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Role of Probiotic in Milk and its Byproducts

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Abstract

Probiotics are living microorganisms which when ingested have beneficial effects on the equilibrium and the physiological functions of the human intestinal microflora. Probiotics have been recently defined as "live microbes which transit the gastro-intestinal tract and in doing so benefit the health of the consumer (Tannock *et al*., 2000). The origin of cultured dairy products dates back to the dawn of civilization. Metchnikoff's studies into the potential life-lengthening properties of lactic acid bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus*, inspired Japanese scientist Minoru Shirota to begin investigating the causal relationship between bacteria and good intestinal health. *Bifidobacterium* were first isolated from a breast-fed infant by Henry Tissier in 1900. live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Roy Fuller,1989). The micro biota of a newborn develops rapidly after the birth. It is initially dependent mainly on: the mother's micro biota, mode of delivery, birth environment and rarely genetic factors. After infancy probiotics are supplied to us by raw foods; lactic acid fermented foods. Now a day, consumers are aware of the link among lifestyle, diet and good health, which explains the emerging demand for products that are able to enhance health beyond providing basic nutrition. The release of different bioactive peptides from milk proteins through fermentation by LAB is now well documented. PUFA (polyunsaturated fatty acids) is higher than saturated fatty acids in meat products. Probiotics are extremely sensitive to heat and other processing conditions. New technologies, like microencapsulation and immobilized cell technologies, offer additional protection to probiotic organisms and new ways to include probiotics in foodstuffs.

Key Words: Dairy Products; Probiotics; Supplement; Peptides; Fermentation; PUFA; Processing.

Introduction

Probiotics are living microorganisms which when ingested have beneficial effects on the equilibrium and the physiological functions of the human intestinal microflora. Probiotics have been recently defined as "live microbes which transit the gastro-intestinal tract and in doing so benefit the health of the consumer (Tannock *et al*., 2000) differing from the earlier definitions which focused on probiotic interactions with indigenous intestinal microbes (Fuller, 1989). These definitions of probiotic bacteria generally agree that probiotic bacteria should be living organisms to confer health benefits. Probiotics have been reported to play a therapeutic role by modulating immunity, lowering cholesterol, improving lactose tolerance and preventing some cancers (Kailasapathy and Chin, 2000). In the recent past, there has been an explosion of probiotic-based health products mostly in the form of fermented dairy products as well as dietary supplements. The markets

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for probiotic products and supplements are increasing worldwide (Playne, 1997). Today there are more than 70 "Bifidus"- and "Acidophilus"-containing products worldwide, including several fermented dairy products (Shah, 2001). Viability of probiotic bacteria in a product at the point of consumption is an important consideration for their efficacy, as they have to survive during the processing and shelf life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine. The consumption of probiotics at a level of 10^8-10^9 cfu/g per day is a commonly quoted figure for adequate probiotic consumption, equating to 100 g of a food product with 10^6-10^7 cfu/g (Kebary, 1996).

History of probiotics

The origin of cultured dairy products dates back to the dawn of civilization; they are mentioned in the Bible and the sacred books of Hinduism. Climatic conditions for sure favoured the development of many of the traditional soured milk or cultured dairy products such as kefir, kumis, leben and dahi. These products, many of which are still widely consumed, had often been used therapeutically before the existence of bacteria was recognized. In 1907, Metchnikoff developed a theory that aging is caused by toxic bacteria in the gut and that lactic acid could prolong life. Based on this theory, he drank sour milk every day. Metchnikoff's studies into the potential life-lengthening properties of lactic acid bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus*, inspired Japanese scientist Minoru Shirota to begin investigating the causal relationship between bacteria and good intestinal health, which eventually led to the worldwide marketing of Kefir and other Fermented milk drinks, or probiotics. And also reported that intake of yogurt containing lactobacilli reduces toxin-producing bacteria in the gut and this increases the longevity of the host.

Bifidobacterium were first isolated from a breast-fed infant by Henry Tissier in 1900 who also worked at the Pasteur Institute. The isolated bacterium named *Bacillus bifidus communis* was later renamed to the genus *Bifidobacterium*. Tissier found that *Bifidobacterium* are dominant in the gut flora of breast-fed babies and he observed clinical benefits from treating diarrhoea in infants with Bifidobacteria. *Bifidobacterium lactis* is used as a probiotic in a variety of foods, particularly dairy products like yogurt, and is also included in baby food. The most important effects noted were that Bifidobacterium is able to alter pro-carcinogenic enzymes, prevent pro-carcinogens and suppress tumours. Although there are many species of *Bifidobacterium*, some of the more important ones are B. *bifidum*, B. *longum*, B. *infantis* and B. *lactis.*

The term *probiotic*, meaning "for life," is derived from the Greek language. *"Substances produced by microorganisms which promote the growth of other microorganisms" (Lilly and Stillwell, 1965).*

Parker (1974) coined the term probiotic and defined the term as "organisms and substances which contribute to intestinal microbial balance".

Roy Fuller (1989), an expert in gut micro-ecology given the modern definition of the probiotic concept and also modified Parker's definition to: "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". This new definition removed the word "substances" which could have included antibiotics. Moreover, Fuller's definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host.

Source of Probiotics

The micro biota of a newborn develops rapidly after the birth. It is initially dependent mainly on: the mother's micro biota, mode of delivery, birth environment and rarely genetic factors. The maternal vaginal and intestinal flora constitutes the source of bacteria, which colonizes the intestine of the newborn. After infancy probiotics are supplied to us by raw foods; lactic acid fermented foods such as yogurt and cheese; and probiotic supplements (Kaur *et al.*, 1995).

Selection of strains for probiotic use should always follow two general principles: safety of the organism, and possessing desirable characteristics for its intended use. Intestinal probiotics are dominated by members of *Bifidobacterium* and *Lactobacillus,* as these two genera have a long history of safe use and have GRAS (generally regarded as safe) status. They are also very suited to augmenting the intestine, as *Bifidobacterium* is a major inhabitant of the large intestine and *Lactobacillus* is a major inhabitant of the small intestine. The origin of the strains used in probiotics can be either freshly isolated from a human or animal host or from a culture collection. Culture collection strains have generally been extensively cultivated in fermentation systems and thus have likely attenuated to suit the *in vitro* environment. However, as stated *by* (Havenaar *et al.*, 1992) the choice of where to get a probiotic strain depends on the specific purpose of the probiotic. For example, if only transient activity of the probiotic is needed, such as for lactose digestion, then it is not necessary for the probiotic to have characteristics that would enable it to colonize the host. Most probiotic effects in the GI system would be enhanced if the probiotic would be able to compete with the indigenous flora. This requires a more careful selection of strains. Although all the criteria for this purpose are not currently known for any intestinal organism, there is one general consensus pertaining to a criterion that is important. That consensus is that the probiotic should originate from the same animal species that it is intended to target. The rationale is that the intestinal environments in different animal species are sufficiently different such that the most competitive bacteria in each host species have evolved specific traits for survival in that host (Freter, 1992). Human probiotics, therefore, should originate from a human source if the objective is to effectively modulate the microbial populations at their target sites. It is noteworthy that when commercial probiotics are fed to human subjects during controlled feeding studies, the probiotic can be detected in high numbers in the faeces during the feeding period, but rapidly disappears following cessation of feeding (Berg, 1998). The rate of decrease of the probiotic such as *Lactobacillus rhamnosus* GG can in some cases persist longer than a week post feeding (Alander, 1998).

Probiotic foods are a group of health-promoting functional foods, with large commercial interest and growing market shares (Arvanitoyannis and Van Houwelingen, 2005). In general, their health benefits are based on the presence of selected viable strains of lactic acid bacteria (LAB), that, when taken up in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001). They are administered mostly through the consumption of fermented milks or yoghurts (FAO/WHO, 2001; Makras, 2004).

In addition to their common use in the dairy industry, probiotic LAB strains may be used in other food products, including fermented meats (Hammes and Hertel, 1998; Incze, 1998). Although the concept is not new, only few manufacturers consider the use of fermented sausages as carriers for probiotic LAB (Arihara *et al*., 2006). Since meat products are seldom perceived as ''healthy foods", due to the perceived image of meat and its controversial nutrient profiling clause with respect to the presence of nitrite, salt, and fat, their marketing potential may be compromised (Lücke, 2000). In addition, the more artisan orientation of sausage manufacturers as compared to the dairy industry, the larger variety of products, and a number of uncertainties concerning technological, microbiological, and regulatory aspects seem to be problematic (Ross *et al.,* 2005).

Probiotic foods are gaining importance due to Peoples having a demand for healthier foods because of health awareness. Now a days peoples are growing about self care movement. Rise in ageing population lead to have awareness concerned with promoting of health. Generally, women are more concerned with body image and health. Parents are also monitoring their children's diets much more closely, as they are becoming more aware of the increasing global prevalence of childhood obesity and the dangers associated with it, such as diabetes, heart disease, and high cholesterol and blood pressure. In order to try and prevent childhood obesity and improve overall health and wellness, parents are purchasing healthier snacks, and more organic fruits and vegetables for their children. Manufacturers are also heavily advertising the nutritional benefits of certain functional ingredients to parents, such as omega-3, which is believed to aid in brain and eye development, and fibre, which is thought to improve digestive health, control hunger and improve heart health. Pregnant women have also become a significant target group, as they increasingly purchase organic and fortified/ functional foods to help ensure a healthy pregnancy and child. According to a recent Mintel study, 62% of children surveyed indicated that they like eating healthy snacks. Snacking has become the 4th meal of the day, and parents are increasingly looking to buy healthy snacks for their children. As the Ageing Population, a significant portion of the world population is now over 55 years of age, especially within North America, Western Europe, and China. This consumer segment has now become a major target group for the health and wellness industry, due to their increasing concern for improving their health and maintaining their lifestyles. The ageing population spends a significant amount of money on healthier foods, exercise equipment, and supplements. This particular group of consumers is highly interested in maintaining a healthy lifestyle and a certain level of vitality long into their retirement. Today's ageing population is also highly educated because of the increasing amount of information available to them (Global Health and Wellness, 2011).

Common Probiotic Candidates

- Lactobacillus spp.
- Bifidobacterium spp.
- Streptococcus salivarius ssp. thermophilus
- Enterococcus faecium
- Lactococcus lactis ssp lactis

- Lactococcus lactis ssp cremoris
- Leuconostoc mesenteroides ssp dexranicum
- Propionobacterium freudenreichii
- Pediococcus acidilactis
- Saccharomyces boulardii

Strains for milk products

- L.*helveticus* cp 790
- L.*rhamnosus* GG
- L. *bulgaricus* ss1
- Lactococcus *lactis ssp cremoris* FT4 (Gobetti *et al*., 2002).

Strains for meat products

- *Lactobacillus sakei* Lb 3
- *Lactobacillus casei/paracasei*
- L. *rhamnosus* (Collins *et al.,* 2007)
- L.*sakei*
- L. *plantarum (*Penacchia *et al.,* 2006*)*
- Pediococcus *acidilactici* PA-2 (Erkkila and Petaja, 2000)
- Yeasts -Candida and Saccharomyces
- Mesophillic lactobacilli (Shameshima *et al*., 1998)

Global scenario of Probiotic foods

Global probiotics demand was worth USD 27.9 billion in 2011 and is expected to reach USD 44.9 billion in 2018, growing at a Compound Annual Growth Rate (CAGR) of 6.8% from 2013 to 2018. Asia-Pacific and Europe dominate the global market in terms of demand, while Asia-Pacific is also expected to be the most promising market in the near future. The global market for probiotics is mainly driven by high demand for probiotic yogurt and growing consumption of functional food. Growing consumer awareness regarding gut health has played a key role in sustained growth of these ingredients (Transparency market research, 2013). The probiotic foods are accepted by 76% of consumers of the world. *Lactobacillus* genus share 61.9% of the total probiotic product produced in the world. Probiotic added meat products marketed since 1998 by German and Japanese producers – Salami (Salami is cured sausage, fermented and air-dried meat) (Arihar, 2006).

Probiotics and Human Health

Now a day, consumers are aware of the link among lifestyle, diet and good health, which explains the emerging demand for products that are able to enhance health beyond providing basic nutrition. The list of health benefits accredited to functional food continues to increase and the probiotics are one of the fastest growing categories within food for which scientific researches have demonstrated therapeutic evidence. Among several therapeutic applications of the probiotics can be cited the prevention of urogenital diseases, alleviation of constipation, protection against traveller's diarrhoea, reduction of hypercholesterolemia, protection against colon and bladder cancer, prevention of osteoporosis and food allergy (Lourens Hattingh and Viljoen, 2001). Ingestion of LAB has been suggested to confer a range of health benefits including immune system modulation (Isolauri *et al*., 2001). Maldonado *et al*., 2009 studied the effect of fermented milk containing *Lactobacillus casei* which induced mucosal immune stimulation reinforcing the non-specific barrier and modulating the innate immune response in the gut, maintaining the intestinal homeostasis. Host immune modulation is one of the suggested benefits of the consumption of probiotic functional food. However, comparative studies on the immunological properties that support the selection of strains of the same species for specific health benefits are limited. Medina *et al*., 2007 evaluated the ability of different strains of *Bifidobacterium longum* to induce cytokine production by peripheral blood mononuclear cells. *B. longum* live cells of all strains induced specific cytokine patterns, suggesting that they could drive immune responses in different directions. Kelly *et al*., 2004 demonstrated the ability of species within the commensal microflora to modulate immune function. Arunachalam *et al*., 2000 studied the dietary consumption of *B. lactis* and concluded that a relatively short-term dietary regime (6 weeks) is sufficient to impart measurable improvements in immunity. Chiang *et al*., 2000 demonstrated that dietary consumption of probiotics in oligosaccharide-rich substrate enhanced immune function by *B. lactis* in a different range for two types of leucocytes. *In vivo* and *in vitro* indices of immunity in healthy mice fed with *Lactobacillus rhamnosus*, *L. acidophilus* and *B. lactis* were examined by Gill *et al*., 2000 and the results suggested that supplementation of the diet with these strains was able to enhance several indices of natural and acquired immunity. Infectious diseases are still the biggest human health problem for the world to solve. Intestinal infection caused by the intake of pathogenic microorganisms with the contaminated water and food are the main causes of death. Under this circumstance, probiotics can assist in part the food borne problematic situation, as it is demonstrated in several studies. Shu and Gill, 2000 demonstrated that *B. lactis* can reduce the severity of infection caused by the enterohaemolytic pathogen *Escherichia coli* O157: H7 and suggested that this reduction may be associated with enhanced immune protection conferred by the probiotic. *B. lactis* also demonstrated the ability to provide a significant degree of protection against *Salmonella* infection by enhancing various parameters of immune function that are relevant to the immunological control of salmonellosis (Shu *et al.*, 2000). Moreover, the same authors suggested that dietary treatment using *B. lactis* could reduce the severity of weanling diarrhoea associated with rotavirus and *E. coli*, possibly *via* a mechanism of enhanced immune-mediated protection. As a consequence, probiotic treatment might be an effective dietary means of preventing or limiting diarrhoea in human infants (Shu *et al.*, 2001). The intestinal barrier maintains the epithelial integrity protecting the organism against bacterial or food antigens that could induce inflammatory processes leading to intestinal disorders such as inflammatory bowel diseases (IBD) (Hooper *et al.,* 2001). *L. casei* ssp. *rhamnosus* has shown to be a promising probiotic in preventing the colonization of the gastrointestinal tract by pathogenic bacteria such as enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and *Klebsiella pneumonia* using in vitro model with Caco-2 cell line (Forestier *et al.,* 2001).

Cultures of lactic acid bacteria, mostly from foods, were tested for their effect on the growth of *Staphylococcus aureus* in Trypticase Soy Broth (BBL). In general, the more effector bacteria there were in the inoculum, the greater was the overall inhibition (or stimulation) of S. *aureus*. Inhibition was most effective at 10 or 15 C, less so at 20 or 25 C, and least at 30 or 37 C, whereas stimulation during early growth was greater at the higher temperatures. Results with different strains of the effectors and with two strains of S. *aureus* were similar, for the most part (Kao and Frazier, 1966).

In the presence of oxygen, LAB produces hydrogen peroxide (H2O2) through electron transport via flavin enzymes. In the presence of H2O2, superoxide anions form destructive hydroxy radicals. This process may lead to peroxidation of membrane lipids (Morris, 1979). The resulting bactericidal effect of these oxygen metabolites has been attributed to their strong oxidizing effect on the bacterial cell as well as destruction of nucleic acids and cell proteins (Piard & Desmazeaud, 1992).

Inoculated pack studies usually found reduced numbers of L. *monocytogenes* after fermentation and drying, however, survivors were also detected. The studies of L. *monocytogenes* survival during fermentation showed several logs decline after the fermentation period of 1 t0 2 days, particularly when starter cultures were used (Farber *et al*., 1993).

Diacetyl is more effective against Gram negative bacteria, yeasts and molds, than against Gram-positive organisms. Diacetyl interferes with arginine utilization by reacting with the arginine binding protein of Gram negative bacteria (Jay, 1996). The growth of salmonella and Staph. *aureus* inhibited when pH decline below 5.3 (Schillinger and Lucke,1989). Decreased pH and water activity-destroys Enterohepatic E.*Coli* (Buchanan and Bagi, 1997).

Effects on composition of milk products

Many of the industrially utilised lactic acid bacteria (LAB) based starter cultures are highly proteolytic. The release of different bioactive peptides from milk proteins through fermentation by LAB is now well documented. It is suggested that some of these benefits can be attributed to biologically active peptides derived from proteins. Peptides are amino acid sequences encrypted within intact protein molecules and are released only upon proteolytic action. Typically, physiologically active peptides can be released during digestion of milk proteins in the gut or during fermentation of milk in the manufacture of yoghurt or cheese (Korhonen and Pihlanto, 2006). Yoghurt bacteria, cheese starter bacteria and commercial probiotic bacteria have been shown to produce different bioactive peptides in milk during fermentation (Gomez-Ruiz *et al*. 2002).

GMP is a C-terminal glycopeptide released from the K-casein molecule by the action of chymosin. GMP is hydrophilic and remains in the whey fraction in the cheese manufacturing process. GMP contains a significant (50-60% of total GMP) carbohydrate fraction which is composed of galactose, N-acetyl-galactosamine and N-neuraminic acid. The non-glycosylated form of GMP is often termed caseinomacropeptide or CMP. Pure GMP can be recovered in large quantities from cheese whey by chromatographic or ultrafiltration techniques. The potential biological activities of GMP have received much attention in recent years. Extensive research has shown that GMP inactivates in vitro microbial toxins of E.*coli* and V. *cholerae*, inhibits in vitro adhesion of carcinogenic bacteria and influenza virus, modulates immune system responses, promotes growth of *Bifidobacteria*, suppresses gastric hormone activities and regulates blood circulation through antihypertensive and antithrombotic activity (Manso and López-Fandino, 2004).

Many studies have demonstrated that Lactobacillus helveticus strains are capable of releasing antihypertensive peptides, the best known of which are ACE-inhibitory tripeptides Valine-Proline-Proline and Isoleucine-Proline-Proline. The antihypertensive capacity of these peptides has been demonstrated in many in vitro and rat model studies (Nakamura *et al*., 1995).

Milk proteins are considered the most important source of bioactive peptides. Over the last decade a great number of peptide sequences with different bioactivities have been identified in various milk proteins. The best characterised sequences include e.g. antihypertensive, antithrombotic, antimicrobial, antioxidative, immunomodulatory and opioid peptides (Gobbetti *et al*., 2007). Milk derived bioactive peptides may exert a number of physiological effects in vivo on the gastrointestinal, cardiovascular, endocrine, immune, central nervous and other body systems. Bioactive peptides are inactive within the sequence of the parent protein molecule and can be released from precursor proteins in the following ways: (a) enzymatic hydrolysis by digestive enzymes (b) fermentation of milk with proteolytic starter cultures, and (c) proteolysis by enzymes derived from microorganisms or plants. In many studies, a successive treatment by (a) and (b) or (a) and (c), respectively, has proven effective in generating bio functional peptides (Korhonen and Pihlanto, 2007).

Effects on composition of meat products

Peptides and amino acids are present in meat at 1% dry matter basis. Bioactive peptides are produced by lactic acid fermentation and having same effect as described above. PUFA (polyunsaturated fatty acids) is higher than saturated fatty acids in meat products. Feeding formula with long-chain polyunsaturated fatty acids as triacylglycerols or phospholipids influences the distribution of these fatty acids in plasma lipoprotein fractions that is usefull for animal health. Cathepsin D (endogenous protease) is activated at pH values around 5.0 and produces peptides which are then further metabolized by the ripening flora. Later in ageing, bacterial enzymes may also play a role in the degradation of peptides formed (Molley *et al*., 1997).

Other beneficial effects

Exopeptidases from lactobacilli with muscle amino peptidases, generates free amino acids, contributing to flavor. Curing colour develops in acidic conditions. Lactic acids-coagulate soluble meat protein that decreases the water binding capacity leads to drying of the product. Protect from detrimental effects of oxygen (Demeyer, 2000).

Effects on the nutritional value of dairy foods

- Increases folic acid, niacin and riboflavin (yoghurt)
- Vit-B_{12} (cottage cheese)
- Vit-B_6 (chhedar cheese)
- Increases digestibility of protein and fat
- Retention of micronutrients
- Synthesis of probiotic compounds

Effects on nutritional value of meats

- Fermentation creates Omega -3 fatty acids
- Trace mineral GTF chromium

GTF (Glucose Tolerance Factor) Chromium is a safe and absorbable form of the essential trace mineral chromium. Minerals can't be absorbed in their pure state, but have to be bound or chelated to something else. In GTF Chromium, chromium is bound to the B-vitamin niacin. Chromium is needed for the optimal function of the hormone insulin, which regulates energy use, storage and metabolism. Chromium deficiency hampers insulin function and can lead to weight-gain and energy loss

- Proteolysis convert simple peptides into essential amino acids
- Lipolysis increase free fatty acids, essential fatty acids (linoleic, linolenic)

Enhancement of immune system

- Improve immune defence responses by increasing Ig A producing plasma cells
- Short Chain Fatty Acids regulate proliferation and apoptosis of lymphocytes

(Kurita ochiai *et al*., 2003)

- Increase no. of T-lymphocytes & natural killer cells
- Enhance specific and nonspecific immune responses
- Promising effects in immunocompromised patients

Anticarcinogenic activity

- Detoxification, bind with heterocyclic amines

- Decrease activity of glucoronidase, nitroreductase, azoreductase
- Alteration of intestinal environment
- Production of metabolites & inhibitors
- Stimulating the immune system

Effect on Biogenic amines

Histamine, tyramine, phenylethylamine, tryptamine, putrescine and cadaverine are not to be exceed 100mg/kg in the body and are mainly derived from bacterial decarboxylation of amino acids. Putrescine and cadaverine are produced by the Gram-negative spoilage flora. Probiotic cultures inhibit rapidly metabolism of Gram negative bacteria and effectively reduce tyramine levels in fermented sausages.

Probiotics: An edge over Antibiotics

Antibiotics:

- Emergence of antibiotic resistance micro-organisms.
- Unpleasant side effects

Probiotics:

- Non- invasive
- Preventive
- Free from undesirable side effects

Factors affecting Probiotic function

- Type of strain
- Dose and viability of probiotic.
- Viability on the shelf and in the digestive tract.
- Length and frequency of the exposure
- Health and age of the person
- Form of the probiotic taken in

Desirable properties of probiotics

Character	Working	Functional properties
Origin	From same source where it is intended to be used	Species specific health effects and viability in GIT.
Stability	Bile salts and gastric acidity	Survival in GIT
Adherence	To the intestinal mucosa	Immune cell modulation and competitive inhibition of pathogenic organism
Implantation	Positive colonization	Growth and multiplication in GIT
Safety	Well documented clinical safety	Identified as non toxic ,non pathogenic, non allergic,non mutagenic, non carcinogenic
Antagonism	Against pathogenic and putrefactive organisms	Prevention of these organisms from their adhesion to intestinal mucosa
Proven health effects	Clinically documented and validated therapeutic effects	Dose response data for minimum effective dosage of pro. Org. in diff formulation
Stability	Viability during processing and storage	Desirable characteristics maintained, genetically stable, no plasmid transfer
Suitability	Technological suitability	For production of acceptable quality finished products with desirable viable counts

- Presence of prebiotics
- Presence of food in stomach
- Composition of the raw materials
- Processing history of the raw material used as substrate
- Possible interaction of probiotics with the starter cultures
- Processing and storage of the final food products
- Physiologic state of the organism added (either from logarithmic state or from stationary state)
- Packaging by active packaging or MAP
- Maintenance of cold chain
- Homogenous distribution of micro organisms in the product

Stability of probiotics is improved by

- Micro encapsulation - Alginate is a linear heteropolysaccharide extracted from different types of algae, with two structural units consisting of D-mannuronic and L-guluronic acids. Calcium alginate has been widely used for the encapsulation of lactic acid- and probiotic bacteria, mainly in the concentration range of 0.5-4%. Blending alginate with starch is a common practice and it has been shown that encapsulation effectiveness of different bacterial cells especially lactic acid bacteria were improved by applying this method (Jankowski *et al.*, 1997).
- Addition of compatible cryoprotectants
- Addition of microbial exopolysaccharides
- Addition of cystein / an oxygen scavenger like ascorbic acid
- Use of oxygen impermeable containers
- Two step fermentations
- Micronutrient incorporation (peptides ,amino acids)

Adverse effects

Their occurrence as normal commensals of the mammalian flora and their established safe use in a diversity of foods and supplement products worldwide supports this conclusion. However, probiotics may theoretically be responsible for three types of side-effects

1. Systemic infections
2. Deleterious metabolic activities
3. Excessive immune stimulation in susceptible individuals

These occurred mainly in very sick patients who received probiotic drugs because of severe medical conditions. Prebiotics exert an osmotic effect in the intestinal lumen and are fermented in the colon. They may induce gaseousness and bloating. Abdominal pain and diarrhea only occur with large doses (Marteau, 2002).

Conclusion

Probiotics are promising healthful dietary ingredients. They may play a role in helping to keep people healthy as well as serve as therapeutic agents for certain conditions.

Probiotic strains of specific species should have specific and well-defined metabolic and functional properties. The probiotic effect should target a particular host function that has been altered through environmental stress, antibiotic utilization, or during specific clinical diseases that result in the alteration of the normal microbiota.

As the industry grows, probiotic products will become more consumer-friendly, with guarantees of product shelf-life, scientifically accurate labeling of contents and truthful descriptions of documented benefits.

Probiotics are extremely sensitive to heat and other processing conditions. New technologies, like microencapsulation and immobilized cell technologies, offer additional protection to probiotic organisms and new ways to include probiotics in foodstuffs.

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