EXTENSION OF SHELF LIFE OF DAIRY PRODUCTS BY BIOPRESERVATION: PROTECTIVE CULTURES

SÜT VE SÜT ÜRÜNLERİNİN RAF ÖMÜRLERİNİN BİYOLOJİK KORUMA İLE UZATILMASI: KORUYUCU KÜLTÜRLER

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ABSTRACT: Protective cultures are food-grade bacteria, which may or may not be strains naturally present in the foods. They are selected for their ability to grow in a product and inhibit a pathogen or undesired microorganism rather than to deliver a desired texture and flavour profile as in fermentation, or health benefits of probiotics. Protective cultures inhibit undesirable microorganisms through the production of low molecular mass compounds, such as organic acids, carbon dioxide, diacetyl, hydrogen peroxide and bacteriocins. This article describes the using possibilities of protective cultures in dairy products with their advantages and limitations. We reviewed previously published data and present new findings that relate to their usage potential for dairy products.
Key words: Dairy foods, food preservation, protective culture

ÖZET: Koruyucu kültürler gıdalarda doğal olarak bulunabilen yada bulunmayan gıda üretiminde kullanıma uygun bakterilerdir. Söz konusu kültürler fermantasyonda istenilen doku ve lezzet profilini sağlama ya da probiyotik olan türlerin sağlığa yararları etkilerinden daha çok bir ürün içinde gelişme kabiliyeti ve patojen ya da istenmeyen baterileri inhibe etme potansiyellerine göre seçilmektedirler. Koruyucu kültürler, ürettikleri organik asitler, karbondioksit, diasetil, hidrojen peroksit ve bakteriyosinler gibi düşük moleküler ağırlıklı bileşikler sayesinde istenmeyen mikroorganizmaları inhibe etmektedirler. Bu makale, koruyucu kültürlerin süt ve ürünlerinde kullanım olanaklarını avantajları ve sınırlamaları ile anlatmaktadır. Çalışmada daha önce yayınlanmış veriler derlenmiş ve söz konusu kültürlerin süt ve ürünlerinde kullanım olanakları ile ilişkili yeni bulgular verilmiştir.
Anahtar kelimeler: Süt ve süt ürünleri, gıda muhafaza, koruyucu kültür

INTRODUCTION

Microbial activity is the first and most dangerous limitation of a food's shelf life. Despite improved manufacturing facilities, safety concepts and implementation of effective process control procedures such as HACCP in dairy industry, the undesired microorganisms (pathogen and spoilage) are still risk factors for dairy products. In the dairy industry today, heat processing is the most prevalent method of preserving quality and subsequently, the shelf life of highly perishable dairy foods (1). Refrigeration is also a preservative-free method against microbial activity, which traditionally has been applied to extend the shelf life of dairy products (2).

In addition to traditional preservation methods (heat treatments, salting, acidification, drying, and chemical preservation), new and various shelf life-extending technologies have been identified and transferred to the dairy industry including high hydrostatic pressure, pulsed electric fields, new packaging systems, natural antimicrobial compounds, and biopreservation. May be the most effective reason for trying to manipulate both

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physical and biological new prevention techniques to food and/or dairy industry is consumer demands. However consumers have been consistently concerned about possible adverse health effects from the presence of chemical additives in their foods. As a result an increasing number of consumers favour minimally processed foods including dairy products with few chemical preservatives (3,4,5). On the other hand it's important to indicate that extending shelf life of dairy products has considerable advantages from financial point of view.

At this point, biopreservation in extending the shelf life of dairy products seems rather applicable than other technologies according to suitability of process steps and product variety. In biopreservation, storage life is extended and/or safety of food products is enhanced by using natural or controlled microflora, mainly lactic acid bacteria (LAB) and/or their antibacterial products (2,5,6,7,8,9). Antagonistic cultures that are only added to inhibit pathogens and/or prolong the shelf life, while changing the sensory properties of the food product as little as possible, are termed protective cultures (PCs) (7,8,9). Similar to probiotic and starter cultures, PCs are food-grade bacteria, which may or may not be strains naturally present in the food type. They are selected for their ability to grow in a product and inhibit a pathogen or undesired microorganism rather than to deliver a desired texture and flavour profile as in fermentation by starter cultures or health benefits of probiotics. PCs should not affect sensorial qualities of product under normal storage conditions and, are of unique advantages such as additive free preservation, "natural" image, temperature responsive inhibition and production of bacteriocins by viable cultures during storage. PCs inhibit undesirable microorganisms through the production of low molecular mass compounds, such as organic acids (e.g. lactic, acetic and propionic acid), alcohols, carbon dioxide, diacetyl, hydrogen peroxide, bacteriocins, reuterin and reutericyclin. Many of these metabolites have a broad activity spectrum against to other species, and their production is largely affected by the food matrix itself (2,3,5,7,8,10).

This review focuses on some properties of protective cultures that contribute to their roles in biopreservation of dairy products and their using possibilities with advantages and limitations. We reviewed previously published data and present new findings that relate to their usage potential for dairy products.

INHIBITORY METABOLITES OF PCs

The genera *Lactococcus, Streptococcus, Lactobacillus, Pediococcus, Leuconostoc, Enterococcus*, and *Propionibacterium*, play an essential role in food fermentations given that a wide variety of strains are routinely employed as starter cultures in the manufacture of especially dairy but also meat and vegetable products (11). Although their first responsibility is producing a fermented food by accelerating and steering its fermentation process (12), some strains among them (PCs) become observed only by their antimicrobial mechanisms for biopreservation including production of organic acids, hydrogen peroxide, carbon dioxide, diacetyl, board-spectrum antimicrobials such as reuterin and reutericyclin.

Organic acids, acetaldehyde and ethanol

The antimicrobial effects of PCs by organic acids such as lactic, acetic and propionic acid are well documented. The antagonism of these weak acids is believed to result from the action of them on the bacterial cytoplasmic membrane which interferes with the maintenance of membrane potential and inhibits active transport and may be mediated both by dissociated and undissociated acid (13). The inhibitory effect of undissociated organic acids is 10-600 times stronger than of their dissociated forms: the extent of dissociation is directly determined by the pH (14).

The antimicrobial activity of each acid at a given molar concentration is not equal. Acetic and propionic acids have higher pKa values (Table 1) than lactic acid and are more effective on yeasts, moulds and bacteria (3,13,15,16,17).

Table 1. The pKa values and inhibitory organisms of weak organic acids (15)

Organic Acid	pKa	Organisms inhibited
Acetic	4,75	Bacteria except lactics and *Acetobacter* species; most moulds
Lactic	3,90	Bacteria (at low pH)
Propionic	4,90	Yeasts-Moulds and Bacteria

Beside these organic acids, the concentration of benzoic acid significantly exceeds the naturally expected value in some fermented dairy products and ranges from 7-11 mg/kg for Gouda cheese, and up to 13-56 mg/kg for low fat yoghurt. This situation is illustrated with the metabolism of hypuric acid by some LAB e.g. *L. casei* and constitutes an additional biopreservation in these products (3).

The contribution of acetaldehyde to biopreservation is minor since the flavour threshold is much lower than the levels that are considered necessary to achieve inhibition of microorganisms. Similarly, although ethanol may be produced by protective cultures, again the levels produced in food systems are so low that the contribution to antibiosis is minimal (13).

Hydrogen peroxide

Another inhibitory metabolite produced by PCs is hydrogen peroxide. Susan ve Nettles (1993) reported that hydrogen peroxide accumulates mainly in cultures of lactobacilli, leuconostocs and pediococci. The way of inhibition attributed to a strong oxidising effect on membrane lipids and to the destruction of basic molecular structures of cellular proteins (13,17). The amount of hydrogen peroxide produced is variable depending on the strain and the presence of oxygen (14).

Diacetyl

Diacetyl which is a product of citrate metabolism has a wide antimicrobial activity spectrum at concentrations of 300-1000 ppm and low pH, but because it produces a marked butter taste and aroma approximately at 2-4 ppm its contribution to biopreservation is limited. Its mode of action is believed to be due to interference with the utilisation of arginine. Diacetyl which is mainly produced by lactic acid bacteria including strains of *Leuconostoc, Lactococcus, Pediococcus* and *Lactobacillus,* is more affective on Gram-negative bacteria, yeasts and moulds than Gram-positive bacteria (13,14,17).

Reuterin and Reutericyclin

Reuterin (β-hydroxypropionaldehyde), a broad-spectrum antimicrobial substance originally produced during stationary phase by the anaerobic growth of *Lactobacillus reuteri* on a mixture of glucose and glycerol or glyceraldehyde, is one of the most intensively studied low-molecular-mass inhibitory compounds of LAB. It is effective against viruses, fungi and protozoa as well as Gram-negative and Gram-positive bacteria, however is not formed in sufficient amounts in the presence of sugars. There is a common estimation that the antimicrobial effect of reuterin is due to the inhibition of ribonucleotide reductase (12,13,14,17).

Reutericyclin is tetramic acid derivative produced by sourdough isolates of *Lactobacillus reuteri.* It is bacteriostatic or bactericidal to many food-related spoilage and pathogen Gram-positive bacteria at concentrations about 0.1-1 mg/L whereas yeasts, fungi and Gram-negative bacteria (because of permeability barrier) are resistant to reutericyclin. Since it is a highly hydrophobic, charged molecule, studies on its mode of action were based on the hypothesis that the cytoplasmic membrane is its cellular target (18,19,20).

Bacteriocins as biopreservatives

The term 'bacteriocin' comprises a large and diverse group of ribosomally synthetized extracellular antimicrobial low molecular mass proteins or peptides which have a bactericidal or bacteriostatic effect on other closely related bacteria (3,9). The target of bacteriocins is the cytoplasmic membrane and because of the protective barrier provided by the lipopolysaccaride and lack of phospholipids at the outer membrane of Gram-negative bacteria, they are generally effective against only Gram-positive organisms (2,13).

Since LAB are used as starter cultures in fermented milk products; today, there is an increasing effort to adapt bacteriocin producers to dairy industry as biopreservatives because of their microbiological, physiological and technological advantages. Bacteriocins produced by LAB are commonly divided in to three or four classes (Table 2). Bacteriocins of the first class are known as lantibiotics (<5kDa) which characterized by their unusual amino acids, such as lanthionine, methyl-lanthionine, dehydroalanine and dehydrobutyrine (5,11,13,21).

Class II includes small (<10kDa), heat-stable, non-lanthionine containing peptides and is divided into there sub-groups. The most common Class IIa comprised of pediocin- like peptides especially effective on *Listeria*. Two peptid bacteriocins which requiring both peptides to be fully active compose Class IIb where as Class IIc includes sec-depended secreted bacteriocins (5,11,13,21).

Finally the last class includes large molecular (>30kDa) proteins which are sensitive to heat and not well characterized (5,13,21).

COMMERCIAL APPLICATIONS OF PCs

Rodgers (2003) reviewed that PCs effective to *Listeria* spp. and *Clostridium* spp. (*Lactobacillus rhamnosus*, *Propionibacterium freudenreichii* spp. *shermanii* and pediocin-producing *L. plantarum*) are commercially available in Australia for applications in yoghurt, semi-hard cheese, Emmental and quark(8). Recently some commercial products have been developed as PCs and/or shelf life extenders described below.

Microgard™

Microgard™ (Wesman Foods, Inc., Beaverton, USA) is the pasteurized product of the fermentation of skim milk by *Propionibacterium freudenreichii* ssp. *shermanii* and its protective action has been associated with diacethyl, propionic, acetic and lactic acid and probably due to a heat stable peptide with a molecular mass of about 700 g/mol. Microgard™ inhibits Gram-negative bacteria such as *Pseudomonas*, *Salmonella* and *Yesinia* as well as yeast and moulds but not Gram-positive bacteria including *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*. It has been approved by the Food and Drug Administration for use especially in Cottage cheese and fruit flavoured yoghurt (4,13,14,15,22,23,24,25). As reviewed by Susan ve Nettless (1993) and Holo ve ark. (2002), 30 % of the cottage cheese produced in the United States was made with Microgard™ as a biopreservative (24).

Table 2. Classification of bacteriocins produced by LAB*

Class	Subclass	Description	Group representatives
I	-	Lantibiotics, small (<5kDa) and heat stable peptides containing unusual amino acids	Nisin Lacticin 3147
II	IIa	Pediocin-like, small (<10kDa) and heat stable peptides	Pediocin PA-1/AcH Leucocin A, Enterocin A
	IIb	Two peptide bacteriocins	Lactococcin G and M
	IIc	Sec-dependent secretion of bacteriocins	Acidocin B Enterocin P and B
III	-	Large molecule (>30kDa), sensitive to heat	Helveticin J

*Adapted from 5,13 and 21.

Salih ve Sandine (1990) observed that Microgard™ inhibited yeasts and preserved commercial yoghurts for over 82 d. at 5°C (22). The same yoghurt samples were also protected from spoilage by Gram-negative psychrotrophs which grew out following pH increases as a result of yeast growth. They also indicate an evident shelf-life extension up to 6-9 days for commercially produced cottage cheese by inhibitory effect of Microgard™ against the same group of bacteria.

In another study, Al-Zareky ve ark. (1991) reported the effectiveness of Microgard™ in inhibiting food spoilage organisms as well as pathogen bacteria (23). They found 100 % inhibition at lower concentrations for all Gram-negative bacteria except *E. coli.* Although Microgard™ was not able to retard the growth of Gram-positive bacteria, variable results were determined for *Listeria monocytogenes*. Researchers stated that preventing activity of Microgard™ can be changed depending on food composition and especially pH (Opt.: 5,3).

Bioprofit™

Another commercial product is Bioprofit™ (Valio, Helsinki, Finland) which contains *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* JS. Used as a protective culture (10^7 cells per gram) the product is reported to inhibit yeasts, moulds in dairy products and *Bacillus* spp. in sourdough bread. The mode of effect is not completely investigated but the protective effects are due to synergistic activities between the two strains (4,13,24,25,26).

A later report claims that when Bioprofit™ used as a protective culture for the production of quark or yoghurt the growth of added yeast is inhibited. Initial levels of $2x10^7$ cells/g of each strain prolonged shelf life, did not interfere with yoghurt starters and improved the sensory qualities of quark (26).

Bioprofit™ can be used in a product range from fermented milks to silage, but only *Lactobacillus rhamnosus* LC705 must take place in semi-hard and hard cheese applications.

ALTA™ 2341, ALC 01 and FARGO™ 23

ALTA™ 2341 (Quest International, USA) is produced from *Pediococcus acidilactici* fermentation and has to rely on the inhibitory effects of natural metabolites, including organic acids and the bacteriocin pediocin. Since its characterization in 1987, there has been considerable scientific literature published supporting the effectiveness of pediocin against Gram-positive bacteria, including *Listeria monocytogenes.* ALTA™ 2341 can serve as an effective barrier to help control the development of *Listeria* in dairy products and successful results were obtained for Queso Blanco type cheese applications (27).

ALC 01 (Niebull, Germany) is also a patented antilisterial culture developed especially for soft cheese production. Its protective activity belongs to pediocin which formed by *Lactobacillus plantarum.* According to supplier, ALC 01 inhibits the growth of Listeria on the surface of artificially and/or naturally contaminated Munster cheese after spray treatment (10^6 cfu/ml).

FARGO™ 23 (Quest International, USA) includes the same metabolites as for ALTA™ 2341, but contains in more the live culture producing pediocin. In France, it is added to raw milk intended for raw milk cheese production.

CONCLUSIONS

In recent years there has been growing interest in the use of bacteria and/or their products such as organic acids, and bacteriocins, as natural preservation agents. PCs are used in the controlled microflora applications. The potential application of PCs as consumer friendly biopreservatives is important. Acceptance of PCs applications will depend on careful selection and application of suitable PCs. The most practical way of a PC delivery is in a freeze-dried form. PC applications could be supply some advantages such as (i) improving the safety of the product without changing processing parameters, (ii) decreasing the severity of processing, (iii) increasing storage temperatures, or extending the product shelf-life, thus reducing waste and increasing convenience. The application of PCs does not require special equipment. Unlike chemical preservatives, PCs are added during or at the end of processing and their microbiological status and aseptic/hygienic handling is critical to prevent contamination of the final product. More research is needed on the usage possibilities of PCs in dairy industry.

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