Review Article

Applications of Bacteriocin and Protective Cultures in Dairy Products: A Mini-Review

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ABSTRACT

The spoilage of food by microorganism is one of the novel topics being tackled today despite the wide range of preservatives present today. Antimicrobial metabolites may have the potential to inhibit the growth of pathogenic bacteria and have been increasing in research due to the rise of human health risk posed by harmful preservatives and the rise of eating raw foods. Bacteriocin is a considerable interest in the field of preservation, is produced by Lactic acid bacteria and considered to be a safe alternative to conventional antimicrobials. Additionally, Lactic acid bacteria naturally occur in dairy products. A number of applications of Bacteriocins have been reported to successfully control and inhibit pathogens in milk, yogurt, cheeses and other dairy products. Bacteriocin poses no threat to human health, supported by research indicating that Lactic acid bacteria are easily degraded by enzymes in the mammalian gastrointestinal tract. Overall, this can be an alternative to satisfy the increasing consumers' demands for safe, fresh-tasting, dairy products. This review will focus on bacteriocin's recent application, advantages and disadvantages.

Key words: Bacteriocin, Protective culture, Dairy products.

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INTRODUCTION

Bacteriocins are a group of antibacterial proteins or peptides that are synthesized by ribosomes. It has the ability to kill or inhibit the growth of closely related producer strains.[1] The said peptides are known to be a biopreservative that has promising applications in the food industry.[2] It also has been a good alternative from chemical preservatives for it promotes high quality and safe preservation of food, but the usage can be limited due to effectiveness of pathogen elimination and its cost.[3]

Many Gram-negative and Gram-positive microorganisms which produce bacteriocins, especially lactic acid bacteria (LAB) are of particular interest to the food industry. Lactic acid bacteria family specifically organisms under the genera Lactococcus, Streptococcus, Lactobacillus, Pediococcus, and Enterococcus, are important group of industrial microorganisms involved in the processing of various fermented foods which include vegetables and sausages, dietary adjuncts, probiotics and even cosmetic ingredients. It is used as a starter culture to improve the texture and the flavor of the products. The ability to inhibit growth of spoilage microorganisms and pathogenic bacteria contribute to the maintenance of hygienic and quality of the products or host health.[4]

In the study of Silva, Silva, & Ribeiro (2018), it is stated that the role of bacteriocin as biopreservative is safe and easily digested by the human gastrointestinal tract.[3]

The different approaches of application of bacteriocin on food was also enumerated: (1) introduction of food with the producer strain; (2) using of purified or semi-purified bacteriocin as food additive; (3) utilizing a previously fermented product with a bacteriocin producing strain. There are three categories that Chikindas *et al.* (2017) divided for food applications involving bacteriocins, specifically, semi-purified bacteriocins,

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dairy and fermented foods with fermentate, and bacteriocin-producing protective cultures.[5] Although considered as safe for being food preservative, in the later year, a study conducted by Kumariya *et al.* (2019) concluded that excessive dosage of bacteriocin can lead to nonspecific bactericidal effects that can be risky to consumers.[6] The study also stated that the reason why only a few out of thousands of bacteriocin can only be applicable to food usage is because some exhibit toxicity. As mentioned, an example is the study of Martins *et al.* (2010) that founds out that galactomannan and nisin coated in Ricotta cheese takes 7 days at 4°C to take effect to reduce the growth of *L. monocytogenes*.[7]

Bacteriocin applications are not limited to food, it has been found to also be applicable to medicines and animals.[8] Over the years, numbers of bacteriocins have been identified, isolated from microorganisms of both gram-positive and gram-negative.[3] Bacteriocins can be used for the improvement of the quality of food and sensory properties. Furthermore, another application is that they can also be used as protective cultures that can improve its safety and shell life by protecting the product from external contamination.[9]

Moreover, Bacteriocin showed an improved protective action after being treated physically, such as processed in different temperatures and pressures, and chemically or bounded to chemical agents such as chelating agents.[10,11] However, Prudêncio *et al.* (2015) stated that the effectiveness of the treatments was affected by certain factors such as pH, temperature, composition of food and the target microorganisms.[11]

MATERIALS AND METHODS

Literature Research

In selecting articles, the databases used were Google scholar, PMC: US National Library of Medicine National Institutes of Health, and PubMed. The keywords used for searching and collecting articles include the mixture of Bacteriocin + Dairy products + effectiveness of Antimicrobial properties of Bacteriocin + Food applications + food packaging. The search filters were set to cover articles published from the year 2001 up to present year 2021. The following were additional inclusion criteria when selecting research articles; (1) the published research, journals and articles must be in the English language; (2) studies on bacteriocin must tackle bacteriocin types, Nisin, Pediocin, Enterocin, lacticin, Lactococcin, and Aureocin; and (3) journals tackling the incorporation of purified bacteriocin on food packaging on dairy products. While the exclusion criteria includes; (1) journals, researches and articles that were not translated in english; and (2) journals that dd not specify the dairy product.

Eligibility Criteria

This study was limited to the data related to the application of bacteriocin in dairy products in terms of its preservative and antimicrobial properties on various dairy products and food packaging or dairy products. Published studies that were written in other languages were excluded to avoid misinterpretation of the data. The articles must be published from the year 2001 to present to be eligible for the study, any older publications than the said timeline were not accepted. This study does include a variety of dairy products that were tested in bacteriocin type Nisin, Pediocin, Enterocin, lacticin, Lactococcin, and Aureocin.

Criteria of the Usage of bacteriocin

The application of bacteriocin was the incorporation of Purified/Semi-Purified Bacteriocins to Dairy Products that serves as protective or adjunct cultures, which are considered as technological alternatives to conventional cheese preservation methods such as chemical additives, excessive salt, etc. Incorporation of isolated bacteriocin producing bacteria will not be accepted.

RESULTS AND DISCUSSION

With the diverse published journals, articles, and research papers being gathered, data and results were being assessed and analyzed to discuss bacteriocin and its application on dairy products and protective cultures. Moreover, the discussion is mainly focused on its bacteriostatic properties against closely related producer strains and its preservative properties.

Bacteriocin Types, Classification and Mode of Action

Karpinski and Szkaradkiewicz (2016) mentioned that there are few hypothetical models available for better understanding of mechanism and actions of some bacteriocin.[12] The study mentioned the wedge model, which was created through the grounds of the sequence and interactions of model lantibiotic, nisin, and target cell's surface membrane. The penetration of the peptides in the middle of the double phospholipid layer activates the local destruction of the ordered bilayer structure. Nissin will then enter the reaction with lipid II, allowing the binding of the lantibiotics to the sensitive bacteria's membrane.[12] The wedge model is also known to be the one accepted for Nisin and lantibiotics pore formation. Other models that represent bacteriocin activity of some classes

Table 1: Mode of Actions of the Classification and Types of Bacteriocin on Dairy Products.

Classification	Bacteriocin	Mode of Action	Product	Reference
I	Nisin	Membrane permeabilization by pore formation	Camembert cheese Semi-hard cheese	Arqués *et al.* 2015[2] Karpiński and Szkaradkiewicz, 2016[12] Kumariya *et al.* 2019[6]
II	Pediocin	Membrane permeabilization by pore formation	Cottage cheese Yogurt Milk Cream Cheese Sauce Munster cheese Semi-hard cheese	Arqués *et al.* 2015[2] Karpiński and Szkaradkiewicz, 2016[12] Kumariya *et al.* 2019[6] Silva, Silva, and Ribeiro, 2018[3]
	Enterocin	Membrane permeabilization by pore formation	Semi-hard cheese Manchego cheese Milk	
	Lacticin	Membrane permeabilization by pore formation	Cottage cheese Semi-hard cheese Buttermilk Infant milk Natural Yogurt	
	Lactococcin	Membrane permeabilization by pore formation	Milk	
	Aureocin	Membrane permeabilization by pore formation	Pasteurized commercial milk	

include toroidal pore model, carpet model, and aggregate channel model (Mani-López, Palou, and López-Malo, 2018).[13] Nisin particularly shows little or limited reduction of pathogens as it does not penetrate the matrix of dairy products, as it effects only for the surface contaminated products, it did not show any antimicrobial property at more than 1-mm depth of the product.[14] Table 1 presents the various modes of action amongst the different classification of bacteriocin within dairy products.

Classification of Bacteriocin can be divided into three, Class I which includes lantibiotics and peptides that are considered thermostable and with molecular weight under 5 kDa, Class II is for the non-lantibiotic bacteriocins which has subclasses A, B, C and D, Class III bacteriocins usually have molecular weight of greater than 30kDa and are considered thermolabile (Karpinski and Szkaradkiewicz, 2016).[12] In the year 2014, Class IV was introduced for some bacteriocin from Class IIc that contains lipids (Gabrielsen, *et al.* 2014).[15] And in a recent study conducted by Lajis (2020), Class V bacteriocins are mentioned to be an emerging family of bacteriocins that has circular shape and described to be peptides that are post-translational non-modified head to tail.[16]

Application of Purified and Semi-purified Bacteriocin to Dairy Products and Protective Cultures

Currently, only two bacteriocins have been commercialized as food preservatives which are nisin and pediocin PA1.[3] Using semi-purified bacteriocin can be applied as an ingredient in food by incorporating an ingredient that was previously fermented with a bacteriocin-producing strain or the use of a bacteriocin-producing culture to replace all or part of starter culture in fermented foods to produce bacteriocin in situ.[9] In another study by Aspri *et al.* (2017), it was also stated that protective or adjunct cultures can serve as a technological alternative to conventional cheese preservation methods such as chemical additives, excessive salt, etc.[17]

Recent applications of bacteriocin include the incorporation to dairy products to extend the shelf-life and limit the growth of pathogenic bacteria (Table 2), and inoculation to products as protective cultures, such as serving as adjunct or starter cultures (Table 3). Favaro *et al.* (2015) stated in their study that applications of bacteriocinogenic cultures have their limitations in controlling the spoilage of microorganisms and cannot be presented as the solution in solving the problems in preservation of the dairy products, and an example is Nisin loses its activity in high pH levels that is why it is only limited to pH values lower than 7.[18] Additionally, it was mentioned that the use of semi-purified bacteriocins could be plausible in solving the issue regarding the storage temperature of cheese which is around below 8°C. Low temperature cannot cause harm to the survival of the Lactic Acid Bacteria (LAB) but it doesn't guarantee to abundantly grow for the suf-

Table 2: Applications of purified/semi-purified bacteriocins in dairy products (2000–2021).

Bacteriocin	Product	Features	Reference
Nisin Z from *Lactococcus lactis* W8	Skim milk, fat milk	Extends shelf-life to 2 months by reducing initial bacterial counts (about 5 log CFU ml^{-1}) to undetectable levels within 8-20 hrs.	Mitra *et al*. 2011[19]
Pediocin PA-1 from Pediococcus pentosaceous NCDC 273	Cheese whey	Used in dairy products	Simha *et al*. 2012[20]
Nisin A, Nisin Z and Lacticin 481 from *Lactococcus lactis*	Cottage cheese	Controls the growth of *Listeria monocytogenes*	Dal Bello *et al*. 2011[21]
Lactobacillus bacteriocins	Turkish dairy products	The inhibitory activity can last until 10 and 20 min at 100°C	Aslim, Yuksekdag, Sarikaya, & Beyatli, (2005) [22]
Nisin (N1 & N2)	Galotyri cheese	N1 and N2 extend the shelf-life of Galotyri cheese stored at 4°C by 7 days and 21 days, respectively.	Kykkidou *et al*, 2007[23]
lacticin 3147 from *Lactococcus lactis* DPC3147 (a natural producer) or *L. lactis*DPC4275 (a lacticin 3147-producing transconjugant)	Cheddar cheese	Limits the proliferation of adventitious gram-positive bacteria.	Ryan, 2001[24]
Lacticin 3147, *Lactococcus lactis* subsp. *lactis* DPC3147	Yogurt and cottage cheese	Reduces *L. monocytogenes* within 2 h in natural yogurt, and reduces up to 85% of the said organism in cottage cheese within the same time frame. Reduction of *B. cereus* by 80% within 3 hrs in presence of 1% (w/w) powdered lacticin 3147.	Morgan *et al.* 2008[25]
lactococcin 972 from *L. lactis* subsp. *Lactis (Plus Nisin A and Nisin Z)*	Starter-free cheeses made from raw milk	Preserves dairy and meat fermented products.	Alegria, 2010[26]
Lacticin 481, *Lactococcus lactis* L3A21M1	Model fresh cheese	Reduction of *L. monocytogenes* adhesion to Caco-2 and HT-29 cells	Ribeiro, 2017[27]
Enterocin AS-48 from *Enterococcus faecalis* A-48-32	Skimmed milk and fresh cheese	Inhibits *S. aureus,* Highest inhibition (4–2 log cfu g^{-1} below controls) obtained within the first storage week.	Mucoz *et al*. 2006[28]
Enterocins from *Enterococcus faecalis* L3B1K3 and L3A21M3	Fresh cheese	Adheres to intestinal cells and prevents the adhesion and invasion of *L. monocytogenes*	Ribeiro *et al.* 2017[27]
lactococcin G and Lactacin F from Lactobacillus plantarum	Yogurt	Inhibits the growth of *P. aeruginosa*	Abo-amer, 2006[29]
Aureocin A70	Skimmed milk	Not extensively lytic, however, is bactericidal against listerial strains. Completely stable at 25°C for a month, at 4°C for 16 weeks, and at -20°C for 20 weeks.	Fagundes *et al.* 2016[30]

Table 3: Applications of protective cultures in dairy products (2000–2021).

Bacteriocin-producing strain on protective cultures	Product	Features	Reference
Lactococcus lactis (L3A21M1) and 7 Enterococcus faecalis	Fresh cheese made from pasteurized cows' milk	The combination of two strains demonstrated great potential protective culture for the cheese against *Listeria monocytogenes with* reduction was of approximately 4 log units	Coelho *et al*. 2014[31]
Lactobacillus rhamnosus LC705 and Propionibacterium freudenreichii JS	Yogurt	Yogurt protection on mold growth	Misirlilar, Kinik and Yerlikaya, 2012[32]
Lactobacillus rham-nosusA238 alone or in combination withBifidobacterium animalissubsp.lactisA026	Cottage cheese	Inhibited mold growth for at least 21 days at 6C, due probably to the production of antimicrobial metabolites	Fernandez, 2017[33]
Enterococcus faecium named ((SD1, SD2, SD3 and SD4)	Goats milk	Potential protective culture because SD1 and SD2 were produced at much higher levels (51200 AU/ml) compared to bacteriocin SD3 (3200 AU/ml) and bacteriocin SD4 (800 AU/ml). additionally, the four bacteriocins remained stable at pH from 2.0 to 12.0, after exposure to 100°C for 120 min and were not affected by the presence of surfactants, salts and other microbial products	Schirru *et al*. 2011[34]

ficient amount of bacteriocin production. However, *L. monocytogenes* can grow and survive in such low temperatures, hence the use of semi-purified bacteriocins as a solution to that issue.

The potential usefulness of bacteriocin, supported by many studies, in food preservation can negate the fact on how low its efficiency due to various factors affecting its activity, such as temperature, pH, composition of food and the target microorganisms. However, compared to chemical preservatives, its potential use on recent trends may actually weigh more advantages than the harmful effects. The main difficulty associated with bacteriocin in dairy products is it cannot penetrate the matrix, as it affects only the surface contaminated area, it did not show any antimicrobial property at more than 1-mm depth of the product.[14]

Altering the bacterial outer membrane through cooling or heating allows bacteriocin to penetrate the microorganism. According to Prudêncio *et al.* (2015), cooling promotes the change in the outer membrane of gram-negative bacteria.[11] During the process, there will be reorganization of the outer membrane of the bacteria, which alters its permeability as the temperature decreases. Thus, allowing bacteriocins to have enough time penetrating through the organisms and do its antimicrobial mechanism. On the other hand, bacteriocin is also an interesting lantibiotic having distinct characteristics such as having the stability against proteases, oxidation, and heating (Dischinger *et al.* 2014).[35] Heat makes gram-negative bacteria vulnerable to antimicrobial action of bacteriocins (Prudêncio *et al.* 2015).[11] However, the emergence of thermal-resistant bacterial spores had become a problem. According to Egan *et al.* (2016), Nisin was observed to have beneficial effects at different concentrations – increasing the sensitivity of bacterial spore to heat and preventing the reproduction of spores that survived the heating treatment.[10] It showed that pre-treatment of food with nisin showed significantly reduced heat resistance thus, compromising the permeability of the spores and allowing another treatment for an easier mechanism of destruction. Temperature treatments and bacteriocin showed synergistic mechanisms; however, the antimicrobial effect still depends upon the type of bacteriocin being used in combination with temperature treatment due to the differences in mode of actions.[36]

Chelating agents, such as EDTA, has been commonly used as an agent combined with bacteriocin to produce a synergistic effect. EDTA shows antimicrobial effect against a wide range of clinically significant pathogens.[37] Khan *et al.* (2015) investigated the synergistic antimicrobial effect of nisin formulated with Na-EDTA observed in a range of pH.[38] Moreover, Na-EDTA exhibits a low inhibition zone against both gram-negative bacteria due to its inability to bind the cations on the bacteria's outer membrane at acidic pH. On the other hand, it was observed on alkaline pH that there is also a decrease in inhibition zone due to the low antimicrobial activity of Nisin as an effect of the alkaline environment.

CONCLUSION

With the mentioned studies and applications of bacteriocin, current trends of its use were being highlighted. Literatures mentioned in the previous sections, revealed that bacteriocin can be inoculated and is effective in preserving and extending the product's shelf-life, and in eliminating possible pathogenic microorganisms affecting the product's safety. However, amidst its proven effectiveness, it also showed that properties of bacteriocin are affected by several factors, making it difficult to fully reveal the association and compatibility of bacteriocin-producing strains and other cultures present in dairy products.

Although there is a continuous trend in research regarding bacteriocin's application, there is still limited data available to have a deeper understanding of its properties and use. Journals and studies mentioned in this mini-review mainly focused on inoculation of bacteriocin to cheese products due to its effectiveness against pathogenic organisms present on cheese. There were also inoculations of bacteriocin to milk, however these were not specified due to limited studies available. The same circumstances were also seen in other dairy products such as yogurts, creams, etc. In line with these, a wider scope of study must be done encompassing different dairy products in the different places in larger regions, such as in Asia, Europe, etc., to evaluate bacteriocins' effectiveness on a wider scale. This is due to the effects of different factors that might affect its properties, such as species of cows and prevalence of microorganisms present in the dairy product in different regions.

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CONFLICT OF INTEREST

The authors declare that the research was made without any commercial or financial relationships that could be construed as potential conflict of interest.

ABBREVIATIONS

LAB: Lactic Acid Bacteria; **PMC:** PubMed Central; **kDa:** Kilodaltons; **EDTA:** Ethylenediaminetetraacetic acid; **Na-EDTA:** Sodium Ethylenediaminetetraacetic acid.

SUMMARY

The review article focused on the recent applications of bacteriocins in food preservation of Dairy products. The collation of articles came from different databases like Google scholar, PMC: US National Library of Medicine National Institutes of Health, and PubMed with the keywords Bacteriocin + Dairy products + effectiveness of antimicrobial properties of Bacteriocin + Food applications + food packaging. With given criteria the study was mainly limited to data related to the application of bacteriocin in dairy products in terms of its preservative and antimicrobial properties on various dairy products. The results mainly discussed its bacteriostatic properties against closely related producer strains and its preservative properties. In finding, the main difficulty associated with bacteriocin in dairy products is it cannot penetrate the matrix of the food, as it affects only the surface contaminated area, it did not show any antimicrobial property at more than 1-mm depth of the product. However, compared to chemical preservatives, its potential use on recent trends may weigh more advantages than the harmful effects, as the potential usefulness of bacteriocin, supported by many studies, in food preservation can negate the fact on how low its efficiency due to various factors affecting its activity, such as temperature, pH, composition of food and the target microorganism.

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