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Bacteriocins Produced by Lactic Acid Bacteria and Their Use for Food Preservation

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Summary

With the shift in consumer preference to foods minimally processed and free from chemical preservatives, bacteriocins produced by lactic acid bacteria (LAB) have received much attention due to their potential use as natural food preservatives. It has been shown that nisin, the only bacteriocin approved so far, and some of the newly isolated novel bacteriocins are effective biopreservatives in certain food systems in which concentrated bacteriocin preparations or the bacteriocin-producing strains have been used. Here, the state of this subject at present is outlined.

Changes in eating habits in developed countries increase the risk of new food-borne illness. A recent estimation suggests that there are 76 million cases of food-borne illness, resulting in about 5,000 deaths, in the United States each year (Mead *et al.*, 1999). In association with the awareness of not only food safety, but also the risk derived from chemical preservatives, there has been an increasing demand for more "natural" and "health-promoting" food (Montville and Winkowski, 1997).

In this context, much attention is being directed to food preservation with the use of lactic acid bacteria (LAB). LAB are the microbes that humans have used for many years unconsciously to make a variety of processed foods in a good state of preservation. However, it has been identified that the preservative effect results from the antimicrobial action of bacteriocin as well as metabolites, such as lactic acid and hydrogen peroxide, produced by LAB (Caplice and Fitzgerald, 1999). Nisin, the bacteriocin produced by *Lactococcus lactis* involved in cheese manufacture, has been well characterized (Ross *et al.*, 2002; Cleveland *et al.*, 2001) and approved as a food preservative in more than 60 countries (Papagianni, 2003). It has already been used as a shelf-life extender in cheese, milk products, brewing, wine manufacture, and confectionary (Ross *et al.*, 2002; Papagianni,

2003). With the emergence of this natural biopreservative, attempts to search for a more effective bacteriocin of LAB origin (Cleveland *et al.*, 2001 ; O'Sullivan *et al.*, 2002) and to develop food preservation technology using the bacteriocin or its producing culture (Ross *et al.*, 1999 ; O'Sullivan *et al.*, 2002) have been made extensively over the last two decades.

Characteristics of the bacteriocins produced by LAB and their application to food preservation are briefly described in the following.

1. Bacteriocins Produced by LAB

Bacteriocins produced by LAB are modified or unmodified ribosomally synthesized peptides, which exhibit a relatively narrow antimicrobial spectrum. They are secreted to combat with competing Gram-positive bacteria, primarily closely related other LAB, for the same ecological niche (Eijsink *et al.*, 2002). However, the bateriocins also show antimicrobial activity against food-borne pathogens such as *Listeria monocytogenes* as well as food spoilage Gram-positive bacteria (O'Sullivan *et al.*, 2002). They generally damage the target cell membrane and/or inhibit cell wall synthesis (Abee *et al.*, 1995). LAB bacteriocins are commonly divided into three main groups (Klaenhammer, 1993 ; Cleveland *et al.*, 2001).

Class I bacteriocins are composed of one or two small, post-translationally modified peptides of approximately 3 kDa. They are referred to as lantibiotics, since they are modified to contain lanthionine, β-methyllanthionine and dehydrated amino acids. The lantibiotics were originally subdivided into two groups, A and B. Type A includes the most well-known bacteriocin, nisin, which is the elongated flexible molecule with a positive charge and the membrane depolarization activity (Sahl and Bierbaum, 1998). Type B includes mersacidin, which is globular in shape and interferes with cell wall synthesis (Sahl and Bierbaum, 1998). In addition to these two groups, some members of the lantibiotics, such as lacticin 3147, are composed of two separate peptides (Papagianni, 2003). In contrast to nisin, which is effective in acidic environments, lacticin 3147 is active at physiological pH (O'Sullivan *et al.*, 2002). Recently, it was found that nisin interacts with a docking molecule, lipid II, a precursor of cell wall biosynthesis, prompting to the hypothesis of dual functionality of nisin (Breukink *et al.*, 1999 ; Wiedemann *et al.*, 2001).

Class II bacteriocins are, in general, small unmodified peptides of <5 kDa and are subdivided into two groups, IIa and IIb. Class IIa bacteriocins, including pediocin PA-1 (AcH), a prototype of this class, have been found in a great variety of LAB (*Lactobacillus*, *Enterococcus*, *Pediococcus*, *Carnobacterium*, and *Leuconostoc*) (Caplice and Fitzgerald, 1999). They have similar amino acid sequences (40-60% similarity) with a characteristic conserved sequence,

YGNGVXC$(X)_4$C, and two cysteine residues forming a disulfide bond in the N-terminal region (Eijsink *et al.*, 2002). This group of bacteriocin is also characterized by their strong anti-listerial activity (*Listeria* active), thus receiving particular attention for an application as biopreservatives (O'Sullivan *et al.*, 2002). Class IIb is composed of two separate peptides including lactococcin G, the first bacteriocin of this class to be isolated, which requires the presence of both peptides for optimal activity (Nissen-Meyer *et al.*, 1992). The primary target of class II bacteriocins is the cytoplasmic membrane of Gram-positive bacteria (Eijsink *et al.*, 2002). Class I and class II bacteriocins are heat-stable.

Class III bacteriocins including helveticin J (Joerger and Klaenhammer, 1986) are the least well-characterized group and consist of a heat-labile protein with a molecular weight of >30 kDa.

Due to their strong antimicrobial activity, wide spectrum, and heat stability, class I and class II bacteriocins have been extensively examined for their application as biopreservatives.

2. Effectiveness of Bacteriocins in Food Systems

Nutritious foods, such as dairy products, fresh meat and fermented meat products, are the environment most susceptible for growth of pathogenic bacteria. Thus, the protective effect of bacteriocin against contaminating pathogens has been assessed most frequently with those food systems. In these examinations, *L. monocytogenes* has been used mainly as a model pathogen. This microorganism is ubiquitous in the environment, proliferates at refrigeration temperatures and has high mortality upon the establishment of infection (Farber and Peterkin, 1991).

The effectiveness of nisin, prepared in the form of a dried concentrated powder from a skim milk-derived fermentate, has been confirmed in a cheese system. The addition of nisin to cottage cheese challenged with 10^4 cfu/g *L. monocytogenes* resulted in a 1,000-fold decrease in the viable cells after 7 days at 20°C, compared to a 10-fold decrease in the control (Ferreira and Lund, 1996). However, the use of nisin in meat as a preservative has achieved little success to date (O'Sullivan *et al.*, 2002), probably due to interference by meat components, such as phospholipid (Henning *et al.*, 1986) and high pH (Rayman *et al.*, 1983) where nisin becomes extremely less soluble and thus ineffective (Liu and Hansen, 1990). This indicates the limitation of nisin application. Meanwhile, an active search for bacteriocinogenic LAB resulted in the discovery of lacticin 3147, another bacteriocin of class I produced by *L. lactis* isolated from an Irish kefir-like grain (Ryan *et al.*, 1996), and its enriched powder has been shown to have an anti-listerial activity even at high pH in a buffer solution system (Ross *et al.*, 1999).

As an alternative to using bacteriocin itself, direct introduction of live bacteriocin-producing culture into foods as a protection starter has been inves-

tigated extensively, and achieved favorable results in some food systems. For example, the nisin-producing starter has been shown to have the potential to inhibit *L. monocytogenes* in Camembert cheese manufacture (Maisnier-Patin *et al.*, 1992). Furthermore, it was reported that *Lactobacillus* or *Pediococcus* strains producing an antilisterial calss IIa bacteriocin could inhibit *L. monocytogenes* growth in meats and meat products (O'Sullivan *et al.*, 2002). Recently, we have isolated a bacteriocinogenic *Carnobacterium* strain from a vacuum-packaged pork, and found that, when inoculated together with *L. monocytogenes* in pork preserved under refrigerated conditions, this strain displays strong bactericidal activity to diminish the pathogen to an undetectable level (Ando *et al.*, in preparation).

The above-described findings demonstrate the potential of LAB bacteriocins and cultures as biopreservatives. Hereafter, it appears that their pertinent exploitation will produce biopreservation systems that are optimum for each food.

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