PROBIOTIC BACTERIA IN DAIRY PRODUCTS

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ABSTRACT

The increase in consumer demand for functional foods in general and probiotic foods in particular may be attributed to the increasing awareness of the link between diet and health. The health benefits of probiotics have resulted in their increased incorporation in dairy foods, leading to creation of a new generation of health foods. Dairy products have been considered as a good carrier for probiotics since fermented foods and dairy products have particularly a positive image. A major advantage is that consumers are already familiar with them and many believe that dairy products are healthy, natural products. Others advantages of dairy products as vehicles for probiotics are that fermentation acts to retain and optimize microbial viability and productivity, while simultaneously preserving the probiotic properties. In addition, consumers are familiarized with the fact that a fermented dairy product contains living microorganisms, and they are also able to protect probiotics through the gastrointestinal transit. When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product and become active when entering the consumer's gastrointestinal tract. These factors include strains of probiotic bacteria, pH of milk, presence of lactic and acetic acids, interactions with other microorganisms, storage temperature and manufacturing conditions. The interactions of probiotics with either the food matrix or the starter culture may be even more intensive when probiotics are used as a component of the starter culture. Some of these aspects are discussed in this paper, with an emphasis on dairy products such as milk, yogurt, cheese , kefir and ice cream.

I.INTRODUCTION

LET food be thy medicine and medicine be thy food', espoused by Hippocrates nearly 2500 years ago, is certainly the tenet of today. With the growing interest in self-care and integrative medicine coupled with our health-embracing baby boomer population, recognition of the link between diet and health has never been stronger. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods, is the small but rapidly expanding arena of probiotics – live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance.

Until recently, probiotics have been used as food supplements. However, it is now common to find commercial probiotics being marketed as capsules, sachets and granules. In recent years, worldwide interest in the use of

functional foods containing probiotic bacteria for health promotion and disease prevention has increased significantly. The dairy sector, which is strongly linked to probiotics, is the largest functional food market accounting for nearly 33% of the broad market, while cereal products have just over 22% (Leatherhead Food International 2006). The word "probiotics" literally means "for life," and refers to living microorganisms that, when consumed in sufficient numbers, exert health benefits beyond basic nutrition. The link between dairy and probiotics can be traced back more than 2,000 years to a time when people consumed large amounts of lactic acid fermented milk products such as kefir and yogurt. In 1908, the Russian Nobel Laureate Elie Metchnikoff attributed the good health and longevity of Bulgarian peasants to sour milk and yoghurt. More recently in a 1981 census, it was noted that Azerbaijan has one of the highest rates of longevity in the world and approximately 48.3 people per 100,000 inhabitants were aged 100 year and above. Current knowledge on probiotics support a number of potential health benefits. They help to maintain good balance and composition of intestinal flora increasing the ability to resist pathogens invasion and maintain the host's well being. Reduction of blood pressure, cholesterol and/or triglycerides levels, reduction of lactose intolerance problems, immune system enhancement, anti carcinogenic activity and improve nutrients utilization are well described in literature. The use of probiotics for preventing and treating illnesses related to gastrointestinal, respiratory and urogenital tracts have been studied. They have been widely used in therapeutic applications as constipation, diarrhea control, bowel syndrome, control of inflammatory processes, prevention of eczema, osteoporosis and food allergy [1,2,3]

Because of the potential health benefits, probiotic organisms are increasingly being incorporated into a number of dairy foods. The following may be attributed to why dairy products -specifically fermented dairy products have been considered as a good carrier for probiotics

- Fermented dairy products such as yogurt already have a record as being healthful.
- Consumers are familiar with the fact that fermented products contain viable microorganisms.
- Probiotics as fermentation organisms combine the positive images of both probiotics and fermentation organisms.
- The image of yogurt like products as healthful foods facilitates recommendation of daily consumption of probiotics
- In addition, there is the important technologic reason for the use of dairy products as carriers of probiotics: many of these products have already been optimized to some extent for survival of the fermentation organisms. Thus, the existing technology can be relatively easily adapted to guarantee sufficient survival of the added probiotic bacteria.

The most commonly used probiotic microorganisms belong mainly to the bacterial genera Lactobacillus and Bifidobacterium [4] . In addition to these genera, the probiotic market contains members from some additional lactic acid bacterial genera, such as Streptococcus and Enterococcus, and members from the genera Bacillus and Propionibacterium. Furthermore, some gram-negative bacteria (e.g. *E. coli* Nissle 1917) and yeast (e.g. Saccharomyces) are being used as probiotic microorganisms [5]

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1.1 Interactions between probiotics and components of fermented foods

Besides their desired health and clinical properties, probiotics must meet several basic requirements for the development of marketable probiotic products. The most important requirements are that probiotic bacteria survive in sufficient numbers in the product, that their physical and genetic stability during storage of the product be guaranteed, and that all of their properties essential for expressing their health benefits after consumption be maintained during manufacture and storage of the product. In addition, probiotics should not have adverse effects on the taste or aroma of the product and should not enhance acidification during the shelf life of the product.

1.2 The variables necessary for or influencing the application of probiotics in dairy products.

- As with all fermented dairy products containing living bacteria, probiotic products must be cooled during storage. This is necessary both to guarantee high survival rates of the probiotic organisms and to ensure sufficient stability of the product. To achieve health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^6–10^7 cfu/g of product.
- Active microorganisms interact intensively with their environment by exchanging components of the medium for metabolic products. Thus, the chemical composition of the dairy product is of paramount importance for the metabolic activities of the microorganisms.
- Essential variables are the kind and amount of carbohydrates available, the degree of hydrolysis of milk proteins (which determines the availability of essential amino acids), and the composition and degree of hydrolysis of milk lipids (which determine the availability of short-chain fatty acids in particular).
- On the other hand, the proteolytic and lipolytic properties of probiotics may be important for further degradation of proteins and lipids. These 2 properties may have considerable effects on the taste and flavor of dairy products
- A major aspect of the production of probiotic fermented dairy products is the interaction between probiotics and starter organisms.
- Antagonism, on the other hand, is often based on the production of substances that inhibit or inactivate more or less specifically other related starter organisms or even unrelated bacteria. Most importantly, antagonism is caused by bacteriocins, which are peptides or proteins exhibiting antibiotic properties. The ability to produce bacteriocins is often discussed as a desirable property of probiotics; however, antagonism to starter cultures and vice versa may be a limiting factor for combinations of starters and probiotics.
- Further antagonistic activities produced by lactic acid bacteria have been described and the substances involved are hydrogen peroxide, benzoic acid (produced from the minor milk constituent hippuric acid), biogenic amines (formed by decarboxylation of amino acids), and lactic acid .
- The intensity of the interactions between probiotics and both the food matrix and the starter organisms depends in large part on the time that probiotics are added to the product, ie, whether they are present during fermentation or are added after

- The physiologic state of the probiotics added may be of considerable importance. This state very much depends on the time of harvesting of the culture (whether during the logarithmic or stationary phase of growth), on the conditions leading to transition to the stationary phase on the treatment of the probiotics during and after harvesting.
- Finally, the composition of the growth medium of the probiotics in relation to the composition of the food to which they will be added. At least some ideas on the handling of probiotics can be taken from the experience of the production of commercial starter cultures .

II.PRODUCTION AND PRODUCT CHARACTERISTICS OF SOME FERMENTED DAIRY FOODS IN RELATION TO USE OF PROBIOTICS

2.1Acidophilus milk

Sweet acidophilus milk and sweet AB milk are probiotic dairy products based on unnfermented milk. Both are produced by adding concentrated probiotic bacteria to intensively heat-treated milk. Heat treatment is necessary to achieve sufficient microbiological stability during storage of the final product. *L. acidophilus* and *L. acidophilus* plus *Bifidobacteria* sp.are added to sweet acidophilus milk and sweet AB milk, respectively. In contrast, acidophilus milk (fermented) is produced by fermentation with *L. acidophilus*. Again, intensive heat treatment before fermentation, yielding almost sterile milk, is necessary for successful fermentation because *L. acidophilus* acidifies slowly and thus can be readily competed out by contaminating bacteria.

2.2Probiotic Yoghurt

Yogurt like products are manufactured with different textures. Natural-set yogurt, stirred yogurt, and drink yogurt differ in their content of nonfat solids: 16–18%, 13–14%, and 11–12%, respectively. Although classic yogurt is produced with a thermophilic protosymbiotic culture of *S. thermophilus* and *L. delbrueckii* subsp.*bulgaricus,* the so-called yogurt mild is produced with a thermophilic culture of *S. thermophilus* and a *Lactobacillus* species, usually *L. acidophilus*. Because of the thermophilic nature of the starter culture, fermentation is usually carried out between 40 and 45°C. The time needed for fermentation may be as short as 2.5 h for the classic yogurt starter culture; this fast fermentation is mainly the result of the protosymbiosis. Because of the rapid acidification and the short time needed, heat treatment is not required with use of the classic yogurt starter culture. Yogurt mild, on the other hand, requires ≈6–8 h for fermentation, mainly because of the use of *L. acidophilus* as the lactobacillus component of the starter. In any case, a pH <4.8 is necessary to guarantee formation of a stable gel from coagulated milk protein . This is especially important for natural-set yogurt. As a result of the method used to manufacture them, stirred yogurt and drink yogurt are well suited to the addition of probiotics after fermentation. Probiotics can be added easily during stirring of the product immediately before filling of the final containers . For natural-set yogurt, probiotic bacteria must be present during fermentation because fermentation takes place in the final containers and subsequent stirring would destroy the product's texture.

2.3Probiotic Kefir

An almost ideal probiotic dairy product may be kefir because probiotic strains have been isolated from several members of the typical flora (eg, *L. acidophilus*, *L. casei*, and *L. reuteri*). However, the market potential of this product is limited because the blown lids of the retail containers (the result of carbon dioxide production after fermentation) apparently signal spoilage to most consumers.

2.4 Probiotic cheese

Today, the probiotics are dominated by yogurts and yogurt drinks. But studies suggest that cheese can be a good alternative food vehicle for the delivery of viable probiotic bacteria [6,7] Cheese (and especially cheddar) may offer certain advantages over other probiotic products such as yogurt or milk. The reduced acidity of cheese compared to yogurt and the high fat content and texture of cheddar cheese may offer protection to microorganisms during passage through the gastrointestinal tract [8] . But Phillips *et al.* [7] adds "Incorporating a probiotic culture into a cheddar cheese would only produce a functional food if the culture remained viable in recommended numbers during maturation and shelf life of the product". The food industry considers 10 6 CFU/ g at the time of consumption as the minimum level of probiotic bacteria necessary to perform their nutritional benefits.[9]

Whereas the coagulation of milk proteins is a consequence of acid production in yogurt, coagulation in cheese is achieved through the proteolytic action of rennet. Less rennet is added for fresh cheese (cheeses that do not undergo ripening) than for ripened cheese. As an example, cottage cheese manufacture will be described. Usually, milk is inoculated with a mesophilic starter culture and incubated at between 20 and 30°C for a relatively short period before rennet is added. Incubation proceeds until the curd has formed. The curd is cut to allow expelling of whey from the coagulated casein. Expelling is reinforced by raising the temperature of the whey-coagulum mixture to 50–55°C for 1–2 h. During this time the coagulum particles shrink (because of further loss of whey) and become more firm. After the whey is drained off, the coagulum is washed with clear water at 7–10°C and then at ≈2°C to remove residual lactose. Finally, cream and salt are added to desired concentrations and the mixed products are poured into retail containers.

Two options exist for adding probiotics to cottage cheese: either with the starter culture or with the cream and salt. Addition with the starter culture is problematic for 2 reasons. First, a considerable number of bacterial cells are lost from the coagulum during draining of the whey. Thus, it is difficult to control exactly the number of the probiotic bacteria in the final product. Second, the scalding temperatures of ≤55°C may negatively affect survival of the probiotic bacteria in the product. For cottage cheese, therefore, it appears to be best to add probiotics with the cream.

Concerning the time of addition of probiotics and impairment of survival by the scalding temperature, the same considerations apply to ripened cheese as to cottage cheese. For cheeses like cheddar that are salted, it is possible to add an exact dose of the probiotics when the salt is added (eg, by spraying a highly concentrated suspension of

the probiotics over the milled curd). An additional problem in ripened cheese is caused by the long period of ripening. It is not yet clear to what extent the different probiotic strains will survive this period and to what extent their functional properties will be affected. One can imagine that the relatively high buffering capacity of the cheese matrix, the high fat content, and the tight matrix may stabilize the probiotic bacteria not only during ripening but also during intestinal passage after consumption.

2.5 Probiotic Ice cream

Among the probiotic dairy products with live probiotics, non-fermented probiotic ice cream is a good vehicle for delivery of live probiotic cells to human intestinal tract because of its neutral pH and high total solids level which provides protection for the probiotic bacteria. Ice cream being the most preference of consumer, probiotic ice cream is a suitable vehicle for delivering beneficial micro organisms such as Lactobacillus and *B.bifidum* to consumers[10] .Eating ice cream containing probiotics-*Bifidobacterium lactis* Bb12 and *L.acidophilus* LA5 rsulted in statistical reduction of *S.mutans* the main bacteria associated with tooth decay [11].

At our institute, freeze dried cultures of *Lactobacillus acidophilus*; *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Bifidobacterium bifidum* were incorporated in the mix at 1% and 2% level before freezing with different combinations of cultures (8 treatments) and were frozen in a batch freezer. The treatments were subjected for physico chemical and sensory evaluation. All the samples found to be acceptable however the ice cream with *Lactobacillus acidophilus* + *Bifidobacterium bifidum* at 1% level culture showed a better acceptability by the Judges which had low acidity as well as higher scores for all the sensory attributes are same to as control ice cream.

Some milk-based probiotic products that have been developed recently by a number of researchers worldwide and that had consumer sensory acceptance are listed in Table 1.

Table.1.Some probiotic dairy products developed worldwide.

Name of the Product	References
Acidophilus "sweet" drink	[12]
Cottage cheese	[13]
Crescenza cheese	[14]
Goat semi-solid cheese	[15]
Biogarde, mil-mil, and acidophilus milk	[15]
Acidophilus butter and progurt	[15]
Dairy fermented beverage	[16]
Nonfermented goat's milk Beverage	[17]
Frozen yogurt	[18]
Argentine fresco cheese	[19]
Canestrato pugliese hard cheese	[20]

Low-fat ice cream	[21] , [22]
Acidophilus milk-based ice cream	[23]
Acidophilus milk drink	[24]
Fermented goat's milk	[25]
Probiotic ice cream	[26]
White-brined cheese	[27]
Whey-protein-based drinks	[28]
Feta cheese	[29]
Cheese from caprine milk	[30]
Semi-hard reduced-fat cheese	[31]
Traditional Greek yogurt	[32]
Dahi	[33]
Banana-based yogurt	[34]
Regular full-fat yogurts	[35]
Low-fat yogurts	[36]
Graviola and cupuassu-based yoghurt	[37]
Guava-based mousse	[38]
Acai yogurt	[39]
Kazar cheese	[40]
Corn milk yogurt	[41]
Synbiotic acidophilus milk	[42]
Frozen synbiotic dessert	[43]
Coconut flan	[44]
Petit-Suisse cheese with prebiotics	[45]
Synbiotic ice cream	[46]
Stirred fruit yogurts	[47]
Turkish Beyaz Cheese	[48]
Homogenized probiotic fermented milk	[49]
Cheddar cheese	[6]
Minas fresco cheese	[50]
Mango soy fortified probiotic Yogurt	[51]
Lactic beverages s with oligofructose	[52]
Peanut milk yogurt	[53]

In this context, several probiotic beverages and yogurts have been developed, both in academic and industrial sectors, to offer new sources of improved products to the consumers throughout the world and some the products are available are listed below.

Table. 2. Probiotic dairy products by country:

Country	Products
Australia	Live fruit chunk yoghurt(F&N), Digestive yoghurt(Yo-Plus), Organic Yoghurt(Wallaby), Bio life Probiotic yoghurt(Bioloife), Probiotic yoghurt(Vaalia), Probiotic chocolate(Ombar)
Brazil	Yakult ,Sofyl(Yakult), Chamyto(Nestle), Activia(Danone), Actimel (Danone), Danito (Danone), Vigor-Club(Vigor), Batavito(Batavo), Bob sponjo(Batavo), Activia-stirred yoghurt (Danone), Acitivia-drinkable yoghurt (Danone), Lectiv-Stirred yoghurt(Vigor), Lectiv-drinkable yoghurt (Vigor), Biofibras-stirred yoghurt (Batavo),Biofibras-drinkable yoghurt(Batavo), Nesvita-stirred yoghurt(Nestle) Nesvite-drinkable yoghurt (Nestle)
Canada	Plant sterol probiotic yoghurt(Biobest)**,** Kraft Live active cheddar cheese(Kraft)**,** Kraft Live Rasberry bars(Kraft), yoghurt(Liberty), Natural no fat probiotic yoghurt(Olympic)
Denmark	Drinkable Yoghurt(Klover),Danimal (Lactobacillus GG),Probio (Arla Cultura)
Germany	Probiotic vitality yoghurt, Soyoghurt
France	Aktiv LGG Yoghurt and drinks (Dukat)
Europe	Kefir(Lifeway), Probiotic infant formula (Culturelle), Bravo Friscus(France), Yoghurt (Yoplait), Activia(Danone), Probiotic Yoghurt (Actimel)
Italy	Probiotic low fat yoghurt(GanedanBC), Probiotic dairy drinks, Laterria Sociale Merano, Fermented dairy beverage (Danocal), Frozen yoghurt (Yolive)
Finland	Milk drink and Yoghurt -Valio Gefilus, ValioKidius Gifilus and Evolus, Yoghurt oat product(Yosa), Valio Villis(Bioferme)
Japan	Yakult(Yakult), Bulgaria yoghurt (Meiji), Yoghurt drink(Meiji), Bifidus Yoghurt (Morinaga), S-120, a probiotic yoghurt drink(Calpis Ameal)
Spain	Kaiku vita, a functional dairy drink from valio ,Bio herbal bifidus active green tea yoghurt (Danone)
Sweden	Yoghurt (ProViva), Frozent yoghurt(Yogunfruz), Biogaia products,Probiotic products
UK	Creamy Yoghurt (Vita Yo), Biolive yoghurt (Yeo Valley), Natural fat free yoghurt (Yeo Valley), Flora-Pro active (Unilever)
USA	Creamy Yoghurt (Activia), GYoplus(Danone), Yoghurt Ice cream(Blue bunny sedona), Chocolate sweet scoops(Blue bunny sedona), Frozen yoghurt (Blue bunny sedona),Probiotic ice cream (Yovation Pierre)

2.6 Probiotic dairy products in India

Amul was the first to make a dent at National level with its probiotic ice creams prolife in February, 2007. Amul, on the other hand, having tasted success in the probiotics category with its ice cream introduced 'Prolife Probiotic Dahi', 'Prolife Probiotic Lassee' and Amul Flaavyo, a probiotic yoghurt. Probiotic products contribute to 10% to its ice-cream sales and 25 per cent of its Dahi (Indian yoghurt) sales. Mother Dairy has the largest

milk (liquid/unprocessed) plants in Asia selling more than 25 lakh liters of milk per day. b-Activ Probiotic Dahi, b-Activ Probiotic Lassi, b- Activ Curd and Nutrifit (Strawberry and Mango) are the company's probiotic products(all the three products contain both *L. acidophilus* and *B. lactis* strain BB12). Probiotic products are contributing to 15% of the turnover of their fresh dairy products. Nestle NESVITA was India's first Dahi with Probiotics – For Healthy Digestion. Heritage Foods (India) Ltd has introduced Probiotic dahi. Yakult Danone India Pvt Ltd (YDIPL) is a 50:50 joint venture between Japan's Yakult Honsha and The French- Danone Group and is offering Yakult, a probiotic drink made from fermented milk, *Lactobacillus casei* Shirota and some sugar. The entry of Yakult is expected to increase the visibility and growth of probiotic category in India.

III.CONCLUSION

Consumers are increasingly being attracted to healthier and functional dairy products. Probiotics have been incorporated into a range of dairy products, including yoghurts, soft, semi-hard and hard cheeses, ice cream, milk powders and frozen dairy desserts. However, there are still several problems with respect to the low viability of probiotic bacteria in GIT and food environments. Probiotics of intestinal origin are difficult to propagate and high survival rate is important for both economic reasons and health effects. Consequently, there is a demand for new technologies such as encapsulation to enhance probiotic viability . Future technological prospects exist in innovations finding solutions for the stability and viability problems of probiotics in new food environments. Current research on novel probiotic formulations and microencapsulation technologies exploiting biological carrier and barrier materials and systems for enteric release provides promising results. Maintenance of low production costs will remain the challenge for future probiotic process and formulation technologies. Exploitation of food-grade raw materials such as native, and physically or enzymatically treated starches, is one example of future technology that has the potential to meet the challenge of broadening the range of food types into which probiotic ingredients can be successfully incorporated. Novel developments for control release systems in foods and pharmaceuticals will also provide new possibilities. Hence, hopefully, the consumption of beverages and foods that contain probiotic microorganisms will be growing worldwide in the days to come.

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