**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 subgroups. 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 produced 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 slightest 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 et al-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).
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 & Næs 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 enzymes(es)) (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, NisK and NisL, 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 lipopeptide, NisA, 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 phenoregulator. 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 (Iwataki 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

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

<table>
<thead>
<tr>
<th>Factor Considered</th>
<th>Bacteriocins</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application</td>
<td>Food/Clincal</td>
<td>Clinical</td>
</tr>
<tr>
<td>Synthesis</td>
<td>Ribosomal</td>
<td>Secondary metabolite</td>
</tr>
<tr>
<td>Bioactivity spectra</td>
<td>Mostly narrow</td>
<td>Mostly broad</td>
</tr>
<tr>
<td>Intensity of bioactivity</td>
<td>Active at nano-to-micro molar range</td>
<td>Active at micro-to-nanomolar range</td>
</tr>
<tr>
<td>Protodiastic enzyme degradability</td>
<td>High</td>
<td>Moderate-to-hardy</td>
</tr>
<tr>
<td>Thermal stability</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Active pH range</td>
<td>Wide</td>
<td>Narrow</td>
</tr>
<tr>
<td>Taste/color/tolerance</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>Ameability to bioengineering</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Class</th>
<th>Features</th>
<th>Representative Bacteriocins</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Antibiotics, small (&lt;5 kDa) peptides containing lantibiotic and 3-methyl-lantibiotic</td>
<td>Nisin A, Nukacins ISK-1</td>
</tr>
<tr>
<td>II</td>
<td>Small (&lt;10 kDa), heat-stable, non-lantibiotic-containing peptides</td>
<td>Pediococin PA-1/AcH, Saracin A and P, Leuconoc A, Camobactericin</td>
</tr>
<tr>
<td>iia</td>
<td>Small heat-stable peptides, synthesized in a form of precursor which is processed after two glycolenic residues, active against Listeria, have a consensus sequence of YGNQVXC in the N-terminus</td>
<td>Lactococins G and F, Lactation F, Plantarinf EF and JK, Brochoxin C</td>
</tr>
<tr>
<td>iib</td>
<td>Two component systems: two different peptides required to form an active portion complex</td>
<td>Enterocin As-48, Lactococin Q</td>
</tr>
<tr>
<td>ii c</td>
<td>N- and C- termini are covalently linked, resulting in a circular structure</td>
<td>Enterocin B, Lacticin Q</td>
</tr>
<tr>
<td>iii</td>
<td>Large molecules heat sensitive peptides</td>
<td>Helicetens J, Acidoph disin A, Lactocins A and B</td>
</tr>
</tbody>
</table>

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*Table 3.* Intensity of bioactivity of bacteriocins (Heng et al. 2007).

<table>
<thead>
<tr>
<th>Intensity of bioactivity</th>
<th>Target cell developing characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active at nano-to-micro molar range</td>
<td>Amino acid, eukaryotic cells</td>
</tr>
<tr>
<td>Active at micro-to-micromolar range</td>
<td>N-terminal region that gives this group a very strong inhibitory ability against the target cell</td>
</tr>
<tr>
<td>Wide</td>
<td>Active pH range</td>
</tr>
<tr>
<td>Narrow</td>
<td>Ameability to bioengineering</td>
</tr>
<tr>
<td>Yes</td>
<td>Mode of action</td>
</tr>
<tr>
<td>None</td>
<td>Antibiotics, small (&lt;5 kDa) peptides containing lantibiotic and 3-methyl-lantibiotic</td>
</tr>
<tr>
<td>Yes</td>
<td>Other class II bacteriocins, including sec-dependent bacteriocins and leaderless bacteriocins</td>
</tr>
</tbody>
</table>
| Relatively low | Class IIa bacteriocins have a distinct conserved sequence YGNQVXC in the N-terminal region that gives this group a very strong inhibitory ability against the highly pathogenic food-borne pathogen Listeria monocytogenes (Emnahar et al. 2000; Finland et al. 2005). This group is often termed as pediocin-like because the first discovered bacteriocin belonging to this group was pediocin PA-1/AcH (Finland et al. 2005; Drider et al. 2006). While class IIa bacteriocins are two-peptide bacteriocins, class IIb bacteriocins that require both peptides to work synergistically to be fully active (Oppgaard et al. 2007; Nissen-Meyer et al. 2010), class IIc bacteriocins, circular bacteriocins (class IIc), and unmodified, linear, non-pediocin-like bacteriocins (class IIId) (Table 2).

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Class Ia bacteriocins (pseudo- and leaderless)

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 (Ganneau 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+, Ca2+, but not divalent cations such as Mg2+ 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 require a receptor on the target bacterial surface to bind specifically to 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 carnosyclic 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, linear 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 lactacin Q, a leaderless bacteriocin, has been well characterized (Yoneyama et al. 2009b). While most bacteriocins require a docking molecule for their antimicrobial action, lactacin Q and its homologue bacteriocins, lacticin 3147 and its homologue bacteriocins, lactacin Q has been found to cause high-level membrane permeabilization of target strains without the need of any specific receptors (Yoneyama et al. 2009a). Lactacin 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 lactacin Q molecules and the negatively charged phospholipid bilayer of the bacterial cell membrane. The negatively charged phospholipid bilayer membrane (i) that results in the formation of HTPs coupled with lipid flip-flop that causes the leakage of intracellular components, including ions, ATP, and small molecules, and (ii), after which, the lactacin Q molecule mass translocates itself from the outer to the inner membrane (Fig. 3) (Yoneyama et al. 2009b). However, the killing mechanism through HTP formation of lactacin Q is selective and highly dependent on the physiological features of the outer membrane of target cells, which explains the non-toxicity of lactacin 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 lactacin Q; 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 toxicity of lactacin 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 lactacin Q.** The highly cationic lactacin Q molecules rapidly bind to the negatively charged phospholipid bilayer membrane that results in the formation of HTPs coupled with lipid flip-flop that causes the leakage of intracellular components, including ions, ATP, and small molecules.

**Fermentation Studies in LAB Growth and Bacteriocin Production**

Bacteriocin production is a growth-associated activity of bacteria. However, the yield of bacteriocin per unit biomass is affected by several factors, including the producing strain, media (carbohydrates 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; Ishiguro-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 source (Mataragas et al. 2004). For Lb. penosus ST151BR, trypophane-stimulated growth and bacteriocin production, whereas lower activity was observed with yeast extract, yeast extract or their combinations. Malose, lactose, and mannose were preferred carbon sources over sucrose, fructose, and glucose. Tween 80 and glycerol also adversely affected bacteriocin production (Todorov & Dicks 2004). Similarly, tryptophane and saccharose increased bacteriocin production by Enterococcus lactici 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 (Izqueez et al. 2002).

Leryn & Vuyts (2001) have done kinetic modeling of the growth of Lb. sakei CTC-494 in the chemically-defined medium of 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 LPC010 was optimized in batch culture using plantaricin C in an integral statistical approach, fractional factorial three-level design experiment (Sanz et al. 2002). Callawett & 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 DCE471. Fed-batch fermentation was also found suitable for Lb. curvatus L442 and L. 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 0.05–0.1 h−1 using glucose as carbon source at a dilution rate of 0.1 to 0.12 h−1 when sucrose or fructose was used (Harcena 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 acidilacticus 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 was concentrated when pH 4.5 to 4.9 sugars, 2% yeast extract and 4% glucose. Results showed that the biomass and bacteriocin production were higher than when MRS broth was used. Another study previously investigated was mussel processing waste (Guerra & Castro 2002). Some bacteriocins from mussel waste have been isolated 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 activity against Gram-negative bacteria, has been used to scavenge the hydroxyl radicals (Li et al. 2013).

**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 hypothesized that LAB are utilized 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 activity against Gram-negative bacteria, has been used to scavenge the hydroxyl radicals (Li et al. 2013).

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 (Schobitz et al. 2006).

The increasing incidence of multidrug resistance bacterial pathogens is one of the most pressing medical problems in recent years (Spellig et al. 2008; WHO 2014). The clinical application of LAB as a potential alternative 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-peak lactic acid bacteriocin 3147 has been found to be active against the methicillin-resistant Staphylococcus aureus (MMSA) strain and vancomycin-resistant Enterococcus faecalis (VRE) strain (Galvin et al. 1999). Furthermore, because bacteriocins are ribosomally synthesized, they have relatively simpler biosynthetic pathways as compared to secondary metabolite antibiotics. The gene-encoded nature of bacteriocins makes them easily amenable to bioengineering to either increase their activity or specify their target organism. Owing to the strength of bacteriocins, 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).

However, there are still some serious bottle-necks 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 (Yufiu 2013). In addition, the use 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 for their further development. The weak potency of many bacteriocins makes them easily amenable to bioengineering to either increase their activity or specify their target organism. Owing to the strength of bacteriocins, 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).
Nevertheless, the Food Safety and Functional Food Biotechnology Program (SFBBP) 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 SFBBP. 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 AAs (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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate/Strain Accession No.</th>
<th>16S rDNA Partial Sequence Homology (%)</th>
<th>papA</th>
<th>PCR Homology [450 bp]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediococcus acidilactici</td>
<td>AA-5a (PNCM 10287)</td>
<td>P. acidilactici DSM 20284</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4E2</td>
<td>P. acidilactici NBRC 12231</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4E4</td>
<td>P. acidilactici DSM 20284</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4E5</td>
<td>P. acidilactici DSM 20284</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4E6</td>
<td>P. acidilactici DSM 20284</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4BL7</td>
<td>P. acidilactici DSM 20284</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3F3</td>
<td>P. acidilactici NBRC12231</td>
<td>papA</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3F8</td>
<td>P. acidilactici NBRC12231</td>
<td>papA</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3F10</td>
<td>P. acidilactici DSM 20284</td>
<td>papA</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3G3</td>
<td>P. acidilactici IMAU20090</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3G8</td>
<td>P. acidilactici UL5</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>IG7</td>
<td>P. acidilactici IZCC0911MX</td>
<td>papA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>B19</td>
<td>P. acidilactici UL5</td>
<td>papA</td>
<td>+</td>
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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 (Sagipio & 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 (Sagipio et al. 2007). Optimization works on bacteriocin production from P. acidilactici AAs 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; Sagipio 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.

REFERENCES


Oppegaard, C., Rogne, P., Emanuelsson, L., Kristiansen, P.E., Finland, G., Nissen-