

# Bacteriocins of Lactic Acid Bacteria: Potential Biopreservative in Dairy Industry

Kanchan Mogha, Sreeja V, Kunal Gawai and J. B. Prajapati

Department of Dairy Microbiology, SMC College of Dairy Science, AAU, Anand-388110.

Email: k\_v\_mogha@yahoo.co.in

Modern technologies implemented in food processing along with stringent microbiological food safety standards have made quality of food easier to achieve, and to a great extent minimize the likelihood of occurrence of food related illness and product spoilage. Until now, approaches towards improvement in food safety have been relied on use of chemical preservatives or on the application of more drastic physical treatments (e.g. high temperatures). Nevertheless, these types of solutions have many drawbacks such as the proven toxicity of many of the commonest chemical preservatives and the possible chances of alteration of the organoleptic and nutritional properties of foods. Additionally consumer awareness is increasing not only related to the risks associated with food borne pathogens, but also from the chemical preservatives used to control these pathogens. This has forced the food industry to look for alternatives which are free from such risks. One possible way to control microbial spoilage and safety hazards in foods can be by use of innovative technologies such as biological antimicrobial systems produced by lactic acid bacteria (LAB) including their bacteriocins singly or in combinations. The use of bacteriocins of LAB with mild physicochemical treatments can be an efficient way of extending shelf life.

It is well known that starter cultures produce a wide range of anti microbial metabolites such as organic acids, diacetyl, acetoin, hydrogen peroxide, antibiotics and bacteriocins during the process of fermentation. Bacteriocins produced by LAB are a heterogeneous group of ribosomally synthesized, extracellularly released, bioactive peptides or proteins which exhibits antimicrobial activity against other bacteria. Bacteriocins get produced in the food matrix during food fermentation *insitu*, although higher amounts of bacteriocins can be produced during *in vitro* fermentations by LAB

under optimal physical and chemical conditions. The bacteriocin production may be of utmost importance when bacteriocin producing LAB are added to foods as starter or adjunct culture. *In situ* bacteriocin production is the most promising for a fast, widespread, and legal use of bacteriocins to achieve desirable fermentation and safety assurance of finished product.

## Classification and properties of LAB bacteriocins

The bacteriocin family includes a wide variety of peptides and proteins in terms of their size, microbial targets, and mechanisms of action and immunity. Based on structural, physicochemical, and molecular properties it can be subdivided into three major classes.

**Class I:** Lantibiotics ([small], cationic, hydrophobic peptides [ $<5$  kDa] containing unusual and posttranslationally modified amino acids [lanthionine, methyllanthionine, dehydrobutyrine, and dehydroalanine]); Type A (elongated and positively charged molecules); Subtype A1 (leader peptides are cleaved by a dedicated serin proteinase), Subtype A2 (leader peptides are cleaved by a dedicated ABC ATP-binding cassette [ATP] transporter), Type B (globular and noncharged molecules)

**Class II:** Nonmodified, heat-stable, small, cationic, hydrophobic peptides ( $<10$  kDa); Subclass II a (pediocin like bacteriocins - active peptides against *listeria spp*) Subclass II b (two peptide bacteriocins), Subclass II c (sec-dependent bacteriocins), Subclass II d (other bacteriocins-leaderless bacteriocins; antimicrobial peptides derived from larger proteins)

**Class III:** Large, hydrophilic, heat-labile proteins ( $>30$  kDa).



**Class IV:** Cyclic, cationic, hydrophobic macromolecules.

The most prominent bacteriocins from Class I is “nisin”. It is the only thoroughly studied bacteriocin and has been permitted use as food additive/biopreservative in certain processed dairy products and canned foods in several countries. Most of the LAB bacteriocins under uses with processed foods are incorporated in class II. Examples of them are pediocins, enterocins, lactococcins, plantaricins, acidocins *etc.*

### Bacteriocins in Food Applications

Many LAB and their bacteriocins have been consumed unintentionally for ages, laying down a long history of safe use. Their spectrum of inhibition, bactericidal mode of action, relative tolerance to technologically relevant conditions (pH, NaCl, heat treatments) and the lack of toxicity study towards eukaryotic cells further support their role as biopreservatives in food. Among the bacteriocins of LAB, Nisin is the single bacteriocin, which has found commercial application as biopreservative. It is considered safe by World Health Organization (WHO) and has received the denomination of Generally Recognized as Safe (GRAS) and also by Food and Drug Administration (FDA). Nisin is produced by *Lactococcus lactis* subsp. *lactis*. Nisin is used in pasteurized, processed cheese products to prevent outgrowth of spores such as those of *Clostridium tyrobutyricum* that may survive heat treatments as high as 85–105°C. Use of nisin allows these products to be formulated with high moisture levels and low NaCl and phosphate contents, and also allows them to be stored outside chill cabinets without risk of spoilage. The level of nisin used depends on food composition, likely spore load, required shelf life and temperatures likely to be encountered during storage. Nisin is also used to extend the shelf life of dairy desserts which cannot be fully sterilized without damaging appearance, taste or texture. Nisin can significantly increase the limited shelf life of such pasteurized products. Nisin is added to milk in the Middle East where shelf-life problems occur owing to the warm climate, the necessity to transport milk over long distances and poor refrigeration facilities. It can double the shelf life

at chilled, ambient and elevated temperatures and prevent outgrowth of thermophilic heat resistant spores that can survive pasteurization. It can also be used in canned evaporated milk. Nisaplin, the commercial nisin, contains 2.5% pure nisin A. It is legally used in more than 50 countries for specific food applications (Khurana and Kanawjia, 2007). Another bacteriocin with a broad host range similar to that of nisin is lacticin 3147, produced by a strain of *Lactococcus lactis*. It is also an effective inhibitor of many Gram-positive food pathogens and spoilage microorganisms, these starters may provide a very useful means of controlling the proliferation of undesirable microorganisms during Cheddar cheese manufacture. Another bacteriocin, Pediocin PA-1, has been observed to inhibit *Listeria* spp. in dairy products such as cottage cheese, ice cream, and reconstituted dry milk (Rodríguez *et al*, 2002). Microgard is commercially produced from grade A skim milk fermented by a strain of *Propionibacterium shermanii*, and has a wide antimicrobial spectrum including some Gram-negative bacteria, yeasts and fungi. It is added to a variety of dairy products such as cottage cheese and yoghurt. In cottage cheese, it inhibits psychrotrophic spoilage bacteria. The inhibitory activity of microgard depends primarily on the presence of propionic acid, but there has also been a role proposed for a bacteriocin like protein produced during the fermentation. The use of milk fermented by a bacteriocin producer as an ingredient in milk based foods may be a useful approach for introducing bacteriocins into foods at low cost. Another commercial product is BioProfit, which contains *Lactobacillus rhamnosus* LC 705 and *Propionibacterium freudenreichii* JS. It is used as a protective culture to inhibit yeasts in dairy products and *Bacillus* spp. in sourdough bread (Khurana and Kanawjia, 2007). Other example of a secondary metabolite produced by lactic acid bacteria which have antagonistic activity include an antibiotic called Reuterocyclin, produced by *Lactobacillus reuteri*. The spectrum of inhibition of this antibiotic is limited to gram-positive bacteria including *Lactobacillus* spp., *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Listeria innocua* (Rattanachaikunsopon and Phumkhachorn, 2010).

Another potential application of bacteriocins is in bioactive packaging, where bacteriocins from



LAB can be incorporated into packaging destined to be in contact with food. This system combines the preservation function of bacteriocins with conventional packaging materials, which protects the food from external contaminants. Bioactive packaging can be prepared by directly immobilizing bacteriocin to the food packaging or by addition of a sachet containing the bacteriocin into the packaged food, which will be released during storage of the food product. Studies investigating the effectiveness of bioactive cellulose based packaging inserts and a vacuum packaging pouch made with polyethylene/polyamide to improve shelf life and safety aspects have proved promising. When considering bioactive packaging, the stability and the ability to retain activity while immobilised to the packaging film is of vital importance.

Bacteriocins may be applied basically in three different formats: i) *in situ* production by primary and/or adjunct starters, ii) as an ingredient (fermentate of a bacteriocinogenic strain), or iii) as an additive in a semi or purified preparation. Nisin producing dairy starters have been designed specifically to inhibit *Staphylococcus aureus* in acid coagulated cheeses and for inhibition of *C. tyrobutyricum* in semi-hard cheeses. Bacteriocins have been shown to accelerate cheese ripening by promoting the release of intracellular enzymes in the cheese matrix and subsequently increase concentration of volatile and other compounds which remained responsible of the good sensory attributes of the matured cheese in addition to preservative effect.

### Applications of bacteriocins in hurdle technology

Combined application of bacteriocins with selected hurdles can further increase the effectiveness of bacteriocin by affecting the outer membrane permeability and thus increasing the effectiveness of use against resistant gram negative cells. Pathogens like *E. coli* O157:H7 and *Salmonella* were controlled when bacteriocins were used with combinations of ethylene diamine tetra acetic acid (EDTA), sodium tripolyphosphate (STPP) or physical methods such as heat and high hydrostatic pressure technique (HHP). The various bacteriocins viz. nisin, pediocin, enterocin were tried along

with other hurdles such as pulsed electric field, modified atmospheric packaging, pH etc.

### Bacteriocins and heat treatments

Bacteriocins can be used to reduce the intensity of heat treatments in foods without compromising microbial inactivation. Nisin and heat acted synergistically to reduce the heat resistance of *L. monocytogenes* in milk. Bacteriocins can also provide an additional protection during food storage against proliferation of endospores surviving heat treatments. Moreover, it has been demonstrated that the intensity of heat treatments against bacterial endospores can be lowered in combination with nisin as well as with enterocin AS-48 which resulted in cost saving and decreasing the impact of heat on the food. Application of sub lethal heat treatment sensitizes many gram negative bacteria towards several bacteriocins such as nisin, pediocin ACH, enterocin AS-48, which resulted in extension of spectrum for action and thereby reducing their impact.

### Bacteriocins and modified atmospheric packaging

Modified atmosphere packaging (MAP) is frequently used in the food industry to prolong the shelf life of perishable food products which ultimately make changes in gaseous environment by creating barriers. Shelf life prolongation of food by MAP have based on retardation of intrinsic food changes and inhibition of spoilage microbiota. These systems have greater effects when applied in combination with bacteriocins and thus ensuring dual safety against food spoilage.

### Bacteriocins and pulsed electric fields

Pulsed electric field (PEF) technology is a non thermal process where microbial inactivation is achieved by application of high-voltage pulses between a set of electrodes. The effect of PEF resembles with bacterial electroporation, but higher intensity of this treatment causes severe damage to the bacterial cell membrane. This technology can only be applied to food products which are low viscous. But it gained attraction in recent years when combined with other hurdles including use of bacteriocins. Since most bacteriocins act on the



bacterial cytoplasmic membrane, the combined application of bacteriocins and PEF is expected to elicit increased bactericidal effects. Moreover, bacteriocins can also provide an additional hurdle against survivors from PEF treatments, such as sub lethally injured cells or bacterial endospores.

### Bacteriocins and other non thermal treatments

Among the various non thermal treatments currently under study for food processing application, only a few reports have been published on their combination with bacteriocins. Irradiation offers a great potential in food preservation. The scale of applications of food irradiation could be expanded in combination with bacteriocins, especially if the radiation doses can be controlled. Low dose of gamma irradiation has minimal undesirable effects on food and may also have better consumer acceptance. It was reported that the combined application of pediocin (as ALTA™ 2341) and low dose irradiation (2.3 kGy) had an increased antimicrobial effect on *L. monocytogenes* in cooked sausages. One of the main limitations of gamma irradiation is the increased resistance of bacterial endospores, requiring the application of combined treatments for a higher effectiveness.

### Conclusion

A large number of bacteriocins from LAB have been characterized till today, and many different studies have indicated the potential usefulness of bacteriocins in food preservation. The need for novel preservation technologies offers new opportunities for application of bacteriocins singly or in combination with different hurdle techniques. Also we need to explore the possibilities of combined application of bacteriocins with some less frequently used techniques such as ultra sonication, irradiation, microwave and ohmic heating, or pulsed electric fields. Additionally, to decide the optimal conditions for application of bacteriocins in foods certain important aspects such as the most effective conditions for application of each particular bacteriocin, antimicrobial effects

of bacteriocins and bacteriocinogenic cultures in food ecosystems need to be studied in detail.

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