

## PROBIOTICS IN HUMAN NUTRITION - PLENARY LECTURE

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The paper reviews the human intestinal microflora and the effects of probiotics on human microecology. The aspects of probiotic strain selection as well as implications of such selection for health and improved quality of life were taken into consideration.

### INTRODUCTION

The word “probiotic” originates from Greek “pro bios” which means “for life”. According to the FAO/WHO definition, probiotics are live microorganisms which, when administered in adequate amounts, exert a health benefit on the host [FAO/WHO Report, 2001]. The site of action of probiotics administered in the form of food is the human gastrointestinal tract, and especially the large intestine. Already in the early twentieth century, in 1908, Eli Metchnikoff, a Nobel Prize winner, emphasised the significance of adequate composition of intestinal microflora for human health. He wrote in his book “Prolongation of life” published in 1907: “The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes” [Metchnikoff, 1907]. Thus, Metchnikoff associated the condition of human health with the presence of specific microorganisms in the gastrointestinal tract. He paid particular attention to lactic fermentation bacteria present in such products as kefir or yoghurt. Since then, the expansion of studies characterising the human intestinal microflora and its effect on health as well as the development of production of so-called functional foods containing health-promoting bacteria or substances stimulating the growth of such bacteria have been observed.

### INTESTINAL MICROFLORA

Up to 1000 different species of microorganisms, at least 50% of which we are unable to cultivate *ex vivo*, i.e. under laboratory conditions, may live in the intestines of different humans. These microorganisms adhere to the intestinal epithelium and are present in the intestinal contents. The total biomass of intestinal microorganisms attains about 1.5-2 kilograms. The highest bacterial concentration is found in the

colon where  $10^{10}$ – $10^{12}$  cells are found per 1 gram of faeces, constituting about 60-80% of its dry mass. Strictly anaerobic bacteria, represented both by Gram-positive as well as Gram-negative strains, predominate numerically in the typical large intestinal microflora of a healthy adult human. Although a few hundred different species are found, in principle about 30–40 of them constitute microflora typical for this environment (about 99% of the total population), with such main genera as *Eubacterium*, *Bacteroides*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus* or *Clostridium*. The number of *Bacteroides*, *Eubacterium* or *Bifidobacterium* species bacteria predominating in the intestines attains the level of  $10^{10}$ – $10^{11}$  cells per 1 gram of intestinal contents. The number of strictly anaerobic bacteria exceeds the number of facultatively anaerobic bacteria 100 to 1000 times [Salminen *et al.*, 1998; Kleessen *et al.*, 2000; Hooper & Gordon, 2002].

Intestinal microflora performs specific metabolic, trophic and protective functions in the host body. Metabolic functions include decomposition and fermentation of undigested food residues, energy storage in the form of short-chain fatty acids (SCFA), vitamin K production and ion absorption. Apart from intestinal pH lowering, short-chain fatty acids stimulate the development of intestinal epithelial tissue (butyric acid), hepatocytes (propionic acid) and peripheral tissues (acetic acid). Fermentation products regulate also glucose and lipid metabolism. Furthermore, SCFA have an effect on mineral metabolism of the human body, enhancing absorption of calcium, magnesium and iron ions from the large intestines. The amount of energy obtained by the human body as a result of fermentation processes conducted by bacteria living in the large intestine is about 7-10% of total energy originating from food. Only 5% of SCFA produced by bacteria in the large intestine is excreted with faeces; the remaining amount is used by host cells. Trophic functions of intestinal microflora involve the control of integrity of the intestinal epithelium and ensuring immune sys-

TABLE 1. Bacteria in the human large intestine [Salminen *et al.*, 1998].

Bacteria	Description	Number of bacteria (log/g dry weight faeces)		Main fermentation products
		mean	range	
<i>Bacteroides</i>	G- rods	11.3	9.2 – 13.5	A, P, S
<i>Eubacterium</i>	G+ rods	10.7	5.0 – 13.3	A, B, L
<i>Bifidobacterium</i>	G+ rods	10.2	4.9 – 13.4	A, L, F, E
<i>Clostridium</i>	G+ rods	9.8	3.3 – 13.1	A, P, B, L, E
<i>Lactobacillus</i>	G+ rods	9.6	3.6 – 12.5	L
<i>Ruminococcus</i>	G+ cocci	10.2	4.6 – 12.8	A
<i>Peptostreptococcus</i>	G+ cocci	10.1	3.8 – 12.6	A, L
<i>Peptococcus</i>	G+ cocci	10.0	5.1 – 12.9	A, B, L
<i>Methanobrevibacterium</i>	G+ cocco bacilli	8.8	7.0 – 10.5	Methane
<i>Desulfovibrium</i>	G- rods	8.4	5.2 – 10.9	A
<i>Propionibacterium</i>	G+ rods	9.4	4.3 – 12.0	A, P
<i>Actinomyces</i>	G+ rods	9.2	5.7 – 11.1	A, L, S
<i>Streptococcus</i>	G+ cocci	8.9	3.9 – 12.9	L, A
<i>Fusobacterium</i>	G- rods	8.4	5.1 – 11.0	B, A, L
<i>Escherichia</i>	G- rods	8.6	3.9 – 12.3	Mixed acids

G+ Gram-positive, G- Gram-negative, A – acetate, P – propionate, B – butyrate, L – lactate, S – succinate, F – formate, E – ethanol

tem homeostasis. The protective role of intestinal microorganisms arises above all from their antagonism against pathogens, mainly as a result of synthesis products such as lactic acid, SCFA (pH lowering), hydrogen peroxide or bacteriocins. Furthermore, these bacteria are competitive against pathogenic microorganisms [Tannock, 1998; Salminen *et al.*, 1998; Tannock, 1999; Guarner & Malagelada, 2003].

Harmful activity of intestinal microorganisms, especially of *Bacteroides* species, anaerobic streptococci or *Escherichia coli*, arises from their production of the so-called faecal enzymes which transform potentially procarcinogenic substances produced as a result of intestinal metabolism or ingested with food into carcinogenic compounds responsible for cancers, especially of the large intestine. Harmful metabolites formed or transformed in the large intestine by intestinal microflora belong to different groups of compound; they may include nitrosamines, phenols, cresols, indols, flavonoid aglycones, heterocyclic aromatic amines and many others. Faecal enzymes of microbiological origin are involved with the formation of these compounds. Particularly active of these enzymes are  $\beta$ -glucuronidases,  $\beta$ -glucosidases, nitroreductase, azoreductase,  $\beta$ -galactosidase or urease. Therefore, the adequate number of lactic acid bacteria *Lactobacillus* and *Bifidobacterium* are considered of utmost importance for maintenance of human health, *i.e.* they are considered to have beneficial effects on health. *Lactobacillus* predominates in the small intestine while *Bifidobacterium* species bacteria predominate in the large intestine. In humans, reduced share of these bacteria in the gastrointestinal tract causes various symptoms ranging from flatulence to serious digestive troubles, gastrointestinal tract disorders and deterioration of human health [Tannock, 1998; Salminen *et al.*, 1998; Tannock, 1999].

The intestinal microflora of each individual is specific and influenced by numerous specific endogenous and exogenous

factors. Particularly marked differences are noted in the quantities of *Eubacterium*, *Lactobacillus*, *Bifidobacterium*, *Clostridium*, anaerobic streptococci or *Escherichia*, the numbers of which may differ inter-individually even by the order of 8 to 10 log. *Bacteroides* species bacteria are subjected to relatively small inter-individual variations (Table 1). Despite varying percentages of individual microorganism groups, their total number does not undergo significant fluctuations. However, excessive growth of microorganisms harmful for human health always results in human health deterioration.

The main factors causing negative changes in the composition and activity of intestinal microflora are antibiotic therapy, gastric and small intestinal surgery, intestinal peristalsis disorders, colitis, renal and hepatic disorders, malignancies or immune system disorders. The structure of microflora is also determined by the host's age, diet used, living conditions, psychological stress and personal features. In the studies by Hopkins *et al.* [2001] faecal microfloras of the following three age groups were compared: children (16 months–7 years), adults (21–34 years) and the elderly (67–88 years). Irrespectively of age, the total number of anaerobic bacteria was maintained at a similar and balanced level of  $10^{10}$  cfu/g wet faeces. On the other hand, the share of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Enterococcus* and enterobacteria was found to be age-dependent. The highest variability was characteristic for *Bifidobacterium*, the largest quantity of which is noted in children – at the level of  $10^{10}$  cfu/g (and sometimes more), decreasing successively with age to the level of  $10^7$  cfu/g in the elderly. Also the number of *Lactobacillus* species bacteria is significantly reduced in the elderly, even by 2 orders of magnitude. On the other hand, an increase in the numbers of enterococci, enterobacteria and *Clostridium* is observed. This may lead as a consequence to many gastrointestinal tract disorders in this age

TABLE 2. Microorganisms commonly used in probiotics [Holzapfel &amp; Schillinger, 2002].

<i>Lactobacillus</i>	<i>Bifidobacterium</i>	Other lactic acid bacteria	Other microorganisms <sup>a)</sup>
<i>L. acidophilus</i>	<i>B. adolescentis</i>	<i>Enterococcus</i>	<i>Bacillus cereus</i> <sup>b)</sup>
<i>L. amylovorus</i>	<i>B. animalis</i>	<i>faecalis</i> <sup>b)</sup>	<i>Escherichia coli</i>
<i>L. casei</i>	<i>B. bifidum</i>	<i>Enterococcus</i>	Nissle 1917 <sup>a)</sup>
<i>L. arispatus</i>	<i>B. breve</i>	<i>faecium</i> <sup>b)</sup>	<i>Propionibacterium</i>
<i>L. gallinarium</i> <sup>b)</sup>	<i>B. infantis</i>	<i>Sporolactobacillus</i>	<i>freudenreichii</i> <sup>b)</sup>
<i>L. gasseri</i>	<i>B. longum</i>	<i>inulinus</i> <sup>b)</sup>	<i>Saccharomyces</i>
<i>L. johnsonii</i>			<i>cerevisiae</i>
<i>L. paracasei</i>			(boulardii) <sup>a)</sup>
<i>L. plantarum</i>			
<i>L. reuteri</i>			
<i>L. rhamnosus</i>			

a) mainly as pharmaceutical products; b) mainly intended for animals

group.

In the course of antibiotic therapy in humans, the number of microorganisms beneficial for the human body, such as: *Lactobacillus*, *Bifidobacterium* or *Bacteroides*, is drastically reduced, and the number of potentially harmful bacteria such as enterobacteria, *Clostridium difficile* as well as of *Candida albicans* yeasts increases [Finegold *et al.*, 2004; Wynne *et al.*, 2004]. Especially marked changes in the composition of the intestinal microflora are associated with viral or bacterial infections. Pathogenic microorganisms (*Salmonella*, *Shigella*, *Staphylococcus*, *Listeria*, *Campylobacter*, *Yersinia*, enteropathogenic *Escherichia coli* strains or some *Bacillus* and *Clostridium* species) which get to the gastrointestinal tract together with the ingested food or as a result of incompliance with hygiene rules may proliferate or even colonise intestinal mucous membranes, causing various types of food poisoning. Furthermore, they produce metabolites which are toxic for human, along with enzymes which may be responsible for formation of carcinogenic substances or for transformation of procarcinogenic substances into carcinogenic ones. All changes in the composition of intestinal microflora are associated with diarrhoea.

The ingested diet may be to a large extent a factor causing positive changes in the proportions of individual microorganisms, in particular it may increase the number of lactobacilli and bifidobacteria. Positive stimulation is based on introduction of specific saccharides called prebiotics (inulin, fructooligosaccharides – FOS, galactooligosaccharides – GOS, IMO – isomaltooligosaccharides, digestion-resistant starch) into the diet [Rastall & Maitin, 2002]. Specific and controlled regulation of the intestinal microflora composition may also be attained by probiosis, *i.e.* ingestion of pharmaceutical preparations or food products containing live bacteria with probiotic properties, mainly *Lactobacillus* and *Bifidobacterium* species (Table 2).

## PROBIOTICS EVALUATION

Nevertheless, not all lactic acid bacteria of these genera cause a similarly potent health promoting effect in the human body. These properties are related to the bacterial strain and not the bacterial species. Obtaining the status of a probiotic strain requires documentation of positive role of the given strain in clinical studies on humans. Although *in vitro* studies are indispensable for characterising the potentially probiotic bacteria, they are not sufficient to foresee the hu-

man health effects of their use. Therefore, such studies are not the basis for using the term of a probiotic strain/product [Reid *et al.*, 2003].

Due to the fact that the term of “probiotic product” often performs a marketing function, FAO/WHO documents precisely define the methods of studying and labelling probiotic products (Figure 1). Strain identification (species categorisation) must be conducted by the currently approved methods. Nowadays, it is recommended to combine methods defining phenotypic features of the bacteria with genetic tests. The method of DNA-DNA hybridisation is considered the most reliable. It may be replaced with characterisation of the 16S rRNA-coding DNA region sequence. Among the basic phenotypic features, it is necessary to determine the profile of fermented sugars and of the end products of glucose fermentation.

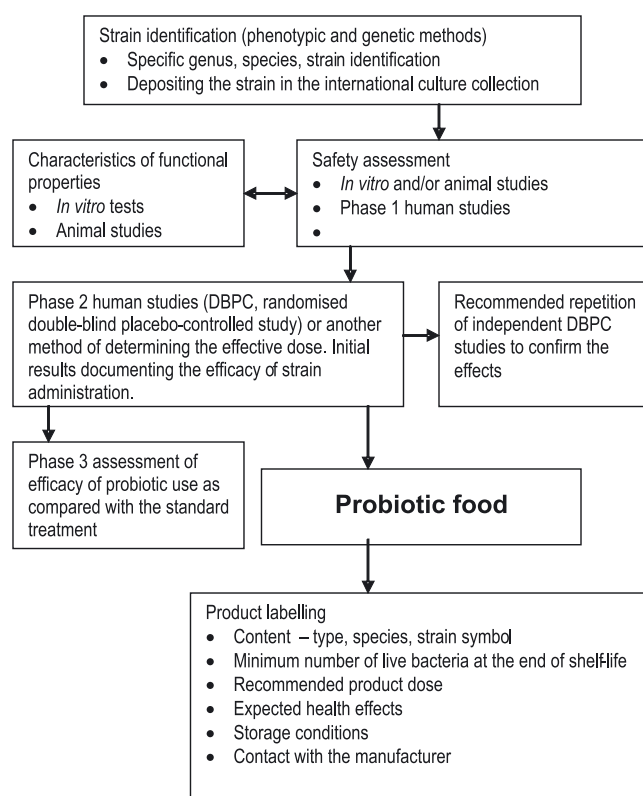


FIGURE 1. Guidelines for the evaluation of probiotics for food use [FAO/WHO Report, 2002].

The applied names of the probiotic bacteria species must be consistent with the updated official list of bacterial names (List of Bacterial Names with Standing in Nomenclature, LBSN) ([www.bacterio.cict.fr](http://www.bacterio.cict.fr)).

Further studies of potentially probiotic bacteria include the assessment of their functional properties (in *in vitro* and animal studies) and also of the degree of safety of strain use (also in *in vitro* and animal studies). The basic functional properties which should be determined include: acid and bile tolerance, adherence ability to mucosa and/or human epithelial cells and cell lines, antagonistic activity against pathogenic bacteria, ability to limit the adhesion of pathogens to epithelial surface, resistance to spermicides (for vaginal probiotics).

Although *Lactobacillus* and *Bifidobacterium* are considered safe microorganisms and have been used in food production for a long time, conducting tests of their safety of use is required. These tests should include determination of the antibiotic resistance profile, some metabolic properties, e.g. ability to synthesise D-lactate, de-conjugation of bile salts and determination of side-effects in human studies. If the strain belongs to a species which may produce toxins, it should be investigated for such properties. The final confirmation of the absence of infectivity is studies in immunodeficient animals. Only obtaining positive results in such studies justifies the purposefulness of conducting clinical studies in animals and then in humans. The requirements for clinical procedures include phase 1 (strain safety assessment), phase 2 (strain efficacy assessment) and phase 3 (efficacy assessment in studies with a large number of persons, comparison of effects with the standard treatment). In principle, the probiotics whose vehicles are food products do not have to undergo phase 3 studies.

Only evidencing the positive effect on human health makes it possible to classify the given strain as a probiotic one. Industrial probiotic strains should be deposited in an international collection authorised to store microorganisms.

The strains of probiotic lactic fermentation bacteria are used in the form of pharmaceutical products, most often in the form of freeze-dried biomass or as food additives. Food supplemented by these bacteria is termed probiotic food and is included in the category of functional food. Milk and milk fermentation drinks are considered especially beneficial vehicles of probiotic bacteria. Milk is a natural environment for the occurrence of lactic acid bacteria. It ensures buffering of gastric content and better bacterial survival in the course of transit through the gastrointestinal tract. Lactose contained

in milk constitutes a bacterial growth substrate. The storage conditions (cooling and the fairly short shelf-life) are beneficial for bacterial survival. Furthermore, milk products introduce additional nutritive value.

Probiotic products should be appropriately labelled. As recommended by FAO/WHO, the label should indicate the name (collection or trade) of the strain, the minimum content of probiotic bacteria at the end of shelf-life, the recommended product dose and the expected health effects. The latter statements must be supported by clinical studies conducted by independent study centres. Consistently with the standpoint of the American Paediatric Society, it is necessary to ingest 1 to 2 billion probiotic bacteria daily to obtain the positive health effect [ADA Reports, 2004]. Examples of several commercial probiotic products available in the European market are given in Table 3.

### PROBIOTICS AND HEALTH EFFECT

Numerous research studies prove probiotic strains to reinstate the natural appropriately functioning intestinal microflora structure, to inhibit the development of numerous pathogenic microorganisms, reduce the incidence of traveller's diarrhoea, to alleviate the course and to shorten the duration of some bacterial and viral diarrhoeas (e.g. caused by *Clostridium difficile*, *Shigella*, *Salmonella*, enterotoxigenic *Escherichia coli* strains or rotaviruses), to prevent the occurrence or to alleviate the course of diarrhoeas due to antibiotic therapy, to reduce the intensity of radiation diarrhoea, to eliminate or reduce symptoms of lactose intolerance, to have hypocholesterolaemic action, to potentially exert therapeutic action in hepatic encephalopathy, and also to normalise intestinal motility disorders in the elderly. Also the studies documenting the antagonism of some probiotic strains against *Helicobacter pylori*, bacteria associated with development of gastric and duodenal ulcers, are interesting. Ingestion of probiotic products may be beneficial in the treatment of functional diarrhoea and may shorten the duration of carriage of *Salmonella*. Furthermore, ingestion of probiotic products after antibiotic therapy allows reinstatement of the normal balance of the natural human intestinal microflora [Crittenden, 1999; Rolfe, 2000; Sanders, 2000; Holzapfel & Schillinger, 2002; Saunier & Dore, 2002; Ouwehand *et al.*, 2002; Picard *et al.*, 2005; Reid *et al.*, 2003; Isolauri *et al.*, 2004; Stanton *et al.*, 2005; Libudzisz, 2006].

Probiotic bacteria enhance specific and non-specific defence mechanisms of humans and animals. As evidenced by

TABLE 3. Commonly used probiotic strains and commercial products [Saxelin *et al.*, 2005].

Species	Strain	Commercial brand name(s)
<i>Lactobacillus casei</i>	DN 114001	Actimel <sup>†</sup>
<i>Lactobacillus casei</i>	Shirota	Yakult <sup>†</sup>
<i>Lactobacillus plantarum</i>	299v	ProViva <sup>†</sup>
<i>Lactobacillus rhamnosus</i>	GG	Actifit <sup>Plus</sup> *, Gafilus*, LGG*, Onaka He GG!, Vifit <sup>†</sup>
<i>Lactobacillus johnsoni</i>	La1	LC1 <sup>†</sup>
<i>Bifidobacterium lactis</i> *	BB12	Various brand names

\*presently *B. animalis* ssp. *lactis*



the studies, daily supplementation of the diet by  $10^9$ – $10^{12}$  cells of probiotic bacteria may result in an increased number of natural killer cells in the blood serum and may increase the activity of macrophages and lymphocytes within only a few weeks. Further effects discovered were stimulation of synthesis of IgA secretory antibodies, production of IL-2, IL-10 and IL-12 interleukins and increasing the level of interferon- $\gamma$  in blood serum [Noverr & Huffnagle, 2004; Madaliński & Szajewska, 2004; Marcinkiewicz, 2005]. Moreover, immune modulating effects of lactic bacteria may additionally reduce allergic symptoms in children [Kalliomaki *et al.*, 2001, 2003, Cukrowska *et al.*, 2006].

The mechanisms of anticancer activity of lactic acid bacteria may be related to stimulation of the human immune system and may result from inhibition of development of bacteria synthesising the enzymes which catalyse intestinal transformation of precursors of carcinogenic compounds into carcinogenic compounds. Furthermore, lactic acid bacteria are able to use (or bind) carcinogenic compounds originating from the diet or created by pathogenic bacteria in the intestines, *e.g.* nitrozoamines, azo dyes, mycotoxins or pyrrolisates of amino acids [Burns & Rowland, 2000; Rafter, 2003].

The ability to assimilate cholesterol evidenced under “*in vitro*” conditions is also a very important feature of some lactic bacteria. The importance of these bacterial abilities for humans has not been still documented and undergoes intense studies. It seems that ingestion of products containing probiotic bacteria may play a role in prevention of atherosclerosis and coronary heart disease [Reid *et al.*, 2003; Salminen *et al.*, 1998; Saunier & Dore, 2002].

Nevertheless, it should be borne in mind that the health effects caused are associated with the specific probiotic strain.

Lactic bacteria reduce also lactose intolerance symptoms in humans. Lactose intolerance is a problem affecting many people. In some countries of Africa and Asia it occurs in almost 100% of the population. In Poland it occurs only in about 20–30% of the population. Lactase, an enzyme hydrolysing milk sugar (lactose) to D-glucose and D-galactose, is responsible for lactose hydrolysis in the small intestine. The hydrolysis products can be subsequently easily absorbed by the intestinal walls.

In healthy humans, the enzyme hydrolysing lactose is naturally contained in the cells of small intestinal epithelium. In the case of lactose intolerance, we deal with a deficiency of this enzyme arising either from a congenital lactase defect or from enzyme activity lowering progressing with age. Lactase activity lowering may also be secondary, for example may be a result of some health disorders. Symptoms of lactose intolerance are diarrhoea and flatulence following lactose or milk ingestion. Persons with these disorders are recommended to consume milk products containing a reduced quantity of lactose, which is achieved, for example, by fermentation. In the milk fermentation process, microorganisms convert 20 to 50% of lactose into lactic acid. Furthermore, microbial cells release in the gastrointestinal tract the active enzyme  $\beta$ -galactosidase which hydrolyses lactose in the intestines.

Due to increasing awareness of the role of the intestinal

microflora system and its importance for the human health, a very intensive development of production of milk drinks fermented with participation of probiotic microflora has been observed in the past few years. For persons intolerant to lactose or milk proteins, vegetable products enriched with probiotic bacteria are offered. This food is classified as functional food, and thus food which apart from its nutritional effect exerts a positive influence on specific body functions, leading to an improvement of the health status and good well-being of the human, or to reduced disease risk.

The importance of probiotics for human health was undoubtedly emphasised by organisation of the inaugural meeting of the International Scientific Association for Probiotics and Prebiotics (ISSAPP) in Canada in 2002.

It can be expected that the foundation of this Association and the development of legal terms for study procedures and labelling of probiotic products will increase trust among researchers and physicians and will make it possible for consumers to ingest probiotic products with full confidence as to the value of such products.

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## **ROLA PROBIOTYKÓW W ŻYWIENIU CZŁOWIEKA – WYKŁAD PLENARNY**

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Artykuł omawia zagadnienia dotyczące mikroflory przewodu pokarmowego oraz wpływu probiotyków na organizm człowieka. Uwzględnia również kryteria doboru szczepów probiotycznych w aspekcie ich wpływu na zdrowie oraz poprawę jakości życia.