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The Use of Bacteriocins Produced by Lactic Acid Bacteria in Food Biopreservation

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ÖZE

Laktik Asit Bakterilerince Üretilen Bakteriyosinlerin Besin Maddelerinin Korunmasında Kullanımı

Metabolik yan ürünler, antibiyotik benzeri maddeler ve bakterisidal peptit yapısında olan ve özelikle son yıllarda oldukça fazla çalışılan bakteriyosinler laktik asit bakterileri (LAB) tarafından üretilen antagonistik maddelerdir. LAB tarafından üretilen bakteriyosinler gıda bozulmasını ve gıdalarda üreyebilen patojen bakterilerin kontrolünü sağlamak amacıyla gıda koruyucu maddesi olarak kullanılma potansiyeline sahiptirler. Bu derleme gıdaların uzun süre korunması için bakteriyosinlerin kullanım potansiyelini ve bununla ilgili son durumu özetlemektedir.

Anahtar kelimeler: Lactik asit bakterisi, bakteriyosin, gıda korunumu

ABSTRACT

Lactic acid bacteria (LAB) produce a variety of antagonistic factors that include metabolic end products, antibiotic-like substance and bactericidal proteins, termed bacteriocins that have recently come under detailed investigations. Bacteriocins of LAB have potential for use as food biopreservatives to control spoilage and pathogenic bacteria. This paper reviews the current status and potential use of bacteriocins for preservation of foods.

Key words: Lactic acid bacteria, bacteriocins, food preservation

GİRİŞ

From past to present: History of food preservation

Although it has been suggested that the food industry started about two million years ago, it is generally assumed that food fermentations developed since the Neolithic times when humans adopted a lifestyle that allowed agriculture to develop. Ever since it is likely that lactic acid bacteria (LAB) have played an important role in the preparation and preservation of fermented foods, although based on recorded history this can be traced back only a few millennia. This time frame is important in considering the extent to which lactic acid bacteria have adapted to their new ecoligical niche, that is, the food environment. In view of the fact that traditional food fermentations, and even modern, large-scale production processes, are operated under nonsterile conditions, it is no surprise that many LAB produce antagonistic compounds that increase their competitive value. This century has been a major effect in describing, cataloging, and characterizing the wide variety of antagonistic compounds produced by lactic acid bacteria. LAB produce lactic acid or lactic and acetic acids, and they may produce other inhibitory substances such as diacetyl, hydrogen peroxide, reuterin (ß-hydroxypropionaldehyde) and bacteriocins (1). Several food-grade lactic acid bacteria, used in food fermentation, are known to have these antimicrobial properties. They provide safety and shelf-stability to the fermented foods. It was assumed that since cells and metabolites of these bacteria have been consumed through different fermented foods for thousands of years without any health hazard, use of these antimicrobial metabolites of LAB may be approved by the regulatory agencies. LAB that grow as the adventitious microflora of foods or that are added to foods as cultures are generally considered to be harmless or even an advantage for human health (probiotics) (2). In

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addition, health conscious consumers view fermented foods and some LAB as natural and healthy since in the United States, they are afforded generally regarded as safe (GRAS) status. Some of the metabolites of the LAB and other starter culture bacteria have already been permitted for use in foods as food additives. Examples are lactic acid, diacetyl, propionic acid and acetic acid. Also, the bacteriocin, nisin, of Lactococcus lactis, has been approved as an antimicrobial biopreservative for use in some foods, particularly some dairy foods in many countries. Besides, pediocin PA-1 of Pediococcus acidilactici strain is the example of bacteriocin from LAB that has found practical applications as food preservative (3).

Bacteriocins which are produced by many food-grade LAB are ribosomally-produced, precursor polypeptides or proteins that, in their mature (active) form, exert an antibacterial effect against a narrow spectrum of closely related bacteria and to which the producer strains show immunity (4-7). Due to the stability of the antibacterial action at high heat and in the environment of many foods, there is an interest in using bacteriocins of lactic acid bacteria as food biopreservatives. While most bacteriocins produced by LAB have a narrow antibacterial spectrum, others are akcive against closely related species and against Listeria and Enterococcus species. Among those with wide antibacterial actions against different spoilage and pathogenic Gram-positive bacteria including Clostridium botulinum, the lantibiotic nisin of some strains of Lactococcus lactis subsp. lactis, and nonlanthionine bacteriocins, pediocin PA-1 and pediocin AcH of Pediococcus acidilactici strains have been thoroughly studied. Nisin and pediocin PA-1 are examples of bacteriocins from lactic acid bacteria that have found practical applications as food preservatives (3).

Currently, artificial chemical preservatives are employed to limit the number of microorganisms capable of growing within foods, but increasing consumer awareness of potential health risks associated with some of these substances has led researchers to examine the possibility of using bacteriocins produced by LAB as biopreservatives. Heightened consumer concern over “chemical” food additives has led to the search for alternative methods for control of food-borne pathogens (8). Therefore, in recent years, “natural” or “close to natural” foods have generated great interest among health conscious consumers. These foods are minimally processed, preserved without or with very little preservatives, and viewed as safe and nutritious as opposed to foods that are harshly processed and preserved with non-food chemicals. Minimally processed foods are attractive to the consumer because they are convenient, have a natural, fresh appearance, are viewed as nutritionally correct, and are generally devoid of added preservatives. More and more consumers now read food ingredients labels and will tend to select foods that do not contain preservatives if given a choice. The “contains no preservatives” label syndrome is quite acute with obvious abuse by marketing strategists. Food experts expect that there will be an increasing trend to produce many convenient and minimally processed refrigerated food products to meet the demand of these health conscious consumers. At present, there are several concerns about the safety of these foods.In general, these foods are refrigerated and vacuumpackaged to have extended shelf-life. However, they may contain pathogenic and spoilage bacteria that could multiply under these storage conditions. Thus, even a low initial population of bacteria can reach a high number during extended storage and make these foods unfit and unsafe for consumption. To control growth of these undesirable bacteria during storage, several techniques, such as reducing water activity, maintaining low pH, low storage temperature and incorporation of suitable preservatives, preferably in combination, have been recommended. The fact that bacteriocins of food grade lactic acid bacteria are produced as normal by-products of microbial metabolism make them attractive as “natural” pereservatives. The scope of current investigations on bacteriocins from lactic acid bacteria is quite extensive, ranging from basic studies on genetic regulation to applications in food preservation. Bacteriocins are particularly attractive preservatives, as they are naturally produced by many strains of lactic acid bacteria used for the production of fermented foods, and thus have been consumed safely by humans thousand of years. In addition, bacteriocins are pro-

tein in nature and therefore should be readily digested in the human gastrointestinal tract. Second, the preservative properties of LAB when used as fermentation agents in food was historically and still is an important means of food preservation. They can function as natural food preservatives through the inhibition of spoilage or pathogenic bacteria and ultimately contribute to food safety. Two relatively recent factors accelerating interest in LAB bacteriocins are the increasing incidence and detection of foodborne disease and the emerging consumer resistance to highly processed foods. Using genetic engineering, the gene(s) encoding bacteriocin production could be transferred into starter cultures used for the production of fermented foods to inhibit the growth of pathogenic and spoilage organisms in situ and extend the shelf-life of the products. Alternatively, bacteriocins could be produced via fermentation by native or genetically engineered organisms, purified and added to foods as pure chemicals. Recent approval by the U.S. Food and Drug Administration (FDA) of the bacteriocin nisin for use in processed cheese spreads has stimulated interest in the potential application of other antimicrobial compounds produced by food-grade microorganisms.

Factors that contribute to the increasing number of applied investigations on bacteriocins of LAB
*Acceptance of nisin as safe and efficacious in the past 35 years
*Approval of nisin by food and drug administration (FDA) as a "generally regarded as safe" (GRAS) in certain applications
*Realization that bacteriocinogenicity is not a rare occurence within the lactic acid bacteria
*Consumer awareness and resistance to traditional "chemical" preservatives
*Justifiable concerns over the safety of existing food preservatives such as sulfites and nitrites
*Possibility of use of bacteriocin production and immunity as selectable genetic markers in starter culture bacteria.
* Improvement in molecular techniques and availability of molecular biology tools to transfer, clone and sequence the genetic determinants and to engineer genetic variants of bacteriocins.
*Willingness of federal funding agencies, food commodity groups, and food processing corporations to find both basic and applied researches.

Application of bacteriocins in food biopreservation

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and/or their antibacterial products. Lactic acid bacteria (LAB) have a major potential for use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods. In milk, brined vegetables, many cereal products and meats with added carbohydrate, the growth of LAB produces a new plant product. In raw meats and fish that are chill stored under vacuum or in an environment with elevated carbon dioxide concentration, the LAB become the dominant population and preserve the meat with a "hidden" fermentation. The same applies to processed meats provided that the LAB survive the heat treatment or they are inoculated onto the product after heat treatment.

Nisin is produced by some strains of Lactococcus lactis subsp. lactis. It is a pentacyclic peptide containing three unusual amino acids in its structure, dehydroalanine, lanthionine and ß-methyl-lanthionine, and has a molecular weight of 3510 Da. It is inactivated by a-chymotrypsin, but is resistant to treatments with pronase, trypsin, and heat under acidic conditions (9). Nisin is effective against Gram-positive pathogens and prevents outgrowth of Clostridium and Bacillus spores. Nisin was first introduced commercially as a food preservative in the UK approximately 30 years ago. First established use was as a preservative in processed cheese products and since then numerous other applications in foods and beverages have been identified. It has been used to inhibit spore-forming organisms in processed cheese spreads, canned foods, and hot-plate products, to extend shelf-life of pasteurised milk, to control lactic acid bacteria in beer production, and to control Clostridium botulinum type E in modified atmosphere packaged fresh fish. It is currently recognized as a safe food preservative in approximately 50 countries (10). Nisin has been approved for use in the United States as the antibotulinal agent in processed cheese spreads (11). More recent applications of nisin inclu-

de its use as a preservative in high moisture, hot baked flour products (crumpets) and pasteurised liquid egg. Renewed interest is evident in the use of nisin in natural cheese poduction. Considirable research has been carried out on the antilisterial properties of nisin in foods and a number of applications have been proposed. Uses of nisin to control spoilage lactic acid bacteria have been identified in beer, wine, alcohol production and low pH foods such as salad dressings (12). Further developments of nisin are likely to include synergistic action of nisin with chelators and other bacteriocins, and its use as an adjunct in novel food processing technology such as higher pressure sterilization and electroporation. Production of highly purified nisin preparations and enhancement by chelators has led to interest in the use of nisin for human ulcer therapy, and mastitis control in cattle (12). Other bacteriocins have not been licensed for addition to foods, but studies have shown that there are other bacteriocins that have potential for use as food preservatives, in particular, pediocin A for its antibotulinal effect (13, 14) and pediocin AcH for its anti-Listeria activity in food preservation (15). Many studies on the activity of bacteriocins against target strains were done in laboratory media and not in foods. There are intrinsic factors in foods that could cause reduced activity of a bacteriocin. Class I and Class II bacteriocins are generally heat resistant, but they can be inactivated by proteolytic enzymes in foods (16). Most bacteriocins are hydrophobic, so they can be bound by fats and phospholipids. Nisin activity against L. monocytogenes is decreased in the presence of increasing fat concentration (17), but inactivation of nisin in the presence of fat was decreased with addition of a nonionic emulsifier such as Tween 80, but not by an anionic emulsifier such as lecithin (17).

Unless fully characterized, the study of bacteriocins as preservatives in foods can be misleading and confusing. This was emphasized by the fact that once the amino acid sequence of pediocin AcH (18), pediocin PA-1 (19), pediocin JD (20) and pediocin Bac (21) as well as mesenterocin 5 (22) was determined, the identity of compounds was realized. Bacteriocins, mesentericin Y105 and leucocin A-UAL187, which are produced by leuconostocs of dairy and meat origin, respectively are being studied for their possible use in preservation of food (23, 24). These two bacteriocins differ from each other only by two amino acids although isolated from unrelated sources. Besides, leucocin B-Tal la produced by Leuconostoc carnosum strain isolated from a vacuum packaged, cured meat in South Africa produces a bacteriocin identical to leucocin A, but there are differences in seven residues of their 24 amino acid N-terminal extension (25). This quite phenomenal distribution of "leucocin A-like" bacteriocins substantiates the observation with nisins A and Z (26) that minor variants of bacteriocins might be quite widespread in nature. This should encourage site-directed mutagenesis studies of bacteriocins as a possible means of influencing their antibacterial spectrum. The potential for structural manipulation with a ribosomally synthesized compound is great. This has yet to become a major emphasis of bacteriocin research, but with a gene replacement strategy such as that developed for nisin by Dodd et al., (27), the opportunity to develop genetically engineered variants of nisin is greatly enhanced. It is frequently stated that studies of bacteriocins in foods are lacking.

1. Starter cultures. Lactic acid bacteria are extensively used for the production of fermented dairy, meat and vegetable products. Bacteriocinproducing strains could be used to enhance the safety of these products, since many have been shown to inhibit Gram-positive pathogens such as Listeria monocytogenes, Staphylococcus aureus, and Clostridium botulimum. Naturally occurring bacteriocin-producing strains could also be used in nonfermented products. Since bacteriocin production and immunity phenotypes are frequently plasmid-madiated traits in the lactic acid bacteria, once identified and characterized, natural gene transfer systems such as conjugation and electroporation could be used to transfer these plasmids to other starter cultures (28, 29).

2. Genetically Engineered Starter Cultures. Alternatively, bacteriocin production and immunity genes could be genetically engineered into dairy and meat starter cultures to inhibit lactic spoilage organisms, or into silage inocula to inhibit competing organisms during fermentation. Bacteriocin production and/or

immunity genes localized on specific DNA fragments could be inserted into cloning vectors using recombinant DNA techniques. Recombinant plasmids can be transferred to bacterial hosts using transformation or electroporation techniques.

Modern approaches towards starter and protective culture improvement rely on advances in molecular biology. For most microorganisms used for food production, gene technological methods have been well developed. By recombinant DNA technology, "tailor-made" starter and protective cultures may be constructed so as to combine technically desirable features. A single strain which normally would fail to accomplish a given 'task' may now be improved so as to meet a set of requirements necessary for a specific production or preservation process (e.g. wholesomeness, no off-flavour production or overproduction of bacteriocins or particular enzymes). In addition, undesirable properties (e.g. mycotoxin or antibiotic production by cheese moulds) may be eliminated by techniques such as "gene disruption" (30).

To increase the acceptability of food products containing genetically modified microorganisms it is necessary to provide in an early stage to the consumers that the product is safe and that the product provide a clear benefit to the consumer. To comply with the first requirement a systematic approach to analyze the probability that genetically modified LAB will transform other inhabitants of the gastro-intestinal (G/I) tract or that these LAB will pick up genetic information of these inhabitants has been proposed and worked out to some degree. From this analysis it is clear that reliable date are still missing to carry out complete risk assessment. However, on the bases of present knowledge, LAB containing conjugative plasmids should be avoided. Various studies show that consumers in developed countries will accept these products when they offer to them health or taste benefits or a better keepability. For the developing countries the biggest challenge for scientists is most likely to make indigenous fermented food products with strongly improved microbiological stability due to broad spectra bacteriocins produced by LAB. Moreover, these LAB may contribute to health (31).

3. Food preservatives. Although nisin is the only approved bacteriocin for use in the United States, there is a great deal of interest in other bacteriocins that have similar properties and exhibit broad-spectrum inhibitory activity. Bacteriocins produced by fermentations could be purified and added to foods as pure chemicals to inhibit food-borne pathogenes and spoilage organisms. Bacteriocins have several characteristics that make them ideal food preservatives. Many bacteriocins are capable of resisting inactivation at the relatively high temperatures used in food processing and can remain functional over a broad pH range. Bacteriocins are usually inactivated by one or more of the proteolytic enzymes present in the digestive tract of humans and would be digested just like any other protein in the diet. Bacteriocins are nontoxic, odorless, colorless, and tateless. Finally bacteriocins may be perceived by consumers to be more natural than chemical preservatives. The efficacy of using bacteriocins as food preservatives will need to be determined for each food system. Solubility, stability, sensory impact, heat and pH tolerance, and types and number of organisms inhibited will need to be evaluated for each bacteriocin in each food product category under a variety of storage conditions.

3.1 Application of bacteriocins in the preservation of dairy products

The earliest use of nisin in food was as a preservative in processed cheese products and this continous to be one of the major applications of nisin to this day (10, 32). The ingredients used in the manufacture of these products are raw cheese, butter, skim milk powder, often various added flavours, phosphate or citrate emulsifying salts, and added water. Spores of anaerobic clostridial species are often present in some of these ingredients, particularly the cheese, and they are usually able to survive the heat process of 85-105°C for 6-10 min which is achieved during the melt process. The composition of processed cheese in terms of the relatively high pH and moisture content combined with low redox potential (anaerobic conditions) can favor the outgrow of these spores, which may the cause subsequent spoilage due to the production of gas, off-odours and liquefaction of the cheese. Clostridium species particularly associated

with the spoilage of processed cheese are C. butrycum, C. tyrobutyricum and C. sporogenes (9). The potential for growth and toxin production by C. botulimum in processed cheese products, particularly spreads, is of considerable significance. Trials have indicated that nisin is effective in these spreads in delaying or preventing the growth and subsequent formation of toxin by inoculated spores of C. botulinum types A and B (33). In dairy practice, nitrate is commonly added to cheesemilk to prevent outgrow of clostridia spores. This cehemical preservative can be very efficiently replaced by nisin A. Outgrow of C. tyrobutyricum spores in nitrate-free Gouda cheese was completely prevented when a nisin A producing strain was added to the starter culture (10% nisin A producers) (34). Nisin A is also an effective inhibitor of L. monocytogenes, and growth of this pathogen was effectively inhibited by Nisin A in camembert (35) and in cottage cheese at 4°C as well as 37°C (36). These results strongly suggest a potentially wider role for nisin A in the future preservation of a variety of dairy products. Recently, the relevant physicochemical and biological properties of nisin A and nisin Z were analysed (3)7. Identical MICs (minimal inhibitory concentration) of nisin A and nisin Z were found with all tested indicator strains of six different species of Grampositive bacteria. However, at concentrations above the MICs, with nisin Z the inhibition zones obtained in agar diffusion assays with all tested indicator strains were larger than those obtained with nisin A. These results suggested that nisin Z has better diffusion properties than nisin A in agar. Whether nisin Z will perform better as a biopreservative in certain foods than nisin A remains to be investigated.

The application of nisin in dairy foods which require lactic acid starter bacteria presents a problem because the wide spectrum of inhibition associated with nisin includes LAB themselves. An alternative approach which could be used to control specific pathogens or spoilage organisms in dairy foods is to employ bacteriocins with a highly specific activity range. The pediocin-like, heat stable bacteriocin enterocin 1146, which is produced by Enterococcus faecium DPC1146, is extremely active against L. monocytogenes at levels which have no effect on lactococcal starters (38, 39). E. faecium DPC1146 was used to ferment milk, which was subsequently pasteurized. The bacteriocin is produced in milk and is unaffected by the heat treatment. This milk was mixed with fresh milk and used for cheese making. The lactococcal starters were shown to grow and produce acid normally in the milk, whereas L. monocytogenes introduced in at the same time was rapidly killed. The inhibitory effect was not observed when a variant of DPC1146 was used which no longer produced the bacteriocin.

Addition levels of nisin to achieve effective preservation depend on the following factors: the spore load present in the formulation, moisture content, pH, salt content, use of flavour additives, cooking process employed and the length and likely temperature of the shelf life required.

Pasteurised liquid egg products (whole, yellow, white) receive heat treatment desired to ensure the destruction of Salmonella. These are typically 62-65°C for 2 to 3 minutes. However, such heat treatment is insufficient to kill of bacterial spores and some species of both Gram-positive and gram-negative bacteria. Many of these surviving bacteria are capable of growth at refrigerated temperatures and pasteurised liquid egg products usually have a limited shelf-life (40). Application of nisin at levels of 2.5 and 5 mg 1-1 has shown to act as an effective preservative giving significant increase in shelflife and providing protection against the growth of psychrotrophic Bacillus cereus. Such use of nisin is of particular interest in the U.S.A. in modified egg products that have greatly reduced cholesterol level. Further unpublished trials indicate that nisin is more effective in liquid white compared to liquid yellow.

3.2 Biopreservation of meat products

Concern on high levels of nitrite in cured meat has lead various workers to consider alternative preservation systems, which include a reduction in nitrite levels, and these have included nisin (41-45). Over the past three decades there has been an increasing research interest in the development of nitrite-free meat curing systems. The principal concern with the use of nitrite for curing of meat is the eventual formation of carcinogenic N-nitrosamines. Recently, attempts have been made to use nisin A as an alter-

native to nitrite. While the use of this bacteriocin alone was not successful, promising results were obtained when it was combined with reduced levels of nitrite: 100-250 ppm nisin A combined with 120 ppm nitrite was more effective than the conventional 156 ppm nitrite (46). Nisin A is apparently not the bacteriocin of choice for meat preservation in contrast to its effectiveness in dairy products. Bacteriocins produced by LAB associated with meat and meat fermentations such as Pediococcus, Leuconostoc, Carnobacterium and Lactobacillus spp. are likely to have much greater potential as meat preservatives (46-48).

L. monocytogenes is a food-borne pathogen which is ubiquitous in the environment and can be isolated from foods of different origin, including meat and meat products. In meat processing plants it may be present in slicing rooms and eventually contaminate pasteurized products during slicing and packaging. Recently, some biopreservation techniques have been applied to meat products and these involved the introduction of a competitive microflora of LAB as protective cultures for chill-stored ready-to-eat meat products, including bacteriocin producing LAB, and the use of purified anti-listerial bacteriocins added directly as natural food additives.

Lactobacillus sake Lb674, a mildly acidifying lactic acid bacterium originally isolated from meat, produces the bacteriocin sakacin 674, which is identical to sakacin P and very similar to pediocin PA-1 (49-51). Yousef et al (48) investigated the growth of L. monocytogenes in packed wiener sausage, a fully-cooked, cured meat product which is susceptible to contamination by L. monocytogenes before packaging. These researchers provided evidence that Pediococcus inoculants or purified pediocin can function as biopreservatives to eliminate Gram-positive pathogenic bacteria in cooked meats during extended refrigerated storage.

3.3 Biopreservation of fish

The application of nisin A in the preservation of fish products has been studied by Taylor et al (52) who showed that nisin treatment of cod, herring, and smoked mackerel fillets inoculated with Clostridium botulinum spores brought about a delay in toxin production of 5 days at 10°C, but only by half a day at 26°C. Nisin treatment did nor interfere with growth of non-pathogenic bacteria and in all samples botulinum toxin was formed before spoilage was evident. The effects of nisin Z, carnocin U149 and bavaricin A on bacterial growth and shelf life of brined shrimp was recently evaluated and compared with those of a benzoate-sorbate solution and a control with no added preservatives (53). Typically this product contains 3 to 6% NaCl and sorbic and benzoic acids in concentrations from 0.05 to 1.0%, with pH ranging from 5 to 6, and is stored at temperatures from 0 to 6°C. The benzoate-sorbate solution preserves the brined shrimp for the whole storage period (59 days). The shelf life of the shrimp in the absence of preservatives was found to be 10 days. Carnocin U149 had no influence on shelf life, while crude bavaricin (a cell-free supernatant of Lactobacillus bavaricus MI 401 extended the shelf life to 16 days. Significantly, when crude or purified nisin Z was applied to the same material the shelf life was extended to 31 days. Such results offer clear perspectives for the biopreservation of certain fish products with nisin Z.

3.4 Vegetable fermenatations

Vegetables that are packaged and ready-to-use as a convenience product generally have a refrigerated storage life of one week and support the growth of a microbial population that is dominated by pseudomanads and Enterobacteriaceae (54, 55). The possibility of preserving ready-to-use vegetables with bacteriocin producing LAB has been investigated (56). Under the conditions of this study it was shown that inoculation of the salads with strains of Lactobacillus casei or Pediococcus pentosaceus resulted in the domination of the vegetables with these bacteria and a dramatic decrease in Enterobacteriaceae that dominated the uninoculated control samples. During the 8-day storage period at 8°C the inoculated LAB grew and the pH of the salads decreased from about 4.8 to 5.2. The study indicated that inoculation of ready-to-use vegetables with LAB is effective, but no evidence was presented to show that bacteriocin production by the LAB was a factor in this application of LAB as biopreservatives.

3.5 Alcohol beverages

Research in europe has demonstrated the potential of nisin is controlling spoilage of lactic acid bacteria in beer (57, 58) and wine (59, 60). Nisin was introduced during fermentation because although the spoilage of lactic acid bacteria are sensitive to nisin, the yeasts are shown to be completely unaffected. Applications identified in the brewing industry are adding to fermenters for controlling and preventing contamination, reducing pasteurisation process and increasing the shelf life of unpasteurised or bottle conditioned beers. Similar applications also occur in the wine industry. However, nisin cannot be used during fermentation of wine that depend on desirable molalactic acid fermantation. Nisin is also used in distilled alcohol production, both for beverages and industrial production. When added to fermantation mashes that is naturally contaminated with LAB, the latter's activity can be controlled and cause increased alcohol yield by allowing the yeast less competition for substarte (61).

4. Food Hygiene. Biofilms have been of considerable interest in the context of food hygiene (62). Of special significance is the ability of microorganisms to attach and grow on food and food-contact surfaces under favourable conditions. Biofilm formation is an dynamic process and different mechanisms are involved in their attachment and growth. Extracellular polymeric substances play an important role in the attachment and colonization of microorganisms to food contact surfaces. Various techniques have been adopted for the proper study and understanding of biofilm attachment and control. If the microorganisms from food-contact surfaces are not completely removed, they may lead to biofilm formation and also increase the biotransfer potential. Therefore, various preventive and control strategies like hygienic plant layout and design of equipment, choice of materials, correct use and selection of detergents and disinfectants coupled with physical methods can be suitably applied for controlling biofilm formation on food contact surfaces. In addition, bacteriocins and enzymes are gaining importance and have an unique potential in the food industry for the effective biocontrol and removal of biofilms. These newer biocontrol strategies are considered important for the maintenance of biofilm-free systems, for quality and safety of foods.

4. Markers for food-grade cloning vector construction. Genes encoding resistance to therapeutic antibiotics (e.g., erytromycin, tetracycline) are frequently used as selectable markers on cloning vectors. These markers are unacceptable for engineering of starter cultures because of the concern over possible transfer of antibiotic resistance to gut microflora. Bacteriocin immunity gene(s) could be used as an altarnative selectable markers for the construction of food-grade cloning vectors. Since bacteriocins are not used therapeutically, transfer of resistance to gut microorganisms would not be an issue.

5. Probiotic organisms. Lactic acid bacteria and their probio-active cellular substances exert many beneficial effects in the gastrointestinal tract (2). LAB prevent adherence, establishment, and replication of several enteric mucusal pathogens through several antimicrobial mechanisms. LAB also release various enzymes into the intestinal lumen and exert potential synergistic effects on digestion and alleviate symptoms of intestinal malabsorption. Consumption of LAB fermented dairy products with LAB may elicit antitumor effects. These effects are attributed to the inhibition of mutagenic activity; decrease in several enzymes implicated in the generation of carcinogens, mutagens or tumor-promoting agents, supression of tumors, and the epidemiology correlating dietary regimes and cancer.

Bacteriocin-producing organisms, particularly lactobacilli that are naturally present in the gut of humans or animals, could be used as probiotics to influence the ecology of the gut. It has been postulated that certain gut microorganisms provide health benefits that include stimulation of the immune system, inactivation of potentially carcinogenic compounds, and reduction of serum cholesterol. Bacteriocins might enhance the ability of these organisms to colonize and compete with indigenous as well as potentially pathongenic gut micoflora.

6. Health care products. Because nisin inhibits a broad spectrum of Gram-positive organisms, it has

been used in tooth dips for prevention of mastitis in cows; in oral health care products, such as toothpaste and mouthwash, for inhibition of dental caries and periodontal disease; and in soap, skin care products, and cosmetics for treatment of acne. The worldwide market for mastitis treatment is approximately $ 100 million and is expected to grow over 30% in the five years. In the oral health care market, the mouthwash market alone is $ 500 million annually in the United States; toothpaste is even larger market. Skin care is also a potentially large market worldwide.

The preparation of highly purified nisin and the observation that both the level and spectrum of activity can be considerably enhanced by combination with chelating agents (63) have each opened up a number of veterinary and pharmaceutical applications for this bacteriocin. Use of nisin with a chelating agent expands the antibacterial spectrum of nisin to include gram-negative bacteria (U.S patent 4,980,163) and studies by Stevens et al., (64, 65) demonstrated a market reduction of enteric bacteria, including Salmonella spp. (3 to 7 log cycle reduction), after one hour exposure to 50 µg of nisin and 20 mM EDTA. The characteristics of nisin molecule that make it suitable for use in food applications also make it suitable for a number of other current and potential opportunities in the veterinary and pharmaceutical area. Nisin is already being used as a preventative agent against bovine mastitis through its use in pre- and post-milking teat dip products. A number of oral care applications are also being actively explored. A nisin based mouth rinse was evaluated in a beagle dog model, and was shown to prevent the build up of plaque and to prevent gingival inflammation (66). The exquisite sensitivity of Streptococcus and Staphylococcus species to the nisin offer opportunities in areas such as topical skin infections and the treatment of MRSA (Methicillin Resistant Staphylococcus aureus) systemic infections.

Bacteriocins: Future prospects

There is currently a large number of research on "natural antimicrobials" for food applications (67), of which bacteriocin comprise one group of compounds that are being studied. Bacteriocins of LAB and other food grade bacteria that have advantage that the organisms generally have GRAS (generally regarded as safe) status with regulatory agencies. Some bacteriocin-producing strains can be applied as protective cultures in a variety of food products. For example, well characterized, homofermentative, mildly, bacteriocinogenic LAB are ideal candidates for biopreservation of meats where modification of the product is undesirable. However, relatively high levels of these cultures may be required for protection against some pathogens. In these cases bacteriocin producers should be selected which do not negatively influence product taste and appearance when incorporated at high numbers. These problems can be avoided if purified bacteriocins or "inactivated cultures" are used directly as natural food additives, however additional hurdles may have to be included in order to prevent bacteriocin-resistant pathogens from growing. Before bacteriocin can be applied in foods their cytolytic abilities should be assessed in detail. This is a very important issue since recently a cytolysin produced by E. faecalis was described that possesses both hemolytic and bacteriocin activities (68). Continued study of the physical and chemical properties, mode of action and structure-function relationships of bacteriocins is necessary if their potential in food preservation is to be exploited. Further research into the synergistic reactions of these compounds and other natural preservatives, in combination with advanced technologies such as PEF and UHP could result in replacement of chemical preservatives, or could allow less severe processing (e.g. heat) treatments, while still maintaining adequate microbiological safety and quality in foods. Although the purified bacteriocins, except for nisin and pediocin PA-1, have not been licensed for addition to foods, it is clear that bacteriocin residues are currently present in the food supply. Two commercial compounds that have been licenced for addition to foods, Microgard and Alta 2341, are ferments of food grade bacteria that impart antibacterial properties to the foods. It is commonly stated that, except for nisin and pediocin PA-1, applied studies on bacteriocins are lacking. This is understandable because no other bacteriocin has been licensed for addition to foods. Convincing evidence of inhibition of pathogens and spoilage bacteria is required to stimulate commercial interest in bacteriocins as agents for biopreservation. Recombinant DNA technology is currently applied, to

enhance production, to transfer of bacteriocin genes to other species, and for mutation and selection of bacteriocin variants with increased and/or broad activity spectra.

REFERENCES

1. Stiles ME: Biopreservation by lactic acid bacteria. Antonie van Leuwenhoek 70: 331 (1996).

2. Naidu AS, Bidlack WR and Clemens RA: Probiotics spectra of lactic acid bacteria (LAB). Crit Rev Food Sci Nutr 39: 13 (1999).

3. Montville TJ and Chen Y: Mechanistic action of pediocin and nisin: recent progress and unresolved questions. Appl Microbiol Biotechnol 50: 511 (1998).

4. Jack RW, Tagg JR and Ray B: Bacteriocins of Gram-positive bacteria. Microbiol Rev 59: 171 (1995).

5. Kolter R and Moreno F: Genetics of ribosomally synthesized peptide antibiotics. Annu Rev Microbiol 46: 141 (1992).

6. Nissen-Meyer J and Nes IF: Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. Arch Microbiol 167: 67 (1997).

7. Miller K, Schamber R, Osmanagaoglu O and Ray B: Isolation and characterization of Pediocin AcH Chimeric protein mutants with altered bactericidal activity. Appl Environ Microbiol 64: 1997 (1998).

8. Gould GW: Ecosystem approach to food preservation. J Appl Bacteriol 73 (Supplement): 58S (1992).

9. Hurst A: Nisin. Adv Appl Microbiol 27: 85 (1981).

10. Delves-Broughton J: Nisin and its uses as a food preservative. Food Technol 44: 100, 102, 104, 106, 108, 111, 112, 117 (1990).

11. Federal Register. Nisin preparation: Affirmation of GRAS status as a direct human food ingredients. Vol. 53, No. 66, April 6. (1988).

12. Delves Droughton J, Blackburn P, Evans RJ, and Hugenholtz J: Applications of the bacteriocin, nisin. Antonie Van Leeuwenhoek. 69: 193 (1996).

13. Okereke A and Montville TJ: Bacteriocin inhibition of Clostridium botulinum spores by lactic acid bacteria. J Food Prot 54: 349 (1991a).

14. Okereke A and Montville TJ: Bacteriocin-mediated inhibition of Clostridium botulinum spores by lactic acid bacteria at refrigeration and abuse temperatures. Appl Environ Microbiol 57: 3423 (1991b).

15. Motlagh AM, Holla S, Johnson MC, Ray B and Field RA: Inhibition of Listeria spp. in sterile food systems by pediocin AcH, a bacteriocin produced by Pediococcus acidilactici H. J Food Protect 55: 337 (1992).

16. Abee T, Krockel L and Hill C: Bacteriocins: modes of action and potentials in food preservation and conrol of food poisoning. Int J Food Microbiol 28: 169 (1995).

17. Jung DS, Bodyfelt FW and Daeschel MA: Influence of fat and emulsifiers on the efficacy of nisin in inhibiting Listeria monocytogenes in fluid milk. J Dairy Sci 75: 387 (1992).

18. Motlagh AM, Bhunia AK, Szostek F, Hansen T, Johnson MC, and Ray B: Nucleotide and amino acid sequence of pap-gene (pediocin AcH production) in Pediococcus acidilactici H. Lett Appl Microbiol 15:45 (1992).

19. Marugg JD, Gonzalez CF, Kunka BS, Ledebore AM, Pucci MJ, Toonen MY, Walker SA, Zoetmulder LCM, and Vanderbergh PA: Cloning, expression and nucleotide sequence of genes involved in production of pediocin PA-1, a bacteriocin from Pediococcus acidilactici PAC 1.0. Appl Environ Microbiol 58: 2360 (1992).

20. Christensen DP and Hutkins RW: Glucose uptake by Listeria monocytogenes Scott A and inhibition by pediocin JD. App Environ Microbiol 60: 3870 (1994).

21. Hoover DG, Walsh PM, Kolaetis KM and Daly MM: A bacteriocin produced by Pediococcus species associated with a 5.5-megadalton plasmid. J food Protect 51: 29 (1988).

22. Daba H, Pandian S, Gosselin JF, Simard RE, Huang J, and Lacroix C: Detection and activity of a bacteriocin produced by Leuconostoc mesenteroides. Appl Environ Microbiol 57: 3450 (1991).

23. Hastings JW, Sailer ME, Johnson K, Roy KL, Vederas JC, and Stiles ME: Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from Leuconostoc gelidium. J Bacteriol 173: 7491 (1991).

24. Héchard Y, Derijard B, Letellier F and Cenatiempo Y: Characterization and purification of mesentericim Y 105, an anti-Listeria bacteriocin from Leuconostoc mesenteroides. J Gen Microbiol 138: 2725 (1992).

25. Felix JV, Papathanasopoulos MA, Smith AA, von Holy A and Hastings JW: Characterization of leucocin B-Tal la: a bacteriocin from Leuconostoc carnosum Tal la isolated from meat. Curr Microbiol 29: 207 (1994).

26. Mulders JWM, Boerrigter IJ, Rollema HS, Siezen RJ. and de Vos WM: Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur J Biochem 201: 581 (1993).

27. Dodd HM, Horn N, Giffard CJ and Gasson MJ: A gene replecement strategy for engineering nisin. Microbiology. 142: 47 (1996).

28. Klaenhammer TR: Bacteriocins of lactic acid bacteria. Biochimie 70: 337 (1988).

29. Kim WJ, Ray B, and Johnson MC: Plasmid transfer by conjugation and electroporation in Pediococcus acidilactici. J Appl Bacteriol 72: 201 (1991).

30. Geisen R and Holzapfel WH: Genetically modified starter and protective cultures. Int J Food Microbiol 30: 315 (1996).

31. Verrips CT and van den Berg DJC: Barriers to application of genetically modified lactic acid bacteria. Antonie van Leeuwenhoek 70: 299 (1996).

32. McClintock M, Serres L, Marzolf JJ, Hirsh A and Mocquot G: Action inhibitrice des streptocoques producteurs de nisine sur le developpement des sporules anaero-

bies dans le fromage de Gruyere fondu. J Dairy Res 19: 187 (1952).
33. Somers EB and Taylor SL: Antibotulinal effectiveness of nisin in pasteurised processed cheese spreads. J Food Protect 50: 842 (1987).
34. Hugenholtz J and de Veer GJCM: Application of nisin A and nisin Z in dairy tchnology. "G Jung and HG Sahl (ed): Nisin and Novel Lantibiotics", p 440, ESCOM, Leiden (1991).
35. Maisnear-Patin S, Deschamps N, Tatini SR and Richard J: Inhibition of Listeria monocytogenes in Camembert cheese made with a nisin-producing starter. Lait 72: 249 (1992).
36. Benkerroum R and Sandine WE: Inhibitory action of nisin against Listeria monocytogenes. J Dairy Sci 71: 3237 (1998).
37. de Vos WM, Mulder JWM, Siezen RJ, Hugenholtz J and Kuipers OP: Properties of nisin Z and distribution of its gene, nisZ, in Lactococcus lactis. Appl Environ Microbiol 59: 213 (1993).
38. Parente E and Hill C: Characterization of enterocin 1146, a bacteriocin from Enterococcus faecium inhibitory to Listeria monocytogenes. J Food Protect 55: 497 (1992b).
39. Parente E Hill C: Inhibition of Listeria of Listeria in buffer, broth and milk by enterocin 1146, a bacteriocin produced by Enterococcus faeceum. J Food Protect 55: 503 (1992b).
40. Delves-Broughton J, Williams GC and Wilkinson S: The use of the bacteriocin, nisin, as a preservative in pasterurised liquid whole egg. Lett Appl Microbiol 15: 133 (1992).
41. Caserio G, Ciampella M, Gennari M and Barluzzi AM: Utilisation of nisin in cooked sausages and other cured meat products. Ind Aliment 18: 12 (1979a).
42. Caserio G, Stecchini M, Pastore M and Gennari M: The individual and combined effects of nitrite on the spore germination of Clostridium perfringens in meat mixtures subjected to fermantation. Ind Aliment 18: 894 (1979b).
43. Rayman MK, Aris B and Hurst A: Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. App Environ Microbiol 41: 375 (1981).
44. Rayman MK, Malik N and Hurst A: Failure of nisin to inhibit outgrowth of Clostridium botulinum in a model cured meat system. App Environ Microbiol 46: 1450 (1983).
45. Taylor S and Somers E: Evaluation of the antibotulinal effectiveness of nisin in bacon. J Food Protect 48: 949 (1985).
46. Shahidi F: Developing alternative meat-curing systems. Trends Food Sci Technol September, 219 (1991).
47. Stiles ME and Hastings JW: Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. Food Sci. Technol 2: 235 (1991).
48. Yousef AE, Luckhansky JB, Degnan AJ, and Doyel MP: Behavior of Listeria monocytogenes in wiener exudates in the presence of Pediococcus acidilactici H or pediocin AcH during storage at 4 or 25°C. Appl Environ Microbiol 57: 461 (1991).
49. Kröckel L: Bacteriocine von Milchsaurebakterien für Fleischerzeugnisse. Mittbl Bundesants Fleischforsch Kulmbach 31: 207 (1992).
50. Tichaczek PS, Vogel RF, Hammes WP: Cloning and sequencing of sakP encoding sakacin P, the bacteriocin produced by Lactobacillus sake LTH 673. Microbiology 140: 361 (1994).
51. Holck AL, Axelsson L, Hühne K, Kröckel L: Purification and cloning of sakacin 674, a bacteriocin from Lactobacillus sake Lb674. FEMS Microbiol Lett 115: 143 (1994).
52. Taylor L, Cann DD and Welch BJ: Antibotulin properties of nisin in fresh fish packaged in an atmosphere of carbondioxide. J Food Protect 53: 953 (1990).
53. Einarsson H and Lauzon HL: Biopreservation of brined shrimp (Pandalus borealis) by bacteriocins from lactic acid bacteria. Appl Environ Microbiol 61: 669 (1995).
54. Huxoll CC and Bolin HR: Processing and distribution alternatives for minimally processed fruits and vegetables. Food Technol 43: 124 (1989).
55. Brocklehurst TF, Zaman-Wong CM and Lund BM: A note on the microbiology of retail packs of prepared salad vegetables. J Appl Bacteriol 63: 409 (1987).
56. Vescova M, Orsi C, Scolari G and Torriani S: Inhibitory effect of selected lactic acid bacteria on microflora associated with ready-to use vegetables. Lett Appl Microbiol 21: 121 (1995).
57. Ogden K and Tubb RS: Inhibition of beer spoilage lactic acid bacteria by nisin. J Inst Brew 91: 390 (1985).
58. Ogden K: Nisin: a bacteriocin with a potential use in brewing. J Inst Brew 92: 379 (1986).
59. Radler F: Possible use of nisin in wine making. I. Action of nisin against lactic acid bacteria and wine yeasts in solid and liquid media. Am J Enol and Vit 41: 1 (1990a).
60. Radler F: Possible use of nisin in wine making. I. Experiments to control lactic acid bacteria in the production of wine. Am J Enol and Vit 41: 7 (1990b).
61. Henning S, Metz R and Hammes WP: New aspects for the application of nisin to foods based on its mode of action. Int J Food Microbiol 3: 135 (1986).
62. Kumar CG and Anand SK: Significance of microbial biofilms in fod industry: a review. Int J Food Microbiol 42: 9 (1998).
63. Blackburn P, Polak J, Gusik S and Rubino SD: Nisin compositions for use as enhanced, broad range bacteriocins. International patent no PCT/US89/02525; international publication number W089/12399. App Microbiology Inc., New York, USA (1989).
64. Stevens KA, Sheldon BW, Klapes NA and Klaenhammer TR : Nisin treatment for inactivation of Salmonella species and other gram-negative bacteria. Appl Environ Microbiol 57: 3613 (1991).
65. Stevens KA, Sheldon BW, Klapes NA and Klaenhammer TR : Effect of treatment conditions on nisin inactivation of gram-negative bacteria. J Food Protect 55: 763 (1992).

66. Howell TH, Fiorellini JP, Blackburn P, Projan SJ, de la Harpe J and Williams RC: The effect of a mouth rinse based on nisin, a bacteriocin, on developing plaque and gingivitis in beagle dogs. J Clin Periodont 20: 335 (1993).

67. Gould GW: Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J Food Protect (Supplement): 82 (1996).

68. Gillmore MS, Segarra RA, Booth MC, Bogie CP, Hall LR and Clewell DB: Genetic structure of the Enterococcus faecalis plasmid pAD1-encoded cytolytic toxin system and its relationship to lantibiotic determinants. J Bacteriol 176: 1335 (1994).