Characteristics and Role of Mesophilic Lactic Cultures

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SUMMARY

The use of lactococci is widespread and has the longest tradition in industrial mesophilic lactic cultures technology. The principal concern of the dairy industry is the reliability and stability of these cultures. This paper describes the major technologically important properties of mesophilic lactic cultures when they are used for fermentation of milk and the production of cheese. This paper includes some of the specific properties of lactococci strains which can help in making the right choice of strains, depending on the product being produced. For example, most dairy fermentation require rapid acid production, ability to produce desired changes in milk and lack of off-flavour production. Certain strains of lactococci have the ability to produce exopolysaccharides or bacteriocines thus contributing to sensory properties of dairy products. In addition, these bacteria make important contributions to proteolysis during ripening and to the development of cheese flavour.

KEY WORDS

mesophilic lactococci culture, aroma, flavour, , texture.

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Karakteristike i uloga mezofilne kulture bakterija mliječne kiseline

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SAŽETAK

Laktokoki u sastavu mezofilne kulture bakterija mliječne kiseline odavno se koriste u mljekarskoj industriji u proizvodnji različitih mliječnih proizvoda. Uspjeh fermentacije primarno je ovisan o upotrebljenoj kulturi od koje se zahtjeva osiguraje poželjnih svojstva na učinkovit i ponovljiv način. U radu su opisana glavna tehnološka svojstva mezofilne kulture laktokoka u fermentaciji mlijeka i proizvodnji sira. Navedena su i specifična svojstva određenih sojeva laktokoka koja mogu poslužiti kao kriterij u odabiru sojeva, za točno određeni mliječni proizvod. Općenito, od sojeva se zahtjeva brzo stvaranje mliječne kiseline i sposobnost stvaranja isključivo željenih promjena u mlijeku. Određeni sojevi laktokoka imaju sposobnost stvaranja egzopolisaharida ili bakteriocina i na taj način povoljno djeluju na senzorna svojstva proizvoda. Osim toga sojevi mezofilne kulture laktokoka imaju sposobnost proteolize nužne za tijek zrenja i formiranje okusa sira. analyses.

KLJUČNE RIJEČI

aroma, mezofilna kultura laktokoka, okus, tekstura

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INTRODUCTION

Mesophilic cultures which contain strains of lactococci are used in the dairy manufacturing of fermented products such as sour milk, cream, butter, buttermilk, fresh cheeses and many varieties of semi-hard cheeses. In other words, of products which are processed at moderate temperatures (~30^{0}C) of treatment (Tamime, 1985). There are two types of starter cultures which are highly selective and precisely defined, and the so-called traditional cultures, which are a poorly defined mixture of bacterial strains of unknown exact composition. (Petterson, 1988). Starter cultures are mostly a combination of two types of bacteria: acid producers and flavour producers. The acid is produced, regardless of the type of culture, by *Lc. lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis*, while the aroma is produced by *Lc. lactis* subsp. *lactis* biovar *diacetylactis* (Cogan,1984). Directly or indirectly, their metabolic products influence a wide variety of textures, flavours and the aroma of final products. They also participate in cheese ripening. These effects are manifested in the fermentation of lactose, reduction of the redox potential, citrate fermentation and protein hydrolysis (Poolman et al., 1995). For example, the production of fresh cheeses requires bacteria properties to be manifested during the first hours of production. Production of semi-hard cheeses, however, demands that bacteria be active throughout the maturing process which, for some types of cheese, takes months (Heap and Lawrence, 1988; Imhof and Bosset, 1994). Successful fermentation of the mentioned products, but also of other dairy products, depends on the selection of the most effective strains.

The aim of this paper is to present a synthesis of experience of the biochemical reactions occurring in fermented products as a consequence of growth and activity of mesophilic lactococci in milk, and to provide a description of criteria allowing for the selection of the most effective strains.

PRODUCTION OF LACTIC ACID, COMPONENTS OF FLAVOUR, AROMA AND TEXTURE

The metabolism of lactic acid bacteria (LAB) and interaction between selected strains are responsible for the production of lactic acid, coagulation of milk proteins and development of other different components which, taken together, provide the specific organoleptic properties of dairy products. Composition of milk, temperature, pH and the presence of oxygen are further factors contributing to the specificity of a product.

Lactic acid reduces the pH value to a level that inhibits the growth of many organisms that cause spoilage or disease and contribute to the flavour of a given product. Biochemically, lactic acid is produced through the combined action of more than 25 enzymes and proteins (Davidson and Hillier, 1995). In such a way, LAB generate energy, while lactic acid is its by- product (Monnet et al., 1996).

The texture of fermented products is a direct result of the production of lactic acid, and they can range from liquid, through semi-solid, to firm. Lactic acid decreasing pH value and though it destabilization of casein micelle into a soluble micellar calcium phosphate. When the pH reaches $\leq$ 5.3 the protein structure is irreversibly precipitated, thus causing the coagulation of milk proteins and the formation of gel or curd. The main difference between acid- and coagulant-induced milk gels is in the property of permeability. While gel induced by lactic acid does not change within a span of 24 hours after gelation, in coagulant-induced gels it increases continuously during the same period (Marshall and Tamime, 1997).

Reduction of pH in the production process of cheese improves the rapidity of whey separation from cheese curd, thus reducing moisture. Low pH and low moisture prevent development of microorganisms causing spoilage (Fox and McSweeney, 1997). At the same time, when used in the production of other fermented products and fresh cheeses, lactic acid gives them a mildly sour taste.

The second important property of the culture is the ability of proteolysis. When used as a culture, lactococci have a significant influence on flavour development in fermented dairy products through the proteolytic system, which is essential to their growth in milk (Law and Haandrikman, 1997). Small peptides and free amino acids, released by hydrolysis, are precursors of the flavour and aroma components of fermented products. Additionally, proteolytic enzymes are responsible for the hydrolysis bitter peptides responsible for bitterness in cheeses (Visser, 1993; Taiwan Tan et al., 1993; Exterkate, 1995). The differing levels of individual proteolytic enzymes in the strains of species *Lc. lactis* can also have an influence on various maturing processes of the same types of cheese (Smid et. al., 1991; Crow et al., 1994; Jullard et. al., 1995). The level of enzyme activity declines with the increase of peptides in milk through technological processes. The reason lies for this in the transcription regulation of proteinase PrtP, which is initiated, or rather controlled, by the peptide content in the media (Meijer and Hugenholtz, 1997). The quality of some fermented products depends on the strains of *Lc. lactis*, subsp. *lactis* biovar *diacetylactis* with ability to produce flavouring compounds and CO_2 by citrate utilization. These are acetate, and C4 compounds: diacetyl, acetoin, and 2.3-butylene glycol, which are responsible for the characteristic aroma of sour cream, butter, buttermilk and fresh

cheese, and CO_2 which is responsible for the texture of some fermented dairy products (Libudizisz and Galevska, 1991; Cachon and Divies, 1993; Cogan, 1995).

Some strains of the *Lc. lactis* species produces exopolysaccharides that modify the texture of the product by increasing its viscosity (Teuber, 1995; Marshall et al., 1995; Cogan et al., 1997). Certain strains of lactococci have the ability to produce a range of bacteriocins which help in the control of certain phatogens and spoilage bacteria in fermented milk products, thus contributing to their sensory properties (Piard et al., 1990).

Through their own metabolic activity, lactococci also directly improve keeping quality, and with regard to health they reduce symptoms of intolerance to lactose and balance the intestinal population (Buttris, 1997).

CRITERIA FOR SELECTION OF STRAINS

The culture used for the fermentation of dairy products is defined as a medium containing specific lactic acid bacteria that are inoculated into milk. Their metabolism have to ensure a microbiologically safe product that defined organoleptic and structural properties in an efficient and repeatable manner (Tamime, 1985). There are two types of mesophilic cultures of the lactic acid bacteria that are used in the dairy industry. The first is traditional and consists of partially defined bacterial species and strains (Petterson, 1988). Traditional mesophilic culture of such unknown composition is still being used in many countries, particularly in Europe and North America. The reason for this lies in its lesser sensitivity to bacteriophages (Crawford and Galloway, 1962; Galeslot et al., 1966; Stadholders, 1975). The stability of this type of culture is also attributed to the symbiotic relationship between species and strains and maybe the fact that those strains are more dependent on their own synthesis of amino acids (Dahiya and Speck, 1962; Weerkamp et. al. 1996; Ayad et. al.,1999.). The second type of culture is a defined or selected culture containing one or more identified strains with specific, but known, properties. Demand for a defined, stable culture imposed itself as a consequence of the rapid development of milk processing technology, which prompted producers to demand a culture with strains possessing foreseeable properties in selected fermentation processes (Leenhouts et al., 1990; Marshall, 1991).

Specific criteria for selection include rate of acid production capacity for polysaccharides production, limited proteolysis, and ability to produce aromatic components. The other criterion for culture selection is based on the ability of the lactic acid bacteria to increase the nutritional and/or physiological value of food. Ultimately, success depends on the genetically stable strains, particularly with regard to the above-mentioned properties (Leenhouts et al., 1990). Stable culture gives the precisely defined quantity of lactic acid, and therefore develops the desired flavour and consistency of the product. The strategy aimed at achieving a stable culture involves the application of established techniques, with special attention being paid to the characterization of strains and the application of molecular genetic methods. Although molecular genetic methods have been applied in the identification and characterization of bacteria only in the past ten years or so, results are staggering. The use of such techniques makes it easier to create a culture with desirable properties (Kok et al., 1985; Nakamura et al., 1992; Powell et al., 1994; Davidson and Hillier, 1995). At the molecular level, the selection of desirable strains demands that the microbiologist possesses significant knowledge, both in the field of individual properties of strains and in the ways they react in a community with other strains or other species. The combination of strains from a mixed lactococci culture demonstrates a different interaction in the course of their growth in milk. The effect varies from a significant stimulatory effect to inhibition (Dahiya and Speck, 1962), which is why a mixed culture, used in milk fermentation, must be tested for compatibility (Salama et al., 1991). A strain with ability to produce bacteriocin can, in mixed cultures, become a dominant one, influencing microbiological balance and preventing development of a product with desired properties (Piard et al., 1990). In opposite, right choise of the bacteriocin producing strains in culture used in cheese manufacture can decrease ripening time and improve sensory evaluation scores (Morgan et. al., 1997).

The success of fermentation depends primarily on rapidity and a predictable rate of acid production (Davidson and Hillier, 1995). This is particularly important in the production of fresh and soft cheeses (Collins, 1977; Gripon, 1997). Thus the culture which contains strains that curdle skimmed milk in 16 hours or less are regarded as good culture for such products (Anderson and Elliker, 1953). Slow milk coagulation as a consequence of the slow strains in the culture can be result of the limited advailability of utilizable amino acids that does not allow growth to reach high cell densities (Smid et al.,1991; Wang et al., 1998).

So-called slow cultures also originate through a process involving the extended use of cultures of different strains of *Lc. lactis* subsp. *cremoris*. After about 100 re-inoculations to a fresh medium the presence of protease negative variant (Prt-) increase to 90-98% of total population. In a pure culture of *Lc. lactis* subsp. *cremoris* strains the Prt- variants occur spontaneously (Hugenholtz et al., 1987).

However, the speed at which it loses proteolytic activity is relatively high, which results in at least 2% of slow strains in relation to the fast strains, which is approximately 10,000 more than 1 in 10^6 of spontaneous mutations (Citti et al., 1964). The process of loss plasmid that encoding proteolysis is irreversible, and it would appear that cells reject it when they no longer require it in the medium (Otto et al.,1982). The culture containing up to 92% of Prt- variants can still sour the milk, but acidification is slow (Hugenholtz et al., 1987). Additionally, many strains of *Lc. lactis* subsp. *cremoris* are ineffective acid producers (Collins et al., 1962; Collins, 1977). In addition to the generated lactic acid, the rate of growth and the proteolytic activities among the strains also differ (Hugenholtz et al., 1984; Stadhoulders et al., 1986; Bruinberg and Limsowtin, 1995). Proteolytic activity plays an equally important a role in the creation of flavour during the maturing period of a cheese. But it is important to know that the Prt- variant can spontaneously become dominant in a culture, regadless of proteolytic activity, if the pH of the medium is ≥ 6 (Otto et al., 1982).

One of the alternative methods of selecting the Prt+ variants is to limit certain amino acids in the medium (Hugenholtz et al., 1987). Proteinase of the Prt+ strains seems to be essential for the hydrolysis of milk proteins and development of flavour. A correlation exists between proteolysis and the production of acid. Rapid strains are four times more numerous in the extended logarithmic phase, and have a four times stronger proteolytic activity. This suggests a possible direct relationship between available nitrogen and the overall quantity of acid produced in the culture. The initially selective strains depend on the available source of nitrogen. When this source is a protein that has not broken down, as is the case with milk, the organism becomes dependent on its own proteolytic enzymes (Citti et al., 1964).

Mesophilic mixed-strain cultures of lactococci manifest population dynamics which cause changes in the composition of the culture. Some of the interaction between different strains occurs extremely quickly. The appearance of nisin or bacteriocin from more than one strain in a culture leads to significant changes in the population. Should the other cells be susceptible to nisin, the nisin producer will become dominant. If the strain in question possesses desirable properties, nothing undesirable will occur in the production of cheese. However, if that strains are produces of bitter peptides, such a culture is unacceptable (Hugenholtz, 1986). Bitter peptides can also be the result of the inability of certain strains to break them down following the release of such peptides from milk protein (Lowrie, 1977). Apart from the absence of aroma, the appearance of bitter peptides remains one of the main problems in cheese production (Bruineneberg and Limsowtin, 1995). In the same way, if the ratio between diacetyl and acetaldehyde in a mixed culture used for cream and butter is lower than 4:1, a yoghurt flavour will appear (Lindsay and Day, 1965).

Agglutination also has an influence on dairy product quality and yield. This occurs as a consequence of interaction between the bacteria of pure cultures and the agglutinin (lactenin-1–the fat globule agglutinin, lactenin-2-lactoperoxidase, lactenin-3-natural immunoglobulins), resulting in long chains and clumps of cells. The consequence is the slow appearance of acid and an inhibitory effect on proteases. The Prt - strains form long chains more often than do Prt+ strains. The strains resistant to agglutination appear in pairs and in shorter chains and have a more prominent proteolytic activity (Irahim, 1995). Generally speaking, the strains with the lowest tendency for agglutination, i.e. those characterized by a heavy coagulum among the *Lc. lactis* strains, are *diacetylactis* strains, where agglutination is most frequent, followed by *cremoris* strains and *lactis* strains (Gosling et al., 1995). It is therefore important in cheese production to choose those cultures consisting of strains with a less tendency to produce heavy coagulum.

The infection of a culture with bacteriophages decelerates the fermentation process and is undoubtedly the most serious problem faced by the dairy industry. Analyses of strains not susceptible to bacteriophages led to the discovery and characterization of a large number of genetic systems ensuring the best resistance to bacteriophages (Fabrizio et al., 1992; Garvey et al., 1995).

The basic problem encountered by scientists in their efforts to select strains is the plasmid encoding of the most important property of *Lc. lactis* species. Bearing in mind the exceptional economic importance of the metabolism of lactose, protein and citrate, and of the resistance to bacteriophages, it is not surprising that numerous scientific researches have focussed on molecular-genetic methods which would ensure the production of strains with stable properties (McKay and Baldwin, 1978; McKay et al., 1980; Anderson and McKay, 1983; Fabricio et al., 1992; Garvey et al., 1995, 1996). There are basically two approaches to the set aim. One is the stabilisation of the plasmid-encoded genes in the chromosome through replacement recombination and the stabilisation of these genes by incorporation of the plasmids in the chromosomes by Campbell-like integration, (Leenhouts et al., 1989; Venema, 1993). Generally speaking, genetic manipulation enables one to achieve the inactivation of a gene or its expression (Guchete et al., 1990; Wells et al., 1993), thereby bringing a direct influence to bear on flavour, aroma and texture, or rather the quality,

of fermented products. Genetic manipulation of lactococci has been made possible through definition of their IS-elements (insertion sequence). Three lactococci IS-elements, ISS1, IS904 and IS981, are linked to the metabolism of lactose and saccharose, to proteinase activity, to nisin production, conjugal transfer determinants and to bacteriophages resistance (Walsh and McKay, 1981; Romero and Klaenhammer, 1993,). IS-elements carry only those genes responsible for insertion, and since they are also present both in the plasmid and chromosome DNA, it is believed that they are responsible for the insertion of plasmids into a chromosome (Brkić et al., 1994).

CONCLUSION

Since about 3200 BC, people have been using lactic acid bacteria for the production of products with various tastes and textures, which differed from the original raw material, without understanding the scientific basis of their effect (Kurman, 1984; Stiles and Holzapfel, 1997). In contrast, the successful production of fermented dairy products today is inconceivable without the use of the most efficient strains with precisely defined and predictable properties and characteristics. Selection of the best strains for a specifically determined type of fermentation is made possible through the genetic modification. However, due to the exceptional complexity manifested by the *Lactococcus lactis* strains comprising the mesophilic culture, even genetic modification cannot always be a guarantee of success. That is why projects focusing on the genetic and technological research of those organisms are always topical.

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