Symposium: Probiotic Bacteria: Implications for Human Health

Considerations for Use of Probiotic Bacteria to Modulate Human Health[1]

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ABSTRACT Oral consumption of probiotic bacteria has the potential to support the health of American consumers. This paper will discuss the rationale of the probiotic theory, several health targets for probiotic bacteria, probiotic products in the U.S. and, finally, issues pertaining to communication about probiotic products to the consumer. J. Nutr. 130: 384S–390S, 2000.

KEY WORDS: • *probiotic* • Lactobacillus • Bifidobacterium

Probiotic definition, scientific basis and rationale

In great number and diversity, microbes inhabit the intestinal tract, skin, urogenital tract, oral and nasal cavities and, in short, any part of the human body that is exposed to the outside world and in which conditions are favorable for bacterial survival. Hundreds of species have been identified as human commensals; bacterial concentrations reach 10^{14} cells on the human body (Drasar and Hill 1974), and the interactions of these colonizing microbes with the host are nothing if not complex. Studies from germ-free animals have proven that animals do not require microbial colonization for survival, but germ-free animals, compared with their conventional counterparts, demonstrate many physiologic and biochemical differences and are more susceptible to infection (Tannock 1998). This is attributed to a poorly primed immune system and perhaps the absence of what has been termed "competitive colonization" (van der Waaij et al. 1972). Competitive colonization is a term describing the interference of virulence by invading pathogens by commensal microbes. The differences between conventional and germ-free animals have provided a basis for the belief that microbial colonization has important health implications for humans.

On rare occasions, microbes develop a pathogenic relationship with a host, and illness or death of the host can result. Negative influences on human health by colonizing or invading microbes need not be acute. Microbial metabolites may possess genotoxic, mutagenic or carcinogenic activity and contribute subsequently to the development of cancer over a period of long-term exposure. It is the recognition of the effects of colonizing microbes in association with the human body, and the combination of wanting to encourage the positive and discourage the negative activities of commensal and invading microbes that have led to the probiotic theory.

Probiotics have been defined as live microorganisms that confer a health effect on the host when consumed in adequate amounts (Guarner and Schaafsma 1998). The concept of probiotics evolved from a theory first proposed by Nobel Prize winning Russian scientist, Elie Metchnikoff (Metchnikoff 1908), who suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products. He believed that when the bacillus was consumed, it carried out the fermentation of this product, positively influencing the microflora of the colon by decreasing the toxic effects of colonic microflora. This concept was developed further through the decades, and today, especially in Europe and Japan, probiotic-focused research, product development and marketing are at an all-time high.

The field of scientific investigation of probiotics is laced with inadequately understood but interesting findings that are difficult to interpret with respect to consumption by a reasonably healthy general population. Research on probiotics consists of experiments done with dozens of different bacterial strains and combinations of strains used at different doses in in vitro, animal or human studies with dozens of different research end points. The positive results from human volunteer or clinical studies, even in the absence of compelling mechanistic studies, provide validity to the probiotic concept. The backdrop to these efforts is the rapidly expanding marketing worldwide of probiotic-containing products. Experts in this field acknowledge that a prerequisite for successful probiotic research and development is developing fundamental knowledge of intestinal bacteria and their interactions with each other and their host (Tannock 1999).

Are these efforts to understand the role probiotic bacteria may play in human health justifiable apart from the interest in yet another functional ingredient to lure purchasing dollars from an increasingly health conscious U.S. consumer? Are benefits from these bacteria going to make a substantive difference in the health of the average consumer? At this point, the responses to these questions are speculative. However, the

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TABLE 1

Mechanisms for probiotic functionality

- Antimicrobial activity
- Colonization resistance
- Immune effects
  - Adjuvant effect
  - Cytokine expression
  - Stimulation of phagocytosis by peripheral blood leucocytes
  - Secretory IgA
- Antimutagenic effects
- Antigenotoxic effects
- Influence on enzyme activity
- Enzyme delivery

emergence of some new public health risks suggests ways in which effective probiotic bacteria may play an important role in maintaining human health.

Some infections, once thought to be benign and self-limiting or readily treatable with antibiotics, are now recognized as more serious health threats. *Campylobacter jejuni*, now believed to be the leading cause of bacterial gastroenteritis (Altekruse et al. 1999), results in Guillain-Barré syndrome (leading to acute neuromuscular paralysis) in 0.1% of cases. Reiter syndrome, a reactive arthritis, can also occur. Other foodborne pathogens have become prevalent and life-threatening, including Shiga-like *Escherichia coli* strains. Multiple antibiotic resistance is a continual threat in the battle against once treatable infections. Vaginosis is now recognized to be associated with low-birth-weight infants, preterm delivery and increased risk for sexually transmitted disease (Hillier et al. 1995, Klebanoff and Coombs 1991, Sweet 1995). Demographic trends have indicated the increase in populations of the immunocompromised, including the elderly, those suffering from AIDS, organ transplant recipients, chemotherapy patients and many others. In the nonindustrialized nations, infections such as rotavirus claim the lives of millions of infants each year (Parashar et al. 1998). Because of these emerging microbial threats, a safe, low risk approach that adds a barrier to microbial infection or to the negative influences of indigenous colonizing microbes may be significant to human health.

Probiotic bacteria have been suggested to play a role in a variety of health effects, and mechanisms proposed for mediating these effects are numerous (**Table 1**). In addition to their proposed direct effects on humans, probiotics may also have implications for human health by their use in animal agriculture. Probiotics have been tested for preventing colonization of food animals, and the products derived from them, with pathogens of animal origin. One product, developed by the USDA and called PREEMPT, blends 29 intestinal bacteria from chickens and is effective at protecting chickens from colonization by *Salmonella*, *E. coli* O157:H7, *Campylobacter*, and *Listeria* (USDA Press Release 0122.98, March 19, 1998). Animal agriculture may also benefit from the improved efficiency that results from greater resistance of farm animals to infectious diseases, increased growth rate, improved feed conversion and increased yield of milk and eggs (Fuller 1998).

More comprehensive reviews of the field of probiotics have recently been published (Fonden 1999, Sanders and Huis in't Veld 1999, Tannock 1999) and are recommended for more in-depth coverage of this area.

Probiotic influence on human health

Key targets for probiotic influence on human health, including influence on gastrointestinal health, immune function and cancer, will be addressed fully in accompanying papers. The focus of this paper will be on targets not covered in these papers. In addition, the reader is referred to an excellent paper that reviews in detail the in vitro, animal and human studies done on the health effects of probiotic bacteria (Fonden et al. 1999).

Epidemiology. Nutritional epidemiology has provided many insights into the association of dietary factors and risk of disease. It is powerful in identifying strong links between risk factors and disease; however, subtle associations are more difficult to identify through this means (Langseth 1996). The complex and interdependent nature of dietary choices also makes these studies difficult. Even recognizing these limitations, epidemiologic links through cohort or case-controlled studies between probiotics and health would provide powerful support for the probiotic theory. Unfortunately, little epidemiologic evidence exists relating probiotics or probiotic-containing foods and disease incidence. These studies would be difficult to control in a manner consistent with our knowledge of probiotic function. Important parameters such as specific strain and dose would be unknown for most probiotic-containing food products.

A few case-controlled studies have been conducted to evaluate the effects of yogurt or fermented milks on some cancer rates. However, neither the type nor level of probiotic bacteria consumed was evaluated in these studies, even though each may have a significant effect on results. Monique et al. (1986) found an inverse relationship between frequency of yogurt consumption and risk of breast cancer in France (1010 breast cancer cases and 1950 controls). Peters et al. (1992) found yogurt to be a protective factor in a case-controlled study of colon cancer incidence in Los Angeles County (746 cases, 746 controls). A case-controlled study of breast cancer in the Netherlands (van't Veer et al. 1989) also suggested that fermented dairy products could be protective (133 cases and 289 controls), although Kampman et al. (1994) did not find a similar relationship between fermented dairy products and colorectal cancer. One intervention trial did show that the recurrence rate for superficial bladder cancer was lower for subjects receiving freeze-dried *Lactobacillus casei* Shirota than a placebo (Aso and Akazan 1992). More such studies will be important in clarifying the role probiotic products play in cancer rates.

In a more general evaluation than that of studies focused on fermented dairy products, a review of 89 epidemiologic studies on dairy foods in general and cancer (prostate, breast, colorectal and others) suggested that there is no significant association (positive or inverse) of dairy food consumption and any cancer, with the possible exception of prostate cancer (Jain 1998). Although prostate cancer incidence showed a weak correlation with milk consumption, available studies were deemed inconclusive. The author concluded that, in balance, current epidemiologic data cannot support a protective or promotional role of dairy foods in cancer rate. Contributing to this conclusion may be the compounding influence of potentially negative components of dairy foods (saturated fat) and putative positive components (bacterial cultures, vitamin D, calcium, conjugated linoleic acids, sphingolipids).

Focused epidemiologic studies using populations consuming defined probiotic products over a long period of time are required to supplement the in vitro and animal studies that suggest a protective influence of probiotic bacteria against cancer. Mechanisms thought to play a role in probiotic-mediated protection of cancer are shown in **Table 2** (Rafter 1995).

TABLE 2

Some proposed mechanisms whereby probiotic bacteria might influence the incidence of cancer, particularly colon cancer

1. Enhancing host's immune response
2. Suppression of growth and activities of intestinal microbes that produce carcinogens and promoters by competitive colonization or production of inhibitors (short-chain fatty acids or bacteriocins)
3. Binding and removal of carcinogens
4. Production of antimutagenic compounds
5. Production of butyrate to stimulate programmed cell death of abnormal cells
6. Inhibition of the conversion of bile salts to secondary bile salts

Hypertension. Although 50 million Americans have been diagnosed with hypertension and its negative effect on health is well documented (Mayo Clinic Web Site, www.mayohealth.org), little is known about any role probiotic bacteria may play in controlling hypertension. One line of research has suggested that bioactive peptides resulting from the proteolytic action of probiotic bacteria on casein (milk protein) during milk fermentation may suppress the blood pressure of hypertensive individuals (Takano 1998). Preliminary studies with spontaneously hypertensive rats (Nakamura et al. 1995 and 1996) and one human clinical study (Hata et al. 1996) provide the evidence. Two tripeptides, valine-proline-proline and isoleucine-proline-proline, isolated from a dairy-based fermentation of milk by *Saccharomyces cerevisea* and *Lactobacillus helveticus* have been identified as the active components. These tripeptides function as angiotensin-I–converting enzyme inhibitors and reduce blood pressure. The Japanese company, Calpis (Kanagawa, Japan), has developed a pasteurized product based on this technology, Ameal-S, which has functional food status in Japan. Unlike many other probiotic-induced effects, it is important to note that this effect is mediated by a fermentation end product, not viable probiotic cells themselves.

Another antihypertensive activity was associated with cell wall fragments of *L. casei* YIT9018 (Sawada et al. 1990). In a placebo-controlled trial with 28 human hypertensive subjects, powdered cell extracts (not viable cells) were administered orally and effects on systolic pressure, diastolic pressure and heart rate were determined. Small, but significant decreases in all three were noted.

An interesting characteristic of these activities is that neither requires viable cells, and they provide novel mechanisms for probiotic-mediated effects. Taken together, they suggest that probiotic bacteria may be effective in mediating an antihypertensive effect.

Urogenital infections. A frequent source of pathogens for urinary and vaginal tract infections in women is the intestinal tract. Pathogens linked to vaginal infections include *Trichomonas*, *Candida* or mixed bacterial infections involving *Gardnerella vaginalis* and *Mycoplasma hominis* (Spiegel 1991). Urinary tract infections are caused by anaerobic gram-negative rods, *E. coli*, *Chlamydia* and *Candida* (Reid et al. 1998). Although effective therapies for curing these infections are available, these infections, once thought benign, can in fact have serious side effects. Vaginal infections are a risk factor for low-birth-weight infants, preterm delivery, pelvic infections leading to infertility and susceptibility to sexually transmitted diseases (Hillier et al. 1995, Sweet 1995). Furthermore, urinary tract and vaginal infections can be recurrent, suggesting that current therapies could be augmented by a prophylactic approach.

A healthy vaginal tract is associated with high populations of lactobacilli (especially hydrogen peroxide–producing lactobacilli) and a pH < 5.0 (Eschenbach et al. 1989, Hawes et al. 1996, Hillier et al. 1992, Klebanoff et al. 1991). This fact, coupled with the intestinal route of transmission of bacteria to the urogenital tract, has led to the theory that oral probiotics may be useful in treatment or prevention of urogenital infections.

Clinical evaluations have been conducted on the influence of lactobacilli on treatment of bacterial vaginosis using intravaginal suppositories and for prevention of recurrent candidal and bacterial vaginal infections (Mallen et al. 1992). Several of these studies have suffered from small numbers of subjects or failure of enrolled subjects to complete the study. Although Nyirjesy et al. (1997) concluded that alternative medicines are unlikely to be of benefit to those with chronic vaginal symptoms, several studies do suggest that administration of lactobacilli, either orally or intravaginally, can play a prophylactic role in the etiology of this disease, presumably through the recolonization of the vaginal tract with lactobacilli (Hallen et al. 1992, Hilton et al. 1992 and 1995, Shalev et al. 1996). In a crossover trial of 46 patients, Shalev et al. (1996) compared the ability of ingestion of yogurt containing live *L. acidophilus* (1.5×10^{10}/d) with pasteurized yogurt to prevent vaginal infections [bacterial vaginosis (BV) and cadidiasis]. Unfortunately, only seven patients completed the entire study protocol. Significant differences were seen in BV infections in those consuming live yogurt compared with pasteurized yogurt or no yogurt. *Candida* infections were decreased during yogurt consumption regardless of the presence of live or heat-killed lactobacilli. Hilton et al. (1992) studied the effect of yogurt consumption on *Candida* vaginitis in a crossover trial with 33 women (13 completed the study). Results indicated a threefold decrease in infections in patients consuming yogurt containing *L. acidophilus* ($>10^{10}$/d). A commercial freeze-dried *L. acidophilus* suppository was administered twice daily for 6 d to women suffering from BV in a placebo-controlled trial (Hallen et al. 1992). After treatment, the patients using the *Lactobacillus* preparation showed a lower level (43%) of BV than did the placebo group (100%), although the effect was short lived (relapse after menstruation).

Lactobacillus applications in urinary tract infections have been evaluated, but not as yet with the use of an oral vehicle of delivery (Reid et al. 1998). Weekly, intravaginal instillations of dried lactobacilli ($>10^9$ colony-forming units/dose) in 10 premenopausal women resulted in the reduction of urinary tract infections from 6.3 per patient in the year before the study, to 1.3 per patient during the study (Reid and Bruce 1995). The mean vaginal pH was 4.8 during the study compared with 5.0 before the study. Reid et al. (1995) reported extended similar results, including 38 women who completed the study. These results suggest that vaginal lactobacilli can decrease the risk of urinary tract infections.

Taken together, these studies suggest a positive role for lactobacilli in controlling vaginal and urinary tract infections in women, and suggest that externally applied probiotic preparations given orally or intravaginally may provide a therapeutic source of lactobacilli to help prevent infections. The lack of negative side effects, the emphasis on prevention rather than cure and the "natural" image surely are positive characteristics of this approach. Hughes and Hillier (1990) concluded that many commercially available foods and dietary supplements containing lactobacilli may be inadequate for vaginal applications. Their conclusions, based largely on the report of im-

proper species being present in the products, must be reconfirmed using modern genetic technologies for lactobacilli (Tannock 1999). Continued research focused on selection of the proper strains for these applications, development efforts to provide products that deliver efficacious levels of these bacteria and clinical trials that substantiate effects will improve the likelihood that probiotics will be used in preventing these infections and their consequences in women.

Lactose intolerance. The inability of adults to digest lactose is widespread, although those deficient in lactase generally tolerate lactose better from yogurt than from milk (Savaiano and Kotz 1988, Shah 1993, Suarez et al. 1995). The effect of lactose maldigestion has been studied by measuring breath hydrogen excretion (Levitt and Donaldson 1970), which has been correlated with colonic fermentation and lactose maldigestion. As accepted as this method is, however, it does not provide a complete understanding of the lactose maldigestion situation because in some cases, the absence of an effect on breath hydrogen has been correlated with improved symptomatology (Montes et al. 1995, Savaiano et al. 1984). The contribution of lactase by the bacterial cultures used to manufacture the yogurt is thought to mediate enhanced lactose digestion; this is evidenced by the inability of pasteurized yogurt or yogurts containing a low cell count to reduce breath hydrogen excretion, although pasteurized yogurt does improve gastrointestinal symptoms (Savaiano et al. 1984). Slower gastric emptying of yogurt compared with milk has also been hypothesized to play a role.

In general, results have indicated that yogurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), present at levels normally seen in yogurt ($\geq 10^8$/g), effectively improve the digestion of lactose in lactose maldigesters. The effect seems to be more cell-density dependent (Lin et al. 1991) than strain specific (Martini et al. 1991, Vesa et al. 1996), suggesting that, in general, most commercial strains of these bacteria likely possess the physiologic and biochemical characteristics necessary for mediating this effect. Defining what exactly these characteristics are, however, has been a research challenge. Total lactase levels in yogurts have not correlated well with breath hydrogen results in human subjects. Martini et al. (1991) found that yogurts made from several different yogurt starters were equivalent in effect, even though total β-galactosidase activity of two of the yogurts studied varied as much as threefold. Kotz et al. (1994) found a similar lack of correlation between reduction in breath hydrogen excretion and lactase content of yogurts. Wytock and DiPalma (1988) reported a difference in effectiveness among commercial yogurts, but no microbiological or enzymatic characterization of the yogurt was conducted in this study, thus making it difficult to judge these results.

The results on probiotic bacteria (*L. acidophilus*, bifidobacteria, among others) are less clear cut. Studies suggest that some dairy products formulated exclusively with probiotic bacteria (e.g., Sweet Acidophilus milk) are not effective (Payne et al. 1981). The low probiotic cell count ($\sim 2 \times 10^6$/mL) in these products presumably contributes to this result. Research also suggests that the physiologic characteristics of these probiotic bacteria may not be as suited to mediating this effect as are the starter cultures. For example, it has been suggested that bacterial cell permeablization in the small intestine after exposure to bile improves lactose digestion by increasing contact between ingested lactose and lactase. Yogurt starter cultures are bile sensitive, whereas probiotic lactobacilli and bifidobacteria are generally bile resistant. Vesa et al. (1996) tested three semisolid fermented dairy products, all containing *S. thermophilus* levels $>10^8$/g, with two of the three products also containing *L. acidophilus* and *Bifidobacterium*. Results indicated no difference in lactose digestion although a fourfold difference in lactase activity was present, leading investigators to attribute enhanced lactose digestion to slower gastric emptying, not microbial lactase.

The roles of bile resistance, acid resistance, cell membrane permeability, specific activity of microbial β-galactosidase and the stability of these factors during storage and on transit through the gastrointestinal tract on alleviation of symptoms of lactose maldigestion must be clarified further to achieve a fuller understanding of the role of starter and probiotic bacteria in enhancing lactose digestion.

Cholesterol. Elevated levels of certain blood lipids are a risk factor for cardiovascular disease. The observation that conventional animals excrete higher levels of cholesterol in feces than germ-free animals suggests that colonizing microbes may influence serum cholesterol levels (Eyssen 1973). The body of research on the effects of culture-containing dairy products or probiotic bacteria on cholesterol levels has yielded equivocal results (Taylor and Williams 1998). Since 1974, 13 studies have been published evaluating blood lipids in human subjects consuming fermented milk products, with a total of 465 subjects (302 of those subjects were in three studies). Statistically significant lowering of total cholesterol ranged from 5.4 to 23.2% and of LDL cholesterol from 9 to 9.8%. The studies conducted to date have been criticized for failure to stabilize baselines before the onset of the feeding protocol, small sample size, short study duration, unreasonably large fermented milk intake requirements and failure to control for diet and physical activity of subjects. Of the studies showing significant results on the lowering of either total cholesterol or LDL, the duration did not exceed 6 wk. One study showed increases in both total cholesterol and LDL cholesterol (Rossouw et al. 1981).

The mechanisms for and effect of probiotic bacteria on reduction of serum cholesterol are unknown. One hypothesis suggests that some strains of *L. acidophilus* can assimilate the cholesterol molecule (Gilliland et al. 1985). This hypothesis has been tested in laboratory assays (Gilliland et al. 1985, Rasic et al. 1992). A criticism of this hypothesis questions the physiologic relevance of assimilation kinetics observed in an in vitro, aqueous assay conducted at pH 6.0 or lower. Rather than assimilation, it has been suggested that the pH-dependent, transient cholesterol precipitation in laboratory media caused the effects (Klaver and Meer 1993, Tahri et al. 1996). Another proposed mechanism is based on the ability of certain probiotic lactobacilli and bifidobacteria to deconjugate bile acids enzymatically, increasing their rates of excretion (De Smet et al. 1994). Because cholesterol is a precursor of bile acids, this could lead to reduction in serum cholesterol because cholesterol molecules are converted to bile acids to replace those lost through excretion. If this mechanism operated in the control of serum cholesterol levels, one concern is the conversion of deconjugated bile acids into secondary bile acids by colonic microbes. These secondary bile acids are known cancer promoters. A potential increased risk of colon cancer may outweigh any benefit of reduction of serum cholesterol levels. This may provide a rationale for the selection of probiotic strains that are bile salt hydrolase negative, although efforts to the contrary have been published (du Toit et al. 1998). Another mechanism, proposed by Mann (1977), postulated that 3-hydroxy-3-methyl glutaric acid (HMG) present in fermented milk inhibits hydroxy methyl glutaryl CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. These hypotheses have not been confirmed in animal or human studies, although Gilliland et al. (1985) established a choles-

terol-lowering effect of a cholesterol-assimilating (but not a nonassimilating) strain in boars. Further research on any mechanisms should be preceded by evidence for clinical effect in at least one thoroughly conducted study.

Other. Additional probiotic effects have also been proposed, but data are either too preliminary or beyond the scope of this article. These include probiotic effects against *Helicobacter pylori* infections in the stomach (Coconnier et al. 1998, Kabir et al. 1997, Midolo et al. 1995), alcoholic liver disease (Nanji et al. 1994), small bowel bacterial overgrowth (Simenhoff et al. 1996, Stotzer et al. 1996), ulcerative colitis (Kruis et al. 1997), allergy to milk protein (Pelto et al. 1996), juvenile chronic arthritis (Malin et al. 1996), antioxidative effects (Ahotupa et al. 1996), asthma (Wheeler et al. 1997), hepatic encephalopathy (Read et al. 1966) and their use as vaccine delivery vehicles (Mercenier 1999).

Probiotic products in the United States

Probiotic product formats and examples. Probiotic bacteria can be found worldwide in a variety of products, including conventional food products, dietary supplements and medical foods. In the United States, the main outlets for probiotic bacteria are dairy foods and dietary supplements (primarily in the form of capsules, powder or tablets). A survey of domestic culture producers suggests that the retail U.S. market for probiotic dietary supplements is between $10 and 20 million. Although this is not a huge market, it has been growing.

Dairy foods containing probiotic bacteria include most major brands of yogurt, culture-containing fluid milks, such as "Sweet Acidophilus Milk" and a few brands of cottage cheese. Dairy foods seem to fit naturally with probiotics because of the traditional association of beneficial fermentation bacteria and fermented dairy products. Consumers naturally associate fermented dairy products with live cultures and perceive a benefit (albeit undefined) in the presence of these cultures.

In Europe and Japan, in addition to dietary supplements in pill form and traditional dairy products, hybrid products are also sold. These products, such as Actimel (Danone, Paris) and Yakult (Yakult, Tokyo), are sold in small (65–100 mL) individual serving size bottles containing a milk-based beverage produced by the fermentation of one or more probiotic bacteria. They are marketed to be consumed daily, as a food supplement, but are not in a size that would be considered, at least in the U.S., a significant component of a meal. Their purpose is to provide a significant dose of functional probiotic bacteria. A comparison of probiotic products in the U.S. and in Europe can be found in Sanders and Huis in't Veld (1999).

Active principle. One issue important to the development and consumption of probiotic-containing products is the concept of "active principle." For the most part, it is assumed that the active component of probiotic products is viable bacteria, and in fact, this is the only measure of probiotic activity noted on U.S. products today. In general, the presumption is that probiotic viability is a reasonable measure of activity. In most cases, even if viability is not required, it is likely correlated with most effects because it is a useful indicator of the number of cells present, regardless of what cell component may be active. However, the literature suggests several situations in which viability is not required for some activities. Improved digestion of lactose (Vesa et al. 1996), some immune system modulation activities (Hosono et al. 1997, Marin et al. 1997, Perdigon et al. 1986, Solis Pereyra and Lemonnier 1993, Tomioka and Saito 1992), and antihypertensive effects (Maeno et al. 1996) have been linked to nonviable cells (cell components, enzyme activities or fermentation products). Some studies have compared nonviable cells as controls in clinical evaluations (Hata et al. 1996, Maeno et al. 1996, Titze et al. 1996).

This discussion leads to the conclusion that definition of the active property of a probiotic product is essential to understand shelf-life issues, and efforts to maximize shelf life must be focused on maintaining optimal levels of this ingredient, whether as the intact, viable cell, some cell component(s), a metabolic end product or a combination of these.

Strain specificity of effects. Not all probiotic bacteria are identical. They differ on the bases of genus, species and even strain. The literature is replete with examples of strain-dependent responses when scientists evaluate characteristics of a multitude of different probiotic bacteria. Strains of the same species could be expected to differ in traits such as stability, expression of enzymes, extent and types of inhibitors produced, carbohydrate fermentation patterns, acid producing ability, resistance to acid and bile, ability to colonize the gastrointestinal tract and, perhaps most importantly, clinical efficacy. Just because strains might differ from one another, it does not necessarily mean that they do. But this microbiological circumstance does impose a burden of proof upon those attempting to commercialize probiotic bacteria. Statements substantiating probiotic activity based on the body of literature on different probiotic strains does not engender a high degree of confidence in the efficacy of inadequately studied strains. Positive research, especially clinical and mechanistic research, conducted on a specific strain is required to prove efficacy. This also contributes substantially to the commercial value of the probiotic strain.

Consumer issues: how do consumers know what they are getting? In general in the U.S., probiotic-containing food products make no mention of the numbers of probiotic bacteria present in the product per serving. Most products list bacterial genera and species added as live cultures, but not levels. California and Oregon are unique in that they legislate a minimum requirement for acidophilus-containing fluid milk products (10^6/mL). In the U.S., yogurt is not required to contain any viable cultures. In response, an industry group, the National Yogurt Association, allows yogurt manufacturers use of its "Live Active Culture Seal" on products that contain 10^8 viable cultures per gram at time of manufacture. However, no distinction is made between yogurt starter cultures used primarily for acid production (*S. thermophilus* and *L. delbreuckii* subsp. *bulgaricus*) and probiotic species (*L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium* species, among others). Therefore, this seal is of little value in assuring consumers of effective probiotic levels. In practice, fluid milk products (with their short shelf life and near-neutral pH) provide the expected levels of probiotic bacteria (10^8 viable cultures per gram), even in states that do not require it. Results from yogurt products show a greater range in levels of viable probiotic bacteria. Some commercial yogurts seem to maintain acceptable levels ($>10^7$/g) (Iturriria-Laverty et al. 1999); others show much lower levels (Dave and Shah 1997, Micanel et al. 1997, Rybka and Fleet 1997). There is clearly a need for industry to provide more useful information to consumers on probiotic content of dairy foods.

Probiotic-containing dietary supplements frequently indicate a viable count per dose contained in the product at time of manufacture, not at end of shelf life. Several reports of misleading labeling of dietary supplements have been published (Hamilton-Miller et al. 1996 and 1999). Labeling has been criticized for overstating the level of viable bacteria, for inaccurately indicating the species of probiotic bacteria present and for the presence of species of bacteria not listed on

the label (e.g., *Enterococcus*). Clearly, there is a need for the probiotics industry to focus on delivery of high potency doses of appropriate bacteria in these products.

SUMMARY

The probiotic theory offers an intriguing approach to controlling negative metabolic or pathogenic activities of microbes to which we are exposed on a daily basis. Throughout the human life cycle, conditions exist that produce increased risk for infection, increased activity of opportunistic pathogens and decreased protection from normal microflora. Old age, treatment with antibiotics and immunocompromised states can all contribute to a disruption of colonizing microbes. When we consider also the increased environmental threats of antibiotic resistant pathogens, emerging new pathogens and serious sequelae of "treatable" infections, an intervention with essentially no risk that may provide another barrier to microbial assault is attractive. Probiotics could provide this benefit. Dietary rather than drug interventions have obvious advantages in terms of cost, reduced side effects and ease of market penetration to large numbers of people.

In the U.S., the market for probiotic products is underdeveloped compared with Europe and Japan. At present, U.S. consumers have little means of determining probiotic levels at time of consumption in probiotic foods and dietary supplements. Probiotics offer a broad range of potential health benefits, but the extent of the effect of specific strains on the health of a generally healthy general population remains to be determined. Equivocal results observed in probiotic efficacy studies in humans may have to do with the testing of ineffective strains or potentially effective strains at doses too low to be effective or poor study design. Research is also required to characterize health benefits further and to define the "active principle" in probiotic preparations.

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