Probiotics in Dairy Products: Their Role Beyond basic Nutrition

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Introduction

Milk and dairy products have always been regarded as pure products that are essential for the health and well being of humans. Based on this perception, many new products appeared that are milk-based. Culture containing milk like buttermilk, sour cream and yogurt are examples of functional and/or probiotic products which are perceived by consumers as nutritious and healthy.

Probiotics, the friendly bacteria, are naturally found in the intestinal tract including the moth, throat, esophagus, small intestine and the large intestine. The first concept of the beneficial effect of these food microbials was accounted for

by a Russian scientist in 1908. The term probiotic which means "for life" was given by an Oregon professor in 1960. (http://www.inventor.ao1.com/gamebiotic/more about probiotic production.htm). A number of definitions have been proposed later to describe probiotics. These include:

a. The word "probiotic" to describe beneficial bacteria (Lilley and Stillwell, 1965);

b. Probiotic as a "live microbial which beneficially affects the host animal by improving its microbial" constitution (Fuller, 1992);

c. Probiotics are a mono- or mixed cultures of live microorganisms which when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora (Haveenar et.al., 1992);

d. Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host (http://www.tteagasc.le/publications/ndc.1999/htm paper 8.htm), and

e. Probiotics are living organisms that upon ingestion in certain numbers exert health benefits beyond basic nutrition (Aimutis, 2001).

Based on these definitions of Probiotics, it appears that consumption of Probiotics affects the composition of the intestinal microflora. This effect of probiotic in the intestinal ecosystem impacts in some beneficial way on the consumer.

In this paper, a review of the lactic acid bacteria (LAB) used in probiotic dairy products available in the market, purported health claims for probiotics, and new trends in probiotic products research and development are presented.

Lactic Acid Bacteria Used in Probiotic Dairy Products

Strains of lactic acid bacteria (LAB) used as probiotic usually belong to the species of the genera *Lactobacillus, Enterococcus*, and *Bifidobacterium* (Klein et.al., 1998). These LAB strains are chemoorganotropic and ferment carbohydrates as a major end product. Survival in the gastro-intestinal tract, resistance to low pH and/or bile and temperature growth ranges, are some of physiological characteristics of LAB. Table 1 shows the LAB species commonly used in present probiotic dairy products.

Table 1. Lactic acid bacteria applied in commercial products*

Lactobacillus sp.	Bifidobacterium	Enterococcus	Other LAB species
L. acidophilus *L. casie shirota* *L. casie immunitas* *L. crispatus* *L. gasseri* *L. johnsonii (L. paracasei)* *L. plantarum* *L. reuteri* *L. rhamnosus* *L. fermentum*	*B. andolescentis* *B. animalis* *B. bifidum* *B. brevi* *B. infantis* *B. lactis* *B. longum*	*Ent. Faecalis* *Ent. faecium*	*L. actoc.lactis* *S. thermophilus*

* Source: Holzapfel et.al. (1998)

Lactobacilli. *Lactobacilli* constitute an important part of the indigenous microbiota of man and animals and distributed in various ecological niches throughout the gastrointestinal/genital tracts (Charteris et.al., 1997). They are gram-positive, non-sporming, non-motile rods, aero tolerant or anaerobic and strictly fermentative. The G + C content of their DNA is usually between 32 and 51 mol%. Technologically used strains are regarded as non-pathogenic and regarded as health promoters, especially in the human gastrointestinal and genitourinary tracts.

Bifidobacteria. *Bifidobacteria* are gram-positive, non-sporming, non-motile catalase-negative anaerobes and saccharoclastic producing acetic and lactic acid and small amounts of formic acid in the molar ratio of 3:2 without CO_2 production except in the degradation of gluconate (Sgorbatii et.al., 1995).

Bifidobacteria play an important role in the large intestine by contributing to pH control through the liberation of lactic and acetic acids, which in turn restrict the growth of many potential pathogens and putreactive bacteria (Moddler et.al., 1990a, Gibson and Roberfroid, 1995).

Enterococcus. Two *enterococcus* strains are used as human probiotic. These are *Enterococcus faecalis* and *Enterococcus faecium*.

Some products available in the local and international markets which contained LAB strains like *Lactobacillus* and *Bifidobacterium* are (http://www.cbt.co.Kr/index-company-e.htm): Everlac-S, Bifidolac, Babylac, Laclean-Gold, Slimlacto, Prolac, Actimel, Actimel Cholesterol Control, Daily Fit, Pro'Ac, Procult 3, Fyos, Fysief, and Silhoutte Plus. These products are additions to popular probiotic products like Yakult, Nestlé LCI, CULTURA, Bifighurt, Biograde, MIL-MIL, KESO-CURT and Yocheese.

Health Claims, Mode of Action and Effects of Probiotics

A number of potential health and developmental benefits arising from changes to the intestinal milieu have been attributed to probiotics. Some of these documented health claims are presented in Table 1.

The role of probiotics, *Lactobacilli* and *Bifidobacteria,* in the intestinal tract which promote health stability of the host include (http://www.cbt.co.kp): antibiotic production; organic acid production; lower pH and oxidation reduction; competitive antagonists; and bile deconjugation and detoxification.

Numerous factors have been attributed to enhance the beneficial effects of probiotics. Immune modulation and strengthening the gut mucosal barrier were two of the important effects of probiotics (Salminen et.al., 1996). These effects are due to: gut microflora modification; adherence to the intestinal mucosa with capacity to prevent pathogen adherence pr pathogen activation; modification of bacterial enzyme capacity especially of those suggested to be related to tumor infection and influence on gut mucosal permeability.

Aimutis (2001) postulated that competitive exclusion by competing for receptor sites by a probiotic strain versus a pathogen in the intestinal membrane may result in potential "washing out" of the intestine. Other proposed antimicrobial mechanisms of probiotics include production of organic acids, hydrogen sulfide, hydrogen peroxide, bacteriocins, fatty acids and deconjugated bile acids.

Recent observation showed that intestinal microflora can influence the expression of epithelial glycoconjugates which may serve as receptors for attachment of (pathogenic) microorganisms. Glycoconjugates comprise the apical surface of the epithelium of the underlying cell coat (glycocalix) which with the mucus gel layer provides the interface between a mammalian host and the intestinal lumen. Bry et.al. (1996) hypothesized that the integration of the ecosystem into the host may be achieved through dynamic host-microbe interactions that allow microbes to modify cellular differentiation.

Certain probiotic strains (LAB) induce specific immune regulators as a result of interaction with mono nuclear phagocytes and endothelial cells of the host. There are indications that specific wall components or surface layers may be involved in the immunodulation effect of probiotics (Holzapfel et.al., 1998). Peptidoglycans and lipopolysaccharide components of the cell wall induce detrimental effects as well as induce a non-specific resistance to microbial infections and irradiation, as well as necrosis of certain tumor type of the host animal (Hamman et.al., 1998).

Table 1. Health claims for probiotics

Health claim	Source
Intestinal disorder: diarrhea-antibiotic induced, constipation, lactic acid intolerance, colitis, Salmonella and Shigella infections, lactose intolerance **Other disorders:** vaginitis, alcohol-induced liver disease, hypercholesteraemia **Other uses:** stabilization of flora, recolonization of bowel after antibiotic treatment, treatment of food allergies, adjuvant for vaccines, and increased weight gain during development	Goldin (1998)
Antimicrobial properties proven to treat diarrhea, improved lactose digestion, cholesterol reduction, immune system stimulation, cancer suppression	Aimutis (2000)
Infants are protected from enteric infection, lowering of intestinal pH by assimilation of sugars, suppression of putreactive and pathogenic bacteriam activation of intestinal function, assistance of digestion and absorption and stimulation of the immune response	Mizota (1996)
Alleviation of lactose intolerance, stimulation of the immune system, prevention of gastrointestinal disorders and reduction of blood ammonia levels	O'Sullivan (1996)
Vitamin synthesis, protein synthesis, assist in digestion and absorption, prevent colonization of pathogens, stimulation of immune response, control of intestinal function, enhancement of mineral absorption, reduction of constipation in the elderly, reduction of diarrhea, ability to exclude competitively *Campylobacter jejuni*, and stimulation of IgA production	Yaeshima (1996)
Degrade certain food components, produce certain B vitamins, stimulate the immune system, produce digestive and protective enzymes involved in the metabolism of some potentially carcinogenic substances, play role in drug efficacy, promote restoration of sublethally damaged *Lactobacillus reuteri*, reduce gastroenteritis, treat acute enteritis and difficile colitis, support intestinal integrity	Holzapfel et.al. (1998)
Anti-tumor and antimetastatic effects on tumor cells, modulation of cellular immune system	Matsuzaki (1998)
Inhibit coliforms in both fermented milk and the intestine of rats, ability to prevent DNA damage and mutations, antagonistic action against intestinal and food pathogens	Streekumar and Hisomo (2000)

Important role in digestion, vitamin and mineral availability, help in breakdown of proteins to amino acids, improve usage of digested fats and minerals like calcium, reduce risk of colon cancer, help maintain the balance between good and bad bacteria during the use of antibiotic	http://www.jwes.nsw.eduau/oldsite/Aus Dairy Corp/ww1720.htm
Acidify colon, normalize bowel movements, improves immune system function, aids in the production of lactose, deactivates various cancer causing compounds, help regulate hormone levels, eliminate intestinal gas and bloating, pH control through liberation of lactic and acetic acids	Moddler et.al. (1990)

Matsuzaki (1998) found out that *Lactobacillus casie* strain Shirota (LcS) showed the potential to ameliorate or prevent a variety of diseases through modulation of the host's immune system, specifically cellular immune responses. This LAB strain has the ability to promote the development of several cytokines which are involved in the regulation of the host's cellular responses.

The anticarcinogenic properties of *Lactobacillus* include the inhibition of tumor cells, the suppression of bacteria which produce enzymes which, in turn, are responsible for the release of carcinogen from the mucous complexes and the destruction of carcinogens which are nitrosamines and nitroreductase (Fuller, 1989). *Lactobacillus acidophilus*, specifically, inhibited the activities of Beta-glucoronidase and Beta-glucosidase. These two enzymes are produced by *E. coli*, bacteriosides and some obligate anaerobes that are implicated in colon carcinogenesis (Streekumar et.al., 2000).

Some LAB species were reported to elicit antimutagenic (Pool-Zobel et.al. 1993a) and antigenotoxic (Pool-Zobel et.al., 1993b) effects. These phenomena were correlated to the ability of such strains to prevent or reduce DNA damage at early stage of carcinogenesis.

A number of commercial probiotic strains exhibit bile salt hydrolase (BSH) activities. This BSH is believed to contribute to reducing serum cholesterol level by deconjugating bile salts and its subsequent excretion (Chikai et.al., 1987). It is an established fact that cholesterol and bile salt metabolism are clearly linked.

New Trends in Probiotic Products Research and Development

The concept of probiotics has been around for nearly 100 years. Yet its impact on human nutrition is still an emerging concept. Obtaining positive scientific validation with the use of suitable probiotic strains and tools to monitor

the performance of these bacteria in the intestines of individuals will bring high impact of probiotics to human nutrition.

The emergence of sophisticated molecular tools that complement the traditional approaches for studying the human ecosystems shows enormous promise for obtaining the necessary insights into the intestinal microflora. Some of these tools include: 1) **differential plating methodologies** which allow differential detection and enumeration; 2) **molecular biological identification methodology** which employs 16S and 23S rRNA probe hybridization for oligonucleotide's detection and identification, restriction enzyme analysis (REA), pulsed field gel electrophoresis (PFGE), ribotyping or restriction fragment length polymorphism (RFLP) of genes for rRNA, polymerase chain reaction (PCR) and randomly polymorphic DNA (RAPD) for genotyping of probiotic microorganisms (Charteris et.al., 1997) and 3) **amplified ribosomal DNA restriction analysis method** (ARDRA) for identifying microorganisms from various environments (Ventura et.al., 2001).

The development of *in vitro* models that mimic the successive *in vivo* gastro-intestinal conditions of humans, e.g. colon model, provided a visible assessment of the survival of ingested lactic acid bacteria (Holzapfel et.al., 1998). Thus, predicted value or function of probiotics are determined and validated.

The recent practice of polyphasic taxonomy is able to differentiate species which are phenotypically very similar, but genotypically quite different (Vandamme et.al., 1996). Similarly, the use of phenotypic fingerprinting techniques, a molecular technique to provide basis for quality assurance, enhanced the safe biotechnology to separate probiotics from potential pathogenic strains.

The ability of *bifidobacteria* to survive through acidic condition in the human stomach and during contact with bile salts in the upper portion of the small intestine was documented (Martin, 1996). This information showed that *bifidobacteria* survive in the normal shelf life of dairy products.

The current emphasis of incorporating both *bifidobacteria* and bifidogenic factors like raffilose, fructo-olosaccharide (FOS), transgalactosyl-oligosaccharide (TOS), lactulose and the enzyme, oxyrase, stimulate growth of the bacteria *in vivo* of human (Martin, 1996). This finding enhanced the choice of *bifidobacteria* as dietary adjunct in dairy foods.

The potential of LAB as live vaccine carriers was being tested for their efficacy (http://www.horizonpress.com/hsp/abs/abspro.htm). The vaccine potential of *Lactobacillus lactis, Streptococcus gordonii*, and *Lactobacillus spp.* was tested to elicit systemic and mucosal antigen-specific immune responses in

rats. Likewise, data on successful oral and vaginal immunizations are also accumulating for *L. lactis* and *S. gordonii*, respectively.

Conclusions

The numerous cited merits of probiotics as therapeutic and medicinal agents bring potential for their incorporation s regular component of the diet for humans. These microbials' relative association to milk and its by-products will enhance further the usefulness of milk as a nutritious food.

It is seen also that probiotic research and development are poised to make great advances in the coming years. The emergence of molecular technologies as means to analyze the intestinal communities in conjunction with the use of biochemical and bacteriological methodologies will provide a detailed understanding of the intestinal microflora. Likewise, the availability and application of these molecular tools will provide critical knowledge of the role of specific bacterial groups in the modulation of the immune system.

It is viewed further that the development of probiotic-type products for administration to other body sites, such as the vagina, will be increasing interest that will produce efficacious products. On the other hand, issues like defining probiotics modes of action and stability *in vivo*, resolving taxonomy issues of probiotic species, developing user-friendly methods for speciation findings in commercial products, defining physiologically relevant biomarkers to assess probiotic effectiveness and confirm efficacy with properly controlled human studies and good epidemiology are to be dealt with to hasten more of the development and marketing of probiotics as component of the diet of human beings.

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