Innovations in Daily



Opportunities for Probiotic Dairy Products

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Note: Research on probiotics and dairy products is conducted worldwide. Some of the information presented in this issue of Innovations in Dairy was extracted from research supported by Dairy Management Inc. TM (DMI), with funding from America's dairy farmers.

Executive Summary

For many years, cultures worldwide have valued live beneficial bacteria consumed in fermented dairy products for the purpose of maintaining health and treating gastrointestinal ailments. Today, tradition meets science as probiotic bacteria are being studied to better understand how and to what extent they may help people stay healthy. Down the road, fermented dairy products may be developed containing new strains of probiotic bacteria for the prevention, management and even treatment of specific diseases and health conditions such as lactose maldigestion, intestinal infections, allergies, inflammatory conditions and perhaps even some forms of cancer. This report discusses commonly used probiotic bacteria, why dairy products may be an excellent delivery system for probiotics, emerging developments in our understanding of the health benefits of probiotics, new genetic-based studies designed to improve the efficacy of probiotic dairy products, probiotic labeling considerations and suggestions for improving the market potential of probiotic dairy products.

Introduction

Probiotics are live microorganisms that, when consumed in sufficient numbers, provide health benefits to the host, according to a report prepared for the Food and Agriculture Organization of the United Nations and the World Health Organization. The human body is host to some 10¹⁴ bacteria. The majority of these bacteria reside in the host's gastrointestinal (GI) tract and are often referred to as gut microflora. Some researchers have claimed that the metabolic activity of gut microflora is potentially greater than that of the liver. Gut microflora play a critical role in health maintenance by modulating the activity of the immune system, protecting the host from invading bacteria and viruses and aiding digestion.

One way to positively affect the composition and activity of gut microflora is to consume probiotics. Another way to assist gut microflora in providing their potential health-enhancing characteristics is to consume prebiotics—nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial bacteria in the colon (Gibson and Roberfroid, 1995).

Not all gut microflora, of course, are beneficial. While bifidobacteria and lactobacilli tend to be beneficial components of gut microflora, some potentially harmful groups of microbes—including Salmonella, E. coli, clostridia, sulfate reducers and amino-acid-metabolizing species—can also colonize the gut. The balance of gut microflora is affected by diet (including, but not limited to, consumption of prebiotics and probiotics), antibiotic intake, infections, food-borne illnesses, environment, stress, diseases and aging.

A trail of scientific discovery has led to our current appreciation of the value of live microbes in our diet. One of the first people to appreciate the health benefits of ingesting beneficial bacteria was the Nobel Laureate Elie Metchnikoff, a Russian immunologist employed at the Pasteur Institute in the beginning of the 20th century. In his book *The Prolongation of Life*, Metchnikoff attributed the longevity of Bulgarian peasants to their high consumption rates of *Lactobacillus*-containing fermented milk. Metchnikoff proposed that prevention of bowel diseases and maintenance of healthy bowel tissue could be achieved by regular consumption of fermented milk containing live *Lactobacillus* organisms.

In 1906, French pediatrician Henry Tissier advised that infants suffering from diarrhea consume bifidobacteria, postulating that the bifidobacteria would favorably compete against the pathogenic bacteria, thereby replacing them.

In the early 1930s, Japanese researcher Minoru Shirota studied beneficial strains of lactic acid bacteria (LAB) that could survive passage through the gut and provide desirable taste qualities to fermented milk products.

The long history of use of probiotic dairy products containing lactobacilli and bifidobacteria attests to their general safety. Some lactobacilli and bifidobacteria are also normal inhabitants of the healthy human GI tract. The safety of bifidobacteria and lactobacilli has been evaluated by several experts (Salminen et al. 1998; Borriello et al. 2003). When considering epidemiological observations and studies done using in vitro models, animal models and human subjects, the general conclusion is that oral consumption of lactobacilli and bifidobacteria poses a very small risk. However, infections caused by some species of commensal lactobacilli and bifidobacteria, although rare, have been documented, and therefore at-risk individuals should consult their health care professional prior to consuming high-potency probiotic products.

Table 1 lists criteria for probiotics used in human foods. While probiotics are predominantly LAB, other types of bacteria (e.g., *Saccharomyces boulardii*, a probiotic yeast) have also been used. Table 2 lists microorganisms currently used in probiotic products around the world.

Table 1: Criteria of Probiotic Bacteria

Nonpathogenic, noncariogenic

Acid- and bile-tolerant (for applications targeting improved GI function)

Stable characteristics and numbers during preparation and over the shelf life of the product

Beneficial physiological effects established in human studies

Ability to survive at the target site (e.g., survive in the intestine for a GI tract target)

Dairy Products as Probiotic Delivery Systems

Probiotics are typically provided in products in one of three ways: (1) as a culture added to a food (usually a dairy product) at medium levels (e.g., 10° colony forming units [cfu] per milliliter), with little or no opportunity for culture growth; (2) inoculated into a milk-based food (or dietary supplement) and allowed to grow to achieve higher levels (>10° cfu/mL) in a fermented food; and (3) as concentrated and dried cells packaged as dietary supplements such as powders, capsules or tablets. Dairy products may be an ideal probiotic delivery vehicle for several reasons:

Protection of probiotic bacteria: To effectively enhance host GI function, probiotic bacteria must arrive in the GI tract alive at efficacious levels. High levels of stomach acids and bile in the small intestine can kill many types of probiotics. Dairy products buffer the high acidic environment in the human stomach and can create a more favo rable environment for probiotic survival.

Refrigeration helps probiotic stability: The typical dairy food carrying probiotics is a short-shelf-life, refrigerated product such as yogurt or fermented milk. In contrast, dietary supplement probiotic pills can sit for one to two years at room temperature on store shelves. Short time and low storage temperature promote probiotic stability.

Positive public image: While the concept of consuming bacteria is not appealing to some consumers, many consumers recognize that "live, active cultures" present in

Table 2: Microorganism	ns Currently	Used in Prob	iotic Products	Around the World
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Lactobacilli	Bifidobacteria	Other LAB	Non-LAB
L. acidophilus	B. animalis	Enterococcus faecium	Bacillus cereus
L. casei	B. breve	Enterococcus faecalis	Escherichia coli
L. johnsonii	B. infantis	Lactococcus lactis	Saccharomyces boulardii
L. reuteri	B. longum		Clostridium butyricum
L. salivarius	B. adolescentis		Propionibacterium freudenreichii
L. paracasei	B. lactis		Bacillius subtilis
L. fermentum	B. bifidum		
L. plantarum			
L. crispatus			
L. gasseri			

Source: Salminen, S.; Ouwehand, A.C. Probiotics: applications in dairy products, in *Encyclopedia of Dairy Sciences*, Volume 4, Roginski, H., Fuquay, J.W., and Fox, P.F., eds., Academic Press, 2003, p. 2316.

fermented dairy foods can be good for their health. Therefore, the probiotic concept may be more acceptable to consumers when associated with dairy foods.

The health advantage: Combining the health benefits of probiotics with a nutritious carrier such as dairy foods creates an appealing, healthy food package. Dairy products are dense in nutrients, providing consumers with vitamins, minerals and high-quality protein. In addition, researchers are discovering other bioactive factors in milk that appear to further enhance the body's function.

Potential Health Benefits of Probiotics

Researchers have been studying the effectiveness of probiotics on human health. While many earlier efforts were marred by poor experimental design and lacked good records, the quality of recent studies has been much improved, as evidenced by studies being conducted employing well-defined probiotic strains, using blinded, placebo-controlled study design when possible, and following guidelines for good clinical practice.

Mary Ellen Sanders, a consultant specializing in probiotic microbiology at Dairy and Food Culture Technologies, Centennial, Colo., points out that while research is rapidly revealing new insights into probiotics, there is still a lack of significant understanding about the types, activities and physiological roles of the microbes that colonize the human GI tract. According to Sanders, one exciting area of research is understanding how the normal microbes in the human body interact with each other, with added probiotics and with the host cells. Microbial and human genomics will provide key tools to examine these interactions.

According to S. Salminen and A.C. Ouwehand of the University of Turku, Finland, the ability of a probiotic strain to influence health may involve a variety of mechanisms, including:

- (a) adherence to intestinal mucosa and mucus.
- (b) production of antimicrobial substances.
- (c) antagonism against pathogens and carcinogens.
- (d) competition for adhesion sites (competitive exclusion).
- (e) interaction with gut-associated lymphatic tissue (immune modulation).
- (f) inactivation of harmful components within the intestinal contents (binding of toxins and regulation of the metabolic activity of the intestinal microflora).
- (g) a trophic effect on the intestinal mucosa (e.g., through the production of butyrate).
- (h) overall normalization of the intestinal microflora composition and activity.

Sanders maintains that the potential health benefits of probiotics are significant. Some benefits have been well established scientifically (e.g., alleviation of certain diarrheal illnesses and reduction of lactose intolerance symptoms), while others are emerging (e.g., relief of allergy symptoms and prevention of cancer) or are anecdotal. Research into the potential health benefits of probiotics has rapidly accelerated in recent years. A recent bibliographic search of the Medline database for "probiotic" showed that only six citations appeared prior to 1990, and no citations were found when the search was limited to clinical

trials involving probiotics. A search from 2002 through Dec. 11, 2004, however, turned up 1,147 probiotic citations and 100 citations limited to clinical trials.

Examples of health benefits of probiotics reported in the literature include the following, which have been excerpted from comprehensive descriptions from www.usprobiotics.org, a Web site maintained by Sanders and sponsored by the California Dairy Research Foundation, Davis, Calif.

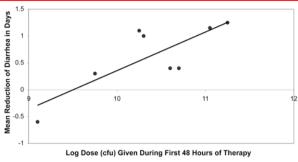
When considering the health benefits of probiotics, it is important to recognize that different strains, species and genera of bacteria may have different effects. For the most part, specific clinical studies on probiotics are done with one defined strain or a defined blend of strains.

Diarrhea: Probiotics have demonstrated the ability to decrease the incidence and duration of some types of diarrheal illnesses (e.g., antibiotic associated, *Clostridium difficile*, traveler's and rotavirus). A meta-analysis by Van Niel et al. (2002) concluded that "*Lactobacillus* is safe and effective as a treatment for children with acute infectious diarrhea." Van Niel and co-workers clearly demonstrated the relationship between *Lactobacillus* dose and reduction of diarrhea in children. The meta-analysis considered nine studies involving various species of lactobacilli. (Figure 1.)

Antibiotics: It appears that people on antibiotic therapy can benefit from probiotic consumption. One negative side effect of antibiotics is that they kill beneficial, as well as undesirable, bacteria. Replenishing the flora with normal/beneficial bacteria during and after use seems to minimize intestinal disruption caused by antibiotic medications. Cremonini et al. (2002) reviewed seven studies (881 total patients) covering probiotic mitigation of antibiotic-associated diarrhea. According to this analysis, probiotics (e.g., *Lactobacillus rhamnosus* GG) can be used to prevent antibiotic-related diarrhea but do not appear to diminish existing diarrhea symptoms.

Irritable bowel syndrome: About 10% to 20% of the adult population suffers from irritable bowel syndrome (IBS), which may include symptoms of abdominal cramps, bloating, diarrhea and constipation. Some symptom relief from probiotic consumption has been reported in studies to date. Research indicates that IBS is an inflammatory response to the host's own colonic bacteria. Supplanting some of the host's bacteria with probiotics (e.g., Lactobacillus plantarum 299V) appears to help relieve IBS symptoms in some cases [Quigley et al. (2002) and Niedzielin et al. (2001)].

Figure 1: Relationship Between Lactobacillus
Dose and Reduction of Diarrhea
in Children



Source: Van Niel, C.W.; Feudtner C.; Garrison, M.M.; and Christakis, D.A. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. Reproduced with permission from Pediatrics, Vol. 109, pages 678-684. Copyright © 2002 by the AAP.

Lactose intolerance: People with true lactose intolerance do not produce sufficient quantities of lactase, the enzyme that hydrolyzes lactose into glucose and galactose. As a result, these people can experience diarrhea, bloating, abdominal pain and flatulence due to undigested lactose reaching the large intestine and being fermented by colonic microbes. It has been demonstrated that people with lactose intolerance can consume yogurt and other fermented dairy products with fewer symptoms, even though these products may contain levels of lactose similar to unfermented dairy products. In addition to the yogurt starter culture, some strains of Lactobacillus acidophilus and bifidobacteria reduce symptoms of lactose maldigestion, although to a lesser extent than yogurt starter cultures (Lactobacillus bulgaricus and Streptococcus thermophilus).

Hypertension: Certain lactobacilli have been shown to produce bioactive compounds that have effective antihypertensive effects. These metabolites include: (a) two tripeptides originating from the proteolytic action of Lactobacillus helveticus on casein; (b) bacterial cell wall components from cell extracts of lactobacilli; and (c) gamma amino butyric acid in fermented milk. Reductions of systolic blood pressure have been documented, suggesting that consumption of certain lactobacilli (or products made from them) may reduce blood pressure in mildly hypertensive people. Interestingly, viability of lactobacilli is not required for the positive effect.

Cancer: Cancer-causing agents can be ingested or generated by metabolic activity of microbes residing in the GI tract. Some researchers suggest that probiotics (e.g., *Bifidobacterium longum* and *L. acidophilus*) might decrease the exposure to chemical carcinogens by

(1) detoxifying ingested carcinogens, (2) altering the environment of the intestine and thereby decreasing populations or metabolic activities of bacteria that may generate carcinogens, (3) generating metabolic products (e.g., butyrate), which improve a cell's ability to die when it should die (i.e., apoptosis or programmed cell death), (4) producing compounds that inhibit growth of tumor cells or (5) stimulating the immune system to better defend against cancer cell proliferation.

Research studying the effects of probiotic consumption on cancer risk has observed the following effects:

- (a) A reduction in the incidence of chemically induced breast and colon tumors in rats.
- (b) A reduction of the activity in fecal enzymes thought to play a role in human colon cancer.
- (c) Degradation of nitrosamines.
- (d) A weakening of mutagenic activity of experimental carcinogens.
- (e) Prevention of damage of DNA in certain colonic cells.
- (f) In vitro binding of mutagens by cell wall components of probiotic bacteria.
- (g) Enhancement of immune system functioning.

A variety of studies have been carried out with rats to determine the effects of specific probiotics on chemical carcinogen induction of putative precancerous lesions in the colon, known as aberrant crypt foci (ACF). Administration of dietary B. longum (1010 live bacterial cfu/day) completely suppressed rat colon tumors induced by the compound 2-amino-3-methyl-3H-imidazo[4,5-f] quinoline, a well-defined experimental carcinogen (Reddy and Rivenson, 1993). McIntosh (1999) reported that L. acidophilus markedly reduced both the number and size of colon tumors induced by 1,2-dimethylhydrazine (DMH). Interestingly, there was also a difference in the type of tumors. In the rats given L. acidophilus, in contrast to the ones given DMH alone, only benign tumors (adenomas) were seen, suggesting that probiotics may inhibit the development of malignant disease.

In view of the promising results demonstrated by animal studies, well-controlled human intervention studies seem warranted. There is a wide range of biomarkers for cancer

risk based on measurements of colon adenoma recurrence and growth, colon biopsies (DNA damage, DNA repair capacity, hyperproliferation, apoptosis) and examination of fecal enzymes, metabolites, genotoxicity and cytotoxicity (Gill and Rowland, 2002). Human intervention studies are the only way to provide persuasive evidence for the anti-cancer activity of probiotics.

Immune function: Improved immune functioning with probiotics is another area that is actively being investigated. The ability of probiotic bacteria to support the immune system could be important to the elderly and other people with a compromised immune system.

"The gut has the highest number of immune cells in the body," Sanders says. Studies show that a properly balanced colon microflora is necessary for optimum immune functioning. For example, studies have shown that lab animals with no bacteria in their gut have very poor immune function, according to Sanders. Studies are under way to determine if the incidence of the common cold, food-borne illnesses and other health problems related to immune dysfunction can be mediated with probiotics. Recent studies have shown that individuals who consume probiotics have an enhanced ability to develop antibodies once they are given a vaccine.

The impact of probiotics on human immune functioning is intricate. Sanders says studies indicate that probiotics can down-regulate the immune response in situations such as inflammatory bowel diseases, stimulate components of the immune system to better protect against pathogens or assist in programming early immune development, thereby reducing the likelihood of developing allergy later in life. Exciting results documenting an impact on immune function have been observed in the elderly, in infants and in patients with inflammatory bowel diseases. However, more studies need to be conducted in healthy, adult subjects to determine what effects probiotics might have on the general population.

Allergies: Probiotic products may be developed to help allergy sufferers control their symptoms. Probiotic strains that have demonstrated an ability to decrease human allergy symptoms include *L. rhamnosus* GG, *Bifidobacterium lactis* Bb12 and *L. rhamnosus* 19070-2/*Lactobacillus reuteri* 122460. Other strains are supported by animal studies.

Enhancing Probiotic Effectiveness Through Genomic Research

Decoding the genome of LAB and other probiotics has profound implications for the dairy industry. Genes express bioactive proteins, including enzymes that determine flavor, body, texture and health-enhancing attributes of fermented dairy products. It should be noted that important commercial benefits from LAB genome sequencing can be realized without resorting to artificial manipulation of LAB genomes to create laboratory-made probiotics for fermented dairy products. The primary application of genetic engineering would be as a tool for research designed to identify the function of LAB genes. If gene expression of probiotics could be better understood, this information could be used to screen LAB in order to select strains with optimum expression of desirable traits and to discard those that express undesirable traits. Genome sequencing studies could also shed light on the biology of specific probiotics in the process and environment in which they are being used-e.g., in studying the production of health-enhancing bioactive proteins in yogurt. This could help researchers adjust processing parameters such as pH and temperature so the environment is more amenable to the expression of desired genes. Sequencing studies could also help dairy scientists better understand stress response in LAB and compare genomic similarities between organisms. In short, the availability of genome sequence data could ultimately revolutionize dairy scientists' ability to control and exploit the beneficial attributes of probiotics to levels that have not previously been possible. Two specific examples are:

Probiotic properties: The ability of probiotic organisms to tolerate stomach acids and bile salts, to survive in the proteolytic environment of the human gut and to exert benefits in vivo will likely be enhanced by careful screening of probiotics for those gene systems that control stress tolerance and express beneficial proteins on the cell's surface. Understanding the genetic code of probiotic cultures is expected to unravel the mechanisms through which these organisms benefit our gastrointestinal ecology and reveal how delivery in milk may enhance their survival and activity.

Lactose reduction: The expression of β -galactosidase (lactase) can be amplified once the gene is identified and stabilized in multiple copies. This could be used to produce a wider variety of lactose-reduced dairy products, a market that is expected to grow substantially in coming years.

Several researchers are currently conducting genomic research on probiotics. For example, DMI-supported researcher T. Klaenhammer at North Carolina State University, Raleigh, N.C., is studying the genomes of the probiotic organisms L. acidophilus **NFCM** Lactobacillus gasseri ATCC 33323. Lactobacilli are widely considered to exert a number of beneficial roles, including immunomodulation, interference with entericpathogens and maintenance of healthy intestinal microflora. Molecular taxonomy has revealed six different Lactobacillus species that comprise what was previously considered a single species, L. acidophilus, a probiotic used in U.S. dairy products. Among these, L. gasseri appears to represent the major homofermentative Lactobacillus species that occupies the human GI tract. L. gasseri demonstrates good survival in the GI tract and has been associated with a variety of probiotic activities and roles, including reduction of fecal mutagenic enzymes, adherence to intestinal tissue, stimulation of macrophages and production of bacteriocins.

Comparative genomic analyses between the various *Lactobacillus* species that occupy the GI tract are expected to identify important regions that are necessary for survival and activity in this environment.

In research funded by DMI, W.M. Russell (now at Mead Johnson Nutritionals, Evansville, Ind.) and Klaenhammer described an efficient method to inactivate genes in the *L. acidophilus* chromosome. This widely accepted integration system (the so-called "Russell integration system") is now being used by many investigators studying genetic features of probiotic lactobacilli.

For example, M.A. Azcarate-Peril (North Carolina State University), Klaenhammer and co-researchers examined the annotated genome of L. acidophilus to identify genes that were likely involved in acid-stress response. These genes were identified, characterized and then inactivated using the Russell integration system. Results indicated that L. acidophilus was quite tolerant to acid, showing no loss of viability when the organism was exposed to pH 3.0 (in hydrochloric acid). When each of the genes suspected to be involved in the acid tolerance responses was inactivated, tolerance to acid was lowered. Interestingly, this sensitivity could be overcome by "turning on" (by growth at pH 5.5) an acid adaptation response that makes the organism even more resistant to acid conditions. These researchers are currently studying the gene sets induced during this acid adaptation period.

In other DMI-supported research, J. Broadbent at Utah State University, Logan, Utah, is studying the genome of *Lactobacillus casei*, an acid-tolerant probiotic. *L. casei* cannot synthesize porphyorins and possesses a strictly fermentative metabolism, with lactic acid as the major metabolite produced. Growth of *L. casei* occurs at 15°C (but not 45°C) and requires riboflavin, folic acid, calcium pantothenate and niacin as growth factors.

L. casei is a remarkably adaptive species and may be isolated from raw and fermented dairy products, fermented plant products and the reproductive and intestinal tracts of humans and other animals. Industrially, L. casei has applications as human probiotics, as acid-producing starter cultures for milk fermentation and as specialty cultures for the intensification and acceleration of flavor development in certain bacterial-ripened cheese varieties.

It is intriguing that even though *L. casei* is not added to cheese starter culture, it is the predominant microorganism in cheese aged four months or longer. Considering *L. casei*'s ability to thrive in low-temperature, high-salt and depleted-lactose environments, it is not surprising that this probiotic grows to such large numbers in cheese. Genomic research will provide insights into *L. casei* that would be difficult to learn otherwise. With these insights, it may be possible to control and enhance *L. casei* subspecies that provide beneficial probiotic attributes so they will outcompete the *L. casei* subspecies that may contribute off-flavor notes and other defects to cheese and cultured dairy products. Once a better understanding is gained of how *L. casei* grows, then processing and ingredient changes can be made to control their levels.

D. O'Sullivan at the University of Minnesota, Minneapolis/ St. Paul, is studying the lactose-reduction properties of various bifidobacteria strains, including *B. bifidum*, *B. breve*, *Bifidobacterium infantis* and *B. longum*. These probiotics demonstrate high activity levels of β -galactosidase and demonstrate tolerance for stomach acid and resistance to bile salts. These properties make *B. longum* an excellent candidate for use in the production of reduced-lactose dairy products.

Suggestions for Developing and Marketing Dairy-Based Probiotics

In selecting an appropriate probiotic, processors need to secure documentation of bacteria type (genus, species, strain), potency (number of viable bacteria needed for a health effect), purity (presence of contaminating bacteria) and the extent of research that has been published for strains on health attributes.

It is important to note that not all probiotics grow well in milk. Lactobacillus GG, for example, does not ferment lactose and Lactobacillus johnsonii La1 requires supplementation with amino acids, nucleotides and iron. Supplementation of milk with glucose, yeast extract or milk protein fractions may enhance probiotic growth.

Industrial production of probiotic dairy products requires consideration of the growth and survival of the bacteria during large-scale processing. Tolerance to acid, shear force and oxygen is essential. The latter is of particular importance when bifidobacteria are used as probiotics.

Sanders emphasizes the importance of delivering effective strains of probiotics at efficacious levels through the end of shelf life. She recommends that appropriate dose levels be based on studies that show a physiological effect. Effective levels may be different with different strains, but daily consumption at levels of 10¹⁰ cfu may be needed for effects. Although yogurt cultures are often delivered at these levels in current dairy products, added probiotic bacteria are frequently not.

Probiotic dairy foods are not currently labeled with probiotic levels or strains in the United States. Sanders maintains that marketers could benefit from labeling products with the actual strains of probiotics used and their levels. At the same time, processors are concerned that assuring label compliance will add to product cost and that providing the information might confuse consumers. Nonetheless, this type of information is needed to ensure that products are delivering the claimed benefits to consumers and to enhance the public image of probiotic dairy foods as reliable, health-enhancing probiotic delivery vehicles. To make claims of probiotic levels on the labels, processors would need to be sure that quality-control testing methods give sufficient information on probiotic levels throughout shelf life.

The key to the future of probiotics in dairy foods is to devote considerable and sustained effort to educating consumers regarding what probiotics are and how they can benefit human health.

8

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