Probiotics and Human Health

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ABSTRACT

Fermentation is one of the oldest methods to preserve food. Lactic acid bacteria with probiotic properties have been selected and used since the 1950s to produce food with special health promoting properties. As a result of fermentation and storage, the components of the substrates are pre-digested and the contents of the nutrients altered. New biologically acting components are produced. The number of probiotic strains as cfu/mL is 100 fold increased, which gives the fermented food preventive and curative properties against many types of diseases in both humans and animals. Most probiotics are consumed as fermented milks, or other fermented foods. However, new approaches such as tablets, lyophilised powders and probiotics inserted in oily suspensions are also prescribed. The beneficial effects of consumption include normalising the intestinal flora composition and improving the intestinal tract health; enhancing the immune system level by stimulating and increasing the numbers and activities of immunomodulatory cells; protecting against many types of infections; synthesizing and enhancing the bioavailability and absorption of nutrients; reducing the symptoms of lactose intolerance by the production of lactase in situ; reducing the prevalence of allergy; preventing and minimising the risk of various cancers; prolonging the individuals healthy state and increasing the healthy life span, among other effects. The methods by which these effects are exerted are among many others, the lowering of the gut pH; action against pathogens by the production of antimicrobial compounds; competing for pathogen binding and receptor sites; competing for nutrients and growth factors. The selection of probiotic bacteria is crucial for the exertion of beneficial effects. This review article covers results obtained by studying the effect of fermentation on the substrate, as well as the tests of probiotic effects on humans and some animal species, concerning their properties for prevention of diseased states, and maintaining health and wellbeing.

Key Words: Lactobacilli, Bifidobacteria, Probiotics, Prebiotics, Synbiotics, Fermented foods, Intestinal flora, Health effects

ÖZET


Anahtar Kelimeler: Lactobacilli, Bifidobacteria, Probiyotikler, Prebiyotikler, Sinbiyotikler, Fermente Gidarlar, Intestinal flora, Sağlıklı etkileri
INTRODUCTION

During the last 100 years scientists in most countries have investigated the effects of various lactobacilli and bifidobacteria for their ability to prevent and cure various diseases in man and animals. The vast amounts of publications witness of a never fading interest to search and succeed. The aim of most of them was to find the long and eternally searched elixir for health and longevity by the means and methods given to the mankind by mother nature.

A fermented milk product, where the proteins are pre-digested, the fat partly hydrolysed, the production of aroma compounds quite high, the vitamin content enhanced, the minerals made easier to absorb and which contains up to one milliard cells/gram of viable, especially isolated friendly bacteria, active and viable when consumed, aimed to protect the consumer's life and health — this is a probiotic food product, maybe the elixir searched so eagerly by so many.

But at the same time, the probiotic food must be palatable and safe to distribute, store and consume, and not too expensive. Everybody should be able to tolerate it and afford a daily portion. The functions desired by the consumer are: to prevent, improve and if possible cure disorders of the gastrointestinal tract directly, or to assist in minimizing health problems indirectly, whatever these problems might be.

This paper tries to illuminate some of the questions concerning the healthfulness of probiotics in the human diet. First, we must know something about the substrate, which is mostly cow’s milk. Then we must know about the lactobacilli of various types, used for conventional products, and the therapeutic lactobacilli, the probiotic microorganisms, used to produce the probiotic foods. We must know how lactobacilli change or affect the properties of nutrients in milk and what this means for the health of the consumers. We must also know how the gastrointestinal tract is affected, how it is colonized by bacteria from the start of a newborn baby’s life and during the whole human lifespan and how it is functioning in health and disease. How many bacteria are there in the intestinal flora compared to the cells in the human body, and also, whom are we protecting? We must know and understand the importance of the intestinal flora for its host, what happens if it is altered in composition or minimized, and how it can be restored in order to remain normal and healthy, until a vital old age.

From prehistoric times, man learned to use milk as food. Fermentation occurred naturally and the product could be consumed without danger for the health of the consumer. Thus, a practical method of preserving a surplus of otherwise perishable milk was probably discovered accidentally. Practically all nations have one or several traditional types of fermented milk products made by the action of lactobacilli and given local names. Preparation became an art handed down from one generation to the next.

The nutritional value of a food is dependent not only upon its content of nutrients, but also upon digestibility and availability of these nutrients. Fermented milk products contain almost the same amount of energy as the milk from which they are made, yet they are in many respects more nutritious because of the transformed nutrients which are made more available.

Fermented milk products have been a part of the human diet since ancient times. Their efficacy in alleviating gastrointestinal (GI) disorders has been exploited in systems of traditional medicine globally. Lactic acid bacteria, the indigenous microfloral flora in fermented milk products and natural inhabitants of the human GI tract, were thought to be responsible for the longevity of their hosts through their prophylactic and curative actions.

The role of lactic acid bacteria in GI microecology has been the subject of extensive research. It is widely believed that probiotic bacteria prevent the growth of putrefactive microorganisms responsible for poor health by competitive inhibition, the generation of an acidic environment and/or by the production of antibiotic-like substances (bacteriocins), and production of metabolites such as the B group vitamins. The proteolytic, lipolytic and beta-galactosidase activities of probiotics improve the digestibility and assimilation of ingested nutrients, thereby rendering them available in convalescent/geriatric nutrition and as adjuncts to antibiotic therapy.

Lactic acid bacteria may also colonize the skin and mucous membranes and play an important role in preventing bacterial and fungal infections of the skin and genito-urinary tract. Lactobacilli play a protective role against vaginal infections. They utilize glycogen in the vaginal epithelial cells to produce lactic acid, which helps to maintain the necessary low pH in this environment between 4.0 and 4.5. This creates a non-conducive environment for the growth of pathogens like Candida albicans, Trichomonas vaginalis and some of the non-specific bacteria, which are responsible for vaginal infections.

THE LEGACY of Elie Metschnikoff

Elie Metschnikoff was the Director of the Institute Pasteur in Paris, recipient of the 1908 Nobel Prize in Medicine for his work on Phagocytosis, which formed the basis for the theory of immunity. He was the first to advocate the consumption of fermented milk, i.e. yoghurt, as beneficial for health and the longevity of the peasants on the Balkan Peninsula [99, 100]. He observed that Bulgarians and other peoples in the region, who consumed fermented milk products, had a much longer and healthier life span. Yoghurt and fermented milk containing live lactobacilli can be regarded as the first types of probiotic foods introduced into the Western countries.

Metschnikoff’s autointoxication theory claims that “the human body is slowly being poisoned and its resistance weakened by the action of wrong type of intestinal flora. Death will come more rapidly to a heavy meat eater;
meat putrifies, whereas milk does not, because of its content of lactose from which the lactobacilli produce lactic acid, which together can affect the number of disease causing microorganisms in milk and in the human organism.

The incubation time of Metschnikoff's ideas has been very long, but today, nobody is denying that we are indeed dependent for our wellbeing on a well functioning intestinal flora and that the presence of probiotic bacteria in the GI tract is necessary. If the intestinal flora is disturbed, altered or minimized for some reason, it must be quickly restored, and this can only be accomplished by the consumption of the right type of probiotics with some type of carrier. Fermented milk alone, however, does not prolong a healthy life span – there is so much more that has to be considered. Metschnikoff himself consumed large quantities of yoghurt, but he might have started the supplementation of his diet with lactobacilli too late in life, and he died only 71 years old.

All mammalian milk can be used for the production of fermented milk products, but most commonly cow's milk is used, with a manipulated fat content and/or supplemented with prebiotics as growth factors. Milk differs in composition, not only among different species but also within the same species and individuals. Some of its constituents, such as milk fat, with its specific fatty acid composition, lactose and casein, are not found elsewhere in nature. The nutritional value of cow's milk is generally accepted as being the highest, providing most of the essential nutrients in a bio-available form. Cow's milk is a good source of most vitamins, except ascorbic acid.

Fermented milk products can be divided into two main groups. The traditional, home made products, where the fermentation is carried out by the lactobacilli present in the environment, these being mesophilic or thermophilic, however, without knowing exactly the composition of the starters. The industrially made products, where the microorganisms are carefully chosen from a culture bank, being investigated and propagated especially for the purpose of producing a typical product, of high quality with good sensoric properties, resembling the home made fermented milk types, but with a more controlled outcome, higher hygienic quality and longer keeping time.

Lactobacilli multiply from one or ten million cells per mL to one thousand million cells per mL in a ready-to-eat probiotic product. The microorganisms are present in the fermented milk, not only as viable cells, but also as dormant cells, together with all the primary and secondary metabolites they have produced and continue to produce until consumption.

A fermented probiotic milk product has much greater advantages regarding its healthfulness compared to acidified milk, or milk just supplemented with probiotic strains (one example being the Sweet Acidophilus milk). Probiotics can be provided consumers also as probiotic tablet types (Dophilus, Trevis tablets, etc.) recommended as preventive medication, or as probiotic cells inserted in some carrier oil recommended also as preventive medication.

**STARTERS IN GENERAL AND SOME DIFFERENCES CONCERNING TECHNICAL AND THERAPEUTIC STARTERS**

Lactic acid bacteria have been used in the production of fermented foods of various kinds for thousands of years. Buttermilk, Ropy milk, Kefir, and Kumyss, are some of the traditional fermented milk types in the world, which have been produced locally for ages and are still produced. Every country where people have cattle and consume milk, they have also one or several types of fermented products with local names, produced in a traditional way since the dawn of their history. The development of probiotic milk products is of recent date and is the answer of the modern dairy industry in collaboration with several other scientific disciplines, such as microbiology, biotechnology, nutrition and medical science. Modern bio-preservation technology and the achieved positive health effects have increased the popularity of fermented products. Metabolic engineering of lactic acid bacteria, in order to control the microbial effect on the substrate, is extensively studied and applied. Examples are the formation of desired lactic acid isomers and the production of exopolysaccharides found initially in ropy milk types.

The starters used in the fermentation can be divided into two main categories. Technical starters, which are good fermenters, produce palatable and safe products, but cannot withstand the acid environment in the stomach, nor the effect of bile acids. The traditional technical starters will grow in milk, produce nutritional transformation of the components present, but will only survive in small amounts in the GI tract. The fermented food is palatable, safe and beneficial, but the cells are unable to attach to the intestinal wall, or influence the intestinal flora in a continuous way.

The therapeutic starters are selected with special care, isolated from healthy individuals, tested thoroughly for their properties, possess the ability to survive in large numbers, are able to attach the intestine and to colonize parts of the GI tract, multiply and produce biologically active components in situ. They can beneficially influence the function of the GI tract and the intestinal flora, thus protecting and improving the health, wellbeing and longevity of the consumer.

**HUMAN ISOLATES AS CANDIDATES FOR PROBIOTICS**

A large range of lactobacilli and bifidobacteria have been isolated, characterized and are used alone or in combination. Various growth factors (prebiotics) are added to the substrate, which can be either milk or, as in some Asian countries, another substrate of vegetable origin, where mostly soymilk is used. The cell density in either type of final product should be high (around 10^8 cfu/mL). Probiotic strains should be host specific. It is important to select human isolates for human probiotic
products and animal probiotics for animal products. This selection measure can provide better protection for the host.

**SOME SELECTION CRITERIA FOR PROBIOTICS AND THEIR DESIRABLE PROPERTIES**

To qualify as beneficial probiotic microflora, the following criteria should be met:
The microflora should:
- grow fast and by fermenting produce a tasty product
- be present in high numbers of viable cells in the product
- be a component of the normal intestinal flora
- not produce any toxic metabolites and not be pathogenic for the host
- have the ability to multiply in the GI tract
- be acid and bile resistant, and metabolically active in the whole GI tract
- be able to adhere to the GI tract
- possess antimicrobial activity against most pathogenic bacteria
- produce organic acids in situ and thereby reduce the intestinal and colon pH.

There are several species of probiotic Lactobacilli and Bifidobacterium ssp. within this environment, possessing complex enzymes and functions that have the potential to benefit the host’s health. However, when there are alterations in the gastrointestinal barrier or in the composition of the microflora of the gut, there is opportunity for creating malfunction and disease.

**PROBIOTICS**

In the late nineteenth century, microbiologists identified microflora in the GI tract of healthy individuals that differed from those found in diseased individuals. These beneficial microflora were termed probiotics already in 1953 by Kollath [72]. In 1992, Fuller described probiotics as “a live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance” [38]. Probiotics are defined as biologically active human or animal-derived components. Literally meaning “for life,” these microorganisms have been proven to exert health-promoting influences in both humans and animals when administered in adequate amounts.

Probiotic microflora display numerous health benefits beyond just providing basic nutritional value. They cooperatively maintain a delicate balance between the GI tract and immune system. When this balance is disrupted, disease and inflammation result. Inflammation and over-stimulation of the immune system by pathogenic bacteria are competitively inhibited by mucosal adherence of normal beneficial microflora. A healthy GI tract with adequate mucus production and appropriate bacterial colonization prevents the overgrowth of pathogenic bacteria, modulates disease processes, and prevents widespread inflammatory disorders. With increasing understanding that beneficial microbes are required for health maintenance and disease prevention, probiotics may be commonly used as therapeutic tools by health care practitioners all over the world.

Already in 1994, the World Health Organization (WHO) deemed probiotics to be the next-most important immune defense system [169], when commonly prescribed antibiotics were rendered useless by antibiotic resistance. The use of probiotics in antibiotic resistance is termed microbial interference therapy. With increasing understanding that beneficial microbes are required and are essential for health, probiotics have become a common therapeutic tool used by health care practitioners when prescribing antibiotic treatment.

**PREBIOTICS**

Prebiotics are the special nourishment foods for probiotics; the non-digestible food components that increase their growth in the gastro-intestinal tract. The interest in probiotic foods in the society is increasing, concomitantly with the problems arising from the overgenerous use of antibiotics, which has led to the development of multi-resistant strains, and along with increased health consciousness among consumers.

Prebiotics are defined as “nondigestible food ingredients” that beneficially affect the host by selectively stimulating the growth and/or activity of probiotic bacteria in the colon [132]. Some examples are inulin-type fructans, such as chicory inulin and its hydrolysate, oligofructose, which are natural food ingredients found in various edible plants, and the nondigestible oligosaccharides i.e. dietary fibers. Prebiotic sugars are: Fructo-oligosaccharides (inulin, FOS, etc); Soy oligosaccharides; Galacto-oligosaccharides; Lactulose; Raffinose [21, 35, 36].

The concept of prebiotics was developed to overcome the technical problems associated with propagation of probiotics. Bacteria that live in our GI tract survive and grow using the partially digested foods that passes down from the small intestine to the large intestine. Detailed research has shown that some bacteria (Bifidobacterium ssp. in particular) have very specific ingredients, it is possible to increase the numbers of the target bacteria in the GI tract. This has the advantage that no external bacteria are necessary to be ingested, but rather the numbers of resident bacteria in the gut are stimulated to multiply and their cell numbers are increased. During the period of consumption of the prebiotic, the numbers of specific bacteria have been shown to increase by up to 100 fold, but similar to the situation with probiotics, when the consumption of the prebiotic stops, the gut bacteria numbers quickly return to their original values [59, 132, 133].

**SYNBIOITS**

Some products, called synbiotics, are now being marketed, containing both probiotic cells and prebiotic sugars. Such products take the advantage of both the
addition of beneficial bacteria and the encouragement of the growth of resident beneficial bacteria. Synbiotics are defined as products (probiotic+prebiotic=synbiotic) that enhance the probiotic effect. By consuming foods that contain probiotic bacteria it is believed that the population of beneficial bacteria will increase – even if it is only temporarily – and improved metabolism and health will be achieved. Since the probiotic bacteria that are consumed do not normally reside in the digestive system, they are soon washed out of the intestines, if consumption of the probiotic product is stopped. The main reason for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive a longer period of time well in the digestive system.

Prebiotics are included to enhance viability, not only in the package on the shelf, but also in the colon. The synbiotic combination allows for much greater attachment and growth rate of the healthy beneficial bacteria. Without the necessary food source for the probiotic bacteria, it will have a greater intolerance for oxygen, low pH, and temperature. In addition, the probiotic will compete against other bacteria that will take over if its specific food source is not available.

FERMENTATION

Fermentation is one of the oldest and most widespread methods of preserving foods, particularly milk. During the fermentation process, most of the substrate (milk) components in which the starter bacteria have been propagated, has been affected and more or less predigested. This pre-digestion process alters the physiological and nutritional value of the components, facilitating a faster absorption rate and rendering especially the protein moieties special biological functions. There are significant differences between non-fermented and fermented milk proteins from a biological and biochemical point of view. The heat treatment before inoculation affects milk proteins in several ways. Heat resistant proteases produced by psychrotrophs may be present in milk even after the heat treatment and can continue to affect proteins during fermentation and storage. Finally, milk proteins are affected by the proteolytic activity of starters used for inoculum [3, 27, 74].

The fermentation process can be characterized as a chemical and biological food preservation, with conditions for an incomplete metabolism of the components, and production of intermediates. The most important components are lactic and other organic acids, as well as many other compounds, with the ability to control the behaviour of various (desirable and undesirable) microorganisms and to some extent also affect the consumer. The lowered pH in all fermented milk products due to the organic acids produced, not only retards the growth of undesirable microorganisms, but also provides milk with a pleasant taste and better keeping properties.

When evaluating the nutritional and physiological properties of fermented milk products in the past, much of the interest has been concentrated on assessing the levels of some sensoric components, produced in a certain product type. The nutritional qualities or the therapeutic value of traditional and probiotic milk types have only more recently been thoroughly studied for their potential impact on human health [3].

THE EFFECT OF FERMENTATION ON MILK CONSTITUENTS

Effects on Proteins

Casein makes up 80% of the total milk protein, and whey protein, mainly as beta-lactoglobulin, alpha-lactalbumin, and immunoglobulins make up the remaining 20%. The average concentration of total proteins in milk is about 3.3% (w/v).

Milk proteins are too large to pass through the cell membranes of lactic starter cells [31] and by the proteolytic action of starters, peptides of various sizes and other types of non-protein nitrogen compounds are formed [131, 166]. The degree of proteolysis depends on the intensity of heat treatment before inoculation [115, 126], the buffering capacity of the milk used for fermentation [139], and the types and amount of proteolytic enzymes excreted by the starters used [102]. By protein denaturation a larger surface is exposed to proteolytic enzymes [15, 73, 113, 140].

The constituents of milk of particular interest are as follows: Lactoferrin (LF) exhibits antibacterial, antifungal, antiviral, antiparasite and antitumor activities. It is protective with regards to the intestinal epithelium, promotes bone growth and accelerates recovery of the immune system. Proline-Rich Polypeptide (PRP) exhibits a variety of immunotrophic functions, including promotion of T-cell maturation and inhibition of autoimmune disorders. PRP improves the status of Alzheimer patients. Casein hydrolysates are protective in diabetic animals, reduce tumor growth and diminish colicky symptoms in infants. Casein derived peptides have been found to possess antihypertensive effects. Glycomacropeptide (GMP), a peptide derived from kappa-casein, exhibits antibacterial and antiinflammatory activities.

Alpha-lactalbumin (LA) demonstrates antiviral, antitumor and anti-stress properties. LA-enriched diets are anxiolytic, lower blood pressure in rats, prevent diarrhoea and led to a better weight gain in malnourished infants. Lysozyme is effective in the treatment of periodontitis and prevention of tooth decay. Milk enriched in lysozyme has been used in feeding premature infants suffering from concomitant diseases. Lactoperoxidases demonstrate antibacterial properties. Milk-derived proteins and peptides are bio-accessible and safe and can be used with high efficiency for prevention and treatment of numerous disorders in humans [172].

The intracellular proteolytic activity of mesophilic and thermophilic lactic acid starters is generally low [10, 114]. Therefore lactic starters might use proteolytic enzymes tightly bound to the surface of the cell membrane, bound
Available biologic evidence suggests that curd size is nearly twice as fast when the ingested feed rate was nearly twice as fast when the ingested feed was fermented, compared to non-fermented milk. Experiments on piglets (Alm et al.) found that 90% of yoghurt is digested within 1 h by baby piglets, as compared to only 30-40% of non-fermented milk. Digestive juices in the gastrointestinal tract act merely on the surface of protein particles and more easily penetrate finely divided protein material. Various fermented milk products show a general decrease of the lactose content, especially in yoghurt, to about 2.3 g/100 g, as compared to 4.8 g/100 g in non-fermented milk. The free galactose content is increased about 2-3 times, resulting in high levels of free amino acids in fermented milk. The small curd size is recognised as an important factor in digestibility [125]. Digestion characteristics of various types of fermented milk proteins were compared with those of human milk [3, 33]. It was found that curd formed following ingestion of fermented milk is composed by smaller particles than that formed by non-fermented milk. Digestive juices in the gastrointestinal tract act merely on the surface of protein particles and more easily penetrate finely divided protein material. Smetinoff [151] found that 90% of yoghurt is digested within 1 h by baby piglets, as compared to only 30% of the non-fermented milk.

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Digestibility of a milk protein, when fermented, depends upon the technological process, i.e. the time, the temperature used for the heat treatment, the type of microorganisms used as the inoculum, whether proteolytic enzymes are released by the inoculum and the type and amount of various organic acids produced. Fermentation brings about various degrees of denaturation of proteins and hydrolysis of protein bonds, resulting in smaller protein particles more easily penetrated by gastric and intestinal juices, when ingested in vivo. Most fermented milk types show an improved digestibility compared with non-fermented milk [75]. Milk types which yield small curd sizes are believed to be tolerated and utilised by infants, children and adults, as they leave the stomach more quickly than milk without this property. Experiments on piglets (Alm unpublished results, 1981) showed that the digestion rate was nearly twice as fast when the ingested feed was fermented, compared to non-fermented milk. Available biologic evidence suggests that curd size is one important factor that influences the rate of digestion of milk proteins, but that many other factors must also be considered.

Effect on Milk Fat

Milk fat is up to 98-99% composed of mixed triglycerides and to 1-2% of phospholipids, sterols, carotenoids, the fat soluble vitamins A, D, E, and K, and traces of free fatty acids. The concentration of milk fat in milk is about 4% (w/v). Triglycerides form 95 to 99% of fat in milk [124]. Of the fatty acids, 60 to 70% are saturated, 25 to 35% are monounsaturated and about 4% are polyunsaturated. Physical and chemical treatment of milk results in lipolysis, which is also induced during fermentation by lipases of microbial origin. Both extracellular lipases from contaminating microorganisms and intracellular lipases from lactic streptococci and lactobacilli have been described. Intracellular lipases are exerting lipolytic activity, which is a basic property of lactic starters, but varies in degree from strain to strain. Short chain fatty acids in fermented milk, i.e. acetic acid, propionic acid and butyric acid as well as other volatile acids in minor quantities, originate mainly from amino acids and lactose. The moderate increase of free fatty acids in fermented milk products caused by the lipolytic activity from the action of the starters indicates some disruption of the fat molecule. The original composition of milk fat and the fermentation process results in improved digestibility of milk fat as compared with fats from other sources.

Effect on Carbohydrate

Lactose is the predominant carbohydrate in milk. The amount of this component does not vary much from the mean value of 4.9% (w/v). During digestion, lactose is hydrolysed into glucose and galactose, a process that is catalysed by lactases normally present in the intestinal mucosal cells, released to the intestinal juice. Hydrolysis of lactose also takes place during fermentation and the monosaccharides produced are assumed to be partly utilized by the fermenting organisms. Various fermented milk products show a general decrease of the lactose content, especially in yoghurt, to about 2.3 g/100 g, as compared to 4.8 g/100 g in non-fermented milk. The free galactose content is increased from only traces in non-fermented milk to 1.3 g/100 g in yoghurt. Other fermented milk products also show decreases down to one third of the original lactose content. Fermented milk products can therefore be recommended without any problems when formulating diets for moderately lactose-intolerant individuals [40, 48, 83].

Low lactase activity is a relatively common abnormality of the small bowel in man [8]. Lactose intolerance is prevalent in most areas where there are few dairy animals or where adults consume little or no milk [30]. About 70% of African Americans and up to 95% of certain Asian people have a more or less advanced low lactase activity [122].
The intestinal flora of the small intestine and large tract and participate in the hydrolysis of the remaining substances. In small amounts, lactic acid is tolerated butyric acid and other volatile and non-volatile substances. In severe lactose intolerance, the acid causes irritation of the intestinal mucosa leading to abdominal cramps, pain and diarrhoea.

In lactose intolerant children, lactose may be toxic and can damage the intestinal mucosa, giving rise to symptoms similar to those of coeliac disease. Steatorrhea has also been reported in individuals with low lactase activity [68]. Fermented milk is tolerated by most lactose intolerant individuals, because of the lower concentration of lactose compared with milk. Lactase containing microorganisms originating from the fermented product continue to be active in the intestinal tract and participate in the hydrolysis of the remaining ingested lactose, minimizing the intolerance symptoms.

Lactic acid, L(+) and D(-) configurations. Lactic acid is the characteristic substance in all fermented dairy products and is generated by both homo and hetero-fermentative microorganisms, by both technical and therapeutic starters [153]. Lactic acid exists in two isomeric forms L(+) and D(-) and the DL racemic mixture. Formation of the isomers depends upon the type of microorganism, substrate composition, temperature, pH, time of incubation and storage time. The presence of lactic acid changes the sensoric and rheological qualities of milk and renders the product with better storage properties. As a nutrient, lactic acid is utilized in the body as a source of energy, thereby providing 3.6 kcal/g or 15.2 kJ/g. It can be transformed through phosphoenolpyruvate and glucose-6-phosphate to glucose and glucogen.

The physiological role of lactic acid isomers in man and animals has been investigated and it has been shown that both isomers are absorbed from the human intestinal tract, although the rate of metabolism of the D(-) isomer is considerably lower than that of the L(+) lactic acid [16]. The L(+) lactic acid is the predominant metabolite formed during fermentation of milk and has the same configuration as the lactic acid formed by the human body. Substantially large quantities of D(-) lactic acid are found in yoghurt [76, 77]. Restricted consumption of high D(-) lactic acid is recommended by the World Health Organisation (WHO), and in infant nutrition, products containing D(-) or DL mixture should be avoided [168]. Production of lactic and other volatile short chain fatty acids causing the pH to decrease is important from a physiological point of view. In many digestive disorders hydrochloric acid secretion is impaired and when fermented milk products are consumed instead of non-fermented, digestion is facilitated without the aid of secretion of higher amounts of hydrochloric acid.

Production of Volatile Components

Volatile, short chain fatty acids (SCFA) in fermented milk products such as acetic acid, etc are of interest in view of their influence on sensoric properties of the product, but they may also be important from a nutritional and therapeutic point of view. The capacity of lactic starters to produce volatile compounds depends on the choice of strain, incubation time, and incubation temperature. Lb. acidophilus and Lb. bifidus (Bifidobacterium bifidum) survive the passage to the lower parts of the ileum, and especially Lb. acidophilus and Bifidobacterium spp. produce large amounts of acetic acid in vitro. It is assumed that the probiotic bacteria continue to produce acetic acid in vivo, following colonization of the intestinal tract. The antimicrobial properties of all fermented milk product, are primarily from the lactic acid content, but seem to be intensified by the other organic acids produced e.g. acetic acid [78]. Consequently, bifidus milk and acidophilus milk may exert bactericidal effects in the intestinal tract, especially following colonization by the fermenting probiotic bacteria. Consumption of fermented products may affect the microbial composition of the intestinal microflora and the concentration of pathogenic microorganisms may be reduced by the presence of probiotics.

Effect on B-Vitamin Content

B-vitamin content and the nutritional value of fermented milk products is assumed to be similar to the milk from which they are made. However, fermentation may lead to somewhat changed concentrations of B-vitamins. During fermentation there is a dramatic increase in cell population representing both colony forming units (cfu) as well as dormant cells. Microbial biosynthesis of some B-vitamins occurs and is a useful method to affect the B-vitamin content of dairy products. A general increase of folic acid was found especially for ropy milk [146]. Milk is also a very good source of riboflavin and vitamin B12. An important question concerning B-vitamins in fermented milk products is their bioavailability. The B-vitamins function as integrated parts of proteins [166] and therefore may occur both in free form and as cell-bound constituents. It is not known whether or where lysis of lactobacilli occurs during the digestion process and whether cell-bound vitamins are made available to the host. Possibly, some types of enzymes derived from the normal intestinal flora participate in the release of cell-bound vitamins, and it may be speculated, that one way in which lactobacilli may influence the metabolic activities of the normal flora is by supplying it with B-vitamins.

Minerals. The ash content of normal milk is about 0.7% (w/v), but it can vary between much higher and much lower values. Milk as known is an especially good source of calcium.

Production of Bilocines

There is conclusive evidence that lactobacilli produce substances that inhibit the growth of pathogens in vitro and in vivo [41, 142, 144, 145]. Lactobacilli as dietary supplement alleviate intestinal infections in the GI tract in both humans and animals [146]. Among the various by-products formed during lactobacilli growth, are certain substances, such as hydrogen peroxide,
Lactolin, Lactocidin, Acidolin and many others with described and pronounced physiologic effects.

The nutritional and physiological effects of fermentation on cow’s milk are:

- digestibility and absorption of milk proteins are enhanced
- digestibility of milk fat is improved
- lactose content is reduced
- better tolerance by lactose malabsorbers
- better tolerance by individuals suffering from low HCl secretion and high pH in the stomach
- enhanced retention of minerals such as calcium
- the content of certain B-vitamins are enhanced
- fermented milk products provide high numbers of viable and dormant cells along with all the metabolites produced during the fermentation and storage.

A probiotic milk can function as:

- carrier of essential nutrients, which can prevent nutritional disorders of many kinds in the very young, as well as the old. There are many examples, such as energy or protein malnutrition, vitamin or mineral deficiencies. By consuming a suitable portion of the probiotic food every day, these disorders can be prevented. The elderly individual may need certain components in larger amounts, due to a lower absorption rate, as is the case with the mineral calcium for prevention of osteoporosis and other bone diseases.
- provider of supplements or increased amounts of certain components, to cover the needs and produce better results in certain sports activities.
- supplement containing large amounts of fiber or non-digestible carbohydrates, for obesity control and to provide a longer period of satiety while consuming an unchanged quantity of the food.
- special food composed in a way that is suitable to special groups, such as those suffering from allergies or intolerances, inherited disorders such as coeliac disease, gluten intolerance, PKU, or various types of disaccharide intolerance, etc.

THE GASTROINTESTINAL TRACT AND ITS FUNCTIONS

The composition of the intestinal microflora is constantly changing, being influenced by factors such as diet, emotional stress, age and treatment with antibiotics or other types of medication. The human GI tract is home to a vast and complex bacterial ecosystem, hosting over 500 different species. In the GI tract in a healthy adult, the average length of the human intestine can be anywhere from 6 to 8.5 m long depending on the size and age of the person and contains approximately 2 to 5 kg living bacteria.

The human intestinal tract contains ten times as many bacterial cells as there are tissue cells in the entire body. The gut microflora plays a vital role in human health and perform important metabolic functions that support the digestive system. Research reveals that the gut lining is primarily nourished by nutrients produced by the favourable bacteria – not by our blood supply, as was previously believed. The species of lactobacilli normally present include Lb. bifidus (Bifidobacterium bifidum), Lb. acidophilus, Lb. casei, Lb. fermentum, Lb. salivarius, Lb. brevis, Lb. leichmanii, Lb. plantarum and Lb. cellobiosus [102]. About one third of the faecal dry weight in adults consists of bacteria. We are completely dependent upon the activities of these bacteria for the manufacture of K vitamins, the assimilation and distribution of nutrients, and for the suppression of pathogenic and putrefactive bacteria. Without bacteria to produce nutrients, cell damage can also occur, leading to a loss of function in the gut lining.

Therefore, maintenance of the proper balance of bacteria in the gut is vital to good health. For example, the short chain fatty acid butyric acid, derived from carbohydrate fermentation, provides the main fuel for colonocytes in the large intestine. Lactobacilli species can prevent food decay, preserve antioxidants and vitamins, remove toxic food components, and prevent pathogenesis of Enterobacteriaceae, S. aureus, and Enterococci eventually found in fermented foods.

In the process of performing their metabolic activities in the human GI tract, these microflora convert complex ingested food constituents into easily digestible forms, perform detoxification processes and are producers of metabolites of nutritional and therapeutic significance to the host. A delicate balance exists in the symbiotic relationship between the microflora and the human host.

Probiotics modulate not only the endogenous flora of the GI tract, but also the immune system [52, 60, 92, 165]. Lactobacilli enhance both cellular and humoral immunity [162, 163]. Lactic acid-producing bacteria stimulate various aspects of the immune system, including the phagocytic function of macrophages, natural killer cells, monocytes, and neutrophils.

Following a rotavirus vaccination, Lb. rhamnosus GG induce IgM-secreting cells and improve IgA seroconversion, which enhances immunoglobulin response to vaccines [165]. In addition, IgA response to rotavirus is enhanced by administration of Lb. rhamnosus GG [161]. These reports confirm the positive effects of probiotics on innate and acquired immune enhancement, most likely resulting from an ability to bind to gut epithelium. After binding, antibody production is stimulated and the complement and reticuloendothelial system is activated. The interaction between probiotics and epithelial cells has been termed bacterial-epithelial cross-talk [165]. Lb. rhamnosus GG has been shown to enhance the antibody response in adults receiving typhoid vaccination [163]. Another example of probiotic enhancement of the immune response, can be seen in the activation of the reticuloendothelial system and complement cascade by...
Saccharomyces boulardii [161]. Interaction between the commensal GI flora and the gut-associated immune system is an important key in maintaining normal immune function.

**INTESTINAL FLORA COMPOSITION AND ESTABLISHMENT**

**Colonization.** The ecosystem of the human GI tract is extremely complex and it is colonized by more than 500 species of bacteria. Although lactobacilli in general represent only a smaller percentage, their metabolic functions make them extremely important. The GI tract of a newborn baby is sterile until the infant ingests vaginal and faecal microflora at delivery and is additionally colonized by the microflora in the environment. The population of microflora in the infant GI tract is further enhanced by feeding. The breast-fed infant contains a colon population of 99% Bifidobacterium ssp. with some Enterobacteriaceae and Enterococci present, but virtually no Bacteroides, Staphylococci, or Clostridia. In contrast [58], Bifidobacterium ssp. does not predominate in the bottle-fed infant. Later on, when breast-fed infants are switched to cow's milk or solid foods, they are colonized by Bifidobacterium ssp., Clostridia, Lactobacilli, Bacteroides, Streptococci [138], among many others.

With increasing age the probiotic flora is slowly disappearing and in the elderly it is almost zero [106]. This decrease may be correlated with age-related diseases also associated with the colon and its function or malfunction. Therefore, it is of greatest importance to daily supplement the diet of the ageing individuals with probiotics in order to retain their health.

The protective and immune barrier of the human GI tract is diverse. It includes the epithelial layer, the mucous layer, the mechanics of peristalsis and desquamation, and actions of secretory IgA, all of which impact bacterial attachment. After attachment, colonic bacteria are prevented from mixing with the host's eukaryotic cells by the epithelial layer, which acts as a vital barrier to invasion. The barrier's healthy structure and proper functioning are essential for ensuring that the GI tract and the microflora are cooperatively maintained.

The type and number of indigenous microflora increase distally along the length of the GI tract. The upper GI tract has relatively fewer bacteria, due to the saliva production and increased intestinal motility, which effectively move bacteria along the intestine and prevent large numbers from adhering to mucosal surfaces. In addition, gastric acid suppresses growth of bacterial cells in the stomach. The relatively sparse flora of the upper intestine generally numbers less than 10^3 colony forming units (cfu) per milliliter (mL) of contents, until the mid-ileum, where the population increases to 10^5 cfu/mL of contents indicating a shift toward the flora that heavily populates the colon.

Favourable characteristics found in probiotics colonizing the human gut are exhibited by Lb. plantarum, Lb. rhamnosus, Lb. reuteri, and Lb. agilis, and Bifidobacterium species, among many others. However, these lactobacilli and bifidobacterium species are scarce in people living in industrialized countries. Lb. plantarum i.e. is only carried by 25 percent of the general population in the United States, whereas nearly 100 percent of the population in Africa and Asia are colonized with Lb. plantarum and other probiotic species. The Western diet is usually heat treated to the extent that it contains decreased amounts of probiotics if any at all, and much lower levels of vegetable fibers than in the diet of developing countries. Therefore, the consumption of probiotic foods in the Western countries is life saving.

**THE EFFECT OF PROBIOTICS ON PATHOGENIC BACTERIA**

Probiotics reduce plasma levels of bacterial endotoxin concentrations, at least in part, by inhibiting translocation of bacteria across the GI lumen into the bloodstream [163]. Lactobacillus colonization in germ-free rats has been shown to decrease gut permeability to mannitol [161]. In addition, administration of Lactobacillus to interleukin-10 knockout mice decreased translocation of bacteria to extraintestinal sites and reduced myeloperoxidase concentrations, often associated with inflammation in the bowel [91].

Decreases in translocation of bacteria may occur as a result of the ability of probiotics to tighten the mucosal barrier [165]. Although very little is known about the specific molecular mechanisms by which indigenous flora tighten the mucosal barrier, this may be accomplished by bacterial-epithelial cross-talk and up-regulation of growth factors and receptor sites. Whatever the method of tighten the barrier to bacterial entry, the net effect is to modulate systemic intestinal allergy and inflammation. Allergy-induced intestinal inflammation mediated by faecal tumor necrosis factor-alpha is decreased by Lb. rhamnosus GG. Lb. rhamnosus GG also increases mucosal regeneration and reduces faecal urease production, a correlate of inflammation associated with chronic arthritis [161].

There are several ways in which probiotic microflora can prevent pathogenic bacteria from adhering to and colonizing of the gut mucosa. Probiotics disallow colonization by disease-provoking bacteria through competition for nutrients, immune system up-regulation, production of antitoxins (bacteriocins) [96] and up-regulation of intestinal mucin genes [90]. Increased mucous production prevents adherence and colonization by competing microflora, thereby preventing imbalances.

Probiotics lower the pH in the colon and promote the growth of non-pathogenic bacteria by production of short chain fatty acids [43]. Acetic acid has antimicrobial activity against molds, yeasts, and bacteria. In addition, probiotics exert protective effects through the production of hydrogen peroxide and benzoic acid, which inhibit many pathogenic and acid sensitive bacteria [1, 13, 82, 116]. Probiotics can also reduce the growth of Clostridium difficile and alter toxin receptors for C.
The inhibition of pathogenic bacteria by probiotics is a combination of structure and function. The bacteriocins-antibacterial compounds produced by Lb. acidophilus are antagonistic within a specific spectrum by inhibiting other strains of Lactobacilli. Therefore, the practice of combining probiotics needs to include beneficial bacteria that do not inhibit other included strains [118]. Adherence of beneficial flora competitively inhibits the colonization of the mucosa by pathogenic bacteria and reduces the over-stimulation of the immune system [146]. A healthy colon with adequate mucus production and appropriate bacterial colonization prevents the adherence of pathogens, modulates disease processes, and prevents widespread inflammatory disorders.

Probiotics exert a positive influence on the health of the population in general, with regards to cardiovascular disease, cancer, infectious diseases, inflammatory diseases and allergies, i.e. most of the common diseases in our society today. The socio-economic potential of the probiotic concept is thus immense.

The consumption of fermented milk products containing probiotic lactobacilli is suggested for gastro-intestinal and other types of infections; in reduced secretion of gastric and intestinal juices; in lactose intolerance; in stress situations; in side effects of long-term use of drugs; liver and bile malfunctions; but also for weight control programs; repair of skin injury; eczema; oral infections and many other diseases.

**SOME PROBIOTIC EFFECTS ON CHILDREN'S HEALTH**

There are many clinical studies investigating the effects of probiotic foods in children and latest results are currently underway. The intestinal flora and the immune defence of both healthy and sick children can be immensely improved by adding probiotics to their daily diets, to improve the status of the immune system, and to combat allergies and certain types of intolerances [25].

For example, a tolerance study of a baby food, fermented with Lb. plantarum 229v and nutritionally adapted to children from the age of 6 months has been carried out with good results. In another study, the effects of lactobacilli on children who suffered from recurrent Clostridium difficile infections were investigated.

Antibiotics are prescribed frequently in children and antibiotic-associated diarrhoea is common in this population. Probiotics may prevent the antibiotic-associated diarrhoea via restoration of the gut microflora. Studies were carried out using Lb. rhamnosus GG, Lb. sporogenes, and Saccharomyces boulardi at 5 to 40 billion colony forming units/day-dose, which showed promising results [65, 170].

**SOME PROBIOTIC EFFECTS ON THE HEALTH OF THE ELDERLY**

In Japan and in some parts of Europe, people are healthier and are living longer than elsewhere, and the average age is still increasing. Advancements in science and medicine, as well as improved living standards, have led to a steady increase in life expectancy, and subsequently a rise in the number of the elderly population. For the well-being of the elderly (65+), an active, healthy and independent lifestyle is of major importance.

The aging demographics of the 21st century will continue to fuel a self-care phenomenon. The number of individuals over the age of 50 might increase by 48% compared with only 16% for the 13- to 24-year-old age group, over the next decade. Also of great importance is however, the increase of the number of individuals >65 years old. By 2035, >70 million people have the possibility be in this age bracket. With a continued increase in the general age of the population, chronic diseases of ageing, such as heart disease, cancer, osteoporosis, Alzheimer’s disease and age-related macular degeneration, among many others, are inevitable, imposing an enormous pressure on the cost of health care. This burden on the society must be minimized at all cost. Preventive healthcare strategies using probiotic foods and other nutraceutical approaches, could considerably reduce the annual healthcare costs. Consumer’s interest in self-care and dissatisfaction with the current healthcare system, will continue to be a leading factor for motivating consumers food purchasing decisions.

The intestinal microflora is important for the maintenance of host health, providing energy, nutrients and protection against invading organisms. Although the colonic microflora is relatively stable throughout adult life, age-related changes in the GI tract, as well as changes in diet and host immune system inevitably affect the GI flora population and composition.

Shifts in the composition of the intestinal microflora may lead to detrimental effects for the elderly host [106, 167]. Increased numbers of facultative anaerobes, in conjunction with a dramatic decrease in beneficial probiotic organisms such as the anaerobic lactobacilli and bifidobacteria, amongst other anaerobes, have been reported. These changes, along with a general reduction in species diversity in most bacterial groups, and changes of diet and digest physiology such as intestinal transit time, may result in increased putrefaction in the colon and a greater susceptibility to disease. Therapeutic strategies to counteract these changes have been suggested in ageing people, which include dietary supplements containing prebiotics, probiotics and a combination of both of these, synbiotics. Limited numbers of feeding trials show promising results with these supplements [57, 70, 89].
PROBIOTICS AS HEALTH PROMOTORS IN SPECIFIC DISORDERS

Advances have been made in understanding the effect of probiotics on specific pathological conditions. Increasing numbers of animal and human studies point to appropriate uses of probiotics as therapeutic agents for a very large number of conditions [66].

High blood pressure. Peptides derived from milk proteins can have angiotensine-converting enzymes inhibiting properties and may be used as an antihypertensive component. Lb. helveticus LBK-16H fermented milk containing bioactive peptides in normal daily use (150 mL/day) has been shown to have a blood pressure-lowering effect in hypertensive subjects [17, 64, 136, 143, 158, 171].

Lipid and cholesterol levels. Results from several studies indicate that the reduction of total plasma cholesterol can lower the incidence of coronary heart disease and several findings indicate that consumption of lactobacilli reduces the serum cholesterol level [94, 95]. Sterols and lipids are altered markedly by the intestinal microorganisms, and bile acids are deconjugated and dehydroxylated. Possibly, the deconjugation of bile acids by the intestinal lactobacilli may function to control the microbial flora composition [67, 85]. Influence of the intestinal flora on sterol and bile acid metabolism appears to be advantageous to the host and may be of significance in human nutrition by preventing the accumulation of excessive cholesterol by using probiotic tablets [84]. The feeding of a milk formula supplemented with probiotic lactobacilli to infants was shown to result in lower levels of blood cholesterol as compared to feeding of milk without lactobacilli.

Hyperlipidemia. Another unexpected benefit of probiotics is serum lipid reduction. Lb. acidophilus NCIMB 1748 and L. sporogenes were found to take up cholesterol in the presence of bile and in the absence of oxygen, both conditions present in the intestinal tract [9, 118, 137]. The effect on cholesterol assimilation is believed to be strain-dependent. Certain strains of Lactobacillus have ability in vitro to assimilate cholesterol, as demonstrated by identification of cholesterol in cells during growth and decreases in the concentration of cholesterol in the growth medium. Uptake of cholesterol occurred only when the culture was grown anaerobically in the presence of bile, such as would be expected to occur in the human gastrointestinal tract. This may have important implications in preventing reabsorption of cholesterol back into systemic circulation.

The effect of probiotics on some carcinogens. The presence of nitrates and nitrites in food and conversion of the nitrates into nitrosamines has been a subject of much controversy. These compounds, also carcinogenic for humans, occur naturally [53] in many foods or can be synthesized in the intestine by certain microorganisms. There is evidence that probiotic intestinal microflora biodegrade nitrosamines [11, 106]. Lower concentrations of amines were found in animals following administration of products containing Lb. acidophilus. In piglets receiving the probiotics, diarrhoea was of shorter duration and less severe, than in control animals. Evidence exist showing that lactobacilli, especially Lb. casei and related microorganisms, biodegrade nitrates and nitrites [37, 38, 58].

Colon Cancer. Epidemiological studies have shown a higher incidence of colon cancer in populations consuming “Western” diets than in those customarily on vegetarian diets. In one such study involving populations in Copenhagen, Denmark (high risk) and Kuopio, Finland (low risk) there was a 4-fold variation in the incidence of colon cancer [128]. Higher intakes of dietary fiber and milk in the low incidence area indicated a possible protective effect that was unrelated to the transit time in the Gl tract. An interesting observation was that in the low incidence area, faecal samples showed a significantly higher population of lactobacilli [71, 107].

The importance of intestinal lactobacilli in the ethiology of colon cancer has been studied by Goldin et al. [47], Reddy [129], Pool-Zobel et al., [123]. Goldin et al. [46] have shown that feeding rats a high beef diet caused much higher levels of bacterial nitroreductase, azoreductase and Beta-glucuronidase, than when the diet was high in vegetables and grain [44]. It was postulated that high beef diet favoured establishment of an intestinal microflora with the metabolic potential to convert procarcinogens to carcinogens, but when the animals received the same diet supplemented with viable lactobacilli, the faecal bacterial enzymes associated with carcinogenesis were substantially reduced [45]. Animals on the beef diet plus lactobacilli had an experimental cancer incidence of 40% versus 70% in the controls not receiving any lactobacilli supplements. The addition of Lb. acidophilus to diets of omnivores resulted in significant decreases in faecal bacterial Beta-glucuronidase, and nitroreductase activities. However, 30 days after discontinuing the supplements, the faecal bacterial enzyme activities returned to normal levels. This tells us that lactobacilli prevention therapies should continue to unlimited times.

Populations at high risk for colon cancer have been found to harbour a gut flora which efficiently metabolizes steroids and hydrolyzes glucuronides. A diet containing large amounts of viable lactobacilli significantly lowered these activities in such individuals. Further effects of probiotics in the Gl tract have been observed in experimental models of colon cancer [51]. In animal studies, the rates of formation of colon tumours were reduced in those receiving Lactobacillus strains [51, 163]. Several species of lactic acid-producing bacteria appear to prevent carcinogenic compounds from inducing the first crucial step of tumour genesis that may ultimately act as a proto-oncogene or inactivate tumor suppressor genes. Administration of Lb. rhamnosus GG has been shown to suppress bacterial enzyme activity such as [beta]-glucuronidase, urease, faecal glycocholic hydrolyse, nitroreductase, and tryptic activity [52]. Of particular interest are reductions in glucuronidase,
ni troreductase, and azoreductase, all of which might play a role in activation of pro-carcinogens in the large intestine.

**Hepatic disease.** Effects of antibiotics, probiotics and prebiotics in the treatment for hepatic encephalopathy is thoroughly investigated and described. It is believed that the preparations can be successfully used to beneficially affect a prevailing hepatic disease [18]. Another case report was published documenting the effect of a high potency, multicultured probiotic preparation in liver cirrhosis [32]. The probiotic preparation contained *Streptococcus thermophilus, Bifidobacteria, Lb. acidophilus, Lb. plantarum, Lb. casei, Lb. debrueckii bulgaricus* and *Streptococcus faecium* at a concentration of 10¹¹ viable cells/g. Oral bacteriotherapy could improve microbial balance and lower the portal pressure with a reduced risk of bleeding. A 76-year-old male patient with liver cirrhosis and esophageal varices was given the probiotic preparation for one month, followed by a one-month washout period and a second one-month cycle of treatment. Blood velocity and flow were measured in the portal, splenic, and superior mesenteric veins before the test and during treatment. The mean blood velocity and flow were greatly increased in the portal vein after one month of treatment, but at the end of the washout period both parameters returned toward baseline levels. After a second cycle of treatment, blood velocity and flow measured in the portal vein were again strongly increased in comparison to baseline. Patients with liver cirrhosis harbour an altered microflora over-populated with urea-splitting bacteria. Oral antibiotics are commonly administered to reduce the production of mediators involved in the pathogenesis of hepatic encephalopathy, portal hypertension, and variceal bleeding. Instead of antibiotics, probiotics should be administered and this could be a safe way to regulate portal pressure [32, 111].

**Inflammation/Arthritis:** Probiotic supplementation has both direct and indirect effects. Probiotics exhibit direct effects locally in the GI tract, including modulation of resident bacterial colonies and vitamin production. There are also indirect effects exerted at sites outside the GI tract, including the joints, lungs, and skin. Indirect effects most likely result from an impact on immunity, via changes in inflammatory mediators such as cytokines. Modulation of inflammatory responses may be related to regulating or modulating the immune system both locally in the GI tract and systemically. It is speculated that inflammation associated with rheumatoid arthritis may be improved by the use of probiotics [93, 117]. Thirty patients with chronic juvenile arthritis were randomly allocated to receive *Lb. rhamnosus* GG or bovine colostrum for a two-week period. Immunological and non-immunological gut defences have been investigated in blood and faeces. It is believed that gut defence mechanisms are disturbed in chronic juvenile arthritis. Orally administered *Lb. rhamnosus* GG has the potential to reinforce normal mucosal barrier mechanisms. When inflamed, the gastrointestinal tract becomes permeable and serves as a link between inflammatory diseases of the GI tract and extra-inflammatory disorders, such as arthritis. Down-regulation of the immune system and subsequent reduction in GI permeability can result from consuming probiotics.

**Allergies/Eczema:** Perinatal administration of *Lb. rhamnosus* GG decreased subsequent occurrence of eczema in at-risk infants by one-half [109]. In newborn infants, the initial bacteria to colonize the sterile GI tract may establish a permanent niche and have a lasting impact on immune regulation and subsequent development of atopic disorders. In infants with already established eczema, significant improvements in dermatitis were noted after a one-month trial with *Lb. rhamnosus* GG-fortified hydrolyzed whey formula. Probiotics may enhance endogenous barrier mechanisms of the gut and alleviate intestinal inflammation, providing a useful tool for treating food allergy [69, 92]. Probiotics have also been found to up-regulate anti-inflammatory cytokines, such as interleukin-10, in atopic children [119, 120]. This is seen both as an immuno-stimulatory effect in healthy subjects and as a down-regulation effect of immuno-inflammatory responses in hypersensitive patients. Probiotic bacteria are important in down-regulating inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy [39, 92, 109].

An adverse balance among intestinal bacteria with marked reduction in lactic acid bacteria and increase in putrefactive pathogens in the faecal flora has been observed in food allergy and eczema. The beneficial role of lactic acid bacteria in preserving intestinal integrity and health has been documented extensively. An adverse balance among intestinal bacteria with marked reduction in lactic acid bacteria and increase in putrefactive pathogens in the faecal flora has been observed in conditions like food allergy and eczema. In hypersensitive patients, probiotics down-regulated a milk-induced inflammatory response [19, 116]. This was secondary to prevention of increased receptor expression in monocytes and neutrophils. Individuals in the same study without milk sensitivity were not found to have receptor down-regulation, when taking probiotics.

**HIV and compromised immunity.** Further evidence of a positive effect of probiotics on the immune system can be seen in a study examining *Lb. plantarum* 299v supplementation in children congenitally exposed to HIV [29]. Children with HIV infections have episodes of diarrhoea and frequently experience malabsorption associated with possible bacterial overgrowth. Seventeen HIV-infected children were randomized to receive either *Lb. plantarum* 299v or placebo. In the treatment group, colonization with *Lb. plantarum* 299v occurred within two weeks, but did not persist after cessation. No adverse effects were reported. Improvements were noted in anthropometric measurements such as height and weight in follow-up visits. A marked increase in immune response was demonstrated by a change from complete anergy to normal immune response which was seen in one patient.
These data suggest that *Lb. plantarum* 299v can be used safely in immuno-compromised hosts, may indeed have a positive effect on immune response, and has the potential to improve growth and development in children. Enhanced immunity and increased resistance to infection has been demonstrated in both animals and humans. In the immunodeficient euthymic mouse model, Lactobacillus spp. and Bifidobacterium spp. decreased disseminated systemic *Candida albicans* [164]. In addition, in a placebo-controlled trial, children with cystic fibrosis had reduced severity of pneumonia when *Lb. rhamnosus* GG was administered. Protection from respiratory disease is related to up-regulation of mucin cells along with the enhanced antibody response. The use of probiotics in immune-compromised patients appears promising [156].

**Disaccharide mal-digestion.** Lactose intolerance occurs frequently in large populations in various parts of the world [96]. Milk contains up to 5% lactose while yoghurt contains less than 3%, the rest is fermented to organic acids. The replacement of milk with yoghurt containing viable lactobacilli or other fermented dairy products, may allow for better digestion and decreased diarrhoea and other symptoms associated with lactose intolerance, due to the release of microbial lactase during the digestion process. A similar benefit was identified in infants with sucrose deficiency, which causes diarrhoea from sucrose ingestion. Enhanced digestion of sucrose was demonstrated when Saccharomyces cerevisiae, a yeast containing the enzyme sucrase, was administered therapeutically.

**Diarrhoea.** Probiotic agents are recommended as an important tool in the treatment of gastrointestinal problems in infants and children. Gastroenteritis and acute diarrhoeal episodes can be related to viral, bacterial, or parasitic pathogens. The prevention and management of acute viral and bacterial diarrhoea as well as the control of antibiotic-associated diarrhoea are areas of significant potential benefit. Probiotic strains, including *Lb. rhamnosus* GG, *Lb. reuteri*, *Saccharomyces boulardii*, several Bifidobacterium species, have been shown to have significant benefit for controlling diarrhoea [43, 90, 96, 134, 135, 149, 157, 162, 163]. In the pediatric population, probiotics appear to benefit viral diarrhoea, possibly by increasing secretory IgA and decreasing viral shedding, suggesting an immunological mechanism [54, 79, 105].

Several studies have demonstrated improvement when acute diarrhoeal disorders, including rotavirus infection, traveller's diarrhoea, and more serious bacterial infections such as those caused by *Clostridium difficile*, were treated with probiotics [42, 91, 121, 134, 135, 149]. Studies using Lactobacillus species or *Saccharomyces boulardii* suggest a beneficial role during *C. difficile*-related infections [14, 50, 80, 97, 98, 121, 127, 155]. In small bowel bacterial overgrowth, and in particular, short bowel syndrome, Lactobacillus species were shown to be effective in improving the symptom complex [160]. The action of probiotics increases the intestinal mucin production, which prevents the attachment of enteropathogens.

**Traveller's diarrhoea.** Travellers all over the world are plagued by many kinds of GI disturbances and some of these can turn out to be long lasting infections resulting in a carrier state. After the first acute phase of the illness, a high number of individuals, although not ill, continue to excrete the pathogenic microorganisms in the faeces. This may cause social, psychological and economic problems to both the individual and society. A preventive and curative measure was to ingest large amounts of viable cells of probiotics, in various types of probiotics (*Lb. acidophilus* 1748; *Lb. acidophilus*, *Lb. rhamnosus* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Bifidobacterium bifidum*) before, during, and after a journey to contaminated areas [4, 5].

**Side-effects of antibiotic treatment.** Therapy with antibiotics, particularly long-term and especially via oral administration, is likely to alter the balance of the intestinal flora. Powerful antibiotics such as Clindamycin, Lincosycin and others may cause drastic changes. Therapy with Clindamycin may eliminate most of the anaerobic microorganisms such as bifidobacteria, lactobacilli, bacteroides, anaerobic cocci with the exception of eubacteria and clostridia, while Lincosycin completely eliminates the normal faecal flora more or less persistently [26]. Antibiotic therapy has often caused intestinal distress subsequent to controlling the causes of infection. Clofils are better able to proliferate and produce acids and gas that cause diarrhoea and flatulence. Administration of probiotic lactobacilli has been used to re-establish the intestinal flora composition and to obtain relief from the intestinal distress. Changes in the normal intestinal flora resulting from antibiotic therapy emphasize the importance of promptly re-establishing the normal microbial balance. The dietary administration of lactobacilli preferably in conjunction with bifidobacterium spp. may help in regeneration of the normal gut flora composition and function.

**Constipation.** In maintaining "good health", the gastrointestinal tract, especially the large intestine and its microflora, is believed to play an important role. A colon that functions well causes frequent bowel movements and prevents constipation, and possibly reduces the risk of colon cancer. A normal microflora is an important determinant of colonic health and thus the wellbeing of the whole individual.

Lactobacilli administration to severely constipated geriatric patients has a beneficial effect on their bowel movement and stool frequency. This was shown in several studies [4, 5, 159]. Regulation of bowel movements and a properly functioning intestinal tract is important in every individual, but it is of utmost importance in old age. The lactobacilli and lactic acid containing products with their antibacterial properties help to correct constipation and diarrhoea as well as intestinal intoxications due to colonic dysfunctions. Consumption of predigested fermented milk products has many advantages for the aged individuals.

**Helicobacter pylori infections.** Evidence for the use of probiotics in the treatment of *Helicobacter pylori*
infection has been conflicting. H. pylori may not always be eradicated with antibiotics and acquisition of resistance is often a serious problem. It was shown [1] that Lb. salivarius is capable of producing high amounts of lactic acid, which can inhibit the growth of Helicobacter pylori in vitro. This was subsequently confirmed in vivo in a murine model and it was concluded that probiotics could be effective against H. pylori. The higher the level of lactic acid production by Lactobacillus, the more potent was the effect on reducing H. pylori urease activity. In addition, the number of H. pylori colonies decreased to an undetectable level with regression of inflammation in some of the treated mice. The anti- ulcer effect of bifidobacteria, lactobacilli and streptococci were examined concerning their ability to produce high levels of polysaccharides, such as rhamnose. The study showed that this polysaccharide had a healing effect on gastric ulcers [110]. When comparing Lb. acidophilus, Lb. casei, and Lb. salivarius, Lb. acidophilus specifically was unable to suppress H. pylori in vivo, possibly due to a low level of lactic acid production, resulting from poor colonization and growth in the stomach. Lb. salivarius being able to produce high levels of lactic acid was found to be most effective [12].

Infections of the genito-urinary tract. Lactobacilli have long been considered to be the protective flora in the vagina. Women with vaginal infections have used various non-prescription products in an attempt to restore their normal vaginal flora. Lactobacillus species capable of producing hydrogen peroxide have been associated with normal vaginal flora. However, much effort must be applied to find strains of Lactobacilli, which are able to produce hydrogen peroxide, in order to obtain positive results [22, 61, 62, 101].

There is a developing role for the use of probiotics in the genito-urinary system, especially for vaginitis, whether of bacterial or fungal etiology [28]. Lb. sporogenes in non-specific vaginitis gave symptomatic relief in the majority of women who used the probiotic vaginally. Lb. acidophilus was found effective in treating vaginal Candida albicans after oral use [9, 34]. The study points to vaginal contamination with faecal flora as the possible rationale for the effectiveness [160]. Both oral probiotics and vaginal suppositories of probiotics have been shown to reduce the incidence of recurrent urinary tract infection [137].

Irritable Bowel Syndrome. Probiotics exhibit a direct effect on the gut in the treatment of inflammatory and functional bowel disorders. In the most common intestinal disorders, irritable bowel syndrome, Lb. plantarum 299v and DSM 9843 strains were shown in clinical trials to reduce abdominal pain, bloating, flatulence, and constipation [160, 112]. Saccharomyces boulardii also decreased diarrhoea in irritable bowel syndrome, but was not effective in alleviating other symptoms [20, 23, 96].

Inflammatory Bowel Disease. It has been reported that probiotic combination therapies may benefit patients with inflammatory bowel disease [24, 141]. Twenty-six patients received a combination product containing several types of probiotics, such as Streptococcus thermophilus, Bifidobacteria, Lb. acidophilus, Lb. plantarum, Lb. casei, Lb. debrecensis bulgaricus, and Streptococcus faecium at 6 g per day for 12 months. 19 patients remained in remission during the whole treatment period. Saccharomyces boulardii in patients with Crohn's disease was found to extend the remission time and reduce relapse rates [49, 55]. Thirty-two patients with established Crohn's disease but in remission were randomized to receive either 3 g daily mesalamine or 2 g daily mesalamine plus Saccharomyces boulardii. Six out of 16 patients enrolled in the pharmaceutical-only arm relapsed while only one of 16 in the probiotic arm relapsed. Both Saccharomyces boulardii and Lb. rhamnosus GG have been reported to increase secretory IgA levels in the gut. Ongoing investigation into the use of probiotics in inflammatory bowel disease has generated considerable interest [130, 147, 154].

Disturbances in severe stress situations. The effect of severe stress on intestinal flora composition in cosmonauts after prolonged space flights [86] and the quantitative composition of the gut microbiota with regards to lactobacilli number and type, before and after the cosmic flights of different durations were investigated [81]. It was found that lactobacilli decreased when an individual is under severe stress. Some individuals experienced diarrhoea, others had only minor symptoms, but if stress periods were of longer duration many types of GI disturbances could occur [81, 87, 88, 152]. Before take-off and landing possibly due to emotional stress, the lactobacilli flora of the cosmonauts showed marked changes regarding lactobacilli. After a space flight, the number of potentially pathogenic enterobacteria, were increased and the lactobacilli almost eliminated. It was also shown that the administration of lactobacilli containing products could be used with success in order to minimize the disturbances caused in cosmonauts during space flights. Everyday stress situations can also cause alterations in the quantity of lactobacilli in the gastrointestinal flora composition. This can be overcome by the daily supplementation of the diet with lactobacilli containing foods.

THE FUTURE OF PROBIOTIC FOODS

Extensive research is currently directed to increase our understanding of functional probiotic foods. Academic, government and private research institutes around the globe are devoting substantial efforts in identifying how functional foods and food ingredients might help to prevent chronic disease and optimize health, thereby reducing healthcare costs and improving the quality of life for most consumers. An emerging discipline that will have a profound effect on future functional and probiotic food research and development efforts is the nutrigenomics, which investigates the interaction between diet and development of diseases based on an individual's genetic profile. Interest in nutrigenomics was greatly augmented by the recent announcement that a
Genetically modified strains. In 2001, the complete sequence of the human genome was announced by Craig Venter and colleagues. This technological breakthrough could eventually make it feasible to tailor a diet for an individual’s specific genetic profile. Nutrigenomics will have a profound effect on future disease prevention efforts, including the future of the functional and new probiotics in the food industry.

Biotechnology thus, will greatly influence the future of probiotic foods. Foods enhanced with other nutritive or non-nutritive substances along with certain types of probiotic strains in adequate numbers per gram food, may even help to prevent most types of infections and several types of chronic diseases such as heart disease, osteoporosis or cancer. Consumer acceptance of genetically modified strains achieved by biotechnology (currently a major issue in Europe) will be important, if the potential of this powerful methodology is to be realized and utilized.

CONCLUSION

Current evidence supports the concept that oral administration of probiotic therapies may be beneficial in a multitude of disorders both inside and outside the GI tract. The direct effects of probiotics in the GI tract are well documented and include up-regulation of immunoglobulins such as IgA, down-regulation of inflammatory cytokines, and enhancement of gut barrier function. Exciting evidence supports indirect, systemic effects of probiotics for a widely divergent set of disorders, including atopic disease, immune compromise, and vaginal infections.

Although many probiotic foods may hold promise for public health, there are concerns that the promotion of probiotic foods and structural/functional claims may not rest on sufficiently strong scientific evidence. Confusion also exists about claims regarding foods and dietary supplements, because of the low number and viability of the cells which may even be of other type than the declared and specified ones, and thus, of lower efficiency.

Claims about the potential health benefits of probiotic foods or food ingredients must be communicated effectively to consumers. The differences between health claims and functional claims must be made clear, to allow consumers to understand the differences in the scientific grounds and proof for such claims.

It is required that any health benefits attributed to functional probiotic foods should be based on sound and accurate scientific criteria, including rigorous studies of safety and efficacy. Interactions with other dietary components and potential adverse interactions with pharmaceutical agents must be clearly imparted.

Thus, probiotics appear to be among the friendliest microorganisms that maintain interactions with humans and animals. Great benefits to the host can be derived from the right types and the right numbers, ingested together with other food ingredients, in promoting a well functioning GI tract. It is important to remember that lactobacilli spp. and bifidobacterium spp. - are essential in the newborn - improve the nutritional status of the adult individual - and in many ways protect the health of the host.

However, consumers must realize that foods no matter how healthy, are in no way “magic bullets” or a panacea for poor health habits. There are no good and bad foods, only good and bad dietary patterns. Consumers should also be aware of many of the promoted or implied benefits of these foods, and must realize that there is no consistent regulation or enforcement of existing regulations in the probiotic foods area.

It must also be remembered that diet is only one aspect of a comprehensive lifestyle approach to good health, which should include regular exercise, tobacco avoidance, stress reduction, maintenance of a healthy body weight and other positive health practices. Only when all of these issues are met, can probiotic foods become part of an effective strategy to maximize health, reduce disease risk and promote a long healthy life.

REFERENCES


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