CHAPTER 1
Lactic acid bacteria as starter cultures

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Introduction

Starter cultures have a basic role: to drive the fermentation process. Concomitantly, they contribute to all the characteristics of products, as well as to their sensorial and safety characteristics. Therefore, the introduction of starter cultures has undoubtedly improved the quality of products and the standardization of the industrial process.

A very important aspect is to have a good knowledge of the metabolic properties required to improve a specific product and to select useful microbial strains. Nevertheless, the limited number of already selected and studied strains that are also able to possess highly technological properties, as well as the constant risk of bacteriophage attacks, are stimulating research into new starter strains, in order to obtain higher quality and product diversification, in response to more and more aware consumers.

General aspects of starter cultures

The production of fermented foods today is based on the use of starter cultures, for example lactic acid bacteria (LAB), which initiate fast acidification of raw material. The great advantage of starter cultures is that they can provide controlled and predictable fermentation.

Starter cultures of LAB can contribute to microbial safety or offer one or more technological, organoleptic, nutritional or health advantages. Examples are LAB that produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins or useful enzymes, or that have probiotic properties (Leroy and De Vuyst 2004).

While starter cultures, chosen on the basis of their good safety and 'functional' characteristics, can benefit the consumer, they must first be able to be

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manufactured under industrial conditions (Saarela *et al.* 2000). Safety aspects of LAB include specifications such as origin, non-pathogenicity, certain metabolic activities (e.g. deconjugation of bile salts), toxin production, haemolytic potential, side effects in human studies (i.e. systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals and gene transfer) and epidemiological surveillance of adverse incidents in consumers (post-market). Functional aspects can be related to viability and persistence in the gastrointestinal (GI) tract, survival at low and high pH and in the presence of bile salts, hydrophobic properties, antibiotic resistance patterns, immunomodulation, and antagonistic and antimutagenic properties. Technological aspects concern growth at different sodium chloride (NaCl) amounts, temperatures, pH values, acidifying ability and metabolism (arginin deamination, esculin hydrolysis, acetoin production) and the ability to produce adequate flavour/texture.

With regard to the effect of salting, the addition of NaCl is a common practice in most fermented dairy foods, and also affects the growth of starter bacteria. Most LAB are partially or fully inhibited by levels of NaCl higher than 5%. However, it is evident that salt tolerance is a strain-dependent characteristic, thus this criterion is important in starter selection (Powell *et al*. 2011).

LAB starters are primarily used because of their ability to produce lactic acid from lactose and for consequent pH reduction, leading also to important effects like inhibition of undesirable organisms, improvement of sensorial and textural properties, as well as contribution to health benefits. A major role of starter cultures in dairy production is the degradation of peptides generated by the coagulant to small peptides and amino acids. Starter cultures are also capable of degrading caseins and converting amino acids to a range of flavour compounds. However, since many of the proteolytic enzymes are intracellular, flavour development in maturing cheese also depends on the release of the enzymes from starter cultures into the cheese matrix through cell lysis. Cell lysis, and the consequent release into the cheese matrix of intracellular enzymes, particularly peptidases and amino acid-degrading enzymes, is an important characteristic for both general protein degradation and also the control of bitterness. Autolysis results from the enzymatic degradation of the bacterial cell wall by indigenous peptidoglycan hydrolases released into the growth medium, although it is still unclear how this process is controlled in the cell. The process is highly strain dependent and is also influenced by factors such as the nutrient status of the growth medium and environmental conditions (Lortal and Chapot-Chartier 2005).

Generally, in maturing cheese there is a positive relationship between the period of starter culture autolysis and the flavour-forming reactions, involving not only proteolysis but also lipolysis. Consequently, various screening assays using buffers or model cheese and milk solutions have been proposed to select highly autolytic strains for use in cheese manufacture. Lysis positively influences the ripening and flavour of the cheese, but the type of peptidases is also very

important, in particular since low peptidase activities and low lytic properties produce bitter cheese. One of the most successful strategies to counteract this defect involves the use of LAB with high peptidase activities, particularly Pep N.

For these reasons, the use of good starter cultures can ensure the safety, quality and acceptability of both traditional and innovative fermented dairy products.

Types of starter cultures

In practice starter cultures may be categorized as mesophilic or thermophilic, according to the incubation and manufacturing temperatures under which they are used. Mesophilic cultures grow and produce lactic acid at optimal levels, at a moderate temperature (about 30 °C), whereas thermophilic cultures optimally function at a higher temperature (about 42 °C). Examples of mesophilic dairy starter cultures are the species *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Leuconostoc mesenteroides* subsp. *cremoris* and *Leuconostoc lactis*. On the other hand, the most thermophilic LAB species are *Streptococcus thermophilus*, *Lactobacillus delbrueckii* and *Lactobacillus helveticus*.

Nevertheless, the most common classification of starter cultures is based on the complexity of the culture and the way it is reproduced. All starter cultures available today are derived in one way or another from **natural starters** (or artisanal starters) of undefined composition (i.e. containing an undefined mixture of different strains and/or species). For some types of products, natural starters have been replaced by commercial **mixed-strain starters** (MSS), derived from the 'best' natural starters and reproduced under controlled conditions by specialized institutions and commercial starter companies, then distributed to the industries that use them to build up bulk starter or for direct vat inoculation. Natural starter cultures and commercial MSS, because of their long history, are called **traditional starters** (Limsowtin *et al.* 1996) as opposed to **defined strain starters** (DSS). DSS are usually composed of only a small number of selected strains and allow greater control over the composition and properties of the cultures. Table 1.1 shows a summary of culture types.

Traditional cultures contain many strains of many microbial species, sometimes including yeasts and moulds as well as bacteria; they all contribute biochemically to the complexity (and the variability) of the final product (Powell *et al.* 2011). Therefore, traditional starter preparation methods are still in use for some particular or traditional products, and have been adapted to a limited industrial scale. Industrial-scale production requires starters that give reproducible performance and are free of undesirable organisms. These goals are difficult to achieve using traditional methods. Thus, DSS have replaced traditional starters in industrial-scale production because of their optimized, highly reproducible performance and their high phage resistance.

Table 1.1 Culture types and their preparation.

Types of starter cultures		Description
Traditional starters	Natural starters	Low cost. Undefined composition. Highly variable composition and performance. Prone to undesirable contamination; microbiologically hazardous
Traditional starters	Mixed-strain starters (MSS)	Undefined composition. Variable composition and performance. With careful handling and some quality control testing, these are still in limited use, but have largely been replaced by laboratory-maintained cultures
Defined strain starters (DSS)		Defined composition, usually composed of only a small number of strains. This gives a high degree of control over starter performance parameters and product properties, as long as strains are carefully selected and managed

Traditional starters: Natural starters

The production of natural starters is derived from the ancient practice of backslopping (the use of an old batch of a fermented product to inoculate a new one) and/or by application of selective pressures (heat treatment, incubation temperature, low pH) (Carminati *et al.* 2010). No special precautions are used to prevent contamination from the environment, and the control media and culture conditions during starter reproduction are very limited. As a result, natural starters are continuously evolving as undefined mixtures composed of several strains and/or species (Carminati *et al.* 2010).

Natural starters are an extremely valuable source of strains with desirable technological properties (antimicrobials, aroma production); for example, they are considered to be highly tolerant to phage infection because they are reproduced in the presence of phages, which leads to the dominance of resistant or tolerant strains (Carminati *et al.* 2010). Also they seem to be advantaged by microbial interactions; in fact, many strains show limited acid-production ability when cultivated as pure cultures (Parente and Cogan 2004).

Traditional starters: Mixed-strain starters (MSS)

MSS, obtained by careful selection of natural starters, are maintained, propagated and distributed by starter companies and research institutions (Parente and Cogan 2004). Like artisanal starters, MSS contain an undefined mixture of strains that differ in their physiological and technological properties (Parente and Cogan 2004).

When undefined cultures are propagated under controlled conditions with a minimum of subcultures, the stability of their composition and performance is greatly improved in comparison to natural strains (Stadhouders and Leenders 1984).

The composition of MSS is undefined, but their reproduction under controlled conditions reduces the intrinsic variability associated with the use of natural starters (Limsowtin *et al.* 1996).

The traditional method for reproduction of MSS, which requires several transfers to build up the bulk starter by using small amounts of stock cultures, has been replaced by the use of concentrated cultures for the inoculation of the bulk starter tank, thus minimizing the need for transfers within the factory and the risk of fluctuations in starter composition and activity (Carminati *et al*. 2010).

Defined strain starters (DSS)

DSS are composed of one or more strains (the dominant species of the traditional product) and are selected, maintained, produced and distributed by specialized companies. Since the strains and/or species ratio in DSS is defined, their technological performance is extremely reproducible and this is a desirable property. In fact, in recent years DSS have replaced traditional starters (Carminati *et al*. 2010). However, as a consequence of the limited number of strains used, a phage infection may cause disruption of lactic acid fermentation. Furthermore, with the subsequent loss of natural microbial diversity, maintenance of the typical features is difficult. Nevertheless, examination of the key properties of each strain (i.e. genetic or biochemical features, growth and acid-production characteristics) can lead to the rational mixing of strains, in order to formulate a culture with a desirable set of properties (Carminati *et al*. 2010).

DSS usually have no defects of flavour, and have a distinctive trait of 'cleaner' aroma and flavour. In order to increase control over their nature and attain a flavour as close as possible to the traditional one, industrial companies are making increasing use of flavour-enhancing adjunct cultures; DSS cultures are added at low levels to the starter, and may themselves be defined or undefined (Powell *et al.* 2011).

Metabolism of lactic acid bacteria

LAB are important in many food fermentations because they contribute to sensory characteristics and preservative effects (Holzapfel 1995) with their physiological features such as substrate utilization and metabolic capabilities. Some LAB are homofermentative and produce lactic acid as the main product of glucose fermentation, while others are heterofermentative and produce carbon dioxide and ethanol in addition to lactic acid (Blandino *et al*. 2003).

It is clear that LAB adapt to various conditions and change their metabolism accordingly. This may lead to significantly different end product patterns, thus LAB metabolism is essential to study when selecting new starter strains.

Lactose metabolism

Lactose, a disaccharide composed of glucose and galactose, is the only free-form sugar present in milk (45–50 g/L). The main pathways for lactose metabolism are shown in Figure 1.1.

The transport of lactose into a cell requires energy. In the lactococci, this energy is sourced via energy-rich phosphoenolpyruvate (PEP), an intermediate of the glycolytic pathway. This is part of a transport mechanism referred to as the phosphoenolpyruvate phosphotransferase system (PEP-PTS), in which the lactose is phosphorylated as it is transported across the cell membrane. Once inside the cell, phosphorylated lactose is hydrolysed by the enzyme phospho-β-galactosidase to glucose and galactose-6-phosphate. The glucose moiety enters the glycolytic pathway, and galactose-6-P is converted into tagatose-6-phosphate via the tagatose pathway. Both sugars are cleaved by specific aldolases into triose phosphates, which are converted to pyruvic acid at the expense of nicotinamide adenine dinucleotide (NAD^+). For continued energy production, NAD^+ must be regenerated. This is usually accomplished by reducing pyruvic acid to lactic acid (Poolman 1993).

In other dairy starter bacteria, including *Strep. thermophilus*, leuconostocs, lactobacilli and bifidobacteria, lactose transport appears to be via a specific protein (a permease) that translocates the lactose into the cell without modification,

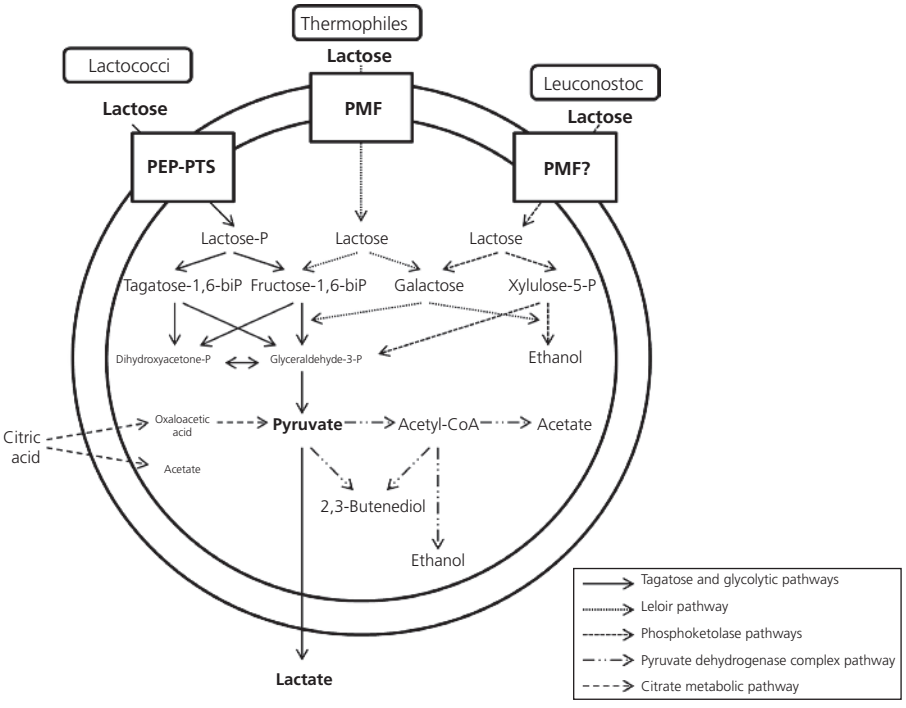

Figure 1.1 General pathways for carbohydrate catabolism by lactic acid bacteria.

although in many of these organisms the exact nature of the system used is still unclear. The lactose is then hydrolysed by β-galactosidase to glucose and galactose (Powell *et al.* 2011). The glucose moiety enters the glycolytic pathway, but galactose is excreted from the cells and accumulates in milk or cheese. Thermophilic lactobacilli that do not excrete galactose and *Lb. helveticus* strains utilize the Leloir pathway to metabolize galactose, while *Lb. delbrueckii* subsp. *bulgaricus* and most strains of *Strep. thermophilus* cannot metabolize galactose. This is a problem in cheese manufacture, since residual sugar can be metabolized heterofermentatively by other bacteria.

It is not known how lactose is transported in cells by *Leuconostoc* species or heterofermentative lactobacilli; however, lactose is known to be hydrolysed by β-galactosidase (Huang *et al.* 1995).

The galactose moiety is transformed into glucose-6-phosphate (Leloir pathway) and, together with glucose, is metabolized through the phosphoketolase pathway.

Lactic acid and ethanol, respectively, are formed during this metabolism to regenerate NAD^+; however, where lactococci are fermenting galactose or lactose at growth-limiting rates, products other than lactic acid can be formed from pyruvate. The enzyme pyruvate formate lyase is able to convert pyruvate to formate, acetate, acetaldehyde and ethanol under anaerobic conditions and at high pH (>7.0). Under aerobic conditions and at pH 5.5–6.5, pyruvate can be converted to acetate, acetaldehyde, ethanol and the minor products acetoin, diacetyl and 2,3-butanediol via the multienzyme pyruvate dehydrogenase complex.

Citrate metabolism

Citrate metabolism in LAB has been reviewed by Hugenholtz (1993). Milk contains 0.15–0.2% citric acid, but not all LAB can metabolize it. However, *Leuconostoc* species, Cit^+ *Lb. lactis* subsp. *lactis* and facultative heterofermentative lactobacilli do metabolize citric acid (Palles *et al.* 1998).

Many LAB use citrate as a substrate for cometabolism with sugars like glucose, fructose, lactose or xylose, providing NADH (citrate + 2 [H]/lactate + acetate + CO_2) (Hache *et al.* 1999) not directly as an electron acceptor, but as a precursor of acetate and oxaloacetate, which will be the final electron acceptor after being decarboxilated. Citrate metabolism is important in *Lc. lactis* and *Ln. mesenteroides* strains, which are often used in the dairy industry.

The latter organism was called *Streptococcus diacetylactis* in the old literature and more recently *Lc. lactis* subsp. *lactis* biovar *diacetylactis*. This name has no taxonomic status and the correct way to refer to it is citrate-utilizing (Cit^+) *Lc. lactis* subsp. *lactis*. Cit^+ strains of *Lc. lactis* differ from non-citrate-utilizing (Cit^-) strains because they contain a plasmid that encodes the transport of citrate. *Leuconostoc* species and Cit^+ *Lc. lactis* subsp. *lactis* strains utilize citric acid and lactose simultaneously and under certain conditions can derive energy via metabolism of citric acid.

Citric acid is transported into the cell by a citric acid permease, which is plasmid encoded in lactococci and *Leuconostoc* (Vaughan *et al.* 1995), and metabolized to pyruvic acid without generation of NADH. The result is an excess of pyruvic acid, which can be used to produce lactic acid to regenerate NAD^+, or in other reactions that regenerate NAD^+ and/or $NADP^+$.

The enzymes involved in these reactions are inducible and their expression is influenced by sugar concentrations and pH; in fact, a low amount of sugar and low pH favour diacetyl/acetoin formation.

Historically, there was a debate on which pathway was the most important. Evidence now clearly prefers the route via α-acetolactate, since α-acetolactate can be detected as an intermediate in cultures producing diacetyl and an α-acetolactate synthase has been identified in several LAB (Hugenholtz 1993).

Diacetyl contributes to typical yoghurt flavours and is produced by chemical decomposition of α-acetolactate (non-enzymatic). This reaction is favoured by aeration and low pH. Acetoin and/or 2,3-butanediol is produced in much larger amounts than diacetyl, but does not contribute to the aroma (Marshall 1987). Hugenholtz (1993) describes the use of genetic engineering to construct strains of lactococci able to produce high levels of diacetyl.

Nitrogen metabolism

Nitrogen metabolism by starters has an enormous impact on their activity and on cheese quality. LAB are fastidious microorganisms and are unable to synthesize many amino acids, vitamins and nucleic acid bases. Depending on the species and the strain, LAB require from 6 to 14 different amino acids (Kunji *et al.* 1996).

The proteolytic system of LAB is very complex and consists of three major components: a cell-wall bound proteinase that promotes extracellular casein degradation into oligopeptides, then peptide transporters that move peptides into the cytoplasm, where finally there are various intracellular peptidases that degrade peptides into smaller molecules and amino acids (Liu *et al.* 2010).

Proteolysis is a major event in cheese ripening: the proteolytic system of primary starter and secondary microflora contributes to the production of hundreds of flavour compounds through the synthesis of low-molecular-weight peptides and amino acids and their subsequent catabolism.

Free amino acids and peptides in cheese can contribute to flavour either directly or indirectly and with positive or negative effects. Cheese flavour development has been the subject of a comprehensive review (Smit *et al.* 2005). A major negative effect of proteolytic products is bitterness, which is believed to be caused by hydrophobic peptides ranging in length from 3 to 27 amino residues (Lemieux and Simard 1992). These peptides are believed to be generated

from casein principally by the joint action of chymosin and LAB proteinases (Broadbent *et al.* 1998) and can be hydrolysed to non-bitter peptides and amino acids by LAB peptidases. In particular, the enzymatic degradation of proteins (caseins) leads to the formation of key flavour components, which contribute to the sensory perception of dairy products.

LAB can catalyse reactions such as deamination, transamination and decarboxylation, and metabolism of their amino acids also contributes to the flavour. As an example, same strains of importance in bakery production convert glutamine to glutamate during sourdough fermentation, imparting taste to the bread (Gänzle *et al.* 2007). The expression of the arginine deaminase pathway in *Lactobacillus* spp. promotes higher production of ornithine, and thus enhances the formation of 2-acetyl pyrroline, which is responsible for the roasty note of wheat bread crumb (Gänzle *et al.* 2007).

The proteolytic activity is also important for other mechanisms; several antihypertensive peptides produced during milk fermentation have a strong activity against angiotensin I-converting enzyme (ACE), a dipeptidyl carboxypeptidase that plays a major role in the regulation of blood pressure within the renine angiotensin system (Riordan 2003), inducing blood pressure increase. *In vivo* studies evidenced a reduction of blood pressure after consumption of fermented milks (Pina and Roque 2008). Moreover, *in vitro* ACE inhibitory (ACEI) activity of different traditional fermented milks has been reported in the literature (Chaves-López *et al.* 2011). Thus, selection of microorganisms to be used in fermented products is gaining in importance, due to the inherent variations in their ability to produce bioactive peptides, particularly those with specific health claims (Ramchandran and Shan 2008).

Recently, LAB-induced proteolysis has been suggested as an efficient method for decreasing the toxicity of wheat and rye flours. Gliadins are among the most affected proteins by food fermentation and the extent of hydrolysis of monomeric gliadins (α-, β-, γ-, ω-gliadins) is strain specific (Di Cagno *et al.* 2002). Di Cagno *et al.* (2002) showed that selected proteolytic LAB could efficiently hydrolyse the 31-43 fragment of the toxic peptide A-gliadin. On the basis of these results, the same authors showed that selected LAB could completely hydrolyse the highly toxic 33-mer peptide over prolonged (12–24 h) and semi-liquid fermentation of a mixture of wheat and non-toxic flours. Breads produced with 12-hour sourdough fermentation retained acceptable quality and when consumed by coeliac individuals, no alterations in the baseline values could be observed. The selected LAB were also successfully used for the detoxification of other fermented foods (De Angelis *et al.* 2006).

A variety of fermented foods, especially protein-rich foods, may contain biogenic amines (BAs). During the fermentation process protein breakdown products, peptides and amino acids, used by spoilage and also by the fermentation microorganisms, represent precursors for BA formation (Bodmer *et al.* 1999). The consumption of foods with high concentrations of BAs can induce adverse

reactions such as nausea, headaches, rashes and changes in blood pressure (Ladero *et al.* 2010). Microorganisms suitable for food fermentation have been examined with regard to their potential to degrade histamine and tyramine (Fadda *et al.* 2001). A low potential for histamine and tyramine degradation among lactobacilli was noticed. In 35 well-known species with a practical function for the fermentation of dairy products and wine, Straub *et al.* (1995) observed a potential to form BAs only for a few strains.

Lipases and esterases

The lipolytic and esterolytic systems of LAB remain poorly characterized. Esterases from lactic acid bacteria may be involved in the development of fruity flavours in foods, and pregastric lipase and esterases are essential for the development of taste perception and typical flavour in Italian cheese. Microbial lipases and esterases may improve quality or accelerate the maturation of cheeses, cured bacon and fermented sausages. However, except for Parmigiano Reggiano, Pecorino and related Italian cheeses and blue cheeses, limited lipolysis occurs in cheese during ripening.

Lipolysis results in the formation of free fatty acids, which can be precursors of flavour compounds such as methylketones, secondary alcohols, esters and lactones. Generally, the role of LAB in lipolysis is less significant, but additional cultures, such as moulds in the case of surface-ripened cheeses, are often highly active in fat conversion. Flavours derived from the conversion of fat are particularly important in soft cheeses like Camembert and Roquefort (Smit *et al.* 2005).

Lipases are chemically defined as glycerol ester hydrolases (EC 3.1.1.3) that hydrolyse tri-, di- and monoglycerides present at an oil–water interface. Esterases (EC 3.1.1.6) hydrolyse esters in solution and may also hydrolyse tri- and especially di- and monoglycerides containing short-chain fatty acids (Medina *et al.* 2004). Esterases have been purified from several starter and LAB, including *Lc. lactis* (Chich *et al.* 1997), *Strep. thermophilus* (Liu *et al.* 2001) and *Lb. plantarum* (Gobbetti *et al.* 1997). All of them are serine enzymes that preferentially hydrolyse butyrate esters and are optimally active at pH 7. Some of them have no activity at pH 5.0; nevertheless, a very small amount of activity over a long time could result in significant hydrolysis of fat during cheese ripening. The major tributyrin esterase of *Lc. lactis* has been cloned, overexpressed and characterized (Fernandez *et al.* 2000).

Some probiotic strains of LAB can hydrolyse triglycerides, releasing most short- and medium-chain and essential fatty acids, which are valuable to today's health-conscious consumer. Medium-chain fatty acids (C6-C14), in particular, have become an accepted treatment for patients with malabsorption symptoms, a variety of metabolic disorders, cholesterol problems and infant malnutrition. These probiotic bacteria could alleviate lipase deficiency in the digestive tract during digestion (Medina *et al.* 2004).

Bacteriocins production

Bacteriocins are peptides produced by various bacteria that inhibit the growth of other bacteria. They could ensure the stability of fermented products, reduce microbial contamination during fermentation, inhibit the growth of moulds and prolong the microbiological spoilage time of baked goods (Juodeikiene *et al.* 2009).

In recent years, interest in starter/probiotic LAB has also grown substantially due to their potential usefulness as a natural substitute for food preservatives in the production of fermented foods with an enhanced shelf life and/or safety. *Lactobacillus* and *Lactococcus* include main strains with probiotic activity (Fuller 1989), producing bacteriocins (Altuntas *et al.* 2010).

The inhibitory host range and the molecular mass can be either large or small. Bacteriocins produced by LAB are divided into three classes: lantibiotics, small heat-stable non-lantibiotics and large heat-stable bacteriocins (Nes *et al.* 1996). Nisin, the best-known bacteriocin, is a lantibiotic that is produced by some strains of *Lc. lactis* and is used commercially in more than 50 countries as a food preservative to control the growth of spoilage and pathogenic bacteria.

Homofermentative *Pediococcus acidilactici* were isolated from spontaneous rye sourdoughs and characterized as producing pediocin Ac807 with antimicrobial activity against *Bacillus subtilis* (Narbutaite *et al.* 2008).

Exopolysaccharide production

Many food-grade microorganisms produce exopolysaccharides (EPS) (De Vuyst and Degeest 1999). EPS act as biothickeners and can be added to a variety of food products, where they serve as viscosifying, stabilizing, emulsifying or gelling agents (Tieking and Gänzle 2005).

They are divided into two classes: homopolysaccharides (HoPS), mainly glucan or fructans polymers; and heteropolysaccharides, with (ir)regular repeating units (De Vuyst and Degeest 1999). Heteropolysaccharide production is an important characteristic of many LAB involved in the production of fermented milks.

Lactic acid bacteria produce either homopolysaccharides, containing fructose or glucose residue, or heteropolysaccharides, composed of repeating units of several different sugars including glucose, galactose, fructose and rhamnose (De Vuyst *et al.* 2001). They may be involved in a wide variety of biological functions, including prevention of desiccation, protection from environmental stresses, adherence to different surfaces, pathogenesis and symbioses (Jolly *et al.* 2002). EPS-producing cultures have also been used to increase the moisture and improve the yield of low-fat Mozzarella cheese (Perry *et al.* 1998).

Glucan and fructans produced by fermenting LAB can strongly influence the quality of wheat bread in terms of bread volume and crumb firmness (Di Cagno *et al.* 2006). In particular, the production of EPS *in situ* is more effective than their addition (Brandt *et al.* 2003).

LAB can also produce gluco- or fructo-oligosaccharides (FOS), among which FOS, together with the fructan inulin, have been well described for their prebiotic effects (Biedrzycka and Bielecka 2004). In addition, the levan produced by *Lactobacillus sanfranciscensis* was proved to stimulate bifidobacterial growth *in vitro* (Dal Bello *et al.* 2001). In sourdough, *Lactobacillus reuteri, Lactobacillus acidophilus* and *Lb. sanfranciscensis* showed the ability to produce the prebiotic FOS 1-kestose (Tieking and Gänzle 2005).

Conclusion

The use of industrial starters has reduced the biodiversity and the organoleptic properties of fermented products. This phenomenon may be explained because the commercial availability of new, interesting starter cultures is very limited. Therefore, the selection of promising and wild strains from raw materials could be an interesting way forward. We can suggest at least three hot topics in selecting new LAB cultures: genome sequencing; interaction with natural microbiota; and functionality (Figure 1.2).

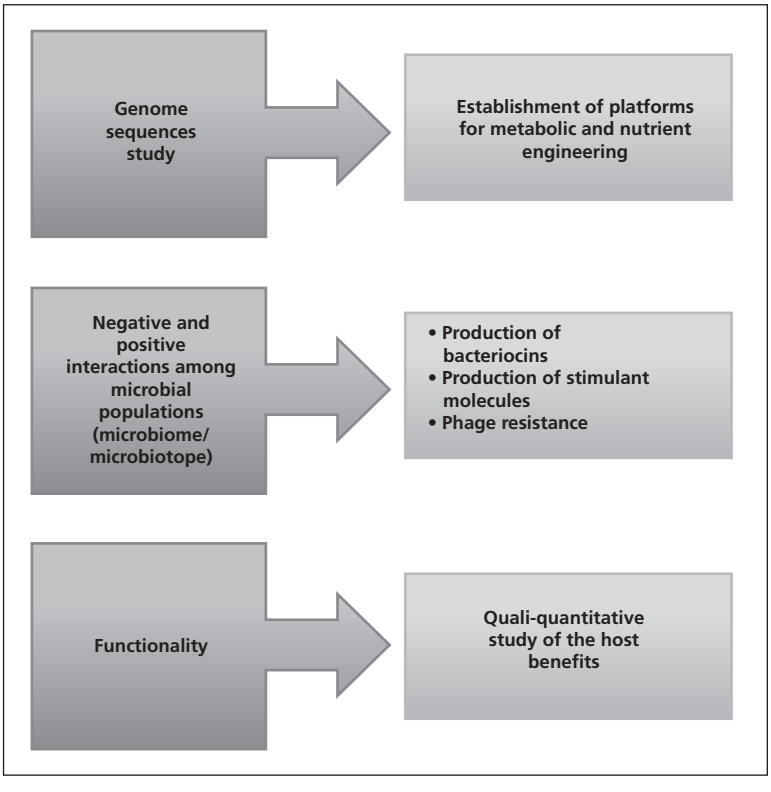

Figure 1.2 A new approach in the selection of microorganisms for innovative food purposes.

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