence on metabolism of lipids

It is considered that metabolism of lipids can be influenced by formation of propionate. Propionate can be formed by decomposition of prebiotics in intestine, and it is absorbed and comes to the liver where it regulates expression of genes which are coded for the occurrence of digestive hormones GLP1, GPI and insulin. Research conducted at humans gives different results. Three of nine clinical studies with inulin and oligofructose supplementation in human volunteers in both normo- and moderately hyperlipidaemic conditions showed no effect on serum levels of cholesterol or triacylglycerol, three have shown significant reductions in triacylglycerol, whereas in four investigations the triacylglycerol and total cholesterol concentration and/or the total and LDL-cholesterol concentration were significantly lowered [30]. These differences can be explained by complexity of metabolic way of decomposition of lipids at people, which complicates research and affects the results [16] [31] [32] [33].

5.6. Prebiotics – future research

Clinical trials to determine the value of prebiotics in managing specific gut mediated disorders are ongoing, as is the use of a quantifiable index to compare efficacy. More human trials are required to prove effect and identify definitive health promoting activities and mechanisms behind them. These should be hypothesis driven and well controlled. Trials in patients suffering from, and/or at risk of, clinical disorders are currently sparse. Varying expertise and techniques now exist however. Exploitation of the latest technologies and collaboration from various disciplines will help to identify outcomes. There are several avenues of research that can be further exploited for prebiotic use. These include [17] [34]:

- Increased functionality. For example, the incorporation of anti-adhesive capacities against gut pathogens and their toxins.
- Preferred use in food products and perhaps defined products for particular target groups (e.g. infants, elderly, different countries, frequent travellers, institutionalized persons, those at particular risk of infection).
- Differential, species level, effects – should individual species of bifidobacteria/lactobacilli resident in the gut be seen to be more beneficial than others.
- Distal colon delivery – as most chronic disease of the colon arise distally, it would be of value to target this region of the bowel.
- Defined health outcomes and mechanisms – a collaborative effort has produced reliable research tools to determine prebiotic efficacy. With further moves into the post-genomic era, more mechanistically driven studies in humans are feasible. These will be hypothesis driven and exploit new approaches such as microarray technology, metabolomics, proteomics. Good biomarkers of effect are already evident (microflora changes, metabolic end product formation).

7. Conclusion

Balanced intestinal microflora is important for human health protection. However, influence of different exogenous and endogenous factors can disrupt intestinal microflora. Increase of useful microorganisms in intestine is possible by oral intake of probiotics and/or prebiotics. Use of prebiotics is more rational approach in regulation of intestinal microflora comparing to probiotics as they can be added to various food and they come into intestine unchanged. Lactobacili and bifidobacteria in intestine ferment prebiotics and make short-chained fatty acids, acetate, propionate, butyrate and biologically active compounds which favorably affect the colon, absorption of minerals, immune system, metabolism of lipids and they have anticancer effects. During last ten years various prebiotics can be found at the market (sorbitol, lactulose, raffinose, fructo-oligosaccharides, inulin), which are added during the process of food production, and sometimes they are combined with probiotic bacteria. However, it has to be pointed out, that functional characteristics of that food still are not very clear. A number of tests done in vitro and in vivo did not give complete and clear evidence how changes in balance and activity of gastrointestinal microecology, caused by application of prebiotics, affect human health. Future research will give more complete answers and at the same time give consumers a guarantee that consuming food with prebiotics means effective way to improve general health.

8. References

[1] Šušković, J. (2009). Why is it established the concept of functional foods?, *Proceedings of 2nd symposium Functional foods in Croatia*, pp. 2-15, Croatia, april 2009, Croatian Chamber of Economy, Zagreb

[2] Siró, I., Kápolna, E., Kápolna, B. & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance - A review, *Appetite*, 51, 456 – 467, ISSN 1095-8304

[3] Donini, L.M., Savina, C. & Cannella, C. (2009). Nutrition in the elderly: role of fiber, *Archives of Gerontology and Geriatrics*, 49 (1), 61 – 69, ISSN 0167-4943

[4] Jones, P.J. & Jew, S. (2007). Functional food development: Concept to reality, *Trends in Food Science & Technology*, 18 (7), 387 – 390, ISSN 0924-2244

[5] Gullón B., Gullón, P., Sanz, Y., Alonso, J.L. & Parajó, J.C. (2011). Prebiotic potential of a refined product containing pectic oligosaccharides, *LWT - Food Science and Technology*, 44 (8), 1687 – 1696, ISSN 1096-1127

[6] Walter, J. (2005): The Microecology of *Lactobacilli* in the Gastrointesinal Tract, In: *Probiotics and Prebiotics: Scientific Aspects*, Tannock, G.W. (Ed.), pp. 51-82, Caister Academic Press, ISBN 1-904455-01-8, Norfolk, USA

[7] Falagas, M.E., Petros, I.R. & Makris, G.C. (2008). Bacterial interference for the prevention and treatment of infections, *International Journal of Antimicrobial Agents*, 31 (6), 518 – 522, ISSN 0924-8579

[8] Samaržija, D., Tudor, M., Prtilo, T., Dolenčić-Špehar, I., Zamberlin, Š. & Havranek, J. (2009). Probiotic bacteria in prevention and treatment of diarrhea, *Mljekarstvo*, 59 (1), 28 – 32, ISSN 0026-704X

[9] Gillian, E., Gardiner, G.E., Ross, P.R., Kelly, P.M., Collins, J.K., Fiztgerald & G. (2002). Microbiology of therapeutic milks, In: *Dairy microbiology handbook*, 3rd edition, Robinson, R.K. (Ed.), pp. 431-478, John Wiley and Sons, Inc., ISBN 0471227560, New York, USA

[10] Prado, F.C., Parada, J.L., Pandey, A. & Soccol, C.R. (2008). Trends in non-dairy probiotic beverages, *Food Research International*, 41 (2), 111 – 123, ISSN 0963-9969

[11] Tamime, A. Y., Božanić, R. & Rogelj, I. (2003). Probiotic fermented dairy products, *Mljekarstvo*, 53 (2), 111 – 134, ISSN 0026-704X

[12] Walstra, P., Wouters, J.T.M. & Geurts, T.J. (2006). *Dairy Science and Technology*, 2nd ed., Taylor and Francis Group, ISBN 0824727630, London, UK

[13] Lacroix, C. & Yildirim, S. (2007). Fermentation technologies for the production of probiotics with high viability and functionality, *Current Opinion in Biotechnology*, 18 (2), 176 – 183, ISSN 0958-1669

[14] Huebner, J., Wehling, R.L. & Hutkins, R.W. (2007). Functional activity of commercial prebiotics, *International Dairy Journal*, 17 (7), 770 – 775, ISSN 0958-6946

[15] Vernazza, C.L., Rabiu, B.A. & Gibson, G.R. (2006). Human colonic microbiology and the role of dietary intervention: Introduction to prebiotic, In: *Prebiotics: Development & Application*, Gibson, G.R., Rastall, R.A. (Eds.), pp. 1-28, John Wiley & Sons, Ltd., ISBN 0470023139, West Sussex, England

[16] Van Loo, J. (2004). Prebiotics promote good health: The basis, the potential and the emerging evidence, *Journal of Clinical Gastroenterology*, 38, S70 – S75, ISSN 0192-0790

[17] Leach, J.D., Rastall, R.A. & Gibson, G.R. (2006). Prebiotics: Past, Present and Future, In: *Prebiotics: Development and Application*, Gibson G.R., Rastall, R.A. (Ed.), pp. 237-248, John Wiley & Sons Ltd, ISBN 978-0-470-02313-6, West Sussex PO19 8SQ, England

[18] Harish, K. & Varghese, T. (2006). Probiotics in humans-evidence based review, *Calicut Medical Journal*, 4 (4), 1 – 11, ISSN 0972-951

[19] Wang, Y. (2009): Prebiotics: Present and future in food science and technology, *Food Research International*, 42, 8 – 12, ISSN: 0963-9969

[20] Mussatto, S.I. & Manchilha, I.M. (2007): Non-digestible oligosaccharides: A review, Carbohydrate Polymers, 68 (3), 587 – 597, ISSN 0144-8617

[21] Šušković, J., Kos, B., Goreta, J. & Matošić, S. (2001): Lactic Acid Bacteria and Bifidobacteria in Synbiotic Effect, *Food Technology and Biotechnology*, 39 (3), 227 – 235, ISSN 1330-9862

[22] Lina, B.A.R., Jonker, D. & Kozianowsky, G. (2002). Isomaltulose (Palatinose): A review of biological and toxicological studies, *Food and Chemical Toxicology*, 40 (10), 1375 – 1381, ISSN 0278-6915

[23] Kelly–Quagliana, K.A., Nelson, P.D. & Buddington R.K. (2003): Dietary oligofructose and inulin modulate immune functions in mice, *Nutrition Research*, 23 (2), 257 – 267, ISSN 0271-5317

[24] Qiao, H., Duffy, L.C., Griffiths, E., Dryja, D., Leavens, A., Rossman, J., Rich, G., Riepenhoff-Talty, M. & Locniskar, M. (2002). Immune responses in rhesus rotavirus-challenged Balb/c mice treated with bifidobacteria and prebiotic supplements, *Pediatric Research*, 51 (6), 750 – 755, ISSN 1530-0447

[25] Lewis, S., Burmeister, S., & Brazier, J. (2005). Effect 1229 of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhoea: a randomized, controlled study, *Clinical Gastroenterology and Hepatology*, 3, 442–448, ISSN 1542-3565

[26] Coudray, C., Tressol, J.C., Gueux, E. & Rayssiguier, Y. (2003). Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats, *European Journal of Nutrition*, 42 (2), 91 – 98, ISSN 1436-6215

[27] Bullock, N.R. & Jones, M.R. (2006). Dietary Intervention for Improving Human Health: Chronic Disorders. In: *Prebiotic: Development and Application*, Gibson, G. R., Rastall, R. A. (Eds.), pp. 181-199, John Wiley & Sons, Ltd., ISBN 978-0-470-02313-6, West Sussex, England

[28] Min-Tze Liong (2008). Roles of Probiotics and Prebiotics in Colon Cancer Prevention: Postulated Mechanisms and In-vivo Evidence, *International Journal of Molecular Sciences*, 9, 854 – 863, ISSN 1422-0067

[29] Hosono, A., Ozawa, A., Kato, R., Ohnishi, Y., Nakanishi, Y., Kimura, T. & Nakamur, R. (2003). Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cell, *Bioscience Biotechnology & Biochemistry*, 67(4), 758 – 764, ISSN 0916-8451

[30] Thammarutwasik, P., Hongpattarakere, T., Chantachum, S., Kijroongrojana, K., Itharat, A., Reanmongkol, W., Tewtrakul, S. & Oraikul, B. (2009). Prebiotics – A Review, *Songklanakarin Journal of Science and Technology*, 31, 401 – 408, ISSN 0125-3395

[31] Delzenne, N.M., Neyrinck, A.M., Bäckhed, F. & Cani, P.D. (2011). Targeting gut microbiota in obesity: effects of prebiotics and probiotics, *Nature Reviews Endocrinology*, 7, 639 – 646, ISSN 1759-5029

[32] Cani, P.D., Dewever, C. & Delzenne, N.M. (2004). Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats, *British Journal of Nutrition*, 92, 521 – 526, ISSN 1475-2662

[33] Waligora-Dupriet, A.J., Campeotto, F., Nicolis, 1597 I., Bonet, A., Soulaines, P., Dupont, & C., Butel, J. (2007). Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre, *International Journal of Food Microbiology*, 113, 1, 108-13, ISSN 0168-1605

[34] Candela, M. Maccaferri, S. Turroni, S., Carnevali, P., Brigidi, P. (2010). Functional intestinal microbiome, new frontiers in prebiotic design, *International Journal of Food Microbiology*, 140, 93 – 101, ISSN: 0168-1605

Photo 070. Clouds / Oblaci