UDC 57.833:579.864.1
ISSN 1330-9862 *review*

(FTB-1020)

Technology Aspects Related to the Application of Functional Starter Cultures

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Received: January 18, 2000
Accepted: April 3, 2000

Summary

The market of pro- and prebiotics as applied in fermented milk drinks is expanding worldwide. More consumers become interested in the potential, health-promoting properties of functional foods. However, the industrial processors need to adapt their production processes and technologies, if they wish to use probiotics in a variety of food products. Indeed, the food matrix composition, the interaction(s) and stability of the culture, the inoculum level, the technological process conditions, etc. influence the viability of the probiotic bacteria considerably. In this paper some problems are discussed related to the application of probiotics in the dairy sector.

In the food sectort there is a fast increasing request for 100 % natural products, not only from the point of view of the consumer but also from that of the authorities. Two examples are given in this paper: the use of natural food preservatives (antimicrobial proteins or bacteriocins) and the application of natural texturisers (microbial exopolysaccharides), both through the application of functional lactic acid bacterium starter cultures. Also, one has to take into account the influence of several factors of the food matrix and the applied process technology on the functionality of the strains used.

Key words: lactic acid bacteria, starter cultures, probiotics, prebiotics, bacteriocins, exopolysaccharides

Introduction

Functional foods came and come into the market; lactic acid bacteria play an important role in this trend (*1*,*2*). Hence, probiotics (life microorganisms such as lactobacilli and bifidobacteria that are added to food and that possess health-promoting properties) and prebiotics (non-digestible food ingredients that stimulate the bifidobacteria present in the colon) may be considered as the driving forces of the functional foods' market (*3*–*8*). Innovation and competition are customary in this sector. Some companies expand worldwide; others occupy strategic positions to guarantee their success. The potential of this growing market is enormous, especially when both the food and therapeutic applications of functional foods are considered. But also the request for 100 % natural products is a rapidly increasing trend, not only from the point of view of the consumer but also from that of the authorities. Both parties are high-demanding for a natural and healthy diet. Applied research is going on to replace chemicals such as nitrite, nitrate, sulfite, etc. by alternative means such as functional starter and/or cocultures of for instance lactic acid bacteria to prolong the shelf-life of foods (*9*,*10*).

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Mammalian milk may be considered as the first »functional food«. It is not surprising then that cow's milk is a natural source of a variety of beneficial, biologically active components (*11*). In addition, it commonly forms the raw material for the production of yoghurts and other fermented milks (*12,13*). Initially, these dairy products were the ideal vectors for the application of probiotics. New applications of probiotics in foods have recently been introduced into the market or are still in the development phase such as frozen yoghurt, soja yoghurt and fermented soya milk, dairy desserts, cheese, ice-cream, bread, chocolate, etc. (*14–16*). Technological aspects related to these complex microbial systems and functional foods are the composition and processing of raw materials, the viability and productivity of the applied starter culture, and the technological and storage conditions of the end-product. Distinction has to be made between *(i)* the processing of starter cultures with desirable, functional properties – further referred to as functional starter cultures – (including probiotics), *(ii)* the processing of foods with desirable, functional characteristics, and *(iii)* the processing of foods containing probiotics (*16*). In this paper, these three production technologies will be dealt with through each other.

Lactic acid bacteria (lactococci, lactobacilli, streptococci, enterococci, etc.) are an important group of starter cultures, applied in the production of fermented foods like yoghurt, cheese, dry sausage, salami, sourdough, etc. (*17*). They display interesting functional properties such as acidification, proteolysis, aroma formation, etc. Lactobacilli, for instance *Lb. acidophilus*, *Lb. casei* subsp. *casei*, *Lb. gasseri*, *Lb. paracasei*, *Lb. reuteri* and *Lb. rhamnosus*, and bifidobacteria, for instance *B. adolescentis*, *B. bifidum*, *B. breve*, *B. infantis* and *B. longum*, constitute a significant proportion of probiotic lactic acid bacterium cultures used in developed countries (*18–21*). These cultures may tolerate acidic conditions of the stomach, and the digestive enzymes and bile salts of the small intestine, hence enabling them to colonise (at least temporarily) the terminal ileum and the colon. However, each strain within these species exhibits unique properties with respect to growth rate, metabolic rate, proteolytic activity and flavour production. It is further important to underline that lactobacilli are aerotolerant or anaerobic, and strictly fermentative, whereas bifidobacteria are anaerobic and saccharoclastic (*19*). Therefore, from a technological point of view, fermented milk products containing *Lactobacillus* and *Bifidobacterium* strains are a microbiologically sensitive group of functional foods. Consequently, special attention is needed to keep stability maximally and to optimise the microbial viability of the applied culture, and to improve the productivity simultaneously. If this is not respected, the ultimate aim of the production of a functional food may not be fulfilled.

Metabolic Properties *versus* Viability of Probiotics

Carbohydrate Metabolism and Nutritional Requirements versus Viability of Probiotics

Carbohydrate Metabolism and Growth of Probiotics

Lactic acid bacteria such as *Lactobacillus*, *Enterococcus* and *Bifidobacterium* are chemo-organotrophic and convert carbohydrates into lactic acid by fermentation (Fig. 1).

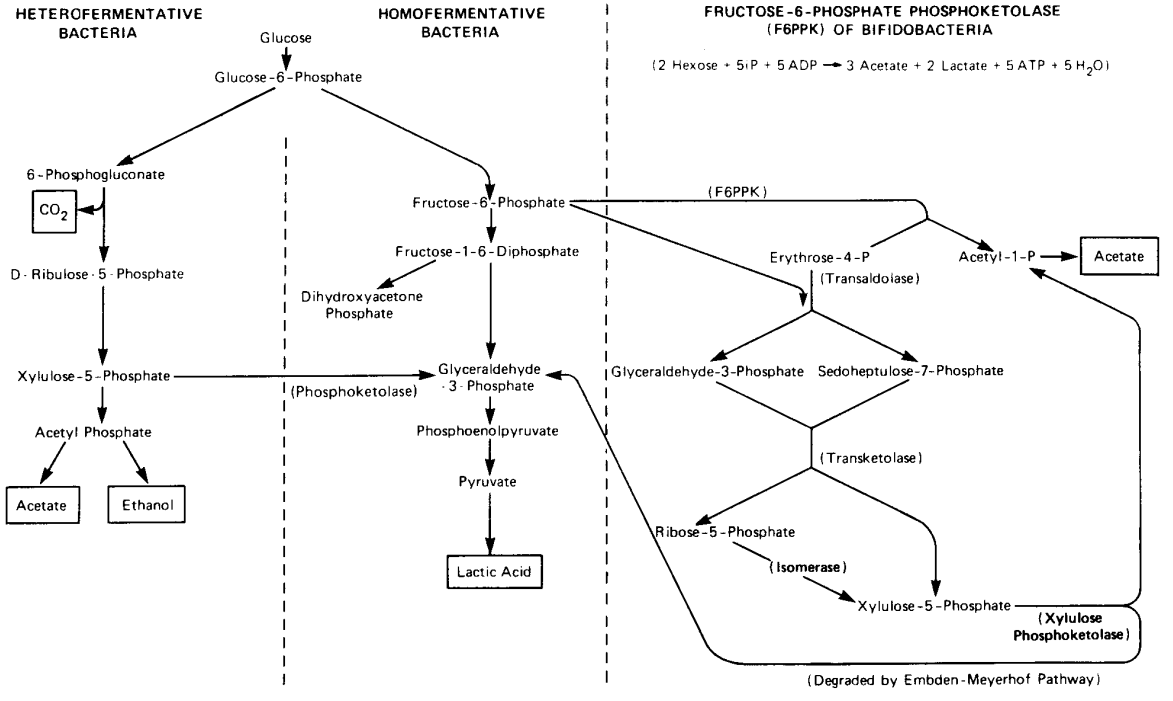

Fig. 1. Schematic overview of the carbohydrate metabolism and the end-metabolites of homo- and heterofermentative lactic acid bacteria (left side) and bifidobacteria (right side) (adapted from *22*)

Lactobacilli and enterococci are strictly fermentative. Glucose is fermented predominantly to lactic acid in homofermentative strains (*Lactobacillus* and *Enterococcus*), or to equimolar amounts of lactic acid, carbon dioxide and ethanol (or acetic acid) in heterofermentative strains (*Lactobacillus*) (Fig. 1, left side). An important member of the probiotic lactobacilli is *Lactobacillus acidophilus.* Although lactose is virtually the only sugar present in milk, *Lb. acidophilus* has been reported to utilise sucrose more effectively than lactose. These observations may be ascribed to differences in activities of the enzymes β-galactosidase and β-fructofuranosidase. While β-fructofuranosidase is a constitutive enzyme, β-galactosidase may be induced in *Lb. acidophilus.* Moreover, both glucose and fructose moieties of sucrose are utilised by *Lb. acidophilus,* whereas the galactose moiety of lactose cannot be metabolised to an appreciable degree. Growth of *Lb. acidophilus* may occur at temperatures as high as 45 °C, but optimum growth occurs within 35–40 °C. The optimum pH is 5.5–6.0. *Lb. acidophilus* is microaerophilic, so surface growth on solid media is generally enhanced by anaerobiosis or reduced oxygen pressure and 5–10 % CO_2.

Bifidobacteria are saccharoclastic organisms that produce acetic acid and lactic acid without generation of carbon dioxide, except during degradation of gluconate (Fig. 1, right side). Heterofermentation is initiated by splitting fructose 6-phosphate into one C_2 and one C_4 moiety. The conversion of the C_2 moiety to acetate is paralleled by the formation of heptose 7-phosphate from the C_4 moiety concomitant with the formation of a triose moiety derived from an additional molecule of fructose 6-phosphate. The heptose 7-phosphate is subsequently split into two molecules of acetate and one molecule of pyruvate. The second triose moiety left from fructose 6-phosphate is converted into lactate. Therefore, the fermentation of two moles of hexose results in three moles of acetate and two moles of lactate. The key enzyme in this glycolytic fermentation is fructose 6-phosphate phosphoketolase. Besides glucose, all bifidobacteria from human origin are also able to utilise galactose, lactose, and, usually, fructose as carbon sources. *Bifidobacterium* spp. are, in some instances, also able to ferment complex carbohydrates. The optimum pH for growth of bifidobacteria is 6.0–7.0, with virtually no growth at pH=4.5–5.0 or below, or at pH=8.0–8.5 or above. The optimum growth temperature is 37–41 °C, with maximum growth at 43–45 °C and virtually no growth at 25–28 °C or below. Bifidobacteria are strictly anaerobic (also requesting carbon dioxide in the atmosphere); strains of *Bifidobacterium lactis* are considered as being oxygen-tolerant.

Nutritional Requirements *versus* Viability of Probiotics

Lactobacilli have complex growth requirements. They require low oxygen tension, fermentable carbohydrates, proteins and their breakdown products, a number of vitamins of the B-complex, nucleic acid derivatives, unsaturated free fatty acids, and minerals such as magnesium, manganese and iron for their growth. An increased amount of thiol groups present in whey protein-enriched milks favours the growth of *Lb. acidophilus*, whereas peptone and trypsin stimulate its acid production. The fact that bifidobacteria can grow in a semi-synthetic medium containing only lactose, three free amino acids (cysteine, glycine and tryptophan), several vitamins and nucleotides, and some minerals contrasts with the generally recognized fastidiousness of lactobacilli with regard to nutritional requirements. A striking difference between lactobacilli and some strains of bifidobacteria is the ability of the latter to grow in a medium containing nitrogen in ammonium form; the remaining bifidobacteria strains require nitrogen from organic sources (*13*).

In contrast with lactobacilli, enterococci and bifidobacteria exhibit a weak growth in milk or do not grow at all in milk. However, milk is an essentially satisfactory medium because it contains all essential nutrients, except that amino acids and small peptides are present at insufficient concentrations to support extended growth of bifidobacteria (*23*). In general, bifidobacteria grow better in rich synthetic media such as TPY and MRS broths, than in milk; however, these media are complex and costly for large-scale propagation of bifidobacteria. Moreover, unless the cells harvested from such media are extensively washed before incorporation, they may confer off-flavours to the finished dairy products. Furthermore, to manufacture a quality product, both in terms of viability of bifidobacteria and texture, a milk-based medium is usually required because the protein (casein) content of milk is higher than that of synthetic media (which are generally low in solids). Thus, improvement of growth conditions for bifidobacteria in milk is necessary from a technological point of view. Yeast extract, a commercial product, was found to be an effective growth promoter. Also the addition of growth-promoting factors such as vitamin-enriched protein hydrolysates, results in desired levels of growth. Furthermore, bifidobacteria invariably require strict anaerobiosis and a low redox potential in the early phase of growth.

The selection of oxygen-tolerant species may be a solution toward minimization of the adverse effect of oxygen in fermented bioproducts containing *Bifidobacterium* spp. and *Lb. acidophilus*. Finally, the fermentation time varies considerably, depending on the bifidobacteria, enterococci or lactobacilli strains applied. For instance, bifidobacteria require long fermentation times and anaerobiosis due to their weak growth and acid production in milk.

Some foods are supplied with bifidogenic factors. Bifidogenic factors are defined as compounds, usually of a carbohydrate nature, that survive direct metabolism by the host and reach the large bowel or cecum, where they are preferentially metabolised by bifidobacteria as source of energy (*24*). Bifidogenic factors fall under the new concept of prebiotics. The latter are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and which may thus improve the health of the host (*25*). In this respect, non-digestible oligosaccharides (NDOs) such as fructo-oligosaccharides, xylo-oligosaccharides, galacto-oligosaccharides, malto-oligosaccharides, manno-oligosaccharides, soya-oligosaccharides, lactosucrose, lactulose, etc. have been used in the diet specifically to

increase relative numbers of bifidobacteria in the gut microflora (*26*). For instance, the bifidobacteria are able to metabolise fructo-oligosaccharides (FOS). These fructose polymers are not digested by the classical digestive enzymes nor metabolised by undesirable gut bacteria, so that including these non-nutritive sweeteners in the diet have been shown to encourage the growth of bifidobacteria in the colon selectively (*27–31*). In the colon of babies the growth of bifidobacteria is selectively stimulated by specific, bifidogenic factors. These are carbohydrates such as *N*-acetylglucosamine and lactulose found in human milk and thermally processed milk products, respectively. The number of bifidobacteria decreases with increasing age of the individual and eventually becomes the third most abundant genus (accounting for cc. 25 % of the total adult gut flora) after the genera *Bacteroides* and *Eubacterium* (*32*).

Acidification Rate, Flavor Generation, and Texture Development in Probiotic Products

The rate of acid development is a critical factor in milk fermentations (*33*). A rapid acidification of the raw material prevents growth of undesirable microorganisms and is also essential for aroma, texture and flavor of the end-product. Acid development by probiotic lactic acid bacterium cultures (for instance *Lb. acidophilus* (A-culture) and *B. bifidum* (B-culture)) is poor in comparison with the traditional yoghurt bacteria *Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (T-culture) (cf. Fig. 2). This is of utmost importance when preparing cultures for probiotic fermented milks. The growth rate of traditional yoghurt cultures is sufficient for them to independently acidify milk for yoghurt making. The probiotic cultures, however, are not so effective. Yet *Lb. acidophilus* may be able to produce good acidity in milk, but a desirable aroma or a good textured end-product may be difficult to achieve. The situation with strains of bifidobacteria is even more critical as, as mentioned above, growth of many of these strains is (very) poor in milk.

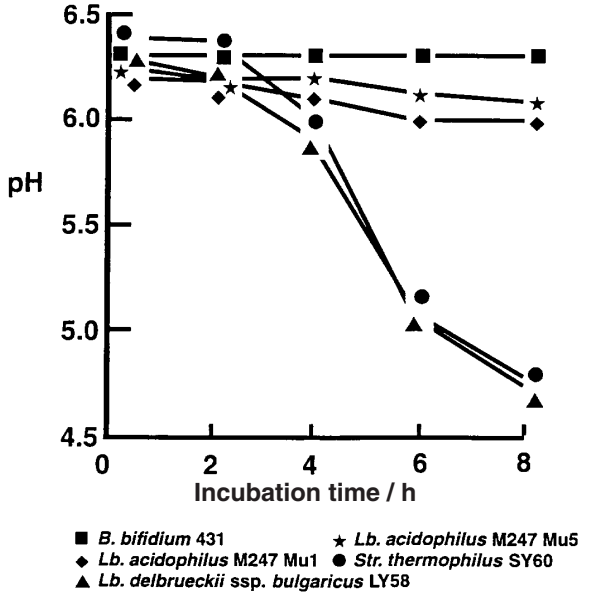

Fig. 2. Rate of acid development (pH) of several lactic acid bacterium strains during fermentation of milk containing 12 grams non-fat-solids per 100 gram of milk (adapted from *33*)

A starter culture consisting of strains of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* produces fermented milk that is acid and has a good texture. Single cultures produce yoghurts of slightly different flavors and aromas. Milk fermented with *S. thermophilus* alone is less acidic and has a buttery aroma, while milk fermented with *Lb. delbrueckii* subsp. *bulgaricus* is quicker to set, has a lower pH and has a pronounced yoghurt (acetaldehyde) aroma (*33*). The cultures used in the preparation of probiotic, fermented milks (*Lb. acidophilus/johnsonii*, *Lb. casei/paracasei*, *Bifidobacterium* spp.) have a decreased ability to produce the required sensory attributes (*33*). Lactic acid bacteria utilizing the heterofermentation pathway are less efficient lactic acid producers and produce unwanted byproducts. Glucose is metabolised to lactic acid and acetic acid. Lactic acid is 'acid-sour' while acetic acid is 'acid-sharp'. Acetic acid acidity results in harshness that is undesirable for milk products. A product with a high acetic acid content will be described as 'vinegary'. Carbon dioxide is produced by heterofermentative strains, which may disrupt the gelling capacity. Finally, acetaldehyde is not generally the end-metabolite for lactic acid bacteria such as *Lb. acidophilus*, as these strains – unlike the traditional yoghurt bacteria – have another enzyme that converts acetaldehyde to ethanol that effectively removes the typcial yoghurt aroma compound. Milks fermented with these strains, therefore, will have less flavor and aroma (*33*).

Besides the rate of acidification, casein hydrolysis (proteolytic activity of the applied starter) and possible exopolysaccharide biosynthesis contribute to texture development of the end-product (*12*). Consequently, careful selection of the strains employed and good monitoring throughout the manufacturing process are therefore compulsory in attempts to control efficiently the metabolic end-products, the final pH, the flavour and aroma, and the texture. The use of combined cultures of bifidobacteria and *Lb. acidophilus* and/or other lactic acid bacteria is another solution for many such problems. Increased growth rates and reduction of fermentation time, absence of certain sensory and texture defects and further improvement of nutritional value of bioproducts are advantages brought about by the latter possibility (cf. infra). Adverse effects with respect to viability have, however, been reported for some strains of *Bifidobacterium* and *Lb. acidophilus*.

Viability and Stability of Probiotics

Several factors influence the viability of probiotic lactic acid bacteria (and lactic acid bacterium starter cultures in general) in yoghurt, other fermented milks and other (dairy) foods (*23,33–36*). These include the strains used, interactions between species present (cf. infra), the degree of acidity, the storage temperature, the presence of microbial inhibitors in the food matrix (e.g. sodium chloride and hydrogen peroxide), etc. Also the chemical composition of the fermentation medium for growth (for instance the carbohydrate source, milk solids content,

availability of nutrients and growth promoters, dissolved oxygen content), the cultivation conditions (for instance the level of inoculation, the incubation temperature, the fermentation time), final acidity, etc. may affect the viability of probiotic organisms in yoghurt. In addition, colonisation abilities of AB-cultures can be adversely affected after multiple transfers and incubations in milk. Other factors that may affect the viability of life microorganisms are heat treatments, homogenisation, and packaging. Finally, the viability of both *Lactobacillus* and *Bifidobacterium* species diminishes drastically during cooling, storage and transport. This may result in low numbers of living cells when approaching the expiry date of the product.

All these observations necessitate careful strain selection and monitoring. In case of functional starter cultures this means strains with a high, initial productivity (in the early phase of the fermentation), a long viability, and strains that are adapted to the food matrix (cf. infra). For probiotics it means a high stability and long viability (think about the passage of the gastrointestinal tract) and a high *in vivo* productivity (for instance competitive exclusion in the colon). In addition, an adapted process technology is required such as the use of mildly-acidifying starter cultures for the production of fermented milk products, adaptation of the substrate, etc. This must finally result in a high viability of the cultures.

Suggested minimum numbers of probiotic bacteria at the time of consumption of a probiotic product are 10^7 viable cells per milliliter or gram of product. The minimum therapeutic dose per day is suggested to be 10^8 to 10^9 viable cells, which may be realisable through an intake of approximately 100 grams of bioproduct containing 10^6–10^7 viable cells per milliliter or gram. However, the 'Fermented Milks and Lactic Acid Bacteria Beverages Association' of Japan has developed a standard which requires a minimum of 10^7 viable cells per milliliter to be present in fresh dairy products. The criteria developed by the 'National Yogurt Association' (NYA) of the United States specifies 10^8 viable cells per gram of product at the time of manufacture, to place a NYA 'Life and Active Culture'-logo on the containers of the products. Anyway, it is considered misleading to describe probiotic fermented milks as having health-promoting properties unless the minimum level of viable cells is present at the expiry date of the product. Cases of marked loss of viability in dairy products have been reported for both *Bifidobacterium* spp. and *Lb. acidophilus*, more often during refrigerated storage at low pH, and reflect, as stressed previously, the need for careful strain selection (cf. infra). New methods for enhancing the long-term survival of probiotic strains, and hence ensure that reasonable numbers of bacteria are delivered to the host, have been investigated either by replacing the carrier food (e.g. cheese instead of fermented milk) or by improving the protection of acid-sensitive strains *via* microencapsulation with polymers (*23*).

Stability of Prebiotics

Supplementation of dairy products with NDOs (such as FOS) may positively affect human health. However, technological conditions of food processing must be taken into account (*35*,*37*). For instance, at pH<4 and process treatments at elevated temperature or during longterm storage at ambient temperature, the oligosaccharides present in the food matrix may undergo hydrolysis. This results in the loss of the nutritional and physicochemical properties of the food. The stability is, however, strongly dependent on the nature and type of the oligosaccharides (fructo-, galacto-, xylo-oligosaccharides, etc.), in turn depending on the sugar residues present, their ring structure, their anomeric configuration, and the type of bonds. β-bonds are usually stronger than α-bonds. In addition, hexoses are more tightly bond than pentoses and deoxysugars, and so are pyranoses as compared to furanoses. Finally, it has to be underlined that NDOs remain substrates for microorganisms, so that they may be fermented during fermentation processes.

Large-scale Production of Probiotics

Production and Addition of the Culture

To achieve the best possible functionalities, the selected probiotic cultures must be produced under the most stringent manufacturing practices. Owing to a slow propagation in milk, they will hardly be competitive in the presence of other microorganisms and will, thus, be easily outnumbered. Therefore, aseptic working conditions and special growth-promoting factors (as discussed above) are prerequisites to ensure high initial viable cell counts. They must further be preserved with maximum viability and activity, and have the stability to meet market demands (*23*).

The common practice in fermented milks production is to use premixed, ready-for-addition, 'direct set'--cultures of *Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, *Lb. acidophilus* and/or *Bifidobacterium* spp. Direct inoculation of processed milk offers great flexibility in control of the desired sensory and microbiological qualities (for instance in terms of ratio of bifidobacteria to lactobacilli). Milk enriched with yeast extract is currently used for their production. Traditional batch fermentations are applied, followed by deep freezing or freeze drying of the cultures. Strains selected for direct-vat-set starters must undergo a concentration of up to 10^{10}–10^{11} CFU/g to permit the desired performance in commercial manufacture of fermented milk products. It is important to underline that the strains must survive these processes too! Apart from these requirements, the starter produced should also display acceptable stability (usually guaranteed for 3–12 months) throughout processing and subsequent storage and distribution.

Lb. acidophilus and *Bifidobacterium* spp. can also be grown separately, either prior to inoculation with the traditional yoghurt cultures, or particularly in the case of non-fermented milks, before incorporation into a product, to provide the retail product with a high 'loading' of the desirable microflora. It is further known that an incubation temperature of 37–40 °C will favor better survival of probiotic strains instead of a temperature of 42 °C traditionally used for yoghurt preparation.

Culture Formulations

To avoid problems of slow acidification, formation of unwanted byproducts, too less flavour and aroma, etc., cultures are often combined to produce fermented milks (*23,33*). One or both traditional yoghurt bacteria are added to reduce fermentation time and to improve taste, aroma and texture of the final product (cf. infra). However, low levels of bifidobacteria in commercial yoghurts were correlated with high populations of *Lb. delbrueckii* subsp. *bulgaricus*. The latter organism not only produces acid fast, but is also responsible for 'over-acidification' of yoghurt at refrigeration temperatures. On the other hand, a stimulatory effect by *Lb. delbrueckii* subsp. *bulgaricus* on *B. bifidum* was observed, possibly due to proteolytic activity of the former microorganism. One of the best combinations is that of *B. longum* and *S. thermophilus*. However, it has been found that cocultivation of species of *Bifidobacterium* with *S. thermophilus* may be disadvantageous for the bifidobacteria, because of the rapid acid production of *S. thermophilus* during the first phase of growth. Whereas *Lb. delbrueckii* subsp. *bulgaricus* produces more acid, so that the milk will have a lower final pH than milk fermented with *S. thermophilus*, and may produce substances such as hydrogen peroxide inhibitory towards AB-cultures, the acetic acid produced by bifidobacteria could have a marginally inhibitory effect on the *Lactobacillus* and *Streptococcus* spp. The level of dissolved oxygen can be reduced and the viability of *Bifidobacterium* in fermented milk may be improved by incorporating *S. thermophilus* having high oxygen utilization ability. It is further known that *Lb. acidophilus* is more tolerant to acidic conditions than *Bifidobacterium*. Also, synergistic growth-promoting effects between *Lb. acidophilus* and *B. bifidum* are known to occur. Hence, ABT-yoghurts (fermented with *Lb. acidophilus*, *Bifidobacterium* spp. and *S. thermophilus*) are widespread, mainly from a technological point of view. Yet, in some fermentations only one strain is applied, for instance *Lb. casei* Shirota. The latter fermentation process takes a whole week. However, what the applied formulation may be, the question still remains whether the viability of several probiotic lactic acid bacterium strains present in the same product (for instance a mixture of *Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, *Lb. acidophilus* or *Lb. casei* and *B. longum*) can be assured till the date of consumption of the product. While the presence of yoghurt cultures may restrict the growth of bifidobacteria, they have comparatively little impact on the long term viability of an existing culture. Thus, as long as an AB-yoghurt manufacturer ensures at least 10^6 viable cells of *Bifidobacterium* spp. per gram of product at the end of fermentation, the number of viable probiotic bacteria should remain stable throughout the anticipated shelf-life.

Functional Properties of Starter Cultures *versus* Productivity

Whereas a probiotic strain must show good survivability, both in the product and after digestion, starter cultures with inherent functional properties of technological importance must display a high initial productivity, *i.e.* in the food matrix and/or during food processing. Besides a fast acidification, important and desirable functional properties may be the production of antimicrobial compounds like bacteriocins (of potential importance in natural food preservation, and in competitive exclusion in the colon in the case of probiotics), the production of exopolysaccharides (sugar polymers that contribute to the texture of foods and that may display potential health-promoting properties), etc. However, it is often observed that the expression of these functional properties works well in the laboratory during *in vitro* experiments, but not in the food matrix itself, let alone that it works in the colon in the case of probiotics.

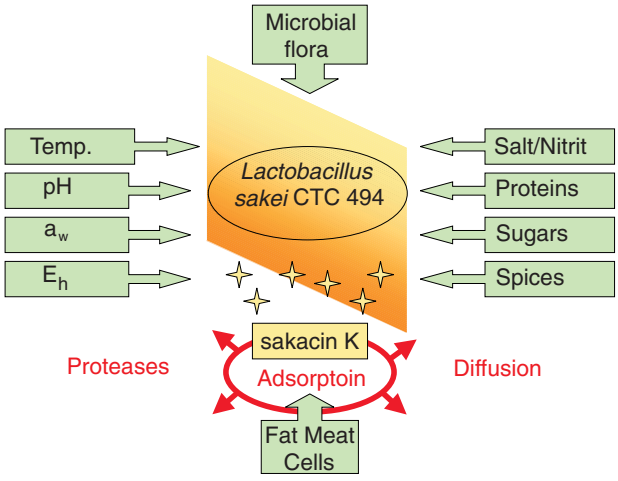

Fig. 3. Interactions between a functional starter culture, the bacteriocin-producing *Lactobacillus sakei* CTC 494 strain, and the food (meat) matrix

As an example, many lactic acid bacterium strains produce antimicrobial peptides or bacteriocins that are clearly active *in vitro* towards food spoilers and/or pathogenic bacteria, but not *in situ*, for instance in the cheese or meat matrix. This is often ascribed to the limited diffusion in and inactivation by certain components of the food itself. Also the process technology of the foods will influence the productivity of the (starter) culture. The choice of the culture is thus of utmost importance. A non-dairy example is given in Figure 3. It concerns a *Lb. sakei* culture that produces the antilisterial bacteriocin sakacin K (*38,39*). The *Lb. sakei* strain was isolated from a spanish naturally fermented dry sausage, and may hence be considered as being adapted to a meat environment. It has further been shown that the temperature and pH conditions that prevail during the sausage fermentation process are optimal for sakacin K production. The presence of salt and curing agent (nitrite) was disadvantageous for the functionality of the *Lb. sakei* meat starter culture. This example indicates that it is of primordial importance to investigate and analyse the influence of the bacterial cell environment on the functionality of the starter culture to be used, to be able to select the most appropriate and performant strains for final practical applications.

A similar problem is the application of exopolysaccharide-producing yoghurt starter cultures for the production of more viscous yoghurts without problems

of syneresis, low-fat yoghurts, creamier yoghurts, etc. To avoid syneresis (separation of water), at present, yoghurt manufacturers still rely on prefermentation processing such as increasing milk solids through the addition of milk powder, whey powder, caseinate, etc. or the concentration of milk (by evaporation, membrane filtration, etc.), heat treatment of the milk prior to inoculation, homogenisation, incubation conditions and handling of the ripened coagulum, and/or addition of stabilisers or viscosifiers (not allowed in all countries) such as chemically modified plant carbohydrates and gelatin. The application of lactic acid bacterium strains excreting heteropolysaccharides is a promising alternative (*40*). However, whereas exopolysaccharide-producing *S. thermophilus* strains are found, producing more than 1.5 grams of the polymer per liter fermentation medium in laboratory fermentors under optimal process conditions, *in situ* production (during yoghurt manufacture) only gives 30–800 milligrams of polymer per liter. Responsible factors are the strains used, the nature of the sugar polymer produced, factors associated with the milk environment, and the applied process technology.

Keeping in mind the examples mentioned above, one has to ask questions concerning the *in vivo* productivity of probiotic strains. Do probiotic lactic acid bacteria express their health-promoting properties in the colon optimally? A lot of research still has to be carried out to give the right answers.

Conclusions

The metabolism of lactic acid bacteria for milk fermentations is well understood at the biochemical and molecular level. As a consequence, selection and manipulation of strains can be done on a more rational basis. However, starter cultures to be used for a certain functional property (for instance bacteriocin production and exopolysaccharide production), as well as probiotics (possessing several health-promoting properties), should be adapted to the fermentable substrate, the food matrix (milk, meat, cereals), etc. In addition, the end-product must have an acceptable shelf-life and the desired sensorial properties such as colour, taste, aroma, and texture. Finally, probiotic lactic acid bacterium strains claimed to be present in the product should remain viable in sufficiently high numbers and retain their metabolic activities even beyond the expiry date of the product. Hence, more information is still needed on, for instance, the productivity and functionality of both probiotics applied in functional foods, and functional starter cultures applied in fermented foods.

Acknowledgements

The author and his research group thank the European Commission (Projects FAIR CT97-3078, FAIR CT97-3227, FAIR CT97-5013, FAIR CT98-4267 and IC15-CT98-0905), the Flemish Institute for the Encouragement of Scientific-Technological Research in the Industry (IWT), in particular the STWW program 'Functionality of Novel Starter Cultures in Traditional Fermentation Processes', the Fund for Scientific Research – Flanders (FWO-Vlaanderen), several Belgian and European companies, and the University Research Council (OZR-VUB) for financing their research.

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Mogućnosti tehnološke primjene funkcionalnih starter kultura

Sažetak

U cijelom je svijetu sve rasprostranjenije tržište prebiotika i probiotika u napitcima od fermentiranog mlijeka. Sve je više potrošača zainteresirano za potencijalna svojstva funkcionalne hrane radi unapređivanja zdravlja. Stoga proizvođači hrane trebaju prilagoditi proizvodne procese i tehnologije ako žele koristiti probiotike u raznim namirnicama. Na život probiotičkih bakterija bitno utječu osnovni sastav namirnice, interakcija sastojaka i stabilnost kulture, razina inokuluma, uvjeti tehnološkog procesa itd. U radu su razmatrani neki problemi vezani uz primjenu probiotika u mljekarstvu.

U području prehrane nadalje je tendencija da budu obuhvaćeni što prirodniji proizvodi, što zahtijevaju ne samo potrošači nego i ovlaštene ustanove. U radu su iznesena dva primjera: uporaba prirodnih sredstava protiv kvarenja hrane (antimikrobni proteini ili bakteriocini) i primjena prirodnih sredstava za poboljšanje konzistencije (mikrobni egzopolisaharidi), oboje primjenom funkcionalnih starter kultura bakterija mliječne kiseline. Također treba uzeti u obzir utjecaj nekih sastojaka iz namirnice i primijenjenog tehnološkog procesa na funkcionalnost upotrijebljenih sojeva.