Probiotic bacteria in fermented foods: product characteristics and starter organisms1–3

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ABSTRACT Probiotic bacteria are sold mainly in fermented foods, and dairy products play a predominant role as carriers of probiotics. These foods are well suited to promoting the positive health image of probiotics for several reasons: 1) fermented foods, and dairy products in particular, already have a positive health image; 2) consumers are familiar with the fact that fermented foods contain living microorganisms (bacteria); and 3) probiotics used as starter organisms combine the positive images of fermentation and probiotic cultures. When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the products and become active when entering the consumer’s gastrointestinal tract. These factors include 1) the physiologic state of the probiotic organisms added (whether the cells are from the logarithmic or the stationary growth phase), 2) the physical conditions of product storage (eg, temperature), 3) the chemical composition of the product to which the probiotics are added (eg, acidity, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content), and 4) possible interactions of the probiotics with the starter cultures (eg, bacteriocin production, antagonism, and synergism). 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 article, with an emphasis on dairy products such as milk, yogurt, and cheese. Am J Clin Nutr 2001;73(suppl):374S–9S.

KEY WORDS Probiotics, lactobacilli, bifidobacteria, starter bacteria, acidophilus milk, yogurt, kefir, cottage cheese, cheese, fermentation, fermented foods, dairy products

INTRODUCTION

Élie Metchnikoff is considered to be the inventor of probiotics. Intrigued by the longevity of the Caucasian population and its frequent consumption of fermented milks, Metchnikoff (1) proposed that the acid-producing organisms in fermented dairy products could prevent “fouling” in the large intestine and thus lead to a prolongation of the life span of the consumer. Although Metchnikoff’s ideas were clearly related to lactic acid bacteria in dairy products, the interest of other scientists soon turned to lactic acid bacteria of intestinal origin. One of the first of these scientists was Henneberg (2) in Kiel, who proposed the use of an intestinal Lactobacillus acidophilus to produce what he called Acidophilus-Milch, or reform yogurt. When this type of fermented product finally became a success under the name of yogurt mild in Germany and some other Western European countries in the early 1980s (3), the health aspects of yogurt mild were far less relevant than was the possibility of producing an acid-reduced, yogurtlike fermented product. Consequently, the lactobacillus species used for fermentation were—and still are—selected solely on the basis of their technologic properties and not their potential health benefits. The probiotic bacteria used in commercial products today are mainly members of the genera Lactobacillus and Bifidobacterium (4–7). Lactobacillus species from which probiotic strains have been isolated include L. acidophilus, Lactobacillus johnsonii, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus gasseri, and Lactobacillus reuteri. Bifidobacterium strains include Bifidobacterium bifidum, Bifidobacterium longum, and Bifidobacterium infantis.

This short excursion into the history of probiotics provides a historical explanation for why dairy products—specifically, yogurtlike products—form the largest segment by far of the market for probiotic products. The consequences of this history with respect to consumer perception are that

• 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 yogurtlike 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. However, it must be pointed out that other fermented products (eg, raw sausages and sauerkraut)
can serve as carriers of probiotic organisms, but few such products are already on the market.

With the apparent market success of probiotic products, questions concerning the nature of probiotic bacteria (4), the definition of the term probiotic (8, 9), and the potential health effects of probiotics (10, 11) are asked by consumers. Of particular importance is whether foods containing probiotics provide added value compared with traditional fermented foods containing living microorganisms, and whether this value is maintained during manufacturing and is provided during the entire shelf life of the product.

In this article, I address the influence of food production technology on the functional properties of probiotics: first, possible interactions between probiotic microorganisms and food components, and second, the effect of product and production characteristics on the functional properties of probiotics. My use of the term probiotic is solely conceptual and is not related to proven health benefits.

**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. Finally, methods should be available to identify probiotic strains unequivocally.

To fully exploit the functional properties of probiotic bacteria, the processes used to manufacture dairy products must be modified to meet the requirements of the probiotics. When this is not possible, other probiotic strains must be tested or, in extreme case, new products must be developed. In this section, I address some of 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 (12, 13). Furthermore, because the intestinal tract is considered to be the natural environment of the probiotic bacteria, the oxygen content, redox potential, and water activity of the medium must be considered (14).

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) (15, 16). On the other hand, the proteolytic (17) 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 (15).

A major aspect of the production of probiotic fermented dairy products is the interaction between probiotics and starter organisms. Although little is known about this interaction, both synergistic and antagonistic effects between different starter organisms are well established. For example, the classic yogurt culture is characterized by a protosymbiosis between Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. This synergism, seen as an accelerated and efficient acidification of the milk and multiplication of the culture organisms and based on cross-feeding of both organisms, is not a property of the 2 species but of specific strains of theses species (18–21). 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 (22, 23). The ability to produce bacteriocins is often discussed as a desirable property of probiotics (10); however, antagonism to starter cultures and vice versa may be a limiting factor for combinations of starters and probiotics (24). 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 (25–29). An overview of the starter bacteria used in dairy fermentations and some of their relevant physiologic properties is given in Table 1.

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. In the latter case, interactions may be minimal because addition may occur immediately before or even after cooling below 8°C and the metabolic activity of starters and probiotics is drastically reduced at these temperatures. However, with extended storage, even small interactions may yield measurable effects. Also, an interruption of the cold chain must be avoided to keep interactions to a minimum.

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 (this will be dealt with in more detail in the following section), on the treatment of the probiotics during and after harvesting, and, finally, on 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 (30).

When probiotic bacteria participate actively in fermentation, the aspects of food composition and of interactions with the food matrix and starters have to be taken into account on a much larger scale. Because antagonisms between probiotics and starter cultures will result in retarded growth or complete inhibition of one of the bacterial components, such cases are relatively easy to identify. One important variable in this respect is lactic acid production and the concomitant reduction in pH during fermentation, which results in inhibition of the probiotic organisms.

The physiologic state of the probiotics is of special importance when considering how fermentation is terminated. Several investigations showed that bacteria from the logarithmic phase are much more susceptible to environmental stresses than are...
bacteria from the stationary phase (31–33). Our own experiments with starter organisms showed that environmental factors that signal to the bacteria the transition from the logarithmic phase to the stationary phase may have a considerable effect on survival rates during the stationary phase (34). Thus, a starvation signal, triggered by depletion of carbon sources, appears to be much more favorable for survival than a low pH in the presence of sufficient carbon sources. However, investigation of stationary phase regulation in lactic acid bacteria is a new discipline. Certainly, much more research is needed to exploit the possibilities for improvement of survival rates of not only probiotic bacteria but also traditional starter bacteria.

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

The base for the production of dairy products is milk, which has a typical composition of ≈87.4% water, ≈4.7% lactose, ≈3.8% fat, ≈3.3% protein, ≈0.2% citrate, and ≈0.6% minerals. The pH of milk is usually between 6.5 and 6.7. The protein fraction is composed of ≈80% casein and 20% whey proteins. Thus, the nonfat dry matter of milk is between 8.5% and 9% (35).

Sweet acidophilus milk and sweet AB milk are probiotic dairy products based on unfermented 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 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 (36). A scheme for acidophilus milk production is presented in Figure 1. (The data presented in the table and figures discussed in this section are based on references 3, 27, and 36 and on information from manufacturers.)

Yogurtlike products are manufactured with different textures (3, 36). Natural-set yogurt, stirred yogurt, and drink yogurt differ in their content of nonfat solids: 16–18%, 13–14%, and 11–12%, respectively.

Considerable variation with respect to the starter culture used is legal in some countries, including Germany. 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth temperature</th>
<th>Lactic acid fermentation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Optimal</td>
<td>Maximum</td>
</tr>
<tr>
<td>L. delbrueckii subsp. bulgaricus</td>
<td>22</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>L. delbrueckii subsp. lactis</td>
<td>18</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>22</td>
<td>42</td>
<td>54</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>27</td>
<td>37</td>
<td>48</td>
</tr>
<tr>
<td>L. kefir</td>
<td>8</td>
<td>32</td>
<td>43</td>
</tr>
<tr>
<td>L. brevis</td>
<td>8</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td>L. casei subsp. casei</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>22</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>Lc. lactis subsp. lactis</td>
<td>8</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Lc. lactis subsp. cremoris</td>
<td>8</td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>Lc. lactis subsp. lactis biovar. diacetylactis</td>
<td>8</td>
<td>22–28</td>
<td>40</td>
</tr>
<tr>
<td>Ln. mesenteroides subsp. cremoris</td>
<td>4</td>
<td>20–28</td>
<td>37</td>
</tr>
<tr>
<td>Ln. mesenteroides subsp. dextranicum</td>
<td>4</td>
<td>20–28</td>
<td>37</td>
</tr>
<tr>
<td>Bifidobacterium (bifidum, infantis, etc)</td>
<td>22</td>
<td>37</td>
<td>48</td>
</tr>
</tbody>
</table>

1 Lb., Lactobacillus; S., Streptococcus; Lc., Lactococcus; Ln., Leuconostoc.
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 (37). 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 (Figure 2). 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.

For the manufacture of yogurt mild, probiotic lactobacilli can even be used as starter cultures because they meet the legal requirements. However, such manufacture is a compromise between full expression of the potential health properties of the probiotic strain and the technologic suitability of the strain. The probiotic strain must meet not only the criteria for good survival but also the criteria for fermentation and harmonious interaction with the \(S.\ thermophilus\) starter strain used. This could mean that the strain with the best combination of functional and technologic properties is the one used, not the strain with the best health properties.

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. A short overview over the manufacture of kefir is presented in Figure 3.

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 (Figure 4). 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 \(\approx 22°C\) to remove residual lactose. Finally, cream and salt (and spices for some products) 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 \(\leq 55°C\) 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.

Many varieties of ripened cheese are known (36), but all of the different manufacturing methods will not be discussed here, especially because ripened cheese is of only minor importance as a carrier for probiotic bacteria. Thus, only some general and critical

<table>
<thead>
<tr>
<th>Yogurt</th>
<th>11–12% nonfat solids</th>
<th>Adjustment of nonfat solids</th>
<th>Drink yogurt</th>
<th>15–14% nonfat solids</th>
<th>Adjusted yogurt</th>
<th>16–16.5% nonfat solids</th>
<th>Natural-set yogurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurization</td>
<td>Down</td>
<td>Postfermentation</td>
<td>Down</td>
<td></td>
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</tr>
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</table>

Addition of starter culture (\(Streptococcus thermophilus\) and \(Lactobacillus delbrueckii\) subsp. \(bulgaricus\))

- Addition of flavor components, fruit, etc.
- Incubation at 40–45°C for 2.5–6 h
- Stirring
- Incubation at 40–45°C for 2.5–6 h
- Cooling to 4–8°C
- Addition of flavor components, fruit, etc.
- Stirring
- Filling of retail containers
- Storage

**FIGURE 2.** The manufacturing process for different types of yogurt.

**FIGURE 3.** The manufacturing process for kefir. CFU, colony-forming unit.
aspects related to survival of probiotics will be presented (Table 2). Pasteurized and prewarmed milk is inoculated with a mesophilic or thermophilic starter culture and incubated at temperatures up to ≈33°C. When the pH of the milk has dropped to a certain value (≈6.0), rennet is added and incubation is continued until the curd has formed. The curd is cut into pieces, the sizes of which differ according to the final product [small pieces like wheat grains for extra hard and hard cheese, medium-sized pieces for semihard cheese, and larger pieces (2–3-cm cubes) for soft cheese]. Depending on the cheese variety, scalding temperatures of ≤55°C may be applied to the curd-whey mixture. After the whey is drained off, the curd particles are placed in molds where they are allowed to coalesce, either by the weight of the curd or by applied pressure. The cheese is then immersed in a brine bath and left for the required period—from a few hours for small and soft cheeses up to 1 mo for large and extra-hard cheeses. Often, cheeses are not immersed in brine baths but are dry salted. Some hard cheeses, like cheddar, are salted during milling of the drained-off curd and are pressed in molds afterward. The duration of ripening under controlled temperature and moisture conditions depends on the type of cheese and can vary from a few days (soft, surface-ripened cheese) to >2 y (extra-hard cheese).

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 (e.g., 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.

Special probiotic products that are obtained by fermentation with a single probiotic strain and that do not use one of the standard dairy products as a carrier will not be dealt with in detail here. With such products, technologic restrictions are kept to a minimum because fermentation is directed toward maximum expression of the functional (health) properties of the probiotic strain. The only “restriction” is to produce a product that will be accepted by the consumer.

TABLE 2
Critical steps in cheese manufacture

<table>
<thead>
<tr>
<th>Step</th>
<th>Conditions</th>
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<tbody>
<tr>
<td>Addition of starter culture</td>
<td>Mesophilic (Lactococcus species, Leuconostoc species)</td>
</tr>
<tr>
<td></td>
<td>Thermophilic (Lactobacillus helveticus, Streptococcus thermophilus, etc)</td>
</tr>
<tr>
<td>Scalding</td>
<td>Up to 57°C for up to 1 h (cheddar: ≈38°C)</td>
</tr>
<tr>
<td>Salting</td>
<td>Brine or dry salting (cheddar: dry salting of milled curd)</td>
</tr>
<tr>
<td>Ripening</td>
<td>At 10–15°C, up to 1 y or more (emmentaler: first 2 mo at 22–25°C)</td>
</tr>
</tbody>
</table>

CONCLUSIONS

With the increasing popularity of probiotic products, consumers frequently demand that the health properties of probiotic strains be preserved in the products sold and that there is at least a theoretical chance that the health effects of the probiotic strains will be evident after consumption. To guarantee this, many important variables must be considered by the dairy industry. One is that sufficient numbers of probiotic cells survive throughout the shelf life of the product. Another is that the probiotic cells survive intestinal passage and establish themselves in the terminal ileum or in the large intestine in sufficient numbers to display their health effects. To ensure this, studies must show that adverse interactions with the food matrix or with the starter organisms of the dairy food do not play any role in this respect. The essential measure must be that the products advertised as being probiotic, and not just the probiotic strains added to the products, have indeed been shown to exhibit probiotic effects. That this is so must be made transparent to consumers by the producers of probiotic products.

REFERENCES


