PROCESSING, CONSUMPTION AND EFFECTS OF PROBIOTIC MICROORGANISMS

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Summary

Humans are surrounded by a huge number of microorganisms and most of them affect our body positively. Naturally, we take up high numbers of different microorganisms with unprocessed food. Thermal treatment and aseptic production of food strongly reduces the amount of microorganisms taken up. This alters the microbial composition in our digestive system. To counteract this development a variety of probiotic microorganisms and related products have been developed and sold already for several years and further growth of the market of these products is expected. In the following text a summary of the large amount of scientific reports about the positive effects of the applications of probiotic microorganisms is presented. Legal aspects and requirements about the advertisement of probiotic properties are summarized. The required survival of the probiotic microorganisms during storage and the stomach passage are discussed referring to the different down-stream processing methods and biological protection methods.

1. Introduction

Microorganisms are present in every part of our environment. Up to now 5000 to 6000 different microorganisms are known. Most of them are harmless or even beneficial for humans. Our skin is covered with bacteria and in our intestinal system more than 800 different microbial strains have been discovered. The total count of bacteria on and in humans outnumbers the count of human cells by the factor 10 to 100. The mass of commensal bacteria in and on our body is presented in Table 1.

Surfaces	**Wet Weight of Bacteria [g]**
Eyes	1
Nose	10
Mouth	20
Lungs	20
Vagina	20
Skin	200
Intestines	1000–2000

Table 1. wet weight of microorganisms on a healthy human body according to Bengmark and Martindale, 2005

Historically, humans consumed large amounts of fermented foods including the microorganisms. A strong symbiosis between humans and microorganisms has developed and we are dependent on a well balanced consortium of microorganisms to maintain our health. These microorganisms help to digest our food, produce essential vitamins and can inhibit the proliferation of hazardous microorganisms.

The increased knowledge about microorganisms and their metabolic function led to the development of several industrial processes in the chemical, pharmaceutical and food industry in the 20th century. Controlled production processes with well characterized strains enabled a reproducible production of food. Furthermore, the knowledge about microorganisms allowed the reduction of spoilage of food by sterilization or pasteurization processes. These techniques help to provide food for the strongly growing population on earth.

On the other side the reduced contact between microorganism and the population of industrial civilizations is discussed to be responsible for the growing number of allergies. To counteract the reduced contact with microorganisms an increasing number of probiotic foods are developed and sold. A summary about the positive effects of pro- pre- and synbiotics is presented in Figure 1.

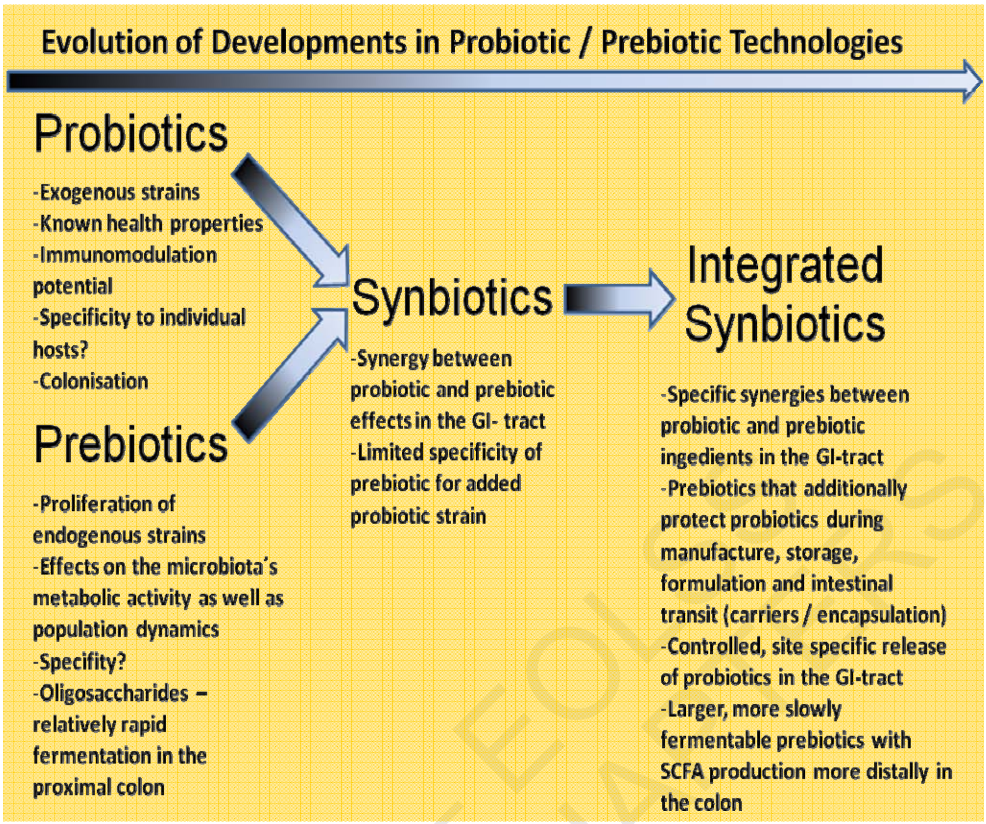

Figure 1. Effects of pro- pre- and synbiotics as well as integrated synbiotics (modified version of Mattila-Sandholm et al., 2002)

2. Definitions: Probiotic, Prebiotics, Synbiotic and Integrated Synbiotics

Probiotic microorganisms are defined according to the *Food and Agriculture Organization* of the United Nation and the World Health Organization as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”.

Prebiotics or dietary fibers are the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (definition according to the American Association of Cereal Chemists).

Synbiotic products are a combination of probiotics and prebiotics. The prebiotic substances are adapted to the demands of the probiotic microorganisms. Hence their survival and proliferation is increased.

Integrated Synbiotics can help to protect the probiotics microorganisms during storage and the passage through the stomach. The capsulation material is selected to improve the microbial metabolism in the large intestine which improves the production of short chain fatty acids (SCFA).

3. Commercial Impact of Probiotic Products

The increasing knowledge about the influence of nutrition and special food ingredients on the human health raises the demand of healthy food. More and more consumers are willing to pay higher prices for food with additional health benefits. In this so called functional foods or "FOSHU" (Foods of Specified Health Use) vitamins, minerals, fibers, antioxidants, amino acids, unsaturated fatty acids and probiotics can be included to improve the effects of the food beyond its normal nutritional value.

The world market of functional food is expected to grow up to more than 90 billion US$ in 2013. Within these products one main product group are the probiotics. In 2008 more than 500 probiotic products with a value of 15.9 billion US$ were sold and it is expected that this market increases up to 28 billion US$ in the year 2014. Probiotic products and manufacturers are summarized in Table 2.

Company	Product name	Applied strain
Marion-Merril-Down Laboratories, Levallois-Perret, France	Bactisubtil	*Bacillus cereus* IP5832
Sanofi-Winthrop SpA, Milan, Italy	Enterogermina	*Bacillus clausii*
Flora-Balance, Montana, USA	Flora-Balance	*Brevibacillus laterosporus+*
Pharmed Medicare, Bangalore, India	Lactopure	*Lactobacillus sporogenes**
Sabinsa Corp., Piscataway, NJ, USA	Lactospore	*Lactobacillus sporogenes**
Hanmi Pharmaceutical Co., Ltd., Seoul, Korea	Medilac	*Bacillus subtilis* R0179
Prodeta, Vannes, France	Paciflor	*Bacillus* CIP 5832 **
Ganeden Biotech, Mayfield Heights, OH, USA	GenadenBC30	*Bacillus coagulans*
Danone Ag, Paris, France	ACTIREGULARIS Lactobacillus casei defensis	*Bifidobacterium lactis* DN 173 010 *Lactobacillus casei* DN 114 001
Nestle S.A., Vevey, Switzerland	Nestlé's LC1	*Lactobacillus johnsonii* (La1)
Danisco	HOWARU Balance FloraFIT® Probiotics	*Bifidobacterium lactis* HN019 *Lactobacillus acidophilus* NCFM® *Bifidobacterium animalis subsp. lactis* 420 (B420) *Streptococcus thermophilus* St-21
Valio Ltd., Helsinki,	Valio Gefilus (LGG)	*Lactobacillus rhamnosus* (ATCC

Finland		53103)
Lallemand Institut Rosell Inc., Montreal, QC, Canada		*Lactobacillus casei* LAFTI L26 (L26) *Lactobacillus acidophilus* LAFTI L10 (L10) *Bifidobacterium animalis lactis* LAFTI B94 (B94)
Yakult Deutschland GmbH	Yakult	*Lactobacillus casei shirota* (DSM 20312)
BioGaia Biologics, Stockholm Sweden	L. reuteri protectis	*Lactobacillus reuteri*
Klosterfrau Healthcare Group, Cologne, Germany	Bactisubtil complex	*Lactobacillus acidophilus* *Bifidobacterium longum*
ARDEYPHARM GmbH, Herdecke, Germany	Mutaflor	*Escherichia coli nissle* 1917
BIOCODEX, Gentilly, France	Florastor	*Saccharomyces boulardii lyo*
Laboratori Turval Srl, Udine, Italy	Turval B	*Kluyveromyces fragilis* B0399

Table 2. Commercially available products with advertised probiotic properties

It is important to be aware of the different legal regulations regarding the declaration and advertising of probiotic properties in different countries.

Historically many probiotic microorganisms are associated with milk or dairy products and a large group of the known probiotics belongs to the group of lactic acid bacteria as *Lactococcus, Lactobacillus, Streptococcus, Enterococcus* and *Pediococcus*.

Another group of bacteria applied in the dairy industry are Bifidobacteria. But also several other groups of bacteria comprise probiotic strains as *Bacillus*, *Clostridium* and *Escherichia*. Probiotic eukaryotic strains belong to the genii of *Kluyveromyces* and *Saccharomyces*.

4. Requirements for Probiotic Microorganisms and Probiotic Food

In general it is assumed that the microorganisms should reach the large intestine alive. Therefore they have to be able to withstand the conditions in the gut with a pH value down to 0.8 and the proteolytic activity of the pepsin. Probiotic strains of *Lactobacillus acidophilus* (Figure 2) belong to the most stable lactic acid bacteria under these conditions. Anyway, many vital bacteria do not reach the large intestine alive and contradicting reports about the survival rate can be found in literature.

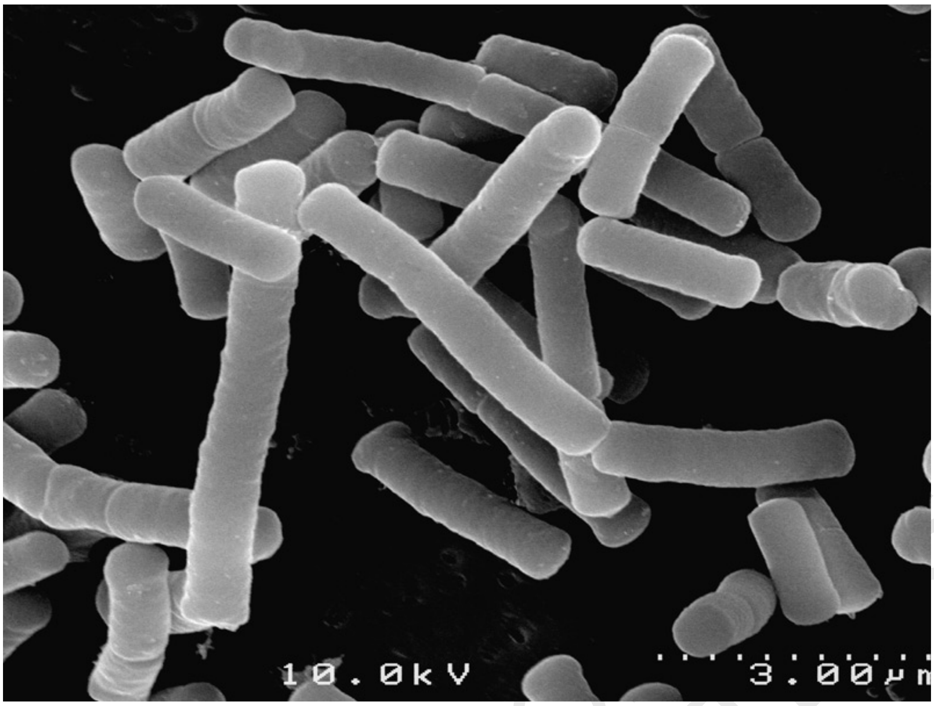

Figure 2. "Electron micrograph image of *Lactobacillus acidophilus* R0418 courtesy of Dr. Sandy Smith, Dept. of Food Science, University of Guelph, Canada. Copyright held by Institut Rosell Inc., Montreal, Canada. http://www.institut-rosell-lallemand.com/uploads/images/souches/lactobacillus-R52_big.jpg

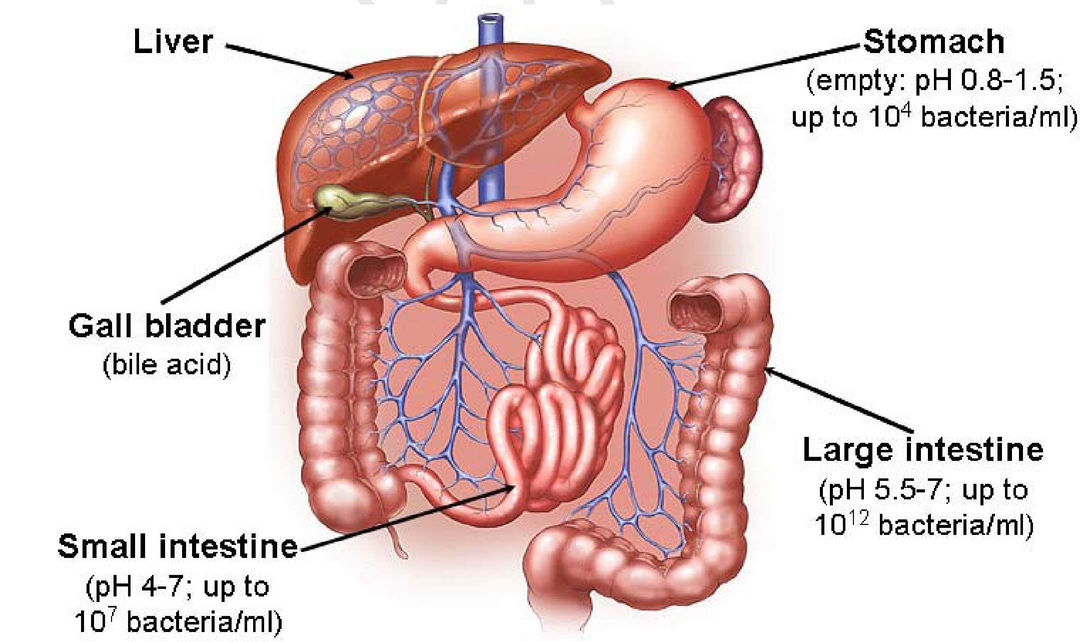

Figure 3. Human intestinal system (Adapted from a graphic of David Carlson: http://www.carlson-art.com/lifescience/index.html)

During the passage though the intestine bile salts, proteases, lipases and amylases can cause an inactivation of vital microorganisms (Figure 3). The survival rate of probiotic microorganisms during the stomach passage is dependent on the strain and the food intake of the human.

Directly after the intake of food the pH value in the stomach can increase from 1.0 up to 4.0 simultaneously the concentration of enzymes and bile salt are diluted which strongly increases survival of vital probiotic strains. *Bacillus* and *Clostridium* form endospores at certain conditions. These spores are much more resistant towards the conditions in the intestinal system. Hence, these spore forming bacteria offer good conditions for the application as probiotics.

If the probiotics are incorporated in food and the probiotic effect is advertised a sufficient number of living bacteria is required and demanded by the lawmaker through the end of shelf live. One portion of probiotic food should contain at least 10^9 living cells or 10^7 spores. The required concentrations can be proved by counting the colony forming units after spreading out of the sample and incubation on nutrition agar plates. It is assumed that these concentrations of microorganisms are sufficient to cause the desired effects in the large intestine like adherence to the epithelial cells, proliferation, and inhibition of unfavorable strains and activation of the immune system. Although there are many reports about the positive effects of probiotic microorganisms there are still no probiotic products that have been approved by the European Food Safety Authority (EFSA).

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Biographical Sketches

Dr.-Ing. Johannes Bader was born in Regensburg, Germany in 1976. After the completion of the study of Biotechnology in 2004 he achieved the degree Master of Science in Biotechnology at the University of Applied Sciences Berlin in 2006. In 2008 he obtained his Ph.D. degree at the Technische Universität Berlin, Germany.He is teaching students of the subjects- biotechnology, brewing technology and food technology at the University of Technology Berlin. Furthermore, he is deputy head of the Research Institute for Microbiology at the Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V. In 2010 he worked as a research associate at the Massachusetts Institute of Technology, Cambridge, USA. His research topics comprise up- and downstream processing of microorganisms in food biotechnology and white biotechnology.

Prof. Dr.-Ing. Milan K. Popović, born in Sremska Mitrovica, Serbia finished his study of Food Technology at the University Novi Sad, Serbia 1965 and obtained his Ph.D. degree in Technical Chemistry at the Technische Universität Berlin, Germany in 1976. He worked as university assistant (teaching and research) at the University Novi Sad, Serbia, as research assistant at the Versuchs- und Lehranstalt für Spirituosenfabrikation Berlin, Germany and as research associate at the University of Waterloo, Canada. After years as senior constructing engineer at Karl Fisher Industrieanlagen GmbH Berlin, Germany he became professor in Biochemical Engineering at the FH Giessen-Friedberg (now TH Mittelhessen) and from 1989 on at the University of Applied Sciences (BHT), Berlin. He retired in 2009. His research and teaching area are chemical and biochemical reactor engineering, environmental engineering and in last years the food and white biotechnology.

Prof. Dr. Dipl.-Ing. Ulf Stahl was born in 1944 in Vienna, Austria. In 1969 he finished his study at the University of Vienna. 1975 he obtained his doctoral degree at the Ruhr- University in Bochum, Germany were he became Professor from 1981 until 1983. In this year he became Professor at the Department of Microbiology and Genetics at the Technische Universität Berlin, Germany. Today he is also head of the Research Institute for Microbiology at the Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V. His research activities comprise genetically modification of industrially relevant microorganisms and their application in food biotechnology and white biotechnology.