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History and Basic of Probiotics

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Abstract: The benefits of probiotics have been recognized and explored for over a century. The pioneering research of Tissier and Moro was elaborated in the Metchnikoff's theory of longevity and converted into commercial reality by Shirota and Kellogg in 1930s and German nutritionists with their probiotic therapy in 1950s. Probiotics are described as live microorganisms, which when administered in adequate amounts confer a health benefit on the host, especially by improving intestinal microbial balance. The major consumption of probiotics is in dairy-based foods form, which is containing intestinal species of *Lactobacillus* and *Bifidobacterium*. A number of potential benefits of probiotics have been proposed, including: Adherence to cells; exclusion or reduction of pathogenic adherence; production of acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth, safe, noninvasive, noncarcinogenic and nonpathogenic characteristics and congregation to form a normal balanced flora. The aim of this study is to pay the tribute to pioneers in the field and provide an overview of the current state of knowledge about probiotics and their impact on our well-being.

Key words: Probiotic, gastrointestinal tract, health effect, bacteria, yeast, microflora

INTRODUCTION

Metchnikoff (1907) first introduced the probiotic concept in 1908, which observed the long life of Bulgarian peasants, who consumed fermented milk foods. He suggested that lactobacilli might counteract the putrefactive effects of gastrointestinal metabolism. In the century, which is elapsed since Metchnikoff's research, scientists and consumers have accepted the probiotic concept throughout the world (Fuller, 1992). In the world, the concept of providing functional foods including beneficial components rather than removing potentially harmful components is gaining ground in recent years. It may consider a functional food with the special property of containing live, beneficial microorganisms. Functional foods and nutraceuticals can prevent and treat diseases. Yogurt and other fermented milks containing probiotics may be considered the first functional foods. More specifically, Fuller (1992) defined probiotics as a live microbial feed supplement that beneficially affects the host beyond correcting for traditional nutrient deficiencies by improving the intestinal balance. The increasing cost of health care, the steady increase in life expectancy and the desire of the elderly for improved quality of their lives are driving factors for research and development in the area of functional foods. Although, the concept of functional foods was introduced long ago with Hippocrates and his motto Let food be your medicine, fairly recently the body of evidence started to support the hypothesis that diet may play an important role in modulation of important physiological functions in the body. Among a number of functional compounds recognized so far, bioactive components from fermented foods and probiotics certainly take the center stage due to their long tradition of safe use and established and postulated beneficial effects. The first clinical trials in the 1930s focused on the effect of probiotics on constipation and research has steadily increased since then. Today probiotics are available in a variety of food products and supplements. Food products containing probiotics are almost dairy products that due to the historical association of lactic acid bacteria with fermented milk. The fermentation of dairy foods presents one of the oldest methods of long-term food preservation. The origin of fermented milk can be traced back long before the Phoenician era and placed in the Middle East. Traditional Egyptian fermented milk products, Laban Rayeb and Laban Khad, were consumed as early as 7000 BC. Their

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tradition claims that even Abraham owed his longevity to the consumption of cultured milk (Kosikowski and Mistry, 1997). Initially, established in the middle and far east of Asia, the tradition of fermenting milk was spread throughout the east Europe and Russia by the Tartars, Huns and Mongols during their conquests. As a consequence, a wide range of fermented dairy products still exists in these regions and some popular products such as yoghurt and kefir are claimed to originate from the Balkans and Eastern Europe.

PROBIOTIC CONCEPT

Although, the preservation role of fermented dairy products was widely recognized and appreciated early, scientists first realized in the late 19th century that a wide range of traditional sour milk products had additional benefits in addition to prolonged shelf-life and pleasant sensory properties. The research of numerous scientists, mainly microbiologists, resulted in important developments and expansion of knowledge pertaining to the microbiology of the human body. Escherich (1885) was the first to recognize the importance of examining bacteria appearing in normal faeces and the intestinal tract and consequently understanding the physiology of digestion and the pathology and therapy of intestinal diseases of microbial origin. Tissier (1990) and Moro (1990), reported their findings of isolates from the faeces of breast-fed infants. Tissier (1990) noted that the anaerobically cultured organism had, in general, staining reactions and morphological appearance similar to those of lactobacilli; however, many of them appeared in bifurcated forms. Thus, he named them *Bacillus bifidus*. Similarly, Moro (1900) postulated that the isolate, which he termed *Bacillus acidophilus* due to its unusual acid tolerance, was derived from the mother's breast and normally resided in the neonate's oral cavity and intestinal content. Later, Tissier (1908) also showed that *B. bifidus* was the predominant organism in the faeces of breast-fed infants approximately 3 days postpartum as opposed to bottle-fed neonates, which predominantly contained *B. acidophilus* (Moro, 1905). At the same time, Nobel Laureate Metchnikoff (2007) noticed that Bulgarian peasants had an average life-span of 87 years, exceptional for the early 1900s and that 4 out of every thousand lived past 100 years of age. One of the major differences in their lifestyle in comparison with the contemporary diet was a large consumption of fermented milk. In his well known auto-intoxication theory, Metchnikoff (2004) suggested that a human body was slowly poisoned by toxins present in the body produced by pathogens in the intestine and body's resistance steadily weakened by proliferation of enteric pathogens, all of which were successfully prevented by the consumption of sour milk and lactic acid producing bacteria. His research was based on an organism previously isolated by Grigoroff (1905), who cultivated it from podkvassa used as a starter for production of the Bulgarian kiselo mleko (sour milk or yahourth) and called it *Lactobacillus bulgaricus*. In the process, Grigoroff (1905) also identified another organism, *Streptococcus thermophilus*, which received no attention since, it was considered a pathogen at that time. Metchnikoff's experiments led him to believe that *L. bulgaricus* could successfully establish itself in the intestinal tract and prevent multiplication and even decrease the number of putrefactive bacteria. However, the research of Herter and Kendall (1908) showed that this organism failed to establish itself in the gut, although, other substantial changes in the gut microflora were observed. Despite the fact that these findings disputed Metchnikoff's theory, scientists continued to investigate possible benefits of bacteria to the human health. Consequently, certain strains of *Lactobacillus acidophilus* were isolated and found to be capable of colonizing human digestive tract where they exerted appreciable physiological activity. Rettger and Horton (1914) and Rettger and Cheplin (1920a, b) reported that feeding of milk or lactose to rats or humans led to a transformation of the intestinal microflora resulting in predominance of acidophilus and bifidus type culture. Other researches followed suit with Minoru Shirota in Japan, who recognized the importance of the preventive medicine and modulation of the gastrointestinal microflora. In 1930, he succeeded isolating and culturing a *Lactobacillus* strain capable of surviving the passage through the gastrointestinal tract. The culture identified as *Lactobacillus casei* strain Shirota was successfully used for the production of the fermented dairy product called Yakult, which initiated the foundation of the same company in 1935 (Yakult, 1998). In the period between late 1930s and late 1950s, the research in this area, lost its pace likely due to extraordinary conditions (depression, war) the world was facing at that time. The rejuvenated interest in the intestinal human microflora was seen in the late 1950s and early 60s that led to the introduction of the probiotic concept.

DEFINITION OF PROBIOTICS

The word probiotics was initially used as an antonym of the word antibiotic. It is derived from Greek words pro and biotos and translated as for life (Hamilton *et al*., 2003). The origin of the first use can be traced back to Kollath (1953), who used it to describe the restoration of

Table 1: Some of the descriptions and definitions of probiotics commonly cited over the years

Description	Source
Probiotics are common in vegetable food as vitamins, aromatic substances, enzymes and possibly other substances connected with vital processes	Kollath (1953)
Probiotics are opposite of antibiotics	Vergin (1954)
Deleterious effects of antibiotics can be prevented by probiotic therapy	Kolb (1955)
A substance secreted by one microorganism which stimulates the growth of another	Lilly and Stillwell (1965)
Tissue extracts, which stimulate microbial growth	Sperti (1971)
Compounds that build resistance to infection in the host but do not inhibit the growth of microorganisms *in vitro*	Fujii and Cook (1973)
Organisms and substances that contribute to intestinal microbial balance	Parker (1974)
Live microbial feed supplement which beneficially affects the host animal by improving microbial balance	Fuller (1992)
Viable mono- or mixed culture of live microorganisms which, applied to animals or man, have a beneficial effect on the host by improving the properties of the indigenous microflora	Havenaar and Huisint'Veld (1992)
Live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host	Salminen *et al.* (1996)
Living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition	Schaafsma (1996)
Microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host	Salminen *et al.* (1999)
A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effect in this host	Schrezenmeir and de Vrese (2001)
Live microorganisms that when administered in adequate amount confer a health benefit on the host	FAO/WHO (2002)

the health of malnourished patients by different organic and inorganic supplements. A year later, Vergin (1954) proposed that the microbial imbalance in the body caused by antibiotic treatment could have been restored by a probiotic rich diet; a suggestion cited by many as the first reference to probiotics as they are defined nowadays. Similarly, Kolb (1955) recognized detrimental effects of antibiotic therapy and proposed the prevention by probiotics. Later on, Lilly and Stillwell (1965) defined probiotics as substances produced by one microorganism that promoted the growth of another microorganism. Similar to this approach, Sperti (1971) and Fujii and Cook (1973) described probiotics as compounds that either stimulated microbial growth or improved the immune response of the host without inhibiting the growth of the culture *in vitro*. Another definition offered by Parker (1974) resembles more recent description of probiotics. Parker (1974) defined them as organisms and substances, which contribute to intestinal microbial balance.

This definition was disputed by many researchers since, various substance even antibiotics might have been included. Late 1980s and 1990s saw a surge of different definitions of probiotics. Most frequently cited definition is that of Fuller's (1992), who defined them as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance. However, his definition was more applicable to animals than to humans. Although, all researchers agreed that probiotics include live microorganisms, Salminen *et al.* (1999) offered their view incorporating non-viable bacteria in the definition. Following recommendations of a FAO/WHO (2002) working group on the evaluation of probiotics in food, the suggested definition describes probiotics as live microorganisms that when administered in adequate amounts confer a health benefit on the host. Consequently, a wide variety of species and genera could be considered potential probiotics (Holzapfel *et al*., 1998); commercially, however, the most important strains are Lactic Acid Bacteria (LAB) (Table 1).

MECHANISMS OF PROBIOTIC ACTIVITY

The mechanism of action of probiotic strains is likely to be multifactor and from existing evidence, appears to be strain specific. Enhancement of colonization resistance and/or direct inhibitory effects against pathogens is likely to be important in situations in which probiotics have reduced the incidence and duration of gastroenteritis. Probiotic strains have inhibited pathogenic bacteria both *in vitro* and *in vivo* through several different mechanisms. Probiotics exert their effects on the host but the mechanisms are still speculative. They may antagonize pathogens directly through production of antimicrobial and antibacterial compounds such as cytokines and butyric acid (De Vuyst and Vandamme, 1994; Kailasapathy and Chin, 2000), reduce gut pH by stimulating the lactic acid to produce microflora (Langhendries *et al*., 1995), compete for binding and receptor sites that pathogens occupy (Fujiwara *et al*., 1997; Kailasapathy and Chin, 2000), improve immune function and stimulate immunomodulatory cells (Isolauri *et al*., 1991, 1995; Rolfe, 2000), produce lactase, which aids in lactose digestion, compete the nutrients and adhere the sites on the gut wall and regulate colonocyte gene expression (e.g., expression of mucin genes) (Fooks and Gibson, 2002; Mack *et al*., 1999; Steer *et al*., 2000). A group of requirements have been identified for a microorganism to be defined as an effective probiotic (Salminen *et al*., 1996). These are:

- Adhere to cells
- Exclude or reduce pathogenic adherence
- Persist and multiply
- Produce acids, peroxide and bacteriocins antagonistic to pathogen growth
- To be safe, noninvasive, noncarcinogenic and nonpathogenic
- Coaggregate to form a normal balanced flora

Applying probiotics to stimulate immune function, especially in individuals with underdeveloped or dysregulated immune function, appears to be sound, considering the positive outcomes of feeding studies targeting viral infections, Inflammatory Bowel Disease (IBD) and allergic diseases. It is still unclear which mechanism or, more probably, which spectrum of mechanisms, is used by probiotics, within the human gut microbiota to bring about improved health. Further human feeding studies are required to confirm probiotic efficacy in specific disease states such as IBD, colon cancer and gastroenteritis. Probiotic activity is likely to be strain specific and that these disease states are of multifactor etiology (Tuohy *et al*., 2003).

SELECTION OF PROBIOTICS

The importance of certain technological and physiological characteristics of probiotic strains was recognized long time ago. Gordon *et al*. (1957) noted that for achieving successful outcome of the *lactobacilli* therapy was necessary for the preparation to fulfil following requirements: the culture must be a normal inhabitant of the intestine, non-pathogenic and must be capable of efficient gut colonization and delivered in substantially high concentrations (10^7-10^9 cfu mL^{-1} of a product). Although, numerous criteria have been recognized and suggested (Mattila *et al*., 2002; Ouwehand *et al*., 1999; Reid, 1999), a general agreement exists with regard to key selection criteria listed in Table 2 (FAO/WHO, 2002). The 1st step in the selection of a probiotic is the determination of its taxonomic classification, which may give an indication of the origin, habitat and physiology of the strain. All these characteristics have important consequences on the selection of the novel strains (Morelli, 2007). The classification and relatedness of probiotics (and other microorganisms) is based on the comparison of highly conserved molecules, namely genes encoding ribosomal RNA (rRNA). Major advances in molecular biology methods have enabled sequencing of 16S/23S rRNA sequences and consequently generation of large sequence databases, which may facilitate a rapid and accurate classification of a desired probiotic strain. Closely, related strains nowadays are successfully distinguished using DNA-based methods such as plasmid profiling, Restriction Enzyme Analysis (REA), ribotyping, Randomly Amplified Polymorphic DNA (RAPD) and Pulse Field Electrophoresis (PFGE) (Holzapfel *et al*., 2001; Vuaghan *et al*., 2005).

Table 2: Key and desirable criteria for the selection of probiotics in commercial applications (Shah, 2006; Morelli, 2007)

General	Property
Safety criteria	Origin
	Pathogenicity and infectivity
	Virulence factors-toxicity, metabolic activity and intrinsic properties, i.e., antibiotic resistance
Technological criteria	Genetically stable strains
	Desired viability during processing and storage
	Good sensory properties Phage resistance
	Large-scale production
Functional criteria	Tolerance to gastric acid and juices
	Bile tolerance
	Adhesion to mucosal surface
	Validated and documented health effects
Desirable	Immunomodulation
Physiological criteria	Antagonistic activity towards gastrointestinal pathogens, i.e., *Helicobacter pylori*, *Candida albicans*
	Cholesterol metabolism
	Lactose metabolism
	Antimutagenic and anticarcinogenic properties

Ouwehand *et al*. (1999) advocated the importance of origin in specific commercial applications. More recently, an FAO/WHO (2001) expert panel suggested that the specificity of probiotic action is more important than the source of microorganism. This conclusion was brought forward due to uncertainty of the origin of the human intestinal microflora since, the infants are borne with virtually sterile intestine. However, the panel also, underlined a need for improvement of *in vitro* tests to predict the performance of probiotics in humans. Dairy and probiotic cultures have been associated with a long tradition of the safe use in commercial applications. Reports on the occurrence of harmful effects associated with consumption of probiotics are quite rare, although, certain Lactobacillus strains have been isolated from bloodstream and local infections (Ishibashi and Yamazaki, 2001; Salminen *et al*., 2006). Another important safety aspect is the antibiotic resistance of probiotics, since antibiotic resistant genes, especially those encoded by plasmids, could be transferred between microorganisms. The information in this regard is rather contradictory; early reports indicated that certain strains of *Bifidobacterium* (Matteuzzi *et al*., 1983) and *Lactobacillus* (Gupta *et al*., 1995) showed a strain dependent resistance to tested antibiotics. On the other hand, a recent study (Moubareck *et al*., 2005) tested 50 strains belonging to 8 *Bifidobacterium* sp. and concluded that these strains were risk-free. The risk of gene transfer depends on the nature of the genetic

material (plasmid, transposons), the nature and concentrations of the donor and recipient strains and their interactions and the environmental conditions, i.e., the presence of an antibiotic may facilitate the growth of antibiotic resistant mutants (Marteau, 2001). Therefore, the probiotic strains need to be tested for their natural antibiotic resistance to prevent the undesirable transfer of resistance to other endogenous bacteria.

The use of lactic acid bacteria as feed supplements goes back to pre-Christian times when fermented milks were consumed by humans. It was not until the beginning of this century that Metchnikoff, working at the Pasteur Institute in Paris, started to put the subject on to a scientific basis. He believed that the flora in the lower gut was having an adverse effect on the host and that these adverse effects could be ameliorated by consuming soured milk. In support of this, he cited the observation that Bulgarian peasants consumed large quantities of soured milk and also lived to a great age; he was in no doubt about the causal relationship and subsequent events have, in part, confirmed his thesis. He isolated what he called the *Bulgarian bacillus* from soured milk and used this in subsequent trials. This organism was probably what became known as *Lactobacillus bulgaricus* and is now called *L. delbrueckii* subsp *bulgaricus*, which is one of the organisms used to ferment milk and produce yoghurt. After Metchnikoff's death in 1916 the centre of activity moved to the USA. The American workers showed that the yoghurt organisms could not colonies the gut and reasoned that if the effect was to be manifested in the gut then a gut micro organism was more likely to produce the required effect. Knowledge available at that time suggested the use of *L. acidophilus* and many trials were carried out using this organism. Encouraging results were obtained especially in the relief of chronic constipation. In the late 1940's interest in the gut microflora was stimulated by 2 research developments. Firstly, the finding that antibiotics included in the feed of farm animals promoted their growth. A desire to discover the mechanism of this effect led to increased study of the composition of the gut microflora and the way in which it might be affecting the host animal. Secondly, the more ready availability of germ-free animals provided a technique for testing the effect that the newly discovered intestinal inhabitants were having on the host. This increased knowledge also showed that *L. acidophilus* was not the only *lactobacillus* in the intestine and a wide range of different organisms came to be studied and later used in probiotic preparations. Some of the more commonly used lactic acid bacteria are shown in Table 3 In the early days, the selection of strains for probiotic use was largely empirical.

Table 3: Lactic acid bacteria used in probiotic products

Lactobacilli, Streptococci, Bifidobacteria
L. acidophilus
L. casei
L. delbrueckii subsp. bulgaricus[a]
L. brevis
L. cellobiosus
L. curvatus
L. fermentum
L. lactis
L. plantarum
L. reuteri
S. cremoris
S. salivarius subsp. thermophilus[b]
E. faecium[c]
S. diacetylactis
S. intermedius
B. bifidum
B. adolescentis
B. animalis
B. infantis
B. longum
B. thermophilum

a- previously *L. bulgaricus*, b- previously *S. thermophilus*, *c*- previously *S. faecium*

However, recently with increased knowledge, attention has been paid to the features, which are involved in colonisation of the gut. On the basis that the effect cannot be reproduced unless the organism is metabolising in the gut, it would seem to be rational to encourage its growth in the intestine. Factors which have been used in this respect are:

- Resistance to pH and bile acids, which can be inhibitory in the gut
- Ability to attach to the gut epithelial lining which aids in preventing the organisms being swept out of the gut by peristalsis

The ideal probiotic would possess these characteristics together with the following features, which the present knowledge of this subject suggest it would be desirable. It should be:

- Non-pathogenic and non-toxic
- Beneficial to the host aimal in some way
- Of high viability
- Stable on storage and in the field
- Able to survive in or colonise the gut
- Amenable to cultivation on an industrial scale

These are the characteristics, which should be aimed at when developing new strains for probiotic use.

COMMERCIALLY IMPORTANT PROBIOTICS

Probiotic cultures have been exploited extensively by the dairy industry as a tool for the development of novel

functional products. While, it has been estimated that there were approximately 70 probiotic-containing products marketed in the world Shah (2004), the list has been continuously expanding. Traditionally, probiotics have been incorporated in to yoghurt; however, a number of carriers for probiotics have been examined recently including mayonnaise (Khalil and Mansour, 1998), edible spreads (Charteris *et al*., 2002) and meat (Arihara *et al*., 1998) in addition to other products of dairy origin, i.e., cheese (Ong *et al*., 2006) or cheese-based dips (Tharmaraj and Shah, 2004). Probiotic organisms are also, available commercially in milk, sour milk, fruit juices, ice cream, single shots and oat-based products. Lunebest, Olifus, Bogarde, Progurt are only some examples of commercial fermented dairy products with probiotics available on the international market with a steady increase in the market shares. The consumption of functional dairy products across West Europe, United States and Japan rose by 12% since, 2005 (Zenith International, 2007). Probiotic products are very popular in Japan as reflected in >53 different types of probiotic-containing products on the market. Commercial cultures used in these applications include mainly strains of *Lactobacillus* sp. and *Bifidobacterium* sp. and some of them are listed in Table 4. The probiotic strains are mainly used as adjunct cultures due to their poor growth in milk which extends the fermentation time (Shah, 2004). *Lactobacilli* are ubiquitous in nature, found in carbohydrate rich environments. They are Grampositive, non-spore-forming microorganisms, catalase negative with noted exceptions, appearing as rods or coccobacilli. They are fermentative, microaerophylic and chemo-organotrophic. Considering the DNA base composition of the genome, they usually have a GC content <54% mol. The genus *Lactobacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae* and its closest relatives are the genera *Paralactobacillus* and *Pediococcus* (Garrity *et al*., 2004). This is the most numerous genus, comprising 106 described species. *Lactobacillus acidophilus*, *L. salivarius*, *L. casei*, *L. plantarum*, *L. fermentum*, *L. reuteri* and *L. brevis* have been the most common *Lactobacillus* sp. isolated from the human intestine (Mitsuoka, 1992). The functional properties and safety of particular strains of *L. casei*, *L. rhamnosus*, *L. acidophilus* and *L. johnsonii* have been extensively studied and well documented. *Bifidobacteria* were first isolated and visualized by Tissier (1900) from faeces of breast-fed neonates. These rod-shaped, non-gas producing and anaerobic organisms were named *B. bifidus* due to their bifurcated morphology. They are generally characterized as Gram-positive, non-spore forming, non-motile and catalase-negative anaerobes with a special metabolic pathway, which allows them to produce acetic acid in addition to lactic acid in the molar ratio of 3:2. Due to their fastidious nature, these bacteria are often difficult to isolate and grow in the laboratory. The taxonomy of bifidobacteria has changed continuously since, they were first isolated. They had been assigned initially to the genera *Bacillus*, *Bacteroides*, *Nocardia*, *Lactobacillus* and *Corynebacterium*, before being recognized as separate genera in 1974. Due to their high (>50% mol) G+C content, bifidobacteria are phylogenetically assigned in the actinomycete division of Gram-positive bacteria. This family consists of 5 genera: *Bifidobacterium*, *Propionibacterium*, *Microbacterium*, *Corynebacterium* and *Brevibacterium*. Presently, there are 32 species in the genus *Bifidobacterium*, 12 of which are isolated from human sources (i.e., dental caries, faeces and vagina), 15 fromanimal intestinal tracts or rumen, 3 from honeybees and remaining 2 found in fermented milk and sewage. *Bifidobacterium* sp. found in humans are: *B. adolescentis*, *B. angulatum*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. dentium*, *B. infantis*, *B. longum* and *B. pseudocatenulatum*. *B. breve*, *B. infantis* and *B. longum* are found in human infants. *B. adolescentis* and *B. longum* are found in human adults (Garrity *et al*., 2004) (Table 4).

Table 4: Some of probiotic strains used in commercial applications (Holm, 2003; Shah, 2004)

Strain	Source
L. acidophilus LA1/LA5	Chr. Hansen
L. delbrueckii sp. *bulgaricus* Lb12	
L. paracasei CRL431	
B. animalis sp. *lactis* Bb12	
L. acidophilus NCFM®	Danisco
L. acidophilus La	
L. paracasei Lpc	
B. lactis HOWARU™/Bl	
L. acidophilus LAFTI®L10	DSM food specialties
B. lactis LAFTIs B94	
L. paracasei LAFTI® L26	
L. johnsonii	Nestle
L. acidophilus SBT-20621	Snow Brand Milk Products Co. Ltd.
B. longum SBT-29281	
L. rhamnosus R0011	Institute Rosell
L. acidophilus R0052	
L. casei Shirota	Yakult
B. breve strain Yakult	
B. lactis HN019 (DR10)	Foneterra
L. rhamnosus HN001 (DR20)	
L. plantarum 299V	Probi AB
L. rhamnosus 271	
L. casei Immunitas	Danone
B. animalis DN173010 (Bioactiva)	
L. rhamnosus LB21	Essum AB
Lactococcus lactis L1A	
L. reuteri SD2112	Biogaia
L. rhamnosus GG1	Valio Dairy
L. salivarius UCC118	University College Cork
B. longum BB536	Morinaga Milk Industry Co. Ltd.
L. acidophilus LB	Lacteol Laboratory
L. paracasei F19	Medipharm

HEALTH EFFECT OF PROBIOTICS

Since, Metchnikoff's era, a number of health benefits have been contributed to products containing probiotic organisms. While, some of these benefits have been well documented and established, others have shown a promising potential in animal models, with human studies required to substantiate these claims. More importantly, health benefits imparted by probiotic bacteria are very strain specific; therefore, there is no universal strain that would provide all proposed benefits, not even strains of the same species. Moreover, not all the strains of the same species are effective against defined health conditions. The strains *L. rhamnosus* GG (Valio), *Saccharomyces cerevisiae* Boulardii (Biocodex), *L. casei* Shirota (Yakult) and *B. animalis* Bb-12 (*Chr. Hansen*) are certainly the most investigated probiotic cultures with the established human health efficacy data against management of lactose malabsorption, rotaviral diarrhoea, antibioticassociated diarrhoea and *Clostridium difficile* diarrhoea.

Disorders associated with the gastrointestinal tract

Prevention of diarrhoea caused by certain pathogenic bacteria and viruses: Infectious diarrhea is a major world health problem, responsible for several million deaths each year. While, the majority of deaths occur amongst children in developing countries, it is estimated that up to 30% of the population even in developed countries are affected by food-borne diarrhea each year. Probiotics can potentially provide an important means to reduce these problems. It should be noted that some of the studies referenced below utilize probiotics administered in a non-food form. The strongest evidence of a beneficial effect of defined strains of probiotics has been established using *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB-12 for prevention (Saavedra *et al*., 1994; Szajewska *et al*., 2001) and treatment (Isolauri *et al*., 1991; Guarino *et al*., 1997; Majamaa *et al*., 1995; Shornikova *et al*., 1997; Perdone *et al*., 1999; Guandalini *et al*., 2000) of acute diarrhea mainly caused by rotaviruses in children. In addition to rotavirus infections, many bacterial species cause death and morbidity in humans. There is good *in vitro* evidence that certain probiotic strains can inhibit the growth and adhesion of a range of enteropathogens (Coconnier *et al*., 1993, 1997; Hudault *et al*., 1997; Gopal *et al*., 2001; Bernet *et al*., 1997) and animal studies have indicated beneficial effects against pathogens such as *Salmonella* (Ogawa *et al*., 2001; Shu *et al*., 2000). There is evidence from studies on travellers' diarrhea, where some of the causative pathogens have been presumed to be bacterial in nature that benefits can accrue with probiotic administration (Hilton *et al*., 1997).

It is important to note that probiotic therapy of acute diarrhea should be combined with rehydration if available. Current WHO (1995) recommendations state that clinical management of acute diarrhea should include replacement of fluid and electrolytes losses along with nutritional support (WHO, 1995). Oral Rehydration Salts (ORS) have been widely used in such disease management and it is within this context that the combination therapy with probiotics is hereby advocated. Effects such as probiotic restoration of the non-pathogen dominated intestinal microflora secondary to infection, maintaining mucosal integrity and improving electrolyte balance could have a significant impact on programs of treatment and prevention of acute diarrhea in developing countries. A major problem associated with antibiotic treatment is the appearance of diarrhea, often caused by *Clostridium difficile*. This organism is not uncommon in a healthy intestinal tract, but the disruption of the indigenous microflora by antibiotics leads to an abnormal elevation of their numbers and subsequent symptoms related to toxin production. The rationale therefore to use probiotics is that in such patients, administration of exogenous commensal microorganisms (that is probiotics) is required to restore the microflora to one that more closely reflects the normal flora prior to antibiotic therapy. Some open ended studies have indeed shown that this approach can alleviate the signs and symptoms of *C. difficile* infection (Gorbach *et al*., 1987; Biller *et al*., 1995; Bennet *et al*., 1986). With respect to antibiotic-associated diarrhea, probiotics have proved useful as a prophylactic regimen and potentially they can also be used to alleviate the signs and symptoms once antibiotic induced diarrhea has occurred (Arvola *et al*., 1999; Vanderhoof *et al*., 1999; Armuzzi *et al*., 2001). It must be recognized that evidence for therapeutic effects against *C. difficile* and other disorders has been obtained using certain probiotic strains, such as *L. rhamnosus* GG. It is important to note that such effects may also be conferred by other strains, but scientific evidence may not yet be available or the microorganisms involved may not be included in the scope of this consultation.

***Helicobacter pylori* infection and complications:** A new development for probiotic applications is activity against *Helicobacter pylori*, a Gram negative pathogen responsible for type B gastritis, peptic ulcers and gastric cancer. *In vitro* and animal data indicate that lactic acid bacteria can inhibit the growth of the pathogen and

decrease urease enzyme activity necessary for the pathogen to remain in the acidic environment of the stomach (Midolo *et al*., 1995; Kabir *et al*., 1997; Aiba *et al*., 1998; Coconnier *et al*., 1998). Human data is limited, but there is some evidence of an effect induced by *L. johnsonii* La1 (Michetti *et al*., 1999). In terms of measuring probiotic effects, feasible end points include the suppression of the infection (which may be reversible upon cessation of treatment), combination treatment with antibiotics leading to fewer side effects such as acid reflux and lower risk of recurrent infection (Michetti *et al*., 1999; Canducci *et al*., 2000; Felley *et al*., 2001). Placebo-controlled trials are needed before specific claims can be made for probiotic anti-*Helicobacter pylori* benefits in humans with respect to prevention and treatment. Such studies are warranted given the preliminary evidence to support these effects.

Inflammatory diseases and bowel syndromes: Inflammatory bowel diseases, such as pouchitis and Crohn's disease, as well as irritable bowel syndrome may be caused or aggravated by alterations in the gut flora including infection (Shanahan, 2000). These are new avenues of investigation, although it is premature to state a firm action of probiotics in these conditions. Some studies support the potential role of probiotics in therapy and prophylaxis and illustrate that combinations of strains may have a role to play in remediation (Gionchetti *et al*., 2000; Gupta *et al*., 2000). The intestinal microflora likely plays a critical role in inflammatory conditions in the gut and potentially probiotics could remediate such conditions through modulation of the microflora. Clinical and mechanistic studies are urgently required to better understand the interface between the microbes, host cells, mucus and immune defenses and to create efficacious interventions. Such studies should include molecular examination of the intestinal (not only fecal) flora and long-term (5-10 years) effects of probiotic microorganisms.

Reduction of the risk associated with mutagenicity and carcinogenicity: Antigenotoxicity, antimutagenicity and anticarcinogenicity are important potential functional properties of probiotics, which received much attention recently. Mutagens are frequently formed during stress or due to viral or bacterial infections and phagocytosis but also commonly obtained via foods. Endogenous DNA damage is one of the contributors to ageing and age-related degenerative diseases. The defence mechanism via leukocytes liberates a range of compounds including NO, O_2^- and H_2O_2 thus, defending an individual from bacterial and viral infections, but these may contribute to DNA damage and mutations. DNA irreversible damage is a critical factor of carcinogenesis and ageing. Antimutagencity could be described as a suppression of the mutation process, which manifests itself as a decrease in the level of spontaneous and induced mutations. Some epidemiological researches have emphasized that probiotic intake may be related to a reduced colon cancer incidence (Hirayama and Rafter, 2000) and experimental studies showed the ability of *lactobacilli* and *bifidobacteria* to decrease the genotoxic activity of certain chemical compounds (Tavan *et al*., 2002) and increase in antimutagenic activity during the growth in selected media (Lo *et al*., 2004). Antimutagenic effect of fermented milks has also been detected against a range of mutagens and promutagens including 4-nitroquinoline-N'-oxide, 2-nitrofluorene and benzopyrene in various test systems based on microbial and mammalian cells. However, antimutagenic effect might depend on an interaction between milk components and lactic acid bacteria. Lankaputhra and Shah (1998) studied the antimutagenic activity of organic acids produced by probiotic bacteria against eight mutagens and promutagens including 2-Nitroflourene (NF), Aflatoxin-B (AFTB) and 2-Amino-3-Methyl-3H-Imidazoquinoline (AMIQ). Among the organic acids, butyric acid showed a broad-spectrum antimutagenic activity against all mutagens or promutagens studied. Moreover, live bacterial cells showed higher antimutagenicity than killed cells against the mutagens studied, which suggested that live bacterial cells were likely to be involved in metabolism of mutagens. The results emphasized the importance of consuming live probiotic bacteria and of maintaining their viability in the intestine in order to provide efficient inhibition of mutagens. Several factors have been identified to be responsible for induction of colorectal cancer including bacteria and metabolic products such as genotoxic compounds (nitrosamine, heterocyclic amines, phenolic compounds and ammonia). Epidemiological studies have shown that diet plays a role in the etiology of most large bowel cancers, implying that it is a potentially preventable disease. Many studies confirm the involvement of the endogenous microflora in the onset of colon cancer. This effect is mediated by microbial enzymes such as β-glucuronidase, azoreductase and nitroreductase, which convert procarcinogens into carcinogens (Goldin and Gorbach, 1984). Experiments carried out in animal models showed certain strains of *L. acidophilus* and *Bifidobacterium* sp. were capable of decreasing the levels of enzymes such as β-glucuronidase, azoreductase and nitroreductase responsible for activation of procarcinogens. This inactivation consequently led to a substantial decline of the risk associated with tumor development. Several

studies have shown that preparations containing LAB inhibit the growth of tumor cells in experimental animals or indirectly lower carcinogenicity by decreasing bacterial enzymes that activate carcinogenesis (Rafter, 2002). Short-chain fatty acids produced by *L. acidophilus* and bifidobacteria were also reported to inhibit the generation of carcinogenic products by reducing enzyme activities. When incubated *in vitro* with 4-nitroquinoline-1-oxide (4NQO), some probiotic strains inhibited the genotoxic activity of 4NQO. *L. casei* was most effective, followed by *L. plantarum* and *L. rhamnosus* (Cenci *et al*., 2002). The most convincing clinical data exist for *L. casei Shirota*, in which the consumption of this organism was associated with the decreased urinary mutagen excretion. Furthermore, it was suggested that the habitual consumption of the fermented milk with this strain reduced the risk of bladder cancer in the Japanese population (Ohashi, 2000). The mechanism of antimutagenicity and anticarcinogenicity of probiotic bacteria has not been clearly understood. It has been suggested that microbial binding of mutagens to the cell surface could be a possible mechanism of antimutagenicity (Orrhage *et al*., 1994). Other proposed mechanisms include alteration of intestinal microecology and intestinal metabolic activity, normalization of intestinal permeability and enhanced intestinal immunity (Shah, 2006).

Constipation: The ability of probiotic therapy to alleviate constipation (difficulty in passing stool, excessive hardness of stool, slow transit through the bowel) is debatable, but may be a feature of selected strains. Randomized placebo controlled efficacy studies aimed at exploring these effects are strongly recommended.

Alleviation of lactose intolerance: The decline of the intestinal β-galactosidase (β-gal or commonly know as lactase) activity is a biological characteristic of the maturing intestine in the majority of the world's population. With the exception of the inhabitants of northern and central Europe and Caucasians in North America and Australia, over 70% of adults worldwide are lactose malabsorbers (De Vrese *et al*., 2001). Lactose upon ingestion is hydrolyzed by lactase in the brush border membrane of the mucosa of the small intestine into constitutive monosaccharides, glucose and galactose, which are readily absorbed in the blood stream. However, the activity of intestinal lactase in lactose intolerant individuals is usually less than 10% of childhood levels (Buller and Grand, 1990). This decline, termed hypolactasia, causes insufficient lactose digestion in the small intestine, characterized by an increase in blood glucose concentration or hydrogen concentration in breath upon ingestion of 50 g lactose, conditions designated as lactose maldigestion (Scrimshaw and Murray, 1988). Hypolactasia and lactose malabsorption accompanied with clinical symptoms, such as bloating, flatulence, nausea, abdominal pain and diarrhoea, are termed lactose intolerance. Symptoms are caused by undigested lactose in the large intestine, where lactose is fermented by intestinal microflora and osmotically increases the water flow into the lumen. The severity of the symptoms depends primarily on the size of the lactose load ingested. The development of the intolerance symptoms also depends on the rate of lactose transit to the large intestine, influenced by the osmotic and caloric load and the ability of the colonic microflora to ferment lactose (Martini and Savaiano, 1988). Numerous studies have shown that individuals with hypolactasia could tolerate fermented dairy products better than an equivalent quantity in milk (Hertzler and Clancy, 2003; Montalto *et al*., 2005; Vesa *et al*., 1996). Various explanations have been suggested in order to clarify this phenomenon. At least 3 factors appear to be responsible for a better tolerance of lactose in fermented milk including starter culture, intracellular β-galactosidase expressed in these cultures and most importantly and oro-caecal transit time. The traditional cultures used in dairy fermentations utilize lactose as an energy source during growth, thus at least, partially reducing its content in fermented products. Furthermore, the bacterial lactase may resist luminal effectors avoiding denaturation and can be detected in the duodenum and terminal ileum after consumption of products containing live bacteria. The presence of this enzyme may lead to lactose hydrolysis and improved lactose tolerance. On the other hand, other studies not supporting this theory found no difference in digestion and tolerance to lactose in several fermented dairy products with substantially different lactase activities (Vesa *et al*., 1996). It was suggested that increased viscosity of fermented milk, in this case yoghurt, slowed gastric emptying and consequently prolonged transit time through the gastrointestinal tract improving absorption and lactose tolerance.

Mucosal immunity: The innate and adaptive immune systems are the 2 compartments traditionally described as important for the immune response. Macrophages, neutrophils, Natural Killer (NK) cells and serum complement represent the main components of the innate system, in charge of the first line of defence against many microorganisms. However, there are many agents that this system is unable to recognize. The adaptive system (B and T cells) provide additional means of defence, while

cells of the innate system modulate the beginning and subsequent direction of adaptive immune responses. Natural killer cells, including gamma/delta T cells, regulate the development of allergic airway disease, suggesting that the interleukins play an important role. Intravenous, intraperitoneal and intrapleural injection of *L. casei* Shirota into mice significantly increased NK activity of mesenteric node cells but not of Peyer's patch cells or of spleen cells (Matsuzaki and Chin, 2000), supporting the concept that some probiotic strains can enhance the innate immune response. A number of studies have been performed *in vitro* and in animals (Gill *et al*., 2000), which clearly show that probiotic strains can modify immune parameters. Correlating these findings with events taking place in the human body is still somewhat unclear, but evidence is mounting that such effects occur. In a series of randomized, double blind, placebo controlled clinical trials, it was demonstrated that dietary consumption of *B. lactis* HN019 and *L. rhamnosus* HN001 resulted in measurable enhancement of immune parameters in the elderly (Arunachalam *et al*., 2000; Gill *et al*., 2001; Sheih *et al*., 2001). Probiotic modulation of host immunity is a very promising area for research. Supportive data is emerging, such as those carried out in humans showing that probiotic microorganisms can enhance NK cell activity in the elderly (Gill *et al*., 2001) and non-specific host defenses can be modulated (Donnet *et al*., 1999; Perdigon *et al*., 1999). There is a need to specify whether the activities being advocated are designed to operate in otherwise healthy people or subjects with known diseases. Some of the critical factors involved in the host's defenses have been identified and include the induction of mucus production or macrophage activation by lactobacilli signaling (Mack *et al*., 1999; Miettinen *et al*., 2000), stimulation of sIgA and neutrophils at the site of probiotic action (for example the gut) and lack of release of inflammatory cytokines or stimulation of elevated peripheral immunoglobulins (Kaila *et al*., 1992; Gardiner *et al*., 2001). It is also, recognized that in some situations, stimulation of factors such as inflammatory cytokines may confer health benefits on the host. Future studies, should focus on the effect in humans and elucidate the mechanisms of action within systems, which simulate the *in vivo* situation and link this to bacterial and human genomics.

Treatment and prevention of allergy: The prevention and management of allergies is another area in which probiotics may potentially exert their beneficial role. The incidence of allergy is on the rise worldwide with a clear difference between developed and developing countries. The hygiene hypothesis postulates that limited childhood exposure to bacterial and viral pathogens would affect the balance between T-helper cells by favoring the Th2 phenotype of the immune system. An insufficient stimulation of Th1 cells cannot offset the expansion of Th2 cells and results in a predisposition to allergy (Yazdanbakhsh *et al*., 2002). A delayed colonization of *Bifidobacterium* and *Lactobacillus* sp. in the gastrointestinal tract of children may be one of the reasons for allergic reactions (Kalliomaki and Isolauri, 2003). Also, the difference in gastrointestinal microbiota may play a role in susceptibility to allergy. Infants with atopic dermatitis had a more adult type *Bifidobacterium* microbiota. Healthy infants, on the other hand, were colonized mainly by *B. bifidum*, typical for breast-fed infants (Ouwehand *et al*., 2001). A recent study also, indicated that early consumption of probiotic preparations containing *Lactobacillus* GG may reduce prevalence of atopic eczema later in life (Gueimonde *et al*., 2006). Similarly, another study suggested that treatment with *Lactobacillus* GG may alleviate atopic eczema/dermatitis syndrome symptoms in IgE-sensitized infants but not in non-IgE-sensitized infants (Viljanen *et al*., 2005a), while a 4-week treatment with *Lactobacillus* GG alleviated intestinal inflammation in infants with atopic eczema/ dermatitis syndrome and milk allergy (Viljanen *et al*., 2005b). The mechanisms of the protective effects of probiotics on allergic reactions are not entirely known; although, the reinforcement of the different lines of gut defence including immune exclusion, immune elimination and immune regulation has been suggested (Isolauri *et al*., 2005).

Cardiovascular disease: There is preliminary evidence that use of probiotic lactobacilli and metabolic by-products potentially confer benefits to the heart, including prevention and therapy of various ischemic heart syndromes (Oxman *et al*., 2001) and lowering serum cholesterol (De Roos and Katan, 2000). While, the consultation believes these findings to be important, more research and particularly human studies are required before it can be ascertained that probiotics confer health benefits to the cardiovascular system.

Urogenital tract disorders: Excluding sexually transmitted diseases, almost all infections of the vagina and bladder are caused by microorganisms that originate in the bowel. There is a strong correlation between presence of commensals, particularly lactobacilli in the vagina with health and an absence of these microorganisms in patients with urogenital infections. Disruption of the normal vaginal flora is caused by broad-spectrum antibiotics, spermicides, hormones, dietary substances

and factors not, as yet, fully understood. There is some evidence that probiotic microorganisms delivered as foods and topical preparations have a role in preventing urogenital tract disorders. The criteria for selection of effective probiotic strains have been proposed (Reid *et al*., 2001c) and should include verification of safety, colonization ability in the vagina and ability to reduce the pathogen count through competitive exclusion of adherence and inhibition of pathogen growth.

Bacterial vaginosis: Bacterial Vaginosis (BV) is a disease of unknown etiology resulting from the overgrowth of various anaerobic bacterial species and associated with the disappearance of lactobacilli, which dominate the normal vagina. Many women with BV are asymptomatic yet are at risk of more serious complications such as endometriosis, pelvic inflammatory disease and complications of pregnancy including pre-term labour. There is some clinical evidence to suggest that oral and vaginal administration of lactobacilli can eradicate asymptomatic (Reid *et al*., 2001a, b) and symptomatic BV (Hilton *et al*., 1995; Sieber and Dietz, 1998). Oral administration of *Lactobacillus acidophilus* and yogurt has been used in the prevention and therapy of candidal vaginitis, although no efficacy data have yet been generated (Hilton *et al*., 1992). The necessity for the lactobacilli to produce hydrogen peroxide has been proposed, but given that these microorganisms are more prone to being killed by spermicides, the combination of 2 or more strains, one of which produces hydrogen peroxide and others which resists spermicidal killing, may prove to be more therapeutic.

Yeast vaginitis: Yeast vaginitis is a very common ailment, often precipitated by antibiotic use, exposure to spermicides or hormonal changes as yet not fully understood. Unlike BV and urinary tract infection, yeast vaginitis is not necessarily due to loss of lactobacilli. Few *Lactobacillus* strains are able to inhibit the growth and adhesion of *Candida albicans* or other *Candida* sp. and there is no solid evidence to indicate that intravaginal administration of lactobacilli can eradicate yeast infection. However, there is some evidence to suggest that lactobacilli ingestion and vaginal use can reduce the risk of recurrences (Hilton *et al*., 1992, 1995) and further studies are warranted since this disease is widespread and debilitating.

Urinary tract infections: Several hundred million women are affected by Urinary Tract Infection (UTI) annually. Uropathogenic *Escherichia coli* originating in the bowel is the responsible agent in up to 85% of cases. Asymptomatic bacteruria is also a common finding in women and sometimes it is followed by symptomatic UTI. There is evidence, including randomized controlled data to suggest that once weekly vaginal capsules of freeze dried Lactobacillus strains GR-1 and B-54 (Reid *et al*., 1995) prepared with addition of skim milk and once daily oral capsule use of *Lactobacillus* strains GR-1 and RC-14 (Reid *et al*., 2001b), can result in the restoration of a lactobacilli dominated vaginal flora and lower risk of UTI recurrences. By creating a lactobacilli barrier in the vagina, it is believed that fewer pathogens can ascend into the bladder, thereby blocking the infectious process.

Hypocholesterolemic effect: It is well established that diet rich in saturated fat or cholesterol would increase the serum cholesterol level, which is one of the major risk factors for coronary heart diseases. Mann and Spoerry (1974) were the first to observe a decrease in serum cholesterol levels in men fed large quantities (8.33 L/man/day) of milk fermented with *Lactobacillus*. As they suggested, this was possibly due to the production of hydroxymethyl-glutarate by probiotic bacteria, which was reported to inhibit hydroxymethylglutaryl-CoA reductases required for the synthesis of cholesterol. Therefore, feeding of fermented milks containing very large numbers of probiotic bacteria would likely cause a hypercholesterolemic effect in human subjects. *In vitro* studies have postulated that the hypocholesterolemic effect of probiotics might be exerted via several possible mechanisms including assimilation by growing cells or binding to the cell surface (Liong and Shah, 2005a, b).

Another mechanism involving the deconjugation of bile by bile salt hydrolase (BSH, cholylglycine hydrolase; EC 3.5.1.24) and coprecipitation of cholesterol with the deconjugated bile has been proposed (Begley *et al*., 2006). The cholesterol is excreted via the faecal route and prior to its secretion the deconjugation of bile results in free bile salts. They are less efficiently absorbed and thus excreted in larger amounts in faeces. This effect is additionally augmented by poor solubilization of lipids by free bile salts, which limits their absorption in the gut leading to further decrease of serum lipid concentration (Begley *et al*., 2006). The largest study that assessed the ability of numerous species and strains of lactic acid bacteria to hydrolyze bile salts showed that BSH activity was common in *Bifidobacterium* and *Lactobacillus* but absent in *Lactococcus lactis*, *Leuconostoc mesenteroides* and *S. thermophilus*. Almost all *bifidobacteria* sp. and strains possessed BSH activity, while it was detected only in selected species of lactobacilli (Tanaka *et al*., 1999). Also, the production of short-chain fatty acids has been

implicated as another potential mechanism for the cholesterol lowering effect of probiotics. In a recent study (Liong and Shah, 2006), serum cholesterol level was reduced via the alteration of lipid metabolism contributed by short-chain fatty acids. This was supported by negative correlation between serum cholesterol levels and caecal propionic acid and However, the findings of some *in vivo* studies have been rather contradictory, i.e., either a lowering effect (Agerholm *et al*., 2000) or no effect was observed (De Roos *et al*., 1999; Lewis and Burmeister, 2005) even though in the latter the strains were able to reduce cholesterol *in vitro*. Despite several human studies, the reduction in serum cholesterol effect is still not considered an established effect and double-blinded placebo controlled human clinical trials are needed to substantiate this claim. Similarly, mechanisms involved in reducing cholesterol level need to be clarified.

Use of probiotics in otherwise healthy people: Many probiotic products are used by consumers who regard themselves as being otherwise healthy. They do soon the assumption that probiotics can retain their health and well-being and potentially reduce their long-term risk of diseases of the bowel, kidney, respiratory tract and heart. Several points need to be made on this assumption and its implications. The consultation recognized that the use of probiotics should not replace a healthy lifestyle and balanced diet in otherwise healthy people. Firstly, there is no precise measure of health and subjects may actually have underlying and undetectable diseases at any given time. Secondly, no studies have yet been undertaken which analyze whether or not probiotic intake on a regular basis helps retain life-long health over and above dietary, exercise and other lifestyle measures. One study of day care centres in finland showed that probiotic use reduced the incidence of respiratory infections and days absent due to ill health (Hatakka *et al*., 2001). The consultation would like studies to be done to give credibility to the perception that probiotics should be taken on a regular basis by healthy men, women and children. Such studies should be multi-centred and require randomization on the basis of age, gender, race, nutritional intake, education, socio-economic status and other parameters. It is currently unclear as to the impact of regular probiotic intake on the intestinal microflora. For example, does it lead to the depletion or loss of commensal microorganisms which otherwise have beneficial effects on the host? While, there is no indication of such effects, the issue needs to be considered. Furthermore, the concept of restoring a normal balance assumes that we know what the normal situation in any given intestinal tract comprises. It was deemed important by the consultation to further study the various contributions of gut microorganisms on health and disease. Another point worthy of note is that, to date, the ingestion of probiotic strains has not led to measurable long-term colonization and survival in the host. Invariably, the microorganisms are retained for days or weeks, but no longer (Tannock *et al*., 2000). Thus, use of probiotics likely confers more transient than long-term effects and so continued intake appears to be required. In newborn children, where a commensal flora has not yet been established, it is feasible that probiotic microorganisms could become primary colonizers that remain long-term, perhaps even for life. While, such probiotic usage can prevent death and serious morbidity in premature, low birth weight infants (Hoyos, 1997), the alteration of flora in healthy babies is a more complex situation. Just so, an implication of the Human Genome Project is that selected probiotics may be used at birth to create a flora that improves life-long health. These issues are very important for the future and will require full discussion including human ethical considerations.

CONCLUSION

Much progress has been made in understanding the breadth, depth and limitations of probiotics. Many strains, including species outwith the traditional *Lactobacillus* and *Bifidobacterium* genera are being examined for probiotic effects. Functionality and human testing will be vital not only to fulfill the requirements for strains to be called probiotic, but to increase our understanding of how products research. Applications in the fields of cancer, cardiovascular disease, inflammation, allergy and infection are currently the main target areas with potential to benefit large numbers of people. With more emphasis on therapy than health retention and augmentation, the parameters within, which probiotics operate or fail to provide benefits must be delineated. The emergence of new molecular, microscopic, nanoscale and imaging technologies will make it feasible to see in real time how probiotic (and indeed indigenous) bacteria influence the host. This will help both humans and animals regain their health when adversely affected by pathogenic microbial damage, antimicrobial treatment and other threats.

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