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Bacteriocin, Antimicrobial as A New Natural Food Preservative: Its Potential and Challenges

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ABSTRACT / ABSTRAK

Seiring dengan pertambahan penduduk dunia, produksi pangan juga harus ditingkatkan. Selain itu, industri makanan harus selalu memperhatikan preferensi pelanggannya untuk meningkatkan kualitas dan penjualan produk makanannya. Oleh karena itu, keamanan pangan dan kualitas pangan akan selalu menjadi perhatian utama dalam industri pangan. Keamanan pangan adalah kegiatan untuk membuat pangan aman dan bebas dari agen penyebab penyakit, misalnya agen infeksi, bahan kimia beracun, dan benda asing. Kualitas pangan mengacu pada semua sifat termasuk rasa, tekstur, dan warna produk pangan yang baik. Masyarakat modern saat ini cenderung memilih makanan yang rasanya segar, bebas dari bahan tambahan kimia, kaya nutrisi, dan seminimal mungkin melalui proses produksi. Bakteriosin sebagai pengawet alami baru, dapat diproduksi oleh bakteri Gram positif, Gram negatif, dan *Archaea.* Sebagian besar bakteriosin dihasilkan oleh Bakteri Asam Laktat (BAL). Baik bakteriosin maupun bakteri penghasil bakteriosin telah lama digunakan sebagai alternatif pengganti bahan tambahan kimia. Penggunaan senyawa antimikroba ini telah terbukti efektif sebagai pengawet alami sehingga seringkali digunakan dalam rantai produksi dan distribusi makanan untuk meningkatkan umur simpan produk pangan. Potensi lain dari penggunaan pengawet alami ini dalam berbagai jenis makanan masih besar. Namun, persetujuan penggunaan bakteriosin dan aplikasi komersialnya masih sangat terbatas. Ulasan ini dibuat berdasarkan studi literatur yang mencakup kriteria bakteri yang dapat digunakan, mekanisme kerja hambatnya, potensi aplikasinya pada industri makanan, farmasi serta potensinya sebagai agen anti-kanker. Selain itu, ulasan ini akan memaparkan beberapa tantangan dalam aplikasi bakteriosin, tahapan proses identifikasi dan karakterisasi hingga isu keamanan dari penggunaan bakteriosin maupun bakteri penghasil bakteriosin khususnya sebagai probiotik.

As the world's population increases, food production must also increase. Additionally, the food industries must always consider their customers' preferences to improve the quality and sales of their food products. Therefore, food safety and food quality will always be a major concern in the food industry. Food safety is an activity to make food safe and free from disease-causing agents, for example, infectious agents, toxic chemicals, and foreign objects. Food quality refers to all characteristics including taste, texture, and color of food products. Modern society today tends to choose food that tastes fresh, free from chemical additives, rich in nutrients, and has a minimal production process. Bacteriocin as a new natural preservative, is produced by Gram-positive, Gram-negative, and Archaea

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bacteria. However, most of the bacteriocins are produced by Lactic Acid Bacteria (LAB). Both bacteriocins and bacteriocin-producing bacteria have long been used as alternatives to chemical additives. The use of these antimicrobial compounds has been proven to be effective as a natural preservative, so it is often used in the food production and distribution chain to increase the shelf life of food products. Other potential uses of this natural preservative in various types of food are still large. However, the approval for the use of bacteriocins and their commercial applications as food preservatives is still limited. This review is based on a literature study covering the criteria for usable bacteria, the mechanism of action of its inhibition, its potential application in the food and pharmaceutical industries, and its potential as an anti-cancer agent. Additionally, this review will describe several challenges in the application of bacteriocins, the stages of the identification and characterization, and the safety issue of using bacteriocins and bacteriocin-producing bacteria, especially as probiotics.

Keywords: Food, Food Industries, Bacteriocins, Gram-positive bacteria, Gram-negative bacteria, Archaea, Lactic Acid Bacteria (LAB), natural preservative, pharmaceutical industry, anti-cancer agent, probiotics.
Kata Kunci: Pangan, Industri Pangan, Bakteriosin, Bakteri Gram positif, Bakteri Gram negatif, Archaea, Bakteri Asam Laktat (BAL), pengawet alami, industri farmasi, agen anti kanker, probiotik.

1. Introduction

Bacteriocins are produced by some group of bacteria and ribosomal synthesized peptides. Bacteriocins show inhibition (bacteriostatic or bactericidal) activity to various groups of undesirable microorganisms. This compound is produced by Gram-negative, Gram-positive bacteria, and some archaebacteria (Lopetuso *et al.*, 2019, p.1). Gram-negative bacteria such as *Escherichia coli*, *Shigella sonnei*, *Shigella boydii*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *Citrobacter freudii* produce bacteriocins, such as colicins and microcins to kill other closely related species to get more nutrients, living space and reduce competitors (Yang *et al.*, 2014, pp. 3-5). However, bacteriocins are mostly known produced by Lactic Acid Bacteria (LAB). This group of bacteria is nonsporulating, Gram-positive, and facultative or anaerobic bacilli and cocci. *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc,* and *Pediococcus* are the major LAB found to produce bacteriocins (Bintsis, 2018, p. 89). Additionally, the Archaea, including extreme halophiles, hyperthermophiles and the methanogens could also produce antibiotics peptides known as archaeocins, halocins and sulfolobicins (O`Connor & Shand, 2002, p. 23).

As the world population's growth increases, food production must also increase. Therefore, food safety and food quality will always be major concerns in the food industry. Food safety refers to the activity to make food safe and free from disease-causing agents, for instance, infectious agents, toxic chemicals, and foreign objects. Additionally, these agents might include microorganisms, pesticides, misuse of food additives, chemical contaminants, biological toxins, and/or adulteration (FAO, 2003, p. 1). Food quality refers to all properties including good taste, texture, and color of food products. These food attributes represent sensory and suitability value as subjective components (Leitzmann, 1993, p. 3).

Furthermore, the food industry must always consider their customer's preferences to increase the quality and sales of their food products. At present, the increasing knowledge of the world community on food quality and safety, creates the customers tend to choose food products with upgraded quality, fresh-tasting, no chemical additives, longer shelf life, minimally processed, and rich of nutrition (Zhang *et al.*, 2018, p. 585). Consequently, reducing the foodborne illness due to the pathogens and spoilage microorganisms in food will be more extraordinary as the customer's preference to have foods without chemical additives (Ben Said *et al.*, 2019, p. 138). Bacteriocin could be used as new alternatives to replace the use of chemical additives. Bacteriocin based strategy might be used to enhance food safety.

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This review article has been focused on the several criteria for the use of bacteria, the potential, and challenges of using bacteriocins as natural bio-preservative agents. Moreover, identification and characterization of new bacteriocin, its approval process, and some related safety issue for the use of bacteriocin-producing bacteria as probiotics should be considered to ensure drug and food safety before marketing.

2. Safety Criteria and How to Apply Bacteriocin

Many antibacterial compounds containing bacteriocins, organic acids, short-chain fatty acids, hydrogen peroxide, and enzymes are produced by probiotics to inhibit gastrointestinal pathogens, regulate the host immune system, and strengthen the intestine barrier (Yang *et al.*, 2014, pp. 3-5; Ben Said *et al.*, 2019, p. 139; Dobson *et al.*, 2012, p. 1). Bacteriocins are considered as one of the probiotics traits. To get the benefits of bacteriocin, bacteriocin-producing bacteria or purified bacteriocin bacteriocins could be used in food products. Nowadays, probiotics are widely used in life, consisting of LAB, non-pathogenic bacilli, *E. coli*, and yeasts (Yang *et al.*, 2014, p. 5). Many recent studies show that LAB is one promising group of bacteria to replace chemical preservatives in food products. LAB includes lactobacilli, lactococci, enterococci, bifidobacteria, and streptococci, which have been used as bio-preservatives in some fermented foods. Considering its potential to protect the foods from undesirable bacteria, prolong the food shelf life and increase fresh tasting in foods, bacteriocin-producing bacteria and bacteriocin must meet some following requirements: 1) non-pathogen or Generally Recognized as Safe (GRAS); 2) show inhibition activity against spoilage microorganism and pathogens; 3) could survive and stay active during production process, storage, and distribution; 4) economical use; 4) show activity in low concentration; 5) and does not affect the taste and texture of foods (Yang *et al.*, 2014, pp. 3-5; Ben Said *et al.*, 2019, pp. 138-139; Holzapfel, Geisen and Schillinger, 1995, p. 346; Gautam and Nivedita, 2009, p. 205).

Bacteriocins could be incorporated into food, feed, or pharmaceutical products in three ways: 1) using bacteriocin-producing bacteria to replace starter culture in fermented food, 2) using bacteriocin-producing bacteria from previous fermented food, and 3) using purified or semi-purified bacteriocins. They could be added to food or pharmaceutical products by surface coating, dipping, or spraying them to the finished products (Ben Said *et al.*, 2019, p. 143). Bacteriocin-producing bacteria has been applied in fermented foods to replace the use of non-bacteriocin-producing cultures, for instance, the use of *Lactobacillus curvatus* DF38 and *Lactobacillus plantarusm* 423 in salami production to inhibit the growth of *Listeria* (Ben Said *et al.*, 2019, p. 143; Todorov *et al.*, 2007, p. 405). Purified bacteriocins could also be added to food products by utilizing edible cellulosic film and polyethylene-based plastic films, directly mixing the food with bacteriocin solution, and using bacteriocin adsorption on different surfaces such as polyethylene and ethylene vinyl acetate, and using bacteriocin-containing packaging (Gautam and Nivedita, 2009, p. 208). Edible film-bacteriocin packaging will inhibit unfavorable microorganisms during storage or distribution. Bacteriocins are bound by covalent binding in the packaging systems (López-Cuellar, Adriana-Inés, and Norberto, 2016., p.1043). The use of purified bacteriocins may be more expensive due to the purification step. Besides, larger doses might be needed, and the loss of efficacy will rapidly occur. However, the use of purified bacteriocin will not affect the food taste and texture as appear of using bacteriocin-producing bacteria (Ben Said *et al.*, 2019, p. 143). Recently, bacteriocins nano-capsules have been developed using nanoparticles, nanoliposomes, nanofibers, and nanoemulsion, increasing the possibility of more effective application in other industrial areas (López-Cuellar, Adriana-Inés, and Norberto, 2016., p.1042).

3. Classification of Bacteriocin

Gram-positive bacteria produce various types of bacteriocins in different sizes, properties, structures, and activity spectrum. Bacteriocins are classified based on their post-translational

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modification or peptide biosynthesis (Ibrahim, 2019, p. 594). Most researchers classify bacteriocins into four classes (Figure 1 and Table 1). In general, class I experiences the post-translational modification, whereas class II, III, and IV are modified during the post-translational process (López-Cuellar, Adriana-Inés, and Norberto, 2016., p.1040).

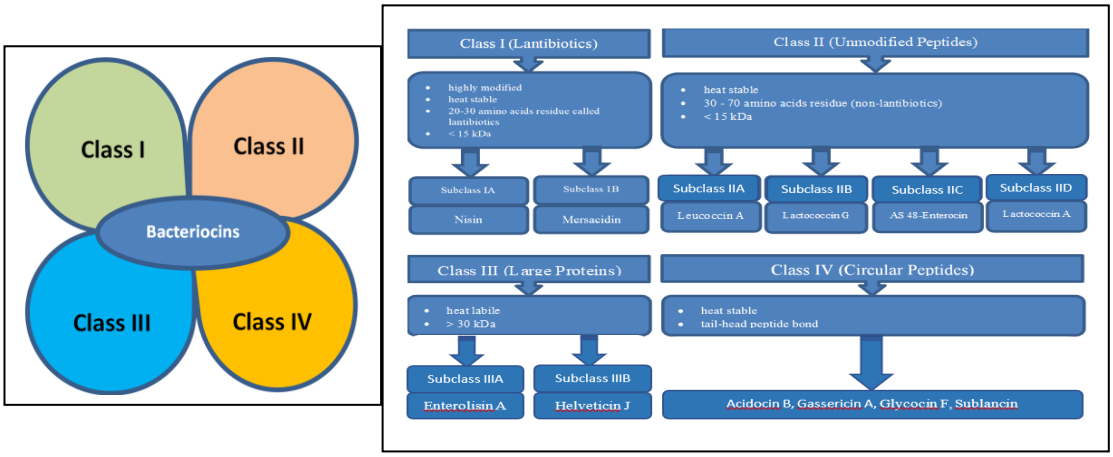

Figure 1. Classification of bacteriocins produced by Gram-positive bacteria (Yang *et al.*, 2014, pp. 3-5; Ibrahim, 2019, pp. 593-602; Slavica *et al.*, 2014, p. 275; Verma *et al.*, 2014, p. 181; Shengyue Ji., *et al.*, 2015, p. 1; Amso Z., *et al.*, 2018, p. 1686).

Table 1. Bacteriocins produced by Gram-positive bacteria.

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Bacteriocin Class	Bacteriocin Name	Producer
Class I (Chen and Hoover, 2003, p. 84)		
Subclass Ia	Nisin	*Lactococcus lactis*
	Lacticin 481	*Lactococcus lactis*
	Lactocin S	*Lactobacillus sake*
	Epidermin	*Staphylococcus epidermidis*
	Gallidermin	*Staphylococcus galinarum*
Subclass Ib	Mersacidin	*Bacillus subtilis*
	Cinnamycin	*Streptomyces cinnamoneus*
	Duramycin	*Streptomyces cinnamoneus*
	Auncovenin	*Streptomyces* ssp.
	Actagardin	*Actinoplanes* ssp.
Class II (Chen and Hoover, 2003, p. 84)		
Subclass IIa	Pediocin PA-1/Ach	*Pediococcus acidilactici*
	Sakacin A	*Lactobacillus sake*
	Sakacin P	*Lactobacillus sake*
	Mesentericin Y105	*Leuconostoc mesenteroides*
	Enterocin A	*Enterococcus faecium*
	Divercin V41	*Carnobacterium divergens*
	Lactococcin MMFII	*Lactococcus lactis*
	Leucocin A-UAL 187	*Leuconostoc gelidum*
Subclass IIb	Lactococcin G	*Lactococcus lactis*
	Lactococcin M	*Lactococcus lactis*
	Lactacin F	*Lactobacillus johnsonii*
	Plantaricin A	*Lactobacillus plantarum*
	Plantaricin S	*Lactobacillus plantarum*
	Plantaricin EF	*Lactobacillus plantarum*
	Plantaricin JK	*Lactobacillus plantarum*
Subclass IIc	Acidocin B	*Lactobacillus acidophilus*
	Carnobacteriocin A	*Carnobacterium pisciola*
	Divergicin A	*Carnobacterium divergens*
	Enterocin P	*Ecterococcus faecium*
	Enterocin B	*Enterococcus faecium*
	Enterocin AS-48	*Enterococcus faecalis*
Subclass IId	Lactococcin A	*Lactococcus lactis*
	Aureocin A53	*Staphylococcus aureus*
	Thuricin S	*Bacillus thuringiensis subsp. entomocidus*
Class III (Yang *et al*., 2014, p. 5; Ibrahim, 2019, pp. 601-602)[2,15]		
Subclass IIIa	Enterolysin A	*Enterococcus faecalis*
	Lysostaphin	*Staphylococcus simulans*
Subclass IIIb	Helveticin J	*Lactobacillus helveticas*
	Caseicin 80	*Lactobacillus casei*
Class IV (Ibrahim, 2019, p. 602; Ji *et al.*, 2015, p. 1; Amso *et al.* 2018, p. 1686)[15,41,42]		
	Glycocin F	*Lactobacillus plantarum*
	Sublancin	*Bacillus subtilis*

Colicin is one of the bacteriocins produced by Gram-negative bacteria that widely studied. Colicins are produced by Gram-negative bacteria without any post-translational modification. Although colicin mostly represents the bacteriocin produced by Gram-negative bacteria, there are some differences in the bacteriocin produced by Gram-negative bacteria (Figure 2). In *Escherichia coli*, colicin is encoded in plasmid replicons, while colicin-like substance from *Serratia marcescens* is encoded in chromosome and plasmid. Additionally, pyocins, colicin-like and phage tail-like bacteriocins produced by *Pseudomonas aeruginosa* is encoded in the chromosome (Simons, Kamel, and Raphaël, 2020, p. 5).

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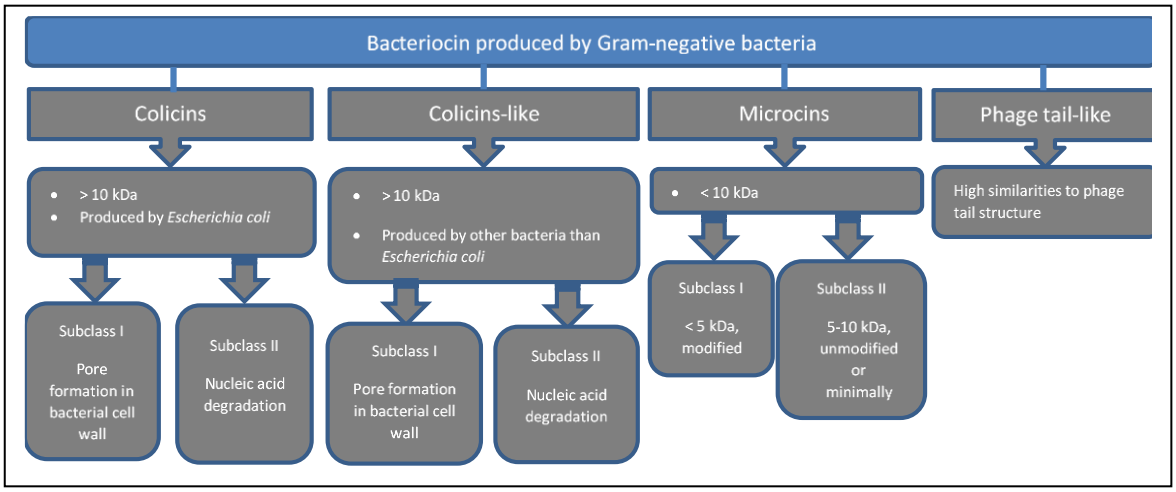

Figure 2. Classification of bacteriocins produced by Gram-negative bacteria (Simons, Kamel, and Raphaël, 2020, pp. 5-13).

Archaea could also produce the bacteriocin-like substance, called archaeocins. Halocin is one of the archaeocins which is produced by halobacteria. Halocins are different in size, ranging from 3,6 kDa as the smallest size and known as microhalocins to 35 kDa as the largest size (O`Connor & Shand, 2002, p. 23). As an example, Halocin S8 contains 36 amino acids and is encoded in mega-plasmid. It is resistant to boiling, organic solvents, desalting, and low temperature for a certain period. Due to its stability, halocins may abide in the environment to diminish the competition (Verma *et al.*, 2014, p.182). Halocins could be detected just before the cultures reach the transition step to the stationary phase. Sulfobicins are archaeocins that produced by *Sulfolobus islandicus*. They are not excreted into the environment; however, it is suspected that they have been associated with small particles from the cell's S-layer (O`Connor & Shand, 2002, p. 23).

4. Mode of Bacteriocin Activity

Bacteriocins have different structures and sizes that could affect bacteriocin activity in inhibiting other bacteria. Of the many studies conducted, most studies show that bacteriocin works by forming pores or channel in the cell membrane and disrupt the energy potential of sensitive pathogens or spoilage microorganisms (Oscariz and Antonio., 2001, p. 16; Hwanhlem and Aran., 2015, p. 183). Naturally, bacteriocin could inhibit cell membrane synthesis by forming pores or interacting with lipids in the cell membrane (Ben Said *et al.*, 2019, p. 140). In general, the mechanism of several bacteriocins is as follows: 1) the bacteriocin directly contact with the cell membrane, 2) this activity will disrupt the membrane potential and the cell becomes weak, 3) membrane instability may affect the formation of holes or pores in the cell membrane through the disruption of PMF (Proton Motivation Force) (Gonzales *et al.*, 1996, p. 2708). The effect of the formation of the pores in the cytoplasmic membrane leads to the changes in the membrane potential gradient (ΔP) and the release of intracellular molecules as well as the entry of extracellular (environmental) substances. The effect causes cell growth to be inhibited and results in a death process in cells that are sensitive to bacteriocins (Drider *et al.*, 2006, p. 571).

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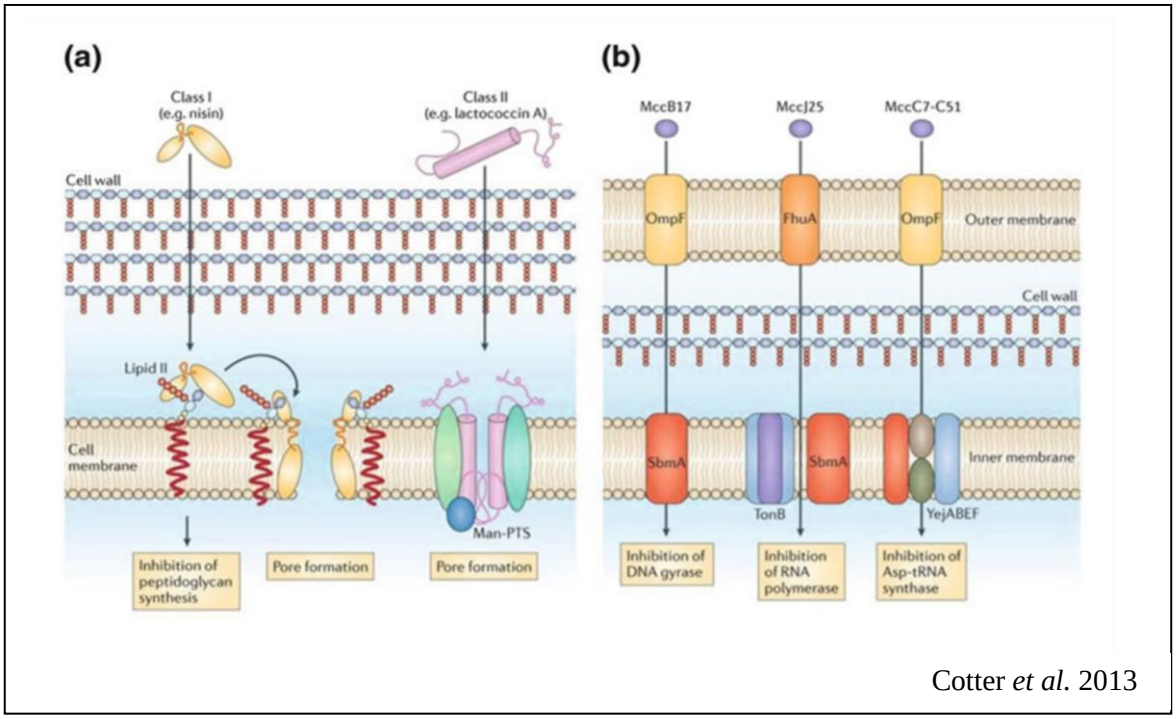

Figure 3. Representatives for mode of bacteriocin activity targeting a) Gram-positive bacteria target and B) Gram-negative target (Cotter *et al*., 2013, p. 102).

4.1 Bacteriocin produced by Gram-positive bacteria

Bacteriocins from LAB could link to lipid II that functions to transport peptidoglycan subunits from cytoplasm to cell wall, thus inhibiting the cell wall synthesis. Furthermore, bacteriocin could utilize lipid II as a docking molecule which then leads to pore formation in the cell membrane (Figure 3a). Nisin and lactacin 3147, as examples of subclass Ia bacteriocin, were known to have these two activities. Differ from those two bacteriocins, mersacidin from subclass Ib bacteriocin could bind to lipid II, but unable to create pores (Cotter *et al*., 2013, p. 97; Zacharof and Lovitt, 2012, pp. 51-52). Class I bacteriocins would destroy lipid bilayer organization when they bind to the cell membrane. Positively charged lantibiotics will interact with negatively charged membrane phospholipids. The interaction between nisin and the bacterial membrane produces ion channels. The pore formation causes an imbalance of ion transfer and leads to the rapid death of other bacteria (Zacharof and Lovitt, 2012, pp. 51-52).

Similar to class I, class II of bacteriocin which consists of several subclasses have the same mechanism of action as nisin. Subclass IIa is a bacteriocin that is specifically capable of killing *Listeria monocytogenes* because of its specific sequence arrangement in the N terminal region. The bacteriocin that belongs to this group involves electrostatic bonds between bacteriocin and membrane, presumably utilized molecular receptors on the membrane. The inhibition mechanism of subclass IIb also involves the change of membrane potential and a reduction of intracellular ATP concentration (Delesa, 2017, p. 181). Another subclass, IIc, has a cyclic structure due to its covalent bond between N and C terminals (Kawai *et al.*, 2004, p. 2906). AS-48 enterocin, reutericin 6, and circularin A are examples of this subclass (Delesa, 2017, p. 181). Circular bacteriocin is mainly produced by LAB and a few from *Bacillus* and *Clostridium*. This compound could inhibit the broad-spectrum of bacteria due to its stability in different pH and temperatures. Circular structure is expected to play an important role in its stability and resistance to proteolytic enzymes (Gabrielsen *et al.*, 2014, p. 6854

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& 6858). Its cyclic structure might decrease the existence of possible cutting sites. Although circular bacteriocin belongs to subclass IIc, some researchers have included it into class IV or new class V of bacteriocin with some debatable issues (Gabrielsen *et al.*, 2014, p. 6854; Heng *et al.*, 2007, p. 52). According to the Klaenhammer's classification, subclass IIc could be more subclassified into two different groups depending on the existence of intramolecular disulfide bonds. Circular bacteriocin with or without cysteine residues shows activity at membrane level by forming pores (Oscariz and Antonio, 2001, p. 15). The broad spectrum of subclass IIc prompt that a specific membrane-bound bacteriocin receptor is not necessary. Positively charged amino acids in the N-terminal region could unspecifically interact with negatively charged phospholipids in the target membrane. A study about AS-48 enterocin has informed that linear forms of this peptide is essential to withstand its antimicrobial activity, while the circle form is only necessary to stabilize the structure and unimportant for antimicrobial activity (Gabrielsen *et al.*, 2014, p. 6858 & 6859). However, similar to other class II, the circular bacteriocins are generally worked by disrupting the membrane integrity of the target cells (Gabrielsen *et al.*, 2014, p. 6859). Subclass IId has a wide diversity in structures and different activity against Gram-positive bacteria. The great examples of subclass IId are lactococcin A, aureocin A53 and thuricin S (Ibrahim, 2019, p. 594 & 600; Netz, 2002, p. 5274; Nissen-Meyer, 2010, p. 52; Chehimi *et al.*, 2010, p. 162). Lactococcin A binds to membrane-embedded part of the mannose phosphotransferase system (Man-PTS) which leads to membrane leakage and causes cell death (Figure 3a) (Nissen-Meyer, 2010, p. 52; Cotter *et al.*, 2013, p. 101). Aureocin A53 produced by *Staphylococcus aureus* A53, interacts with acidic and neutral membranes of target cells, disrupts the membrane by causing imbalance membrane permeabilization without forming the pores (Ibrahim, 2019, p. 600; Netz, 2002, p. 5274). In a different way, thuricin S forms pores in sensitive cells, thus the cells lose their membrane integrity (Chehimi *et al.*, 2010, p. 162). Generally, the class II bacteriocins have amphiphilic helical structures, which enable them to enter the cell membrane of the target cell and cause depolarization and cell death (Cotter, Hill, & Ross, 2005, p. 781).

Class III consists of large size (>30 kDa) and thermolabile bacteriocins with complex structure and activity. This class could be divided into: 1) subclass IIIa, for example, enterolysin A and lysostaphin, and 2) subclass IIIb, for example, helveticin J and caseicin 80 (Yang, 2014, p. 4; Ibrahim, 2019, p. 602). Their mode of action is dissimilar to other class of bacteriocins. They work by inhibiting cell wall synthesis or creates lysis of the cell wall of the target cells. The C terminal is necessary for recognizing the target cells, whereas the N-terminal is an endopeptidase homologous that involved in cell wall synthesis (Delesa, 2017, p. 181).

Class IV is a circular bacteriocins that contains other non-protein moieties such as lipid and carbohydrate. These moieties are necessary for their action and still need to be more characterized (Delesa, 2017, p. 181).[22] Two bacteriocins from this class, Glycocin F and sublancin have been elucidated and grouped into different subclasses. Glycocins shows bactericidal function for a broad range of Gram-positive bacteria, while sublancin is effective against *Staphylococcus aureus* (Ibrahim, 2019, p. 602; Ji *et al.*, 2015, p. 1; Amso *et al.*, 2018, p. 1686).

4.2. Bacteriocin produced by Gram-negative bacteria

Colicins, bacteriocins produced by Gram-negative bacteria, similarly act like bacteriocin from Gram-positive bacteria as also shown in Figure 2. Colicins have three main regions, an amino-terminal translocation (T) region, a central receptor-binding (R) region, and a carboxy-terminal cytotoxic (C) region that shows antimicrobial activity (Yang, 2014, p. 1). Colicins have been classified into groups A and B based on the translocator system. Group A utilize Tol system to enter the outer membrane of sensitive cells, for instance colicins E1 to E9, colicins A, K, etc. Group B utilize the Ton system to enter the outer membrane of sensitive cells, for instance, colicins 5,10, B, D, etc. (Dimov *et al.*, 2005, p. 5). According to their mode of action after entering the target cells, colicins are grouped into pore-forming type and nuclease-type, and peptidoglycanase type. Pore-forming type will create pores in the cell membrane which cause ion loss and cell death, for instance,

colicin A, B, Ia, Ib, E1, N, and K. Nuclease type colicin acts by digesting DNA or RNA non-specifically. It consists of colicins E2 to E9. Lastly, peptidoglycanase type will digest the precursor of peptidoglycan, therefore the target cells could not be able to synthesize peptidoglycan (Cascales *et al.*, 2007, p. 161). Other examples with different modes of action, microcin J25 (MccJ25) could inhibit RNA, MccB17 could inhibit DNA gyrase, and MccC7-C51 could inhibit aspartyl-tRNA synthetase (Figure 3b). Contrary to these microcins, MccE492 function through pore formation (Cotter *et al*., 2013, p. 102).

4.3 Bacteriocin produced by Archae

Additionally, micro halocins, as one member of archaeocins, consist of the huge number of neutral and non-cationic residues, that the specific mode of action is not fully understood. Therefore, the inhibition mechanism needs to be more elucidated because it was reported that micro halocins could affect several halo archaeal genera and *Sulfolobus* species (O'Connor & Shand, 2002, p. 23).

5. Potential Application of Bacteriocin

5.1 Food Industry

Either bacteriocin-producing bacteria or bacteriocin itself could be used as natural bio-preservatives to prolong the food shelf life (Lulietto, 2018, p. 849). Fermentation is the most common way of bio-preservation using natural or controlled bacteriocin-producing bacteria. Bacteriocin-producing bacteria or also known as bioprotective cultures have long been used as natural bio-preservation in many fermented foods like yogurt, cheese, and fermented meat since old-time (Ben Said, *et al.*, 2019, pp. 140-142; Yang *et al.*, 2012, p. 1). During fermentation, bioprotective cultures will degrade a complex compound to produce acids or alcohol, synthesize various vitamins or precursors, enhance the food quality by producing aroma and flavor compounds and synthesize bacteriocin to inhibit the growth of pathogens and spoilage or undesirable microorganisms (Delesa, 2017, p. 181).

Table 2. Commercial Bacteriocin.

Bacteriocin	Commercial Name	Applications	Target Microorganisms	Company
Nisin A	Nisaplin®	Dairy, meat, bakery, culinary products and beverages	*Listeria* spp., *Bacillus* spp., *Clostridium* spp.	Danisco, Copenhagen, Denmark
Nisin A, Nisin Z	Nisin A® Nisin Z®	Dairy and bakery products, beverages, delicacies, meat	*Listeria* spp., *Clostridium* spp., *Bacillus cereus*	Handary, Brussels, Belgium
Nisin	Chrisin®	Meat, sausages and spore forming bacteria in cheese	*Clostridium botulinum*, *Listeria monocytogenes*	Chr. Hansen, Horsholm, Denmark
Natamycin	Natamax®	Cheese, fresh dairy products, beverages, processed meat	Yeast and Molds	Danisco, Copenhagen, Denmark
Micocin	Micocin®	Meat products	*Listeria monocytogenes*	CanBiocin, Edmonton, Canada
Pediocin	ALTA®2351 2341	Meat products	*Listeria monocytogenes*	Kerry Bioscience, Carrigaline, Co. Cork, Ireland
Pediocin	Fargo 23®	Meat products	*Listeria monocytogenes*	Quest International, B.V., Naarden, The Netherlands
Pediocin, Sakacin	Bactoferm F – LC®	Meat products	*Listeria monocytogenes*	Chr. Hansen, Horsholm, Denmark

(López-Cuellar, Adriana-Inés, and Norberto, 2016, p. 1046)

Some bacteriocins are already commercially available (Table 2), for instances, nisin and pediocin PA-1 were marketed as Nisaplin® and Alta®2341 (Yang *et al.*, 2014, pp. 6-7; López-Cuellar, Adriana-Inés, and Norberto, 2016, p. 1046). Nisin was the first bacteriocin that has been approved to be used as a bio-preservative in many kinds of food and licensed in more than 45 countries (Yang *et al.*, 2014, pp. 6-7; Settanni and Corsetti, 2008, p. 124). Nisin has broad-spectrum activity against Gram-positive bacteria including *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus*, *Clostridium botulinum*, bacterial spores, spoilage-associating LAB, and Gram-negative bacteria (Ibrahim, 2019, p. 603; Prudêncio, Miriam, and Maria, 2015, p. 5410; Scott and Steve, 1981, p. 117). Pediocin PA-1 is known effective against *Listeria monocytogenes* in meat products (Yang *et al.*, 2014, p. 7; Settanni and Corsetti, 2008, p. 132; Dabour *et al.*, 2009, p. 226). Similar to pediocin PA-1, aureacin A53 is also proved in inhibiting *Listeria monocytogenes* (Ibrahim, 2019, p. 600). In European cheese products, *Enterococci* is used as bioprotective cultures or co-cultures to prevent microbes contamination (Yang *et al.*, 2014, p. 7; Foulquié Moreno, 2006, p. 2). Many LABs have

been used in the food industry as starter, co-cultures, probiotics as well as bioprotective cultures in many fermented and non-fermented foods and vegetables due to they could produce bacteriocins and some natural inhibitors. These bacteriocins and bacteriocins-producing LAB show potential applications in food preservation to improve food safety and quality (Table 3) (Yang *et al.,* 2014, p. 6; Delesa, 2017, p. 186).

Nisin and pediocin PA-1 have another potential application as food packaging material, either vacuum or modified atmosphere packaging. Some studies about the use of 5000 IU/g nisin sprayed on fresh meats before being vacuum-packed and refrigerated, showed the successful inhibition to the growth of *Listeria* spp. Along with the technology development, nisin could be also microencapsulated to avoid its degradation by proteolytic enzymes (Ibrahim, 2019, p. 603; Narsaiah *et al.,* 2014, p. 4054).

Table 3. Research on bacteriocin against pathogen and spoilage microorganisms in foods.

Food Products	Target Microorganisms (Pathogen or Spoilage Microorganisms)	Bacteriocins
Milk and milk products	*Salmonella* spp.	Enterocin AS-48
	Clostridium botulinum	Nisin Z, Thermophilin
	Bacillus cereus	Nisin, Enterocin AS-48
	Listeria monocytogenes	Nisin Z, Lacticin 3174, Pediocin PA-1/AcH, Enterocin CRL35, Propionicin PLG1
	Staphylococcus aureus	Enterocin A, Enterocin CCM 4231, Enterocin 226NWC
	Vibrio parahaemolyticus, Yersinia enterocolitica, Corynebacterium sp., *Pediococcus* spp.	Propionicin PLG-1
	Pseudomonas fluorescens	Variacin
Egg and egg products	*Salmonella enteritidis, Listeria monocytogenes*	Nisin, Pediocin PA-1/Ach
	Listeria innocua, Escherichia coli, Bacillus cereus	Nisin
Meat and meat products	*Staphylococcus aureus*	Enterocin AS-48, Lacticin 3147
	Listeria monocytogenes	Pediocin PA-1, Lactocin AL705, Enterocin AS-48, Sakacin-P, Piscicolin
	Listeria innocua	Enterocin As-48, Lacticin 3147, Sakacin-P
	Brochothrix thermosphacta	Nisin, Pediocin Ach, Lactocin AL705, Sakacin-P
	Leuconostoc, Lactobacillus	Nisin
Cereals and pulses	*Bacillus subtilis*	Nisin
Fruits and Vegetable	*Clostridium sporogenes, Bacillus stearothermophilus, Bacillus macerans, Bacillus coagulans, Salmonella*	Nisin
	Alicyclobacillus acidoterrestris	Nisin, Enterocin AS-48
	Bacillus cereus	Enterocin AS-48
	Staphylococcus aureus	Enterocin AS-48, Enterocin CCM4231
	Pediococci	Thermophilin 110
	Oenococcus oeni	Pediocin PD-1
	Clostridium tyrobutyricum	Bovicin HC5, Nisin
Fish and other seafoods	*Lactobacillus, Photobacterium phosphoreum, Clostridium, Listeria innocua*	Nisin
	Listeria monocytogenes	Nisin, Sakacin P

(Choyam *et al.*, 2019, pp. 4-7)

5.2 Pharmaceutical Industry and Medical Treatment

Since the discovery of antibiotics until now, the frequent inappropriate use and abuse of antibiotics in humans or animals has led to the serious effect of multiple-drug resistance pathogens (Yang *et al.*, 2014, p. 7). Several studies of bacteriocin effect in plant, animal, and human pathogens have been performed and demonstrated that bacteriocin is effective to inhibit *Agrobacterium*, *Brenneria* spp., enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), Vancomycin-Resistance *Enterococcus* (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA) (Yang *et al.,* 2014, p. 7; Cotter *et al.,* 2013, p. 99; Grinter, Milner, and Walker, 2012, p. 1499). It was also reported that 20 strains of *Escherichia coli* has the ability to produce colicins that could inhibit *E.coli* O157:H7, O26, O128, O111, O145 which are responsible for Hemolytic Uremic Syndrome (HUS) and diarrhea in human. In vivo experiment in cattle rumen, STEC was successfully inhibited by colicin E1, E8, E4, K, and J (Yang *et al.,* 2014, p. 7; Jordi *et al.,* 2001, p. 332). In vitro experiments of bacteriocin against ETEC showed that colicin E1 and N were able to inhibit ETEC (Yang *et al.*, 2014, p. 7; Stahl, 2004, p. 3119). OR-7, a class II bacteriocin, was proved to be able against *Campylobacter jejuni*, a human gastroenteritis pathogen (Yang *et al.*, 2014, p. 7; Stern, 2006, p. 3115). These all results have showed the potential application of bacteriocin to overcome the problem of multiple drug-resistance pathogens. Aureacin A53, a subclass IId bacteriocin, has a broad activity against multidrug-resistance *Staphylococcus* spp (Ibrahim, 2019, p. 600; Zhao, 2015, p. 1548). Aureacin A53 has potential applications in pharmaceutical biotechnology as an alternative to antibiotics or a supplement as medical treatment for human and animals which infected by antibiotic-resistance pathogens like MRSA (Ibrahim, 2019, p. 600).

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Bacteriocin has many characteristics to be used as new antibiotic replacement drugs in the future. Bacteriocins could also neutralize endotoxin. The idea about bacteriocins as alternative antibiotics is now being developed by several companies of pharmacy and biotechnology. By utilizing a technology of recombinant DNA, a peptide structure obtained could be used as a starting point of new drug development (Fitri, 2012, p. 64; Bals, 2000, p. 145). Some bacteriocins have been clinically tested from phase 1 to 3 in humans. Bacteriocins has also several important roles relieve inflammation and stimulate the immune system (Fitri, 2012, p. 64; Beisswenger and Bals, 2005, p. 255; Zaiou, 2007, p. 321). According to those reasons, bacteriocins might be qualified to be utilized as antibiotics, inflammation relieving or anti-lipopolysaccharides (Fitri, 2012, p. 64; Bals, 2000, p. 141). Up to now, bacteriocins have been developed for topical use due to safer than systemic use. One leading pharmaceutical company has also established bacteriocins to trigger the immune system without inflammation which indirectly inhibits *Staphylococcus aureus* (Fitri, 2012, p. 66).

Several studies in cancer therapy demonstrated that bacteriocins could act against tumor cells. Bacteriocins produced by Gram-negative bacteria like colicin A and E1 could inhibit the growth of human fibroblast line MRC5 and 11 human tumor cell lines (Yang *et al.*, 2014, p. 7). Colicin A, E2, E3, and D exhibit inhibition function to the murine leukemia cells P388, whereas colicin E1 and E3 inhibited v-myb-transformed chicken monoblasts (Yang *et al.*, 2014, p. 7; Lancaster, Wintermeyer and Rodnina, 2007, p. 16). Another study on fesses from carcinoma and healthy patients indicated bacteriocin-producing *E.coli* may reduce the human colorectal carcinoma.

Microcin E492 secreted by *Klebsiella pneumoniae* RYC492 has been reported to have a toxic effect on a variant of Burkitt's lymphoma (RJ2.25), T cell leukemia (Jurkat), human cervical adenocarcinoma (HeLa), and colorectal carcinoma cells. Pyocin obtained from *P. aeruginosa* was lethal to mice fibroblast (L6OT) cell line. It was reported that purified and partially purified of pyocin from *P. aeruginosa* 42A exhibit cytotoxic effect on human hepatocellular carcinoma and human immunoglobulin derived from multiple myeloma. Nonetheless, both purified and partially purified were entirely non-toxic to the human fetal foreskin fibroblast (HFFF) cell line (Kaur and Sukhraj, 2015, pp. 4-5).

Nisin and other bacteriocins produced by Gram-positive bacteria were also reported to be effective to suppress the growth of cancer cells as shown in Table 4 (López-Cuellar, Adriana-Inés, and Norberto, 2016, p. 1043; Joo *et al.*, 2012, p. 302). However, due to its natural and harmless, bacteriocins show a great potential to be applied as anti-cancer or anti-tumor agents by also performing further investigation of their biological side effects. According to the fact that bacteriocins-producing bacteria are probiotics, the use of bacteriocins-producing probiotics will be potential in cancer prevention or medical treatment.

Table 4. Research of bacteriocin application in pharmaceutical industry and medical treatment.

Bacteriocin	Producer	Application	Target	Treatment	Result
Nisin	*Lactococcus lactis*	Bacterial infection	Methicilin-Resistant *Staphylococcus aureus* (MRSA) strains Xen 30 and Xen 31	Combination nisin and DHBA (dihydroxybenzoic acid) was incorporated with nanofiber	Reducing approximately 88% of bacteria biofilm formation
Nisin	*Lactococcus lactis*	Cancer treatment	Skin cancer in mice	Combining nisin and DOX (doxorubicin)	Reducing tumour size and tumour burden
Nisin ZP and AP	*Lactococcus lactis*	Cancer treatment	Head and Neck Squamos Cell Carcinoma (HNSCC)	*In vitro* and *in vivo*	*In vitro*: reducing viable cells during culture *In vivo*: significantly reduced tumour volume after three weeks
Lacticin 3147	Lactococcus lactis subsp. lactis DPC3147	Systemic Infection	*Staphylococcus aureus* Xen 29	Lacticin 3147 was intraperitoneally applied after infection	Significant reduction of *Staphylococcus aureus* growth in liver, spleen, and kidney of mice
Fermenticin HV6b	Lactobacillus fermentum HV6b MTCC 10770	Contraception	Human spermatozoa	Observation of motility and immobilization of spermatozoa	Reducing motility of human spermatozoa
Fermenticin HV6b	Lactobacillus fermentum HV6b MTCC 10770	Cancer treatment	Hepatocarcinoma, breast carcinoma, perpetual cervical, spleen lymphoblast, kidney embryonal	Fermenticin was exposed to cancer cells	Reducing each of cancer tested in different inhibitory concentration of fermenticin

(Ahire J.J., and Dicks L.M.T. 2015; Preet *et al.*, 2015; Kamarajan *et al.*, 2015; Kaur *et al.*, 2013; Piper *et al.*, 2012; Kaur and Sukhraj, 2015, cited in López-Cuellar, Adriana-Inés, and Norberto, 2016, p. 1043)

6. Challenges of Bacteriocins Application

Bacteriocins are known as harmless and thermostable substances. Therefore, bacteriocins will not alter the microbiota in the gastrointestinal tract is caused by antibiotics and will survive during thermal processing of foods. Some bacteriocins are also reported to be used as suitable food additives for acid and cold-processing foods. Moreover, the bacteriocin-encoding genes of some well-known bacteriocins were already fully characterized and located on naturally movable elements, thus it

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enables to transfer of these genes to other strains to enhance bacteriocin production (Simons, Kamel, and Raphaël, 2020, p. 185).

Despite these all advantages, there are several challenges will be faced in the implementation of bacteriocins. This review has summarized some of the challenges, as follows.

1. Easily degraded by protease enzymes.
   The presence of protease enzymes secreted by some species in foods could break down peptides. Based on bacteriocin structure, which is composed of peptides, increases the possibility of being degraded by proteolytic enzymes (Ben Said *et al.*, 2019, p. 142; Simons, Kamel, and Raphaël, 2020, p. 186; Fitri, 2012, p. 65).
2. Bacteriocin activity may be affected by food matrix and environmental factors.
   Food properties and quality like food structure and composition will potentially inactivate bacteriocin activity (Ben Said *et al.*, 2019, p. 142; Slavica *et al.*, 2014, p. 276). Bacteriocins are hydrophobic and relatively small size molecules. Their hydrophobic characteristics may interact with lipids or easily diffuse to the water phase in food products (Ben Said *et al.*, 2019, p. 142; Simons, Kamel, and Raphaël, 2020, p. 185). Furthermore, several environmental factors like manufacturing condition such as cooling, freezing, high temperature and pressure, pH, homogenization, etc. may indirectly affect the growth of bacteriocin-producing bacteria and their bacteriocin synthesis (Slavica *et al.*, 2014, p. 278; Gálvez *et al.*, 2007, p. 55). Optimization of influencing factors must be performed to obtain an optimum manufacturing condition.
3. Arising of bacteriocin-resistance pathogens
   Certain pathogens are found more resistant to bacteriocins. It could be accidentally or spontaneously occurred (Macwana and Muriana, 2012, p. 8). These resistance pathogens might arise in the prolonged exposure of certain level of bacteriocins (Fitri, 2012, p. 65; Giuliani, Pirri, and Nicoletto, 2007, p. 22; Bastos, Coelho, and Santos, 2015, p. 7). A high level of bacteriocins is not recommended to be utilized in foods. However, researchers have identified this problem, and bacteriocins were proved to be effectively used in some foods like meats and cheese (Gálvez *et al.*, 2007, p. 183 & 184). Additionally, some bacteriocin-producing bacteria could produce more than two kinds of bacteriocins that reduce the arising of bacteriocin-resistant pathogens (Ennahar *et al.*, 2000, p. 89).
4. Costly
   It was reported that the production cost of bacteriocin could be more expensive than antibiotics (Fitri, 2012, p. 65; Giuliani, Pirri, and Nicoletto, 2007, p. 15). This fact will reduce the purchasing power of society and might be unprofitable for producers. However, recombinant technology has been widely developed and could be one of the cheapest production alternatives. By using certain bacteriocin-resistant microorganisms as expression vectors may overcome the costly problems of bacteriocin production (Fitri, 2012, p. 65).

7. Identification and Characterization of New Bacteriocin and Bacteriocin-Producing Bacteria

Identification and characterization are two important parts of the full strategy to discover new bacteriocin and bacteriocin-producing bacteria (Figure 4). Several studies have been performed on the identification of bacteria using morphological, biochemical, and genotypic-based methods. Isolates of bacteria could be characterized by observing their growth on different kinds of media (Abdelhadi *et al.*, 2016, p. 78). As an example, commonly, there are three kinds of specific media utilized for detecting the three different genera of LAB. Isolation of *Lactobacillus* could be performed on MRS Agar, while Streptococci uses KAA and lactic Streptococci uses M17 (Abdelhadi *et al.*, 2016, p. 78; Terzaghi and Sandine, 1975, p. 807). After isolation of bacteria, phenotypic characterization or biochemical test must be performed to ensure the species used in the products. This biochemical test could be replaced by using some commercially available biochemical-based-rapid test kit methods (Benkerroum *et al.*, 2007, p. 483). Biochemical test using

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sugar-based fermentation profile are mostly performed as presumptive identification. According to many studies, phenotypic characterization is not reliable due to the similar results of morphological and biochemical observation that may be obtained during identification testing. Recently, bacterial identification and classification have been mostly conducted using the molecular biology approach. Genotype-based methods are now the most commonly used by many researchers to identify the bacteria based on 16S rDNA for partial or total sequencing techniques. These methods are more robust and rapid compared to traditional culture methods (Abdelhadi *et al.*, 2016, p. 80; Amor, Vaughan, and de Vos, 2007, p. 742S). Thus, it could be an alternative for the culture method. Moreover, a genotype-based method is useful to be used as a screening or confirmation step on the identification of bacteria. Both culture and genotype-based methods are possible to be used in the identification of other bacteriocin-producing bacteria.

To be commercially produced, after isolation of bacteria, identification of antimicrobial activity from bacteria must be performed using the agar well diffusion method. In study of identification antimicrobial activity, some bacteria were used as indicator strains. As an example, for dairy product, eight bacteria such as *E.coli* ATCC 19404, *Salmonella* Typhimurium *ATCC 14028, Staphylococcus aureus* ATCC 25923, *Bacillus subtilis ATCC 6633, Bacillus cereus ATCC 33018, Listeria monocytogenes* ATCC 7644 *Pseudomonas aeruginosa* ATCC 9027, and *Lactococcus lactis* ATCC 11454 were used (Abdelhadi *et al.*, 2016, p. 77). Many studies reported that lactic acid bacteria have been known for their ability to produce various antimicrobial compounds like bacteriocin, organic acids, and hydrogen peroxide. In the identification of antimicrobial activity sources, it is very important to remove compounds other than bacteriocin to ensure that antimicrobial activity is only originated from bacteriocin. Besides, it is also possible that bacteriophage shows inhibition activity (Kormin *et al.*, 2001, p. 64). According to some studies, the Flip plate method could be used to prove this possibility. Furthermore, after eliminating these compounds, antimicrobial activity resulted from the experiment is suspected from bacteriocin that showed by the occurrence of inhibition zones around the growth of producing strains (Kormin *et al.*, 2001, pp. 64-66). The antimicrobial activity is calculated from the reciprocal of the highest dilution which exhibits definite antimicrobial activity and is stated as AU/mL (Arbitrary Unit/ml) (Kormin *et al.*, 2001, pp. 65). Quantitative determination of maximum activity could be performed by growing the culture for 24 hours incubation, monitoring its growth every 2 hours interval by colony counting method from serial dilution, and determining antimicrobial activity from every interval. Moreover, to prove that antimicrobial activity is coming from bacteriocin, cell-free supernatant (CFS) containing maximum activity of bacteriocin must be treated by protease enzymes and other enzymes like amylase, lipase, etc. The use of protease enzymes will break down and inactivate bacteriocin since it is composed of peptides. Furthermore, it was demonstrated that some bacteriocins may consist of carbohydrate or lipid moieties that also responsible for their antimicrobial activity, thus the adding of amylase or lipase will degrade these moieties and bacteriocin become inactive. This characterization result is then compared to standard nomenclature for bacteriocins to identify the bacteriocin type (Kormin *et al.*, 2001, pp. 65; Enan *et al.*, 1996, p. 199). Recently, mass spectrometry has been utilized for the rapid detection of bacteriocin like nisin, pediocin, enterocins A and B, brochocins A and B, etc., which is called as MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy) (Chen and Hoover, 2003, p. 83).

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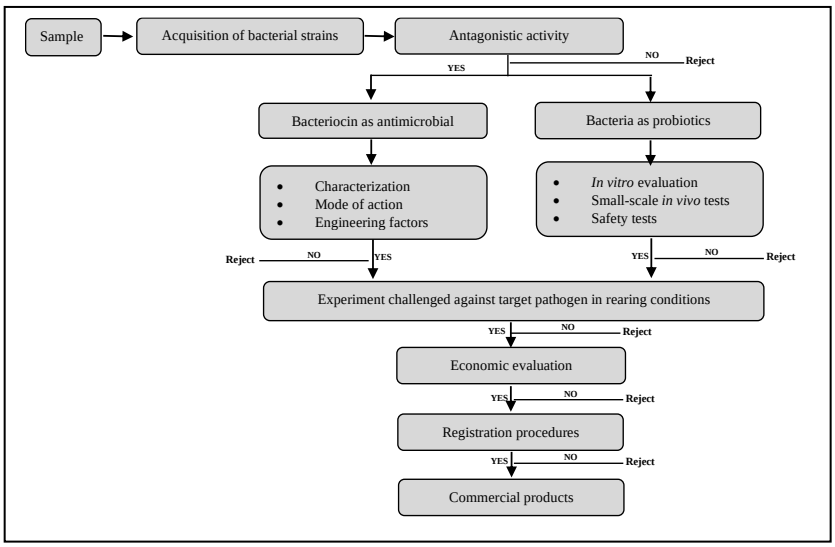

Figure 4. Full strategy to discover new bacteriocin and bacteriocin-producing bacteria for probiotics (Desriac *et al.,* 2010, p. 1167).

8. Approval of Bacteriocin-Producing Bacteria and Bacteriocin as A New Bio-Preservative

Generally Recognized as Safe (GRAS) are substances or microorganisms that are considered safe by the US Food and Drug Administration (FDA). This GRAS status would be given to substances or microorganisms that have a long history of safety or have been determined as safe for their intended use by qualified experts (Desriac *et al.,* 2010, p. 1166; von Wright, 2005, p. 20).

To be approved as GRAS, the safety and efficacy of GRAS candidates are assessed by the regulatory body (Desriac *et al.,* 2010, p. 1166). In general, regulator would evaluate general requirements, as follow: 1) microorganisms have been identified by culture, biochemical and molecular-based method, 2) manufacturing and stabilization method are mentioned in detail including their optimum growth condition, 3) the concentration and the amount of new bio-preservative must be stated, intended for proper use, and the procedure, suggestion, recommendation as proposed, 3) data of efficacy from the use of bio-preservative for its proper use, 4) availability of method for regulatory purpose to determine final concentration of bio-preservative and possibility of any compounds resulted from their use in the finished food, 5) complete report about the safety of new bio-preservative including the existence of pathogenic genes, biogenic amines production, allergenic potential, digestibility, haemolytic, and the profile of antibiotic resistance, etc., 6) A proposed maximum level of concentration in the finished product, and 7) data of permitted residual concentration in the finished product. Approval would then be released to the substance with proven efficacy. After it is approved, the substance would be added to the list of permitted food additives and used commercially. To get approval for the use of the same component in other foods, it is sufficient to add evidence of the efficacy data of this component in new foods (Ben Said *et al.*, 2019, p. 144).

9. Safety and Quality Control Issues of Bacteriocin-Producing Bacteria as Probiotics

According to the Food and Agriculture Organization (FAO) of the World Health Organization Probiotics (WHO) could be defined as live microorganisms that grant a health benefit to the host if giving an inadequate amount (De Simone C, 2019, p. 809). Probiotics show the capabilities to produce anti-microbial substances, compete for nutrients, eliminate pathogens, and modulate the

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immune system. Many antibacterial substances, like bacteriocins, hydrogen peroxide, and short-chain fatty acids, are produced by probiotics to inhibit pathogens or gastrointestinal microorganisms (Yang *et al.*, 2014, p. 4). Therefore, it is considered that bacteriocins are one of the characteristics of probiotics. Recently, several probiotics such as LAB, bacilli, non-pathogenic *E. coli*, and yeasts are used in daily life (Yang *et al.*, 2014, p. 5).

Probiotics could be used as probiotic foods including dietary supplements, foods, and food ingredients), probiotics for animal use, and genetically modified probiotics. If a probiotic will be used as a drug, it must follow the regulatory process as a drug, like any new therapeutic agent. The probiotic drug must be proved as safe and effective for its intended use before marketing. If a probiotic will be intended as a dietary supplement, it is placed under the "foods" term and does not need approval before being marketed. However, the producers must notify FDA before marketing a product. If a dietary supplement consists of a new ingredient that was not sold before October 15, 1994, the manufacturer needs to inform and prove to FDA about the safety of the new ingredient to be used in a dietary supplement before it is marketed (FDA, 2020).

Contrary to chemical food additives, there are no validated or established testing criteria to ensure the safety of a micro-organism. In most cases, the safety of novel strains has been determined mostly from their common existence in foods or the human gut. A new safety aspect that must be considered is that of transmissible antibiotic resistance. Multi-resistant strains like enterococci with transferable vancomycin resistance some of lactococci and lactobacilli with antibiotics resistance are examples of this issue. *In vitro* studies showed that these enterococci could transfer this resistance to other genera or species (von Wright, 2005, pp. 17-18).

For probiotic issues, besides fulfilling the required elements, a commercial product with the claim on health benefits to patients must be labelled with the number of live bacteria at a specific concentration by also considering the number of dead bacteria. The number of dead bacteria in a probiotic product is ignored when evaluating the process of product safety. During the production process, dead bacteria and their fragments could not be removed or separated from the live bacteria. Hence, the final product would consist of live and dead bacteria, and several microbe-associated fragments and molecules. Only the live bacteria could be cultivated and counted on agar plates as colony-forming units (CFU) and appeared on the product label. In subjects with dysreactive immune disorders, this live or dead, fragmented, or entire, these bacteria could be harmful at certain numbers and affect the imbalance between pro-and anti-inflammatory cytokines as well as other cell functions (De Simone C, 2019, p. 811). Besides, to ensure food safety, dairy products containing probiotics could be generally tested by counting undesirable microorganisms using simply total plate count method (Peraturan BPOM No. 13 Tahun 2019, p. 11).

Ideally, the quality controls for the probiotics for medical treatment should not be limited to viability, acid and bile stability, adhesive properties, but should also contain an assessment of the immunological and biochemical profile of the product, and if there are any differences, the products should be new tested in animals and then in humans (Trinchieri *et al.*, 2017, p. 2).

The accurate identification of the bacteria including the strain level is also a fundamental need. Initially, the identification of bacteria was determined according to their morphological and biochemical characteristics, but recently, it has been developed by using modern genomic techniques. By understanding the way to identification and characterization both of bacteriocin-producing bacteria and bacteriocin itself, for the quality control purposes of a product containing known bacteriocin-producing bacteria, culture method, PCR-based method with specific primer or partial and total sequencing of 16S rDNA are basically could be conducted for identification. If necessary, to ensure that bacteriocin works effectively to eliminate pathogens, detection of certain pathogens could also be performed by culture, PCR, and sequencing methods, depending on the needs.

For the bacteriocin, despite the conventional and mass spectrometry method for identification of antimicrobial activity that has been mentioned above, bacteriocin could also be tested by the ELISA method. As an example, nisin as a worldwide use of bacteriocin, could be detected by the ELISA

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technique using purified anti-nisin immunoglobulin to bind nisin and anti-nisin peroxidase that link to the substrate (Diwas Pradan, 2015). Nonetheless, mass spectrometry is the most used recently.

The method to measure bacteriocins efficacy is quite similar to measure antibiotics efficacy, which could use the diffusion method (Kormin, 2001, p. 65) as mentioned above or optical density measurement (Diwas Pradan, 2015). Nevertheless, the measurement of bacteriocins efficacy would be difficult since it is product dependent (Choyam *et al.*, 2019, p. 8). Nisin was the first approved bacteriocins by FDA. Different countries determine different maximum levels of nisin used in certain foods including Indonesia (Peraturan BPOM No. 11 Tahun 2019, p. 861). Until now, nisin is still considered as safe bacteriocins and the maximum level requirements set by each country may differ in different foods.

10. Conclusion

Undoubtedly, both bacteriocins and bacteriocin-producing bacteria have a lot of potential to be developed as natural bio-preservatives in foods, alternatives to antibiotics, or disease treatment. Nonetheless, some challenges must also be considered, thus their potential would be effectively applied. Bacteriocin may lose its activity during interaction with other components in the products and may not be easily maintained (Simons *et al.*, 2020, p. 186). Gram-negative bacteria are mostly found to be often resistant to bacteriocins from LAB. Considering their role as foodborne pathogen, this resistance could be the main problem in the future (Mathur *et al.*, 2017, p. 11). However, this resistance could be diminished by combining the use of bacteriocins with chelating agents or physical treatment. Additionally, some bacteria could produce two or more bacteriocins, which could reduce the arising of bacteriocin-resistance pathogen (Ben Said *et al.*, 2019, p. 142). Additionally, bacteriocins as anti-cancer must be further elucidated including the use of genetically engineered protein to develop more stable and higher efficacy for medical treatment (Ibrahim, 2019, p. 604). High cost is also still an important issue; thus, it prevents the widespread use of bacteriocins. Consequently, not only discovering new and more effective bacteriocins, development and optimization of existing bacteriocins must be concerned biologically and economically (Simons, Kamel, and Raphaël, 2020, p. 186).

Furthermore, to be recognized as GRAS, safety assessment to new bacteriocin must be conducted including complete characterization of the substance, mechanism of action, its efficacy, their effect on the product, manufacture and stabilization procedures, availability of method for regulatory purpose, safety report, and proposed maximum level of concentration in the finished product (Ben Said *et al.*, 2019, p. 144). Nisin is the first and mostly applied in many countries. However, the patented bacteriocins have increased in recent years (López-Cuellar, Adriana-Inés, and Norberto, 2016, p.1045). Moreover, the research to discover and engineer new and more effective bacteriocin is still on going, which allows a wider application in many areas. The trends of research and application of bacteriocin and bacteriocin-producing bacteria reflect the current and future demands in food, pharmaceutical, health care, biomaterials, and biomedicine (López-Cuellar, Adriana-Inés, and Norberto, 2016, p.1047). However, the potentialities of bacteriocin and probiotics application are very promising. In the future, research could also focus on minimizing the safety issues and overcoming existing challenges. Hence, the application of bacteriocin and bacteriocin producers will be more effective and sustainable.

References

Abdelhadi A. A., Nagwa E., Rasha G. S., Ahmed N. S. and Nivien A. 2016. Characterization and Genetic Improvement of Bacteriocin Producing Lactic Acid Bacteria. *Biotechnology*, 15 (3-4): 76-85.

Ahire J.J., and Dicks L.M.T. 2015. Nisin Incorporated with 2,3-dihydroxybenzoic Acid in Nanofibers Inhibits Biofilm Formation by A Methicillin-Resistant Strain of *Staphylococcus aureus*. *Probiotic Antimicro Proteins.*, 7: 52–59.

ISSN 2747-2493

Amor K.B., E.E. Vaughan and W.M. de Vos. 2007. Advanced Molecular Tools for The Identification of Lactic Acid Bacteria. *J. Nutr.*, 137: 741S-747S.

Amso Z., Sean W. B., Sung-Hyun Y., Paul W. R. H., Tom H. W., Claudio D. N., Mark L. P., Gillian E. N., Margaret A. B. 2018. Total Chemical Synthesis of Glycocin F and Analogues: S-glycosylation Confers Improved Antimicrobial Activity. *Chemical Science* 9(6): 1686-1691.

Bals R. 2000. Epithelial Antimicrobial Peptides in Host Defense Against Infection. *Respir. Res.*, 1: 141-150.

Bastos M. do C., Coelho M.L., and Santos O.C. 2015. Resistance to Bacteriocins Produced by Gram-positive Bacteria. *Microbiology*, 161: 683–700.

Beisswenger C. and Bals R. 2005. Functions of Antimicrobial Peptides in Host Defense and Immunity. *Curr. Protein Pept. Sci.* 6(3): 255-264.

Ben Said L., He´le`ne G., Laurent D., Miche`le T., and Ismail F. 2019. Bioprotective Culture: A New Generation of Food Additives for the Preservation of Food Quality and Safety. *Industrial Biotechnology*., 15(3): 138-147.

Benkerroum N., Yasmine G. and Hakim G. 2007. Screening for Bacteriocin-producing Lactic Acid Bacteria from Various Moroccan Food Products and Partial Characterization of Putative Bacteriocins. *Biotechnology*, 6(4): 481-488.

Bintsis T. 2018. Lactic Acid Bacteria: Their Applications in Foods. *J. Bacteriol. Mycol.*, 6(2):89–94.

Boziaris I.S. and Adams M.R. 1999. Effect of chelators and nisin produced in situ on inhibition and inactivation of Gram-negatives. *Int. J. Food Microbiol.* 53: 105–113.

Cascales E., Susan K. B.,Denis D.,Colin K.,Roland L., Kathleen P., Margaret R., Stephen S., and Danie`le C. 2007. Colicin Biology. *Microbiol.Mol.Biol.Rev.* 71: 158–229.

Chehimi S., Anne-Marie P.A., Sophie S., Mohamed-Rabeh H., Férid L. 2010. Mode of Action of Thuricin S, A New Class IId Bacteriocin from *Bacillus thuringiensis*. *Canadian Journal of Microbiology*, 56(2): 162-167.

Chen H. and Hoover D.G. 2003. Bacteriocins and Their Food Applications. *Compr. Rev. Food. Sci. Food. Saf.* 2: 82–100.

Choyam S., Alok K. S., Jae-Ho S., Rajagopal K. 2019. Ocins for Food Safety. *Frontiers in Microbiology*: 1-13.

Cotter P.D., Hill C., Ross R.P. 2005. Bacteriocins: Developing Innate Immunity for Food. *Nat. Rev. Microbiol.*, 3:777–788.

Cotter P.D., Ross R.P., and Hill C. 2013. Bacteriocins—A Viable Alternative to Antibiotics? *Nat.Rev.Microbiol.*, 11: 95–105.

Dabour N., Annina Z., Ehab K., Christophe L., Ismail F. 2009. In Vivo Study on The Effectiveness of Pediocin PA-1 and *Pediococcus acidilactici* UL5 at Inhibiting *Listeria monocytogenes. International Journal of Food Microbiology* 133(3): 225-233.

De Simone C. 2019. The Unregulated Probiotic Market. Clinical Gastroenterology and Hepatology (17): 809–817.

Delesa D.A. 2017. Bacteriocin as an advanced technology in food industry. *Int. J. Adv. Res. Biol. Sci.*, 4(12): 178-190.

Desriac F., Diane D., Nathalie B., Benjamin B., Patrick Le C. and Yannick F. 2010. Bacteriocin as Weapons in the Marine Animal-Associated Bacteria Warfare: Inventory and Potential Applications as an Aquaculture Probiotic. *Mar. Drugs* (8): 1153-1177.

Dimov S., Ivanova P., Harizanova N., and Ivanova I. 2005. Bioactive Peptides Used by Bacteria in the Concurrence for The Ecological Niche: General Classification and Mode of Action (Overview). *Biotechnol. Biotechnol.Eq.* 3: 3–22.

Diwas Pradan. 2015. Bacteriocin-Based Strategy for Enhanced Food Safety. National Dairy Research Institute.

Dobson A., Paul D. C., Ross R.P., and Colin H. 2012. Bacteriocin Production: A Probiotic Trait? *Applied and Environmental Microbiology.*: 1– 6.

Drider, D., Fimland, G., Hechard, Y., McMullen, and H. Prevost. 2006. The Continuing Story of Class IIa Bacteriosins. *Microbiology and Molecular Biology*: 562-582.

ISSN 2747-2493

Enan G., Ei-Essawy A.A., Uyttendaele M., Debevere J., 1996. Antibacterial Activity of *L. plantarum* UG1 Isolated from Dry Sausage: Characterization, Production and Bactericidal Action of Plantaricin UG1. *Int. J. Food Microbiol.*, 30: 189-215.

Ennahar S., Sashihara T., Sonomoto K., Ishizaki A. 2000. Class IIa Bacteriocins: Biosynthesis, Structure, and Activity. *FEMS Microbiol Rev*., 24: 85–106.

Fitri N. 2012. *Antimicrobial Peptides* sebagai Obat Alternatif pada Resistensi Antibiotik. Jurnal Kefarmasian Indonesia, 2(2): 62-67.

Food and Agriculture Organization (FAO). 2003. Important Food Issues. http://www.fao.org/3/y8705e/y8705e03.htm (diakses pada 3 September 2020).

Food and Drug Administration (FDA). 2020. New Dietary Ingredients in Dietary Supplements-Background for Industry [Online]. Available at: https://www.fda.gov/food/new-dietary-ingredients-ndi-notification-process/ (Accessed: 12 September 2020).

Foulquié Moreno M.R., Sarantinopoulos P., Tsakalidou E., and De Vuyst L. 2006. The Role and Application of Enterococci in Food and Health. *Int.J.Food Microbiol.* 106: 1–24.

Gabrielsen C., Dag A. Brede, Ingolf F. Nes, Dzung B. Diep. 2014. Circular Bacteriocins: Biosynthesis and Mode of Action. *Applied and Environmental Microbiology* 80(22): 6854–6862.

Gálvez A., Abriouel H., López R.L., and Omarn B. 2007. Bacteriocin-Based Strategies for Food Biopreservation. *Int. J. Food Microbiol.*, 120: 51-70.

Gautam N. and Nivedita S. 2009. Bacteriocin: Safest Approach to Preserve Food Products. Indian *J. Microbiol.*, 49: 204–211.

Giuliani A., Pirri G., and Nicoletto S.F. 2007. Antimicrobial Peptides: An Overview of A Promising Class of Therapeutics. *CEJB*, 2(1): 1-33.

Gonzales, B.E., E. Glaasker, E.R.S. Kunji, A.J.M. Driessen, J.E. Suarez, and W.N. K. Onings. 1996. Bactericidal mode of Action of Plantaricin S. *Appl. Environ. Microbiol.*, 62: 2701-2709.

Grinter R., Milner J., and Walker D. 2012. Bacteriocins Active Against Plant Pathogenic Bacteria. *Biochem.Soc.Trans.* 40: 1498–1502.

Heng N.C.K., Wescombe P.A., Burton J.P., Jack R.W., Tagg J.R. 2007. The Diversity of Bacteriocins in Gram-Positive Bacteria, p 45–92. *In* Riley MA, Chavan MA (ed), Bacteriocins: Ecology and Evolution. Springer-Verlag, Berlin, Germany.

Holzapfel W.H., Geisen R, Schillinger U. 1995. Biological Preservation of Foods with Reference to Protective Cultures, Bacteriocins and Food-Grade Enzymes. *Int J Food Microbiol.*, 24(3):343–362.

Ibrahim O.O. 2019. Classification of Antimicrobial Peptides Bacteriocins, and the Nature of Some Bacteriocins with Potential Applications in Food Safety and Biopharmaceuticals. *Applications in Food Safety and Bio-Pharmaceutical. EC Microbiology*, 15(7): 591-608.

Ji S., Weili L., Abdul R.B., Meng W., and Binyun C. 2015. Improved Production of Sublancin via Introduction of Three Characteristic Promoters into Operon Clusters Responsible for This Novel Distinct Glycopeptide Biosynthesis. *Microbial Cell Factories*, 14:1-10.

Joo, N.E., Ritchie, K., Kamarajan, P., Miao, D., and Kapila, Y.L. 2012. Nisin, An Apoptogenic Bacteriocin and Food Preservative, Attenuates HNSCC Tumorigenesis via CHAC1. *Cancer Med.* 1: 295–305.

Jordi B.J., Boutaga K., van Heeswijk C.M., van Knapen F., and Lipman L.J. 2001. Sensitivity of Shigatoxin-Producing *Escherichia coli* (STEC) Strains for Colicins under Different Experimental Conditions. *FEMS. Microbiol. Lett.* 204: 329–334.

Kamarajan P., Takayuki H., Bibiana M., Yang L., Theodora D., Ayyalusamy R., Francis W., Sunil K., Yvonne K. 2015. Nisin ZP, A Bacteriocin and Food Preservative, Inhibits Head and Neck Cancer Tumorigenesis and Prolongs Survival. *PLoS One* 10: 1-20.

Kaur B., Praveen P. B., Bharti M., Balvir K., and Neena G. 2013. Biomedical applications of fermenticin HV6b isolated from Lactobacillus fermentum HV6b MTCC10770. *Biomed. Res. Int.*: 1-9.

ISSN 2747-2493

Kaur S., and Sukhraj K. 2015. Bacteriocins as Potential Anticancer Agents. *Frontiers in Pharmacology*, 6: 1-11.

Kawai Y., Yasuyuki I., Kensuke K., Koichiro U., Boku S., Junko N., Haruki K., Yukiko Y., Yoshio T., Takatoshi I., and Tadao S. 2004. Structural and Functional Differences in Two Cyclic Bacteriocins with The Same Sequences Produced by Lactobacilli. *Applied and Environmental Microbiology*, 70: 2906-2911.

Kormin S., Gulam R., Son R. and Foo H. L.. 2001. Bacteriocin-Producing Lactic Acid Bacteria Isolated from Traditional Fermented Food. *Malaysian Journal of Medical Sciences,* 8(1): 63-68.

Lancaster L.E., Wintermeyer W., and Rodnina M.V. 2007. Colicins and Their Potential in Cancer Treatment. *Blood Cells Mol.Dis.,* 38: 15–18.

Leitzmann C. 1993. Food Quality-Definition and a Holistic View. *Safeguarding Food Quality* pp 3-15.

Lopetuso, L.R., Maria E. G., Angela S., Franco S., Antonio G. and Giovanni C. 2019. Bacteriocins and Bacteriophages: Therapeutic Weapons for Gastrointestinal Diseases?. *Int. J. Mol. Sci.,* 20 (1): 11-12.

López-Cuellar M., Adriana-Inés R. H., and Norberto C.H. 2016. LAB Bacteriocin Applications in The Last Decade. *Biotechnology & Biotechnological Equipment,* 30(6): 1039–1050.

Lulietto M.F., Paola S., Elisa C., Luca G., Margherita C., Ahmad R. Al A., Bilge I., Haluk M.A., and Beniamino T.C. 2018. Inhibition of Listeria monocytogenes by a formulation of selected dairy starter cultures and probiotics in an in vitro model. *Italian Journal of Animal Science*, 17:4, 845-850.

Macwana S., and Muriana P.M. 2012. Spontaneous Bacteriocin Resistance in *Listeria monocytogenes* as A Susceptibility Screen for Identifying Different Mechanisms of Resistance and Modes of Action by Bacteriocins of Lactic Acid Bacteria. *J. Microbiol. Meth.,* 88:7–13.

Mathur H., Field D., Rea M.C., *et al*. 2017. Bacteriocin-Antimicrobial Synergy: A Medical and Food Perspective. *Front Microbiol.,* 8: 1-18.

Narsaiah K., Shyam N.J., Robin A.W., Harshad M.M., Musuvadi R.M. 2014. Optimizing microencapsulation of nisin with sodium alginate and guar gum. *Journal of Food Science and Technology*. 51(12): 4054-4059.

Netz D.J.A, Maria do Carmo de Freire Bastos, and Hans-Georg S. 2002. Mode of Action of the Antimicrobial Peptide Aureocin A53from *Staphylococcus aureus*. *Applied and Environmental Microbiology* 68(110): 5274-5280.

Nissen-Meyer J., Camilla O., Per R., Helen S.H., Per E.K. 2010. Structure and Mode-of-Action of the Two-Peptide (Class-IIb) Bacteriocins. *Probiotics & Antimicro. Prot.,* 2: 52–60.

Noraphat Hwanhlem and Aran H-Kittikun. 2015. Biopreservation of Seafood by Using Bacteriocins and Bacteriocinogenic Lactic Acid Bacteria as Potential Bio-control Agents. *Microbiology monographs*: 183-213.

O'Connor E.M. and R F Shand. 2002. Halocins and Sulfolobicins: The Emerging Story of Archaeal Protein and Peptide Antibiotics. *J. Ind. Microbiol. Biotechnol.,* 28: 23-31.

Oscariz J.C. and Antonio G. P.. 2001. Classification and mode of action of membrane-active bacteriocins produced by Gram-positive bacteria. *Int. Microbial.*, 4: 13-19.

Peraturan Badan Pengawas Obat dan Makanan Nomor 11 Tahun 2019 tentang Bahan Tambahan Pangan.

Peraturan Badan Pengawas Obat dan Makanan Nomor 13 Tahun 2019 tentang Batas Maksimal Cemaran Mikroba dalam Pangan Olahan.

Piper C., Pat G.C., Colin H., Paul D. C., Paul R. 2012. The Lantibiotic Lacticin 3147 Prevents Systemic Spread of *Staphylococcus aureus* in A Murine Infection Model. *Int. J. Microbiol.*: 1-6.

Preet S., Sanjay B., Anshul P., Rupinder T., Praveen R. 2015. Effect of Nisin and Doxorubicin on DMBA-Induced Skin Carcinogenesis–A Possible Adjunct Therapy. *Tumour Biol.,* 36: 8301–8308.

Prudêncio, C.V., Miriam T. & Maria C.D.V. 2015. Strategies for The Use of Bacteriocins in Gram-negative Bacteria: Relevance in Food Microbiology. *Journal of Food Science and Technology* 52(9): 5408-5417.

Scott V.N and Steve L.T. 1981. Effect of Nisin on the Outgrowth of *Clostridium botulinum* Spores. *Journal of Food Science* 46(1): 117-127.

Settanni L., and Corsetti A. 2008. Application of Bacteriocins in Vegetable Food Biopreservation. *Int. J. Food Microbiol.,* 121: 123–138.

Simons A., Kamel A., and Raphaël E. D. 2020. Bacteriocins, Antimicrobial Peptides from Bacterial Origin: Overview of Their Biology and Their Impact against Multidrug-Resistant Bacteria. *Microorganisms*, 8: 1-31.

ISSN 2747-2493

Slavica M., Vesković M., Dragutin A. Đ., Nurgin R. M. 2014. Bacteriocins Produced by Lactic Acid Bacteria-A Review. *APTEFF*, 45: 271-283

Stahl C.H., Callaway T.R., Lincoln L.M., Lonergan S.M., and Genovese K. J. 2004. Inhibitory activities of colicins against *Escherichia coli* Strains Responsible for Post Weaning Diarrhea and Edema Disease in Swine. *Antimicrob. Agents. Chemother.* 48: 3119–3121.

Stern N.J., Svetoch E.A., Eruslanov B.V., Perelygin V.V., Mitsevich E.V., Mitsevich I.P., . 2006. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacterjejuni* in the chicken gastrointestinal system. *Antimicrob.Agents.Chemother.* 50: 3111–3116.

Terzaghi B.E., and W.E. Sandine. 1975. Improved Medium for Lactic Streptoccocci and Their Bacteriophages. *Applied Environ. Microbio.,* 29: 807-813.

Todorov S.D., Koep K.S., Van Reenen C.A., Hoffman L.C, Slinde E., Dicks L.M.T. 2007. Production of Salami from Beef, Horse, Mutton, Blesbok (Damaliscus dorcas philipsi) and Springbok (Antidorcas marsupialis) with Bacteriocinogenic Strains of *Lactobacillus plantarum* and *Lactobacillus curvatus*. *Meat Sci*., 77(3):405–412.

Trinchieri V., Luca L., Beatrice V., Carola P., Ilaria G., Daniela C., Paola M., Claudio D. 2017. Efficacy and Safety of a Multistrain Probiotic Depends from Manufacturing. *Front Immunol.,* 8:1474.

Verma A.K., Banerjee R., Dwivedi H.P, Juneja V.K. 2014. Bacteriocin. Potential in Food Preservation. *Encyclopedia of Food Microbiology*, 2nd: 180–186.

von Wright, A. 2005. Regulating the Safety of Probiotics - The European Approach. *Curr. Pharm. Des.,* (11): 17–23.

Yang E., Fan L., Jiang Y., Doucette C., and Fillmore S. 2012. Antimicrobial Activity of Bacteriocin-Producing Lactic Acid Bacteria Isolated from Cheeses and Yogurts. *AMB Exp.,* 2 (48): 1-12.

Yang S., Chih-Hung L., Calvin T.S. and Jia-You Fang. 2014. Antibacterial Activities of Bacteriocins: Application in Foods and Pharmaceuticals. *Frontiers in microbiology,* 5: 1-10.

Zacharof M.P. and Lovitt R.W. 2012. Bacteriocins Produced by Lactic Acid Bacteria a Review Article. *APCBEE Procedia*, 2: 50–56.

Zaiou M. 2007. Multifunctional Antimicrobial Peptides: Therapeutic Targets in Several Human Diseases. *Journal of Molecular Medicine,* 85(4): 317-329.

Zhang Y, Zhu L, Dong P, et al. 2018. Bio-protective Potential of Lactic Acid Bacteria: Effect of *Lactobacillus sakei* and *Lactobacillus curvatus* on Changes of The Microbial Community in Vacuum-Packaged Chilled Beef. Asian-Australas. J. Anim. Sci., 31(4):585–594.

Zhao G., Huiming Z., Mao Z., Yucai H. 2015. Effects of Antimicrobial Peptides on *Staphylococcus aureus* Growth and Biofilm Formation In Vitro Following Isolation from Implant-Associated Infections. *International Journal of Clinical and Experimental Medicine,* 8(1): 1546-1551.