REVIEW

Bacteriocins from Lactic Acid Bacteria: A Review of Biosynthesis, Mode of Action, Fermentative Production, Uses, and Prospects

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Abstract—Bacteriocins are antimicrobial peptides that help bacteria fight competing bacteria in microecological systems. Bacteriocins of lactic acid bacteria (LAB) have attracted much interest in recent years because of their properties that make them suitable as natural food preservatives against specific food pathogens, and as possible supplement to antibiotics against drug resistant bacterial strains. LAB bacteriocins are generally classified into the lantibiotics and non-lantibiotics, the latter divided into four sub-groups. To date, only nisin and to a lesser extent, pediocin are the commercially applied bacteriocins for food use. Clinical applications are still limited to animal health. One of the more exciting prospects on the use of bacteriocins is the possibility of subjecting them to bioengineering to either increase antimicrobial activity or further specify their target microorganism. The latter would make it less damaging to the natural gut microflora, which is a common drawback of conventional antibiotic therapy.

This paper focuses on the nature, biology, and applications of bacteriocins based on knowledge gained abroad and in the Philippines during the last two decades.

Keywords—lactic acid bacteria, antimicrobial peptides, bacteriocin, bacteriocin application, bacteriocin biosynthesis, bacteriocin prospects

INTRODUCTION

In microbial ecosystems, some microorganisms synthesize antimicrobial compounds, such as bacteriocins, that destroy or inhibit the growth of other microorganisms (Cleveland et al. 2001). It has been suggested that more than half to almost all bacterial species synthesize bacteriocins (Riley & Wertz 2002; Cotter et al. 2005). Bacteriocins comprise a huge family of ribosomally synthesized peptides that usually show antimicrobial activity to strains that are closely-related to the producer strain (narrow-spectrum bioactivity) and sometimes to strains across genera (broad-spectrum bioactivity) (Diep & Nes 2002; Perez et al. 2014).

Bacteriocins from lactic acid bacteria (LAB) have attracted a lot of attention from many industries due to their various attributes that have potential for applications. Bacteriocins are a new trend in food packaging, as these substances can be incorporated into the extruder when the antimicrobial film or co-extruded film is produced (Deshmukh & Thorat 2013). The "generally regarded as safe" (GRAS) distinction of LAB and their by-products given by the U.S. Food and Drug Administration (FDA) highlighted the applicability of bacteriocins as safe food preservatives (U.S. Federal Register 1988). Although bacteriocins, in a sense, can be considered as antibiotics, they differ from conventional antibiotics in numerous aspects (Zacharof & Lovitt 2012; Perez et al. 2014). The subtlest differences are summarized in Table 1. Bacteriocins are inherently tolerant to higher thermal stress and are more active at a wider pH range than conventional antibiotics. These antimicrobial peptides are colorless, odorless, and tasteless, making them ideal for use as food preservatives. Development of resistant strains among their target bacteria is unlikely as they have fast-acting antimicrobial mechanisms that are highly potent even at very low concentrations. Furthermore, their proteinaceous nature minimizes resistance development as they are easily degraded by proteolytic enzymes, thus lessening the chances of target strains developing any resistance machinery. Perhaps the most promising advantage of bacteriocins over conventional antibiotics is their primary metabolite nature that makes them easily subjected to bioengineering to either increase their bioactivity or to specify their target microorganisms (Perez et al. 2014).

Despite the tremendous application potential of bacteriocins in various fields, they have remained underutilized. Currently, nisin and to a lesser extent pediocin PA-1/AcH are the only bacteriocins that are commercially used in food applications; and their use in clinical settings has been limited to animal, not extending to human health (Cotter et al. 2005). On the other hand, the approval of nisin for application in food by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives, as well as the approval by the US Food and Drug Agency (FDA) for its use in pasteurized, processed cheese spreads, should establish a legal precedent for the use of other bacteriocins as food preservative. In clinical settings, the increasing number of researches done on the development of bioengineered bacteriocins with enhanced bioactivity against clinical pathogens should fast-track their widespread use as therapeutic agents.

In this review, the nature and biology of bacteriocins, their applications and prospects in various fields are discussed. The status and potential of bacteriocin research in the Philippines are also highlighted.

LAB Bacteriocin Classification

Over the years, various classification schemes of LAB bacteriocins have been suggested (Klaenhammer 1993; Diep & Nes 2002; Cotter et al. 2005; Heng et al. 2007). The classification scheme suggested by Cotter & co-workers (2005) is the most widely accepted, limiting the grouping in just two classes (Table 2); the lantibiotics (class I) and non-lantibiotics-containing bacteriocins (class II). The

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Table 1. Main differences between LAB bacteriocins and conventional antibiotics (Perez et al. 2014).

Factor Considered	Bacteriocins	Antibiotics
Application	Food/Clinical	Clinical
Synthesis	Ribosomal	Secondary metabolite
Bioactivity spectra	Mostly narrow	Mostly broad
Intensity of bioactivity	Active at nano-to-micro molar range	Active at micro-to-milli molar range
Proteolytic enzyme degradability	High	Moderate-to-hardly
Thermal stability	High	Low
Active pH range	Wide	Narrow
Taste/color/odor	No	Yes
Amenability to bioengineering	Yes	No
Possible mechanism of target cell developing resistance	Adaptation through changes in cell membrane composition	Genetically transferable determinant that inactivates the active compound
Mode of action	Pore formation on cell membrane, inhibition of cell wall biosynthesis	Cell membrane or intercellular targets
Toxicity towards eukaryotic cells	Relatively no	Yes

most notable change in this new scheme is the suggested exclusion of class III bacteriocins and renaming them as *bacteriolysins* since they are lytic enzymes rather than peptides. Recently, Heng & co-workers (2007), although agreeing broadly with this classification scheme, suggested a further modification in which circular bacteriocins should be grouped into a different class.

Class I bacteriocins or lantibiotics (lanthionine-containing antibiotics) are small peptides (<5 kDa) that possess unusual post-translationally modified amino acid residues such as lanthionine or 3-methyllanthionine (McAuliffe et al. 2001). These unusual amino acid residues form covalent bridges between amino acids that result in the formation of internal "ring" or cyclic structure that gives the stability to the whole molecule (Willey & van der Donk 2007; Asaduzzaman & Sonomoto 2009). The first reported and most extensively studied bacteriocin, nisin A, belongs to this class.

Class II bacteriocins or the non-lantibiotics are the most naturally-occurring bacteriocins. These bacteriocins are relatively small (<10 kDa), heat-stable, non-lanthionine-containing peptides, which unlike lantibiotics, do not undergo extensive post-translational modification. This group is further divided into four sub-classes by Cotter et al. (2005): "pediocin-like" bacteriocins (class IIa), two-peptide bacteriocins (class IIb), circular bacteriocins (class IIc), and unmodified, linear, non-pediocin-like bacteriocins (class IId) (Table 2).

Class IIa bacteriocins have a distinct conserved sequence YGNGV in the N-terminal region that gives this group a very strong inhibitory ability against the highly pathogenic food-borne pathogen *Listeria monocytogenes* (Ennahar et al. 2000; Fimland et al. 2005). This group is oftentimes called pediocin-like because the first discovered bacteriocin belonging to this group was pediocin PA-1/AcH (Fimland et al. 2005; Drider et al. 2006). While class IIb bacteriocins are two-peptide bacteriocins that require both peptides to work synergistically to be fully active (Oppegard et al. 2007; Nissen-Meyer et al. 2010), class IIc bacteriocins, arguably the most poorly understood, are grouped based on the circular configuration of their structure. The N- and C- termini of class IIc bacteriocins are covalently linked that results to a very stable molecular structure (Maqueda et al. 2008; van Belkum et al. 2011). On the other hand, class IId bacteriocins comprise the remaining bacteriocins that are combined as miscellaneous or one-peptide non-pediocin linear group (Cotter et al. 2005; Iwatani et al. 2011; Masuda et al. 2012). *Sec*-dependent bacteriocins (Cintas et al. 2000) and the growing number of bacteriocins that do not have leader peptides and therefore known as leaderless bacteriocins (Fujita et al. 2007), belong to this class.

Bacteriocin Biosynthesis

Bacteriocins have relatively simpler biosynthetic machinery because they are primary metabolites (gene-encoded and synthesized through the ribosome) while conventional antibiotics are secondary metabolites. Bacteriocin coding genes are often associated with transferable elements such as conjugative transposons or plasmids (Klaenhammer 1993; Riley & Wertz 2002). Genes related to the biosynthesis of bacteriocins are generally clustered together with the minimum genetic machinery consisting of the structural gene and the cognate immunity

Table 2. Classification of Bacteriocins (Cotter et al. 2005).

Class	Features	Representative Bacteriocins
I	Antibiotics, small (<5 kDa) peptides containing lanthionine and 3-methyllanthionine	Nisin A, Nukacin ISK-1
II	Small (<10 kDa), heat-stable, non-lanthionine-containing peptides	
IIa	Small heat-stable peptides, synthesized in a form of precursor which is processed after two glycine residues, active against *Listeria*, have a consensus sequence of YGNGVXC in the N-terminal	Pediocin PA-1/AcH, Saracens A and P, Leucocin A, Carnobacteriocin
IIb	Two component systems: two different peptides required to form an active portion complex	Lactoccins G and F, Lactation F, Plantaricin EF and JK, Brochocin C
IIc	N- and C- termini are covalently linked, resulting in a circular structure	Enterocin As-48, Lactocyclicin Q
IId	Other class II bacteriocins, including *sec*-dependent bacteriocins and leaderless bacteriocins	Acidocin, Enterocin P, Enterocin B, Lacticin Q
III[†]	Large molecules heat sensitive peptides	Helveticins J, Acidophilucin A, Lactacins A and B

[†] suggested that they be called *bacteriolysins* and no longer considered bacteriocins.

genes (Klaenhammer 1993). Bacteriocins are synthesized as biologically-inactive precursor peptides that contain the N-terminal leader peptides attached to the C-terminal propeptides. These would then undergo enzymatic processes (often referred to as bacteriocin maturation) to yield the "mature" active bacteriocins (Klaenhammer 1993; Diep & Nes 2002; Riley & Wertz 2002). The leader peptide (i) functions as a recognition site for the biosynthetic enzymes involved in the maturation process and its transport to the extracellular space, (ii) protects the producer strain from the bacteriocin's inhibitory activity by keeping the bacteriocin in an inactive state (precursor peptide form) while inside the producer cell, and (iii) interacts with the propeptide domain of the precursor peptide to ensure a suitable conformation essential for the enzyme-substrate interaction (precursor peptide and the biosynthetic enzyme(s)) (Klaenhammer 1993; van der Meer et al. 1994; van Belkum et al. 1997; Oman & van der Donk 2009).

To illustrate further the mechanism of bacteriocin biosynthesis, a schematic diagram of the biosynthesis of the most extensively studied bacteriocin, nisin A, is shown in Fig. 1 (Perez et al. 2014). Nisin A is synthesized through the ribosome of the producer strain as an inactive peptide NisA, a gene product of the structural gene nisA. NisA consists of an N-terminal leader peptide attached to the propeptide moiety. The biosynthetic gene cluster that encodes the biosynthetic machinery responsible for the modification, transport, immunity, and production regulation, is encoded directly upstream of the *nisA* structural gene. The modification enzymes, NisB and NisC, dehydrate and cyclize the propeptide, respectively, and subsequently the ABC transporter, NisT, translocates the modified precursor peptide into the extracellular space. The protease, NisT, then recognizes the leader peptide of the modified precursor peptide and cleaves off the leader peptide, releasing the mature (active form) nisin A. The lipoprotein, NisI, can bind to the nisin A molecules around the producer cell, thereby providing protection from its antimicrobial action. The multi-protein ABC transporter complex, NisFEG, provides additional protection to the producer cells by expelling the nisin A molecules away from the cell.

The production regulation of nisin biosynthesis is controlled by a two-component regulatory system, composed of a histidine kinase, NisK, and a response regulator, NisR, where the nisin A molecule itself serves as the peptide pheromone. The NisK protein senses the nisin A molecule, causing it to autophosphorylate and subsequently transfers the phosphoryl group to NisR; the phosphorylated NisR then initiates the transcription of the *nisA* gene cluster by activating the promoters (Fig. 1).

Recently, a growing number of bacteriocins lacking the leader sequences have been reported (Iwatani et al. 2011; Masuda et al. 2012). This group of new bacteriocins is very interesting as they are in their active forms right after translation. Many questions arise about the details of their biosynthetic mechanism such as how the producer cell protects itself from the inhibitory action of the bacteriocin while it is inside the cell. Do the producer cells have a unique mechanism of transport of the bacteriocin molecule to the extracellular space? From an application perspective, these bacteriocins offer promising commercial potential applications as they can be readily synthesized without having the need to cleave off the leader peptide. This makes them easily applicable for scaling up production using different systems, even possibly using eukaryotic heterologous production systems (Perez et al. 2014).

Killing Mechanisms of Bacteriocins

Bacteriocins are known for their very high potency against their target strains. In general, bacteriocin activity is limited to strains that are closely related to the bacteriocin producer strain (narrow-spectrum bioactivity) although recently, many

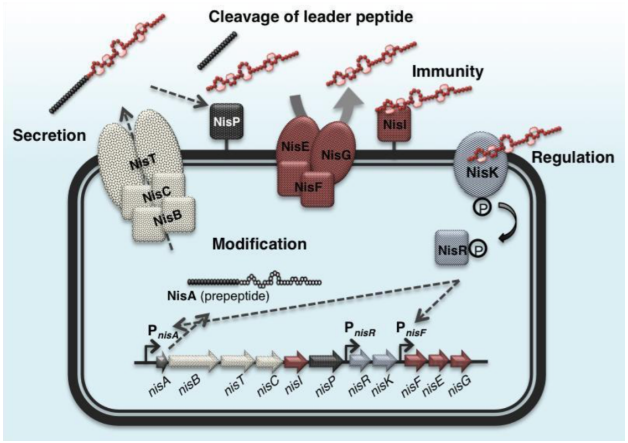

Figure 1. A schematic diagram of the biosynthesis of nisin A. Nisin A is ribosomally synthesized as an inactive prepeptide, NisA, consisting of an N-terminal leader sequence attached to a propeptide moiety. Modification enzymes, NisB and NisC, dehydrate and cyclize the propeptide respectively, and subsequently the ABC transporter, NisT, translocates the modified prepeptide into the extracellular space. The protease, NisP, then cleaves off the leader peptide, releasing the mature (active form) nisin A. The lipoprotein NisI that can bind to nisin A, and the multi- protein ABC transporter complex NisFEG, which expels nisin A from the cell, comprise the self-immunity system for nisin A. The two-component regulation system that is responsible for the up-regulation of the nisin gene cluster is composed of a histidine kinase, NisK, and a response regulator, NisR. Nisin A serves as the signal peptide that activates this regulation system.

bacteriocins have shown antimicrobial activity against a wide range of genera (broad-spectrum bioactivity). The killing mechanism of bacteriocins is specific to each group of bacteriocins. Moreover, the general cationic nature of bacteriocins plays a very important role in their initial interaction with the cell membrane of their target strains. The negative charge of bacterial cell membranes and the positive charge of bacteriocin molecules create an electrostatic interaction between them, thereby facilitating the approach of the molecules to the membranes. However, this interaction is not responsible for the killing of the target bacterial cells. This interaction is also responsible for the inactivity of most bacteriocins toward Gram-negative bacterial strains. The composition of Gram-negative bacterial membrane differs from that of Gram-positive bacterial membrane in that the former contains a lipopolysaccharide outer membrane. Bacteriocins from LAB only become active against Gram-negative bacteria when combined with other agents that compromise the integrity of the outer membrane such as surfactants (Stevens et al. 1991; Zhang & Mustapha 1999). As mentioned above, the mode of action of bacteriocins is different among classes. To apply their killing mechanism, bacteriocins require a receptor molecule or a "docking molecule" found in their target bacterial cell membrane, which differs among different classes and subclasses.

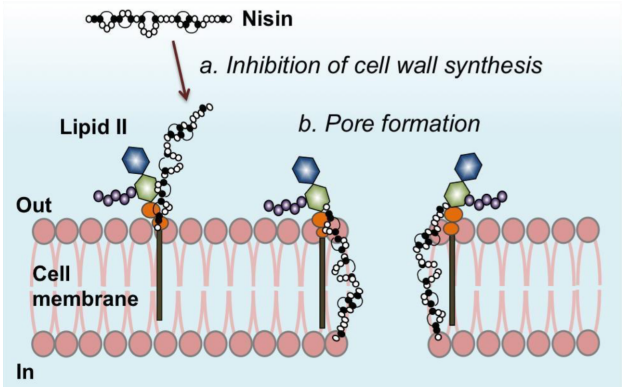

Figure 2. Killing mechanism of class I bacteriocins. Class I bacteriocins, such as nisin, have a dual mechanism in killing their target cells through (a) inhibition of cell wall synthesis and (b) pore formation. Both mechanisms are facilitated by the binding of the nisin molecule to the lipid II, which is the main transporter of peptidoglycan subunits – the building blocks of the bacterial cell wall. At lower concentrations, nisin prevents the proper cell wall synthesis, which leads to cell death. At higher concentrations, nisin binds to lipid II that initiates membrane insertion and pore formation, which leads to the leakage of cellular components such as ions and ATP.

Class I bacteriocins (lantibiotics)

Lantibiotics, such as nisin, have two known killing mechanisms, although both systems share a common denominator (Breukink et al. 1999; Wiedemann et al. 2001; Hsu et al. 2004). It has long been shown that the lantibiotic nisin disrupts the integrity of the bacterial cell membrane by forming pores that would lead to the dissipation of the membrane potential and the efflux of small metabolites such as ions, amino acids, nucleotides and other cytoplasmic solutes, resulting in the termination of all biosynthetic processes, leading to cell death (Ruhr & Sahl 1985; Sahl et al. 1987). Nisin is extremely potent against its target bacterial strains, showing antimicrobial activity even at a single-digit nanomolar concentration. At lower concentrations, nisin has been shown to kill target bacteria through enzyme inhibition. The nisin molecule has been shown to bind to lipid II, which is the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall (Fig. 2). Peptidoglycan is the main component of the bacterial cell wall. The binding of nisin to lipid II results in the prevention of proper cell wall synthesis, thereby causing cell death. Furthermore, at higher concentrations, the nisin-lipid II molecule complex initiates membrane insertion that creates pores in the bacterial cell membrane. Thus, the binding of nisin to lipid II facilitates its dual mode of preventive action involving cell wall synthesis and membrane pore formation (Breukink et al. 1999; Wiedemann et al. 2001).

Class II bacteriocins (non-lantibiotics)

Non-lantibiotics are the most commonly occurring bacteriocins. Most members of class II bacteriocins exert their antimicrobial action by inducing membrane permeabilization that subsequently leads to the leakage of cytoplasmic molecules, causing cell death of the target bacteria. The mechanisms of antimicrobial action of class II bacteriocins differ among subclasses (Perez et al. 2014).

Class IIa bacteriocins (pediocin-like bacteriocins)

Members of class IIa bacteriocins are known for their high potency against *L. monocytogenes*, a highly pathogenic and robust food-borne bacterium. Class II bacteriocins have been shown to bind to mannose phosphotransferase system (Man-PTS) proteins, the sugar-uptake system of target bacteria, as docking molecule for their killing mechanism. The conserved YGNGV amino acid sequence at the N-terminal region of class IIa bacteriocins is responsible for the anti-listerial antimicrobial activity, whereas the less conserved C-terminal domain is responsible for their antimicrobial activity against other target strains (Fimland et al. 2005; Johnsen et al. 2005).

Class IIb bacteriocins (two-peptide bacteriocins)

Two-peptide bacteriocins require the synergistic activity of both peptides to promote their killing activity against their target bacteria. These peptides display very low, if any, bacteriocin activity when tested individually. Thus, the two peptides of class IIb bacteriocins should be considered as one antimicrobial unit instead of two independent antimicrobial peptides that show synergistic activity (Oppegard et al. 2007). Members of class IIb bacteriocins can be classified into two types: type E (enhanced) and type S (synergistic) peptides (Garneau et al. 2002). The killing mechanisms of class IIb bacteriocins involve membrane permeabilization of their target bacterial strains, which results in the leakage of small cytoplasmic molecules such as the monovalent cations, Na^+, K^+, Li^+, Cs^+, Rb^+, but not divalent cations such as Mg^{2+} or phosphates (Oppegard et al. 2007). Membrane pores formed as a result of class IIb bacteriocins are relatively smaller in size than those of lantibiotics.

Class IIc bacteriocins (circular bacteriocins)

Compared to other classes of bacteriocins, circular bacteriocins display a broader spectrum of antimicrobial activity towards various Gram-positive bacteria, including many food-borne spoilage and pathogenic bacteria. Circular bacteriocins are bactericidal toward their target bacterial cells. Similar to many other bacteriocins, circular bacteriocins apply their killing mechanism toward their target bacteria by permeation of the bacterial cell membrane, resulting in the leakage of ions, dissipation of membrane potential, and eventually in cell death (Gabrielsen et al. 2014). Studies on the mode of action on enterocin AS-48, gassericin A, subtilosin A, and carnocyclin A have suggested that circular bacteriocins do not require a receptor molecule for their activity. It has been thought that basic amino acid residues of circular bacteriocins that patch on the surface of their compact hydrophobic globular structure were responsible for the electrostatic interaction between the bacteriocin and the surface membrane of the target cell (van Belkum et al. 2011). However, a later study on garvicin ML, a new member of circular bacteriocins, has suggested that garvicin ML has a dual mode of action as in the case of nisin A. Aside from the non-receptor electrostatic interaction killing mechanism, a maltose ABC-transporter protein serves as a target receptor of garvicin ML, which facilitates the efflux of intracellular solutes that eventually leads to cell death (Gabrielsen et al. 2012).

Class IId bacteriocins (miscellaneous bacteriocins)

The killing mechanisms of one-peptide non-pediocin linear and leaderless bacteriocins are still poorly understood. Unlike the other classes where bacteriocins within the same grouping share a similar mechanism of antimicrobial action, as discussed above (*i.e.* lantibiotics use lipid II whereas pediocin-like bacteriocins utilize the Man-PTS as receptor molecules respectively), class IId bacteriocins do not share any common system for their killing mechanisms. This is primarily because of the fundamental diversity of their primary structures (Iwatani et al. 2011).

On the other hand, the unique killing mechanism of lacticin Q, a leaderless bacteriocin, has been well characterized (Yoneyama et al. 2009b). While most bacteriocins require a docking molecule for their antimicrobial action, lipid II for nisin A and other lantibiotics, and mannose ABC transporter, MptD, for pediocin PA-1/AcH and its homologue bacteriocins, lacticin Q has been found to cause high-level membrane permeabilization of target strains without the need of any specific receptors (Yoneyama et al. 2009a). Lacticin Q forms a huge toroidal pore (HTP) from around 4.6 to 6.6 nm in size, enough to cause leakage of intracellular components such as ions and ATP as well as large molecules such as proteins,

thereby causing cell death (Yoneyama et al. 2009b). It has been shown that the mechanism of HTP formation starts with the electrostatic interaction of the cationic lacticin Q molecules and the negatively charged membranes. The rapid binding of lacticin Q to the phospholipid bilayer membranes results in the formation of HTPs coupled with lipid flip-flop. Intracellular components then escape from these pores,leading to cell death. The pores formed in the membrane are short-lived because these HTPs close back as the lacticin Q molecular mass translocates itself from the outer to the inner cell membrane (Fig. 3) (Yoneyama et al. 2009b). However, the killing mechanism through HTP formation of lacticin Q is selective and highly dependent on the physiological features of the outer membrane of target cells, which explains the non-toxicity of lacticin Q against Gram-negative bacteria (Yoneyama et al. 2011). Furthermore, very recently, it was suggested that another mechanism is responsible for the selective antimicrobial activity of lacticin Q, and this is the accumulation of hydroxyl radicals through Fenton reaction, with variations within species, and in some cases, even within strains. It was inferred that the selective toxicity of lacticin Q would depend on the strains' ability to scavenge the hydroxyl radicals (Li et al. 2013).

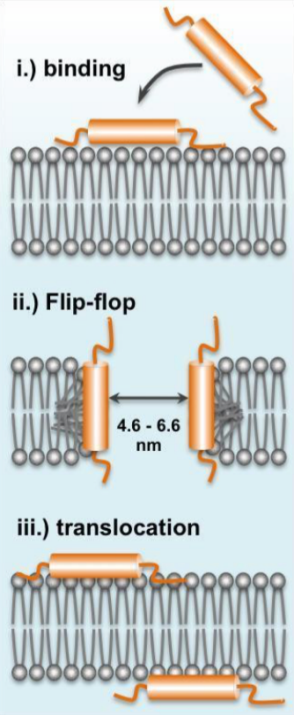

Figure 3. Huge toroidal pore (HTP) model of the antimicrobial action of lacticin Q. The highly cationic lacticin Q molecules rapidly bind to the negatively charged phospholipid bilayer membrane (i) that results in the formation of HTPs coupled with membrane lipid flip-flop that causes the leakage of intracellular components, including ions, ATP, and small proteins (ii), after which, the lacticin Q molecular mass translocates itself from the outer to the inner membrane as the pore closes (iii) (Yoneyama et al. 2009b).

Fermentation Studies in LAB Growth and Bacteriocin Production

Bacteriocin production is growth-associated but the yield of bacteriocin per unit biomass is affected by several factors, including the producing strain, media (carbohydrate and nitrogen sources, cations, etc.) and fermentation conditions (pH, temperature, agitation, aeration and dilution rate in continuous fermentations) (Anthony et al. 2009; Thirumurugan et al. 2015). Continuous fermentation processes with cell recycle or immobilized cells can result in a dramatic improvement in productivity over batch fermentations (Parente et al. 1997; Bhugaloo-Vial et al. 1997; Liu et al. 2005; Schobitz et al. 2006).

Growth and bacteriocin production in *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442 were found to be directly proportional to the carbon to nitrogen ratio (Mataragas et al. 2004). For *Lb. pentosus* ST151BR, tryptone stimulated growth and bacteriocin production, whereas lower activity was observed with meat extract, yeast extract or their combinations. Maltose, lactose or mannose were preferred carbon sources over sucrose, fructose and glucose. Tween 80 and glycerol also adversely affected bacteriocin production (Todorov & Dicks 2004). Similarly, tryptone and saccharose increased bacteriocin production by *Enterococcus faecium* ST311LD by two-fold (Todorov & Dicks 2005). For *Lb. salivarius* CRL1328, optimal growth and bacteriocin production in MRS broth were recorded at an initial pH of 6.5 and 37 °C. In LAPTg (chemically defined medium composed of 1.5% peptone, 1% tryptone, 1% glucose, 1% yeast extract, 0.1% Tween 80), maximum bacteriocin activity was obtained after 6 h at 37 °C and at initial pH of 6.5 and 8.0 (Juarez et al. 2002).

Leroy & de Vuyst (2001) have done kinetic modeling of the growth of *Lb. sakei* CTC 494 in the chemically-defined medium de Man Rogosa Sharpe (MRS). They found that growth was inhibited by the accumulation of lactic acid and other toxic products, and the depletion of nutrients. Bacteriocin production by *Lb. plantarum* LPCO10 was optimized in batch culture using plantaricin C in an integral statistical approach, fractional factorial three-level design experiment (Sanchez et al. 2002). Callewaert & De Vuyst (2000) have also found that fed-batch fermentation helped stabilize the conditions of the culture broth and the added nutrients improved growth and bacteriocin production of *Lb. amylovorus* DCE 471. Fed-batch fermentation was also found suitable for *Lb. curvatus* CWBI-B28. On the other hand, the bacteriocin from *Lb. plantarum* LL441, was optimally produced in chemostat or continuous culture at pH 5.0, 30 °C, 150 rpm and a dilution rate of 0.05 h—1 using glucose as carbon source at a dilution rate of 0.10 to 0.12 h—1 when sucrose or fructose was used (Barcena et al. 1998).

Biomass and bacteriocin production of some strains of lactic acid bacteria using natural basal media have been done. Guerra & co-workers (2005) tried fed-batch pediocin production by *Pediococcus acidilactici* NRRL B-5627 on whey. Diluted whey supplemented with 2% yeast extract and 1% glucose was used as initial medium and the fed-batch medium used was concentrated whey (4.8 % total sugars), 2% yeast extract and 4% glucose. Results showed that the biomass and bacteriocin production were higher than when MRS broth was used. Another substrate previously investigated was mussel processing waste (Guerra & Castro 2002). Some processes to recover bacteriocins from the culture media have also been devised. Several simple recovery processes, based on adsorbing bacteriocins on resins or silica compounds, have been developed and can be used to build integrated production processes (Parente & Ricciardi 1999).

Bacteriocin Application and Prospects

LAB have been associated with food fermentations dating all the way back to ancient times due to their beneficial influences on nutritional, organoleptic, and shelf-life properties of foods (De Vuyst & Leroy 2007). It is, however, their ability to produce bacteriocins that have made them particularly promising in both food and pharmaceutical industries. In the food industry, bacteriocins have been widely utilized for the biopreservation of various foods, either alone, or in combination with other methods of preservation known as hurdle technology (Chen & Hoover 2003; Ghrairi et al. 2012). Incorporation of bacteriocins into the food packaging film or surfaces has been explored as well (Galvez et al. 2007). The antimicrobial activity of many bacteriocins, especially the class IIa bacteriocins, against the highly pathogenic food-borne *L. monocytogenes* can be an ideal solution to the problem on *L. monocytogenes* contamination on ready-to-eat refrigerated food products (Chen & Hoover 2003).

There are three common approaches in which bacteriocins can be applied in food systems: (i) direct inoculation of bacteriocin-producing LAB into the food system, (ii) addition of the bacteriocin in its purified form as a food preservative, and (iii) utilization of the product, fermented by a bacteriocin-producing LAB, as a raw material for food processing (Schillinger et al. 1996).

The increasing incidence of multidrug resistance bacterial pathogens is one of the most pressing medical problems in recent years (Spellberg et al. 2008; WHO 2014). The clinical application potential of LAB bacteriocins has been the subject of on-going investigations by many scientists in many countries all over the world due to the activity of some bacteriocins against Gram-positive human and animal pathogens including some multi-drug resistant (MDR) pathogens (Cotter et al. 2005). Bacteriocins have been considered to be viable candidates to supplement the arsenal of antibiotics targeting MDR-associated infections (Cotter et al. 2013). For example, the two-peptide lantibiotic lacticin 3147 has been found *in vitro* to be active against the methicillin-resistant *Staphylococcus aureus* (MRSA) strain and vancomycin-resistant *Enterococcus faecalis* (VRE) strain (Galvin et al. 1999). Furthermore, because bacteriocins are ribosomally synthesized, they have relatively simpler biosynthetic mechanisms than that of secondary metabolite antibiotics. The gene-encoded nature of bacteriocins makes them easily amenable through bioengineering to either increase their activity or specify their target microorganism. Owing to this feature of bacteriocins, antibiotic therapy can become less damaging to the natural gut microflora, which is a common drawback of conventional antibiotic therapy.

However, there are still some serious bottlenecks hindering the large-scale application of LAB bacteriocins. It remains a huge challenge to establish a cost-effective system for large-scale production and down-stream processing systems (Yusuf 2013). In addition, cultivation of lactic acid bacteria requires more expensive complex media compared to other antimicrobial compound-producing microorganisms. The weak potency of most bacteriocins against Gram-negative pathogens is also considered a disadvantage since there are numerous Gram-negative food-borne pathogens that are common concerns in many food products. In order to address this limitation, combinational strategies (hurdle technology) of food preservation have been studied (Chen & Hoover 2003; Mills et al. 2011). Moreover, the use of bioengineered bacteriocins, especially for food applications, can face resistance by misinformed consumer, as in the case of other genetically-modified organisms (Perez et al. 2014).

For clinical applications of bacteriocins, their mode of administration still requires more in-depth studies. Although a variety of administration methods have been proven successful, such as subcutaneous, intravenous, intranasal, intragastric, intraperitoneal, and topical, their efficacies have not yet been directly compared (Jabes et al. 2011; Lohans & Vederas 2012). Moreover, some of these methods are unnecessarily invasive (Lohans & Vederas 2012). Drug therapy for humans usually prescribes oral administration; however, in such a case, the fate of bacteriocin molecules in the gastro-intestinal environment still remains a huge question. Due to its proteinaceous nature, enzymatic degradation of the bacteriocin in the gut is highly possible.

Nevertheless, these issues have not discouraged many scientists from looking at bacteriocins for their large-scale applications. This is clearly evident in the rapidly increasing number of research studies done on their application as food preservatives and as therapeutic agents (Lohans & Vederas 2012; Arthur et al. 2014; Nigam et al. 2014; Yang et al. 2014).

Bacteriocin Research in the Philippines

The Philippines is one of the countries blessed with the world's most abundant biodiversity. It is highly possible that the Philippines has highly diverse bacterial strains producing novel bacteriocins with exceptional properties. This is evident in the variety of indigenous fermented food products that are unique to each of the three-main island groups of the Philippines, namely Luzon, the Visayas, and Mindanao. These fermented foods often rely entirely on the natural microflora of the raw material and surrounding environment (Banaay et al. 2013).

Some efforts on the isolation and screening for bacteriocin-producing LAB strains have been done by the National Institute of Molecular Biology and

Biotechnology (BIOTECH) and the Institute of Biological Sciences at the University of the Philippines Los Baños (UPLB) in Luzon and the Philippine Rootcrops Research and Training Center (PhilRootcrops) at the Visayas State University in the Visayas, but no novel bacteriocins have been discovered. Most bacteriocins produced from the lactic acid bacterial strains were already known and had been reported elsewhere by foreign scientists (Banaay et al. 2013).

Nevertheless, the Food Safety and Functional Food Biotechnology Program (FSFFBP) of BIOTECH, UPLB has been doing basic research on the isolation and characterization of bacteriocin-producing lactic acid bacteria for more than a decade and has established a collection of identified and well-characterized LAB isolates from indigenous sources. To date, around thirty (30) bacteriocin-producing LAB isolates have been characterized genetically and biochemically by the FSFFBP. Most of these isolates are positive for the *papA* gene, the pediocin structural gene (Table 3). Out of the 23 isolates tested for *papA* gene, 20 have been found PCR-positive for the gene (Perez et al. 2012). Some of these bacteriocins have been purified to homogeneity and their properties, such as their tolerance to low pH, high temperature and bile and adhesion to porcine intestine, thoroughly elucidated. Two of these promising isolates, namely *Lb. plantarum* BS (deposited at the Philippine National Collection of Microorganisms (PNCM 10287)) and *P. acidilactici* AA5a (PNCM 10289), have also been fingerprinted for IPR and patenting purposes (Elegado et al. 2004a and 2004b).

Table 3. Genetic screening for *papA* (pediocin structural gene) in the lactic acid bacteria (LAB) collection of the Food Safety and Functional Food Biotechnology Program, BIOTECH, U.P. Los Baños.

Species	ISOLATE/STRAIN/ ACCESSION NO.	16S rDNA PARTIAL SEQUENCE HOMOLOGY [99%]	*papA* PCR [450 bp]
Pediococcus acidilactici	AA-5a (PNCM 10287)	*P. acidilactici* DSM 20284	*papA*$^+$
	4E2	*P. acidilactici* NBRC 12231	*papA*$^+$
	4E4	*P. acidilactici* DSM 20284	*papA*$^+$
	4E5	*P. acidilactici* DSM 20284	*papA*$^+$
	4E6	*P. acidilactici* DSM 20284	*papA*$^+$
	4BL7	*P. acidilactici* DSM 20284	*papA*$^+$
	3F3	*P. acidilactici* NBRC12231	*papA*$^+$
	3F8	*P. acidilactici* NBRC12231	*papA*$^+$
	3F10	*P. acidilactici* DSM 20284	*papA*$^+$
	3G3	*P. acidilactici* IMAU20090	*papA*$^+$
	3G8	*P. acidilactici* UL5	*papA*$^+$
	IG7	*P. acidilactici* 8D2CCH01MX	*papA*$^+$
	B19	*P. acidilactici* UL5	*papA*$^+$
	K_2A_1-1	*P. acidilactici* DSM 20284	*papA*$^+$
	K_2A_2-1	not determined	*papA*$^+$
	K_2A_2-3	not determined	*papA*$^+$
	K_2A_2-5	*P. acidilactici* DSM 20284	*papA*$^+$
	K_3A_2-1	not determined	*papA*$^+$
	K_3A_2-2	*P. acidilactici* UL5	*papA*$^+$
	K_3A_2-3	not determined	*papA*$^+$
	S3	*P. acidilactici* DSM 20284	*papA*$^+$
P. lolii	4E10	*P. lolii* 0510Q	*papA*$^-$
P. pentosaceous	B- Acc. 1225	[100%] *Pediococcus pentosaceous* PP	*papA*$^+$
L. plantarum	LP1	*L. plantarum* M01210	*papA*$^-$
	ATCC 8014 (B-Acc. 1074)	*L. plantarum* P10	*papA*$^+$
	BS (PNCM 10289)	*L. plantarum* P10	*papA*$^+$
Lb. brevis	4B1	*L. brevis* 0945	*papA*$^+$
	A	*L. brevis* M0121	*papA*$^-$
	C	*L. brevis* strain SC13	*papA*$^-$
	D	[100%] *L. brevis* KLDS1.0411	*papA*$^+$
	F	*L. brevis* 0945	*papA*$^+$
	G	*L. brevis* 0945	*papA*$^+$
	H	[100%] *L. brevis* 0945	*papA*$^+$
Lb. casei/ paracasei	030	*Lactobacillus paracasei* M0116	*papA*$^-$
Lb. fermentum	FM7	[97%] *L. fermentum* IMAU50008	*papA*$^-$
Enterococcus durans	NSRI 1171	*E. durans* M.D.E.MRSA-5	*papA*$^-$
	ML15	*E. durans* KLDS 6.0606	*papA*$^+$
E.faecium/ faecalis	3B2	*Enterococcus faecium* JZ1-1	*papA*$^-$
	IF2	*E. faecalis* YH-10-3	*papA*$^-$
	B-Acc. 1072	Not determined	*papA*$^+$
Lactococcus lactis	SC1	*Lactococcus lactis* LC	*papA*$^+$
Leuconostoc mesenteroides	B-Acc. 1493	Not determined	*papA*$^+$

Several possible application studies have been done to explore the suitability of each of these probiotic and bacteriocinogenic LAB isolates as adjunct or sole inoculum to improve the quality of probiotic foods. Sensory and health-functional attributes and the keeping quality against pathogenic listeria and staphylococci have been targets of research (Elegado et al. 2007). Examples of products being developed using the above probiotic/bacteriocinogenic LAB isolates are probiotic white cheese from carabao's milk using BIOTECH microbial rennet and selected probiotic LAB, probiotic drinks based on carabao's milk, soybean, vegetable extracts and selected fruit flavor, and the development of fermented meat sausages (Marilao et al. 2007). Non-dairy probiotic LAB, preferably with bacteriocin-like inhibitory substances, are also being tested as to their complementation and enhancement of preventative or therapeutic attributes of herbal plants (Saguibo & Elegado 2012), Results have shown in vitro that the bacteriocin of *P. acidilactici* K2a2-3 has inhibitory effects against human colon adenocarcinoma (HT29) and human cervical carcinoma (HeLa) cells (Villarante et al. 2010).

A few applications of purified or semi-purified bacteriocins have also been done, such as the use of bacteriocins as sanitizing agent against *L. monocytogenes* on stainless steel food vessels (Sagpao et al. 2007). Optimization works on bacteriocin production from *P. acidilactici* AA5a and *Lb. plantarum* BS using various cheap carbon sources such as molasses, coconut water, cheese whey, sago starch hydrolyzate and extract of spent distillery yeasts have also been conducted (Elegado et al. 2002; Elegado et al. 2004c; Sagpao et al. 2007).

SUMMARY OF REVIEW & RECOMMENDATIONS

Bacteriocins are antimicrobial peptides produced by many bacterial strains that inhibit the growth of competing bacterial species in microecological systems. The potential of bacteriocins produced by lactic acid bacteria (LAB) as natural biopreservatives for food against resistant Gram-positive pathogens is huge. Once harnessed, this can result in the minimal use of antibiotics and chemical preservatives in foods, as preferred by well-informed consumers. Moreover, due to their strong potency against antibiotic-resistant pathogens, bacteriocins may be a viable solution to the growing problem of multidrug-resistant pathogens. Nonetheless, more research still needs to be done in the isolation and

characterization of bacteriocins to maximize their potential in food and pharmaceutical applications.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

RHP and FBE outlined the topics of the review. RHP, FBE and MTP drafted the manuscript. All authors read and jointly approved the final manuscript.

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