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A THEMATIC REVIEW ON POTENTIAL ANTIMICROBIAL POTENTIAL APPLICATIONS OF BACTERIOCINS FROM LACTIC ACID BACTERIA

Nimra Riaz, Saleha Mahnoor Faheem and Muhammad Usman Ahmad*

Institute of Industrial Biotechnology, GC University Lahore-54000, Pakistan.

***Corresponding Author: Dr. Muhammad Usman Ahmad**

Institute of Industrial Biotechnology, GC University Lahore-54000, Pakistan.

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ABSTRACT

The world-wide emergence of resistant pathogenic strains against the currently available antibiotics and the toxicity associated with them has compelled scientific community to increase their quests in finding other natural antimicrobial alternatives. In this regard recent discoveries on antimicrobial arsenals secreted by bacterial species against competing strains are gaining utmost importance not only because of their antimicrobial but also due to their anti-cancer potential. These natural bacterial arsenals are ribosomally synthesized low-molecular weight, heat stable, membrane active, proteolytically degradable and pore-forming, cationic peptides known as "Bacteriocins". Owing to their anti-bacterial, anti-viral, anti-fungal, and anti-biofilm potential, bacteriocins seem to be the immediately available promising option for mitigation of AMR (antimicrobial Resistance) crisis and minimization of toxic effects of antibiotics on Host's microbiome. The non-toxic and Generally Regarded as Safe (GRAS) status of Lactic acid bacteria (LAB) bacteriocins signifies their huge commercialization potential, and research on their biology is currently the most extensively studied subject of biotechnology. Their intrinsic food preservative and therapeutic potential can be enhanced by combinatorial approaches with other antimicrobial substances. However, their potential to be used as AGP (antimicrobial Growth Promoter) in animal production must also be evaluated. The initial half of this review focuses on the significance and biological aspects of LAB bacteriocins, while the latter half addresses the potential antimicrobial aspects of bacteriocins in various sectors.

KEYWORDS: Bacteriocins, Antimicrobial Resistance, Biopreservation.

INTRODUCTION

Since the discovery of penicillin by Alexander-Fleming in 1929 there has been continuous increase in the quests for new antimicrobial compounds from micro-organisms. Penicillin and many other antibiotics are successfully being utilized by medical and veterinary community in treatment against specific diseases caused by micro-organisms. It is a globally accepted fact, that antibiotic's discovery has considerably improved the quality and expectancy of life. But, unfortunately antimicrobial resistance emerged a few years later since their use was started. This issue has become a global health concern. Its continuous spread among currently sensitive strains is even more alarming. In hospital settings, multidrug and even pan drug resistant strains of both gram-positive and gram-negative bacteria have been observed against the most potent and core antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococci* (VRE) being the most fatal ones are continuing to increase the morbidity and mortality rates (Simons et al., 2020).

One source of such unremitting increase in AR (Antibiotic Resistance) lies in widespread unrestricted utilization of antibiotics in food producing animals as antimicrobial growth promoter. This action is potentially transmitting multidrug resistant (MDR) pathogens to humans. In the swine industry, tetracyclines, aminoglycosides, trimethoprim–sulphonamides, and ampicillin resistant enterotoxic *Escherichia coli* strains are found. Similarly in poultry industry resistant genes have been found in *Salmonella* serovars, *Enterococcus* spp., and *Clostridium perfringens* collected from feces and ceca of broiler chickens (Lagha et al., 2017). These findings show whenever an antibiotic is added in animal feed for production enhancement purpose it establishes a selective pressure for advent of antibiotic-resistant bacteria. Thus, the utmost need of hour is exploitation of other anti-bacterial substance of biological origin that can be safely used in animal production and against which bacteria won't develop resistance easily and immediately.

On similar basis the requirement of natural antimicrobial substances for food preservation and safety is inevitable in food industries but the utilization of therapeutic antibiotics against food-borne pathogens and food spoilage bacteria is generally not recommended by FDA to food industries as they can induce antibiotic resistance in Food-borne pathogenic bacteria, decrease the number

of beneficial gut micrbiota and can also pose many other serious public health problems (Baltork et al., 2019). The alternative approach used by food industries is addition of antagonistic additives to foods that exhibit both preservative and anti-microbial properties (Chen and Hoover, 2003). Though, these synthetic chemical-preservatives prolong the shelf life of food and aid in delaying/stopping the growth of undesirable food borne microbes, but unfortunately recent findings indicate that their addition in food can cause more harm than benefit as many of them are capable of causing behavioral changes (Gultekin et al., 2013) such as allergies, skin rashes, asthma, urticaria dermatitis and even carcinogenisis in cosumers (Sharma, 2015). These facts are raising health concerns among public and consumers are becoming increasingly worried about the impacts of chemical preservatives in food they consume. Therefore, increasing public awareness and demand regarding naturally preserved (biopreserved) foods or foods with no chemical preservative is compelling researchers to develop a novel method of food preservation, entirely different from the traditional ones. Increased number of insistences by dairy plants to prolong shelf-life and preclude deterioration of dairy products is another reason for finding other natural antimicrobial alternatives for biopreservation of foods (Silva et al., 2018).

To overcome all these issues researchers are trying to utilize another class of recently discovered antimicrobial substances i.e. bacteriocins, that cannot only act as potential natural biopreservatives without compromising the organolepticproperties of processed food but can also serve as a best substitute of conventional antibiotics in therapeutics and clinical settings. Their anticancer potential is also being emerging as a novel tool in combating cancer. The main objective of this review is to discuss the general characteristics of bacteriocins, significance of LAB bacteriocins their mode of action and potential applicactions in various fields. A brief overview on the safety profiles of few bacteriocins has also been presented towards the end of discussion.

GENERAL DESCRIPTION OF BACTERIOCINS

Bacteriocins are ribosomally synthesized low molecular weight extracellular peptides that exhibit bacteriostatic and bactericidal potential against members of closely related species and different strains of similar species. Most of them show narrow range of inhibitory activity. More or less all types of gram-positive and gram-negative bacteria secrete them under stress conditions to kill neighbouring cells when nutrients in environment are depleting, the producer cell remains unaffected due to its simultaneous production of immunity protein against respectively produced bacteriocin. The genes encoding bacteriocins structural proteins, immunity proteins, associated regulatory proteins, transport and modification (in case of Lantibiotics) proteins are located on different genetic determinants in the form of operon clusters. It is proposed by Riley and Gordon (1999) that the primary role of these potent AMP's is to mediate intra-specific or population-level interactions (Riley and Gordon, 1999). These cationic, hydrophobic peptides kill sensitive strains by either forming pores or by inserting their amphipathic helical structures inside the targets cell's plasma-membrane to cause depolarization of cell membrane and efflux of essential metabolites. The consequent dissipation of PMF (Proton motive force) results in cell death of target bacteria (Da Costa et al., 2019).

Bacteriocins seem to be like antibiotics in their mode of action but they cannot be termed as antibiotics as they restrict their inhibitory activities against strains of species identical with bacteriocin secreting species and more particularly against strains of producer bacterium. While therapeutic antibiotics have broader spectrum of antimicrobial activity and if they occasionally exhibit narrow inhibitory range they never obstruct proliferation of associated strains of same species. Secondly, antibiotics are secondary metabolites while bacteriocins are produced by ribosomes during primary growth phase as small peptides that are often subjected to post-translational modifications. This feature makes them easily amenable for gentetic engineering applications. An additional benefit that allow bacteriocins to be used as safe food-preservatives is their biodegradability in Human gastro-intestinal tract by proteolytic digstetive enzymes. This feature renders them safe for human consumption (Zacharof and Lovitt, 2012) as well as reduces the risk of pathogenic strains to come in prior contact with them as they don't persist in human bodies and environment. It is an initial point in development of antibiotic resistance and bacteriocins intrinsically stop this from occuring. Their other notable differences with conventional antibiotics include: bacterial species as only source organism; non-toxicity towards eukaryotic cells; broad diversity in terms of molecular size; structure; mode of action and number of target bacteria; high thermal stability; wide range of active pH; strong antimicrobial effect even at nano to micro molar ranges; lacking of properties like odour, colour and taste; strong antibiofilm potential; potential amenability to bio-engineering; pore-formation/inhibition of cell-wall biosynthesis being the prominent modes of action; broad spectrum of applications (not only clinical) and unlike the case of antibiotics, their inability to transfer resistant genes in other bacterial species. This posses least chances for sensitive strains to attain resistance against bacteriocins (Cleveland et al., 2001; Meade et al., 2020; Perez et al., 2014).

BACTERIOCINS FROM LAB

Among gram positive bacteria representatives of group LAB are considered to be of particular importance as they have been used by many populations since centuries for fermenting different food products due to their natural food preserving nature and for their ability of imparting significant texture, aroma and nutrional values to food products. The discovery of novel bacteriocins from Lactic acid bacteria and their exploitation in food

industry is currently the most extensively studied subject of food biotechnology. This group comprises species of *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus and Weissella.* The genus *Lactobacillus* with more than 100 species makes the largest LAB group. It is abundantly present in carbohydrate rich enivronments (Mokoena, 2017).

Members of LAB are known for producing an array of anti-microbial compounds i.e., Organic-acids, Diacetyl, Hydrogen-Peroxide, acetoin and bacteriocins. Due to these defining roles in food safety and food preservation they have attained GRAS statuts and are regarded as nontoxic/safe for human ingestion. Since the bactericions from LAB pose no health risk concerns, they can serve as great alternative to conventional chemical preservatives in dairy products either in their purified semi-purified forms (Silva et al., 2018). Furthermore, most of the bacteriocin producing strains from LAB are normal food-isolates that's why are considered preferably the best source of bacteriocin production. Hence food industry shows particularly high interest in provision of bacteriocins from Lactic acid bacteria (Deegan et al., 2006).

MODE OF ACTION

The cationic nature of bacteriocins is the main factor responsible for cell death of target strains. The anionic surface components like phosphatidylethanolamine (PE), phosphatidylglycerol (PG), lipopolysaccharide (LPS), lipoteichoic acid (LTA) and cardiolipin (CL) on target bacterial cell-surface, serve as main targets for positively charged bacteriocins. The Bacteriocins assume conformations that are suitable for electrostatic binding with target cell surface. The traversing of bacteriocins through lipid bilayer occurs through association of their hydrophobic regions with host cells plasma-membrane (Kumariya et al., 2019). These initial electrostatic interactions lay the foundation of subsequent events. The mechanism of pore formation is evident in cases of both lantibiotics and non-lantibiotics but the manner in which they form differs significantly For Instance Lantibiotics are known for creating pores in "wedge-like model", whereas class II bacteriocins are known to intensify host's membrane permeability by formation of pores in "barrel stave or carpet manner" (Moll et al., 1999). Above all these facts it is important to note that bacteriostatic and bactericidal potential of any bacteriocin is highly dependent on factors like physiological conditions, amount of bacterioicin being used, Indicator/Target pathogen and environmental factors (Jozala et al., 2015).

Nisin is very potent class I AMP (antimicrobial Peptide) It is effective against sensitive strains even at lower concentrations. The proposed mechanism of its action is that it can stop cell-wall synthesis of sensitive bacterial strains by binding with lipid II molecule which is the chief transporter of peptidoglycan subunits and moves from cytoplasm to the cell wall. This binding prevents cell wall biosynthesis, thus causing cell death. However, at greater concentrations, the nisin-lipid II docking-complex directs nisin to insert into host's membrane and form pores in their plasma membrane (Perez et al., 2015). This shows Lipid-II acts not only as binding receptor for Nisin but also enable it to exert its renowned targeted pore forming action (Moll et al., 1999). Pore formations that result due to latter case are responsible for leakage of essential ions (K^+, H^+, Phosphate), cell contents and ATP by disturbance of PMF, pH equilibrium of target cell's plasma membrane (Deegan et al., 2006). Peptidoglycan precursor lipid II acts as docking molecule not only for class I bacteriocins but also for antibiotic vancomycin, although binding-site is different there (Breukink and de Kruijff, 2006).

Like all other LAB Bacteriocins Class IIa bacteriocins are also bactericidal in their modes of action. Their well known representatives like Pediocin PA-1 (Chikindas et al., 1993) Mesentericin Y105 (Maftah et al., 1993)and Bavaricin MN (Kaiser and Montville, 1996) have demonstated that sensitive bacteria can be killed by permeabilization of cell-membrane of target bacterium through formation of poration-complexes, resulting in subsequent ionic imbalance and leakage of inorganic phosphate. These all factor collectively contribute for dissipiation of PMF which can further be partial or total disturbance of transmembrane potential and pH gradient. Only newly discovered Mutacidin shows complete dissipation of transmembrane potential like lantibiotics (Bennik et al., 1998).

Dissimilarly with lantibiotics class IIa Bacteriocins donot seem to cause an ATP leakage from sensitive target strain might be due to smaller pores form by them. The sensitive cell shows depleted ATP concentrations because of its quicker utilization of ATP to restore its original PMF and Protecting cell from ATP exhaustion due to P efflux or due to incompetence of cell to produce ATP due to inorganic Phosphate efflux. pediocin-PA-1-induced ATP reduction and inorganic phosphate efflux were observed by Chen and Montville, 1995 and found that loss in ATP concentration is attributed to cell's ability to regenerate reduced PMF (Chen and Montville, 1995). While Nisin Z shows opposing behavior (Abee et al., 1994). Similarly to lipid II, mannose-phosphotransferase systems (man-PTS) is suspected to serve as receptor molecule for some class II bacteriocins (Jozala et al., 2015).

UTILIZATION OF BACTERIOCIN

With the growing population and advancement in science, people need food in terms of quality and quantity. Using bacteriocin as food preservative not only increases the shelf life of food, is also a potent source of eradicating an established infection too, as with the emerging antibiotic resistance put researchers in a stress

condition to find an alternative of antibiotics which are used for therapeutic purpose, and probiotic are found as a safe alternative for the treatment.

Besides this, Multi Drug Resistance (MDR) pathogen, the hurdle of selection of resistant bacteria to antibiotics is a growing problem of the 21st century (Kapil, 2005; Chopra et al, 1998) which is a prime concern as they weekend the ability of antibiotic arsenal to fight with them. To find the possible solution to this growing problem researchers are focusing on the usage of bacteriocin due to its high specific activity even against MDR pathogens. The diversity in the applications of bacteriocin is owing to its recognition of GRAS (Generally Regarded as Safe) substance by the US (United State) of food and FDA (Federal Drug Administration) and the European legislation for pharmaceutical and food industries uses. LAB producing bacteriocin is used as a potential 'Natural-bio preservatives' which reduce chemicals' side effect thus helping in the improvement of human health as these peptides are active against pathogen (Moreno et al., 2006).

In the current realm of the procession, the research on bacteriocin is, however, gaining importance because of its natural defense system mechanisms ability to compete with the microorganisms in the same environment and hence its widespread potential applications in bio-nonmaterial, veternity, dairy products (Nisin is used for this purpose which is approved GRAS Bacteriocin), crop management and solutions to problems of systematic infection, oral care, cancer, vaginal infection, skincare, and contraception. The primary function of bacteriocin is food preservation and the continuous development of newer classes of antimicrobial agents has become of emerging importance for medicine (Kumar and Schweiser, 2005; Fisher et al., 2005). Here we will discuss the applications of bacteriocin in detail, which are shown in the form of tabulation as given below in Fig. 1.

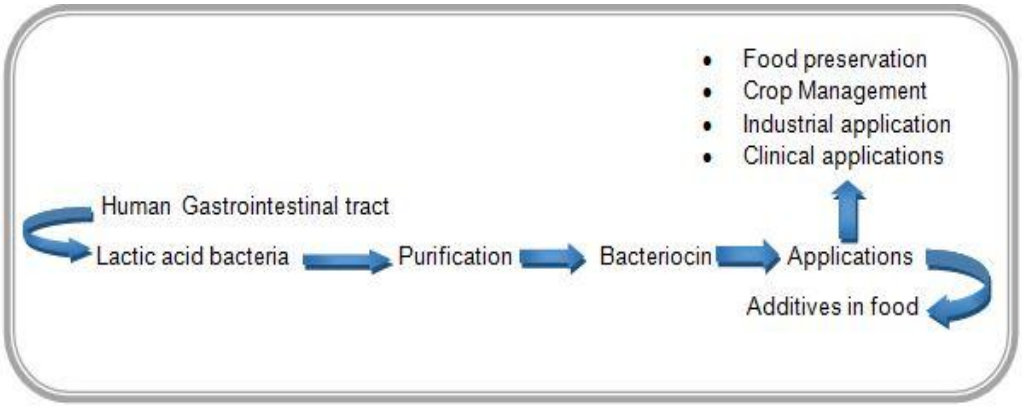
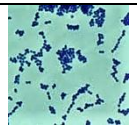
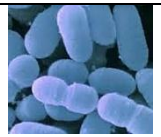
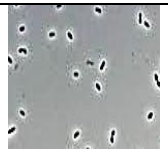


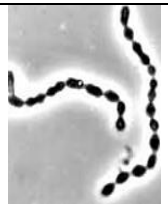
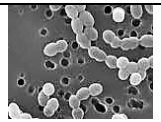
Figure 1: Overview of Applications of Bacteriocin (Evangelin et al., 2015).

IMPORTANT BACTERIOCIN PRODUCED FROM LAB AND ITS APPLICATION

A vast array of bacteriocin has been produced by lactic Acid Bacteria (LAB) Gram positive bacteria, cationic, heat stable, permeabilizing membrane peptide, amphiphilic, and can work at lower pH; owing to their properties they are exploited for utilization in food preservation as a natural preservative and for therapeutic purpose. The most important bacteriocin 'Nisin' belongs to class-I and Acidocin, Lactacin, Lactococcin, and Pediocin belong to class-II. The bacteriocin produce from LAB can be used in both purified and crude form. Because of a non-toxic effect of LAB bacteriocin, it's been regarded as safe which highlights its importance. LAB producing bacteriocin is used as a potential 'Natural-bio preservatives' which reduce chemicals' side effect and its probiotic properties are used in treatments thus helping in the improvement of human health as these peptides are active against pathogen (Moreno et al., 2006). Therefore, the increasing interest in these compounds has encouraged the researchers for the isolation of LAB producers and the characterization of many novel peptides (Deraz et al., 2005). In the Table 1 given below, some important bacteriocins produced from LAB are mentioned with description.

Table 1: Comprehensive comparative elaboration of different features of Bacteriocins.

Lactic Acid Bacteria (LAB)	Morphology	Micrograph	Bacteriocin	Source	Characteristics of Bacteriocin	Indicator organism	Potential Application	References
Enterococcus faecium	Gram positive, sometime motile, cocci spherical		Enterocin A and B	Naturally present in the GIT	Class II bacteriocin, 4,8 kDa, 47 amino-acid residues, heat-stable	*Listeria monocyt-ogenes, Pediococcus, Enteroc-occus spp.* , VRE	Active against food borne pathogens and have antilisterial effect.	(Lauková and Czikková, 1999)
Lactobacillus acidophilus CH5	Gram positive, fermentative, spiral or straight rod or coccobacill-ary form		Acidocin B	Member of normal intestina-l microflo-ra	Class II bacteriocin, forms high molecular weight aggregates	Inhibiting strains of the genus *Lactoba-cillus, Bacillus, Microco-ccus* and *Coryneba-cterium* Gram-positive bacteria	Used in food industry And kills food spoilage bacteria. Used as probiotic too.	(Chumchalova et al., 1995)
Lactobacillus plantarum	Have rounded end and rod shape, gram positive, occurring singly or in pair		Plantaricin	Ferment-ed food products like kimchi	molecular mass of 4347.8467 Da,wide pH stability, high thermal stability, and surfactants stability	*Staphylo-cocccus aureus E. coli, Streptococc-us mutants* etc.	Anti listerial cultures for fermented sausages and cooked ham and future application in food preservation	(Olasupo N.A., 1996)
Lactobacillus casei	Non-motile, gram positive rod shape, mesophilic		Lactocin 705	Normally in gut, fermen-ted food yogurt	Class II two-component bacteriocin (33 amino-acids each component), 3,4 kDa,	*Listeria monocyt-ogenes, Lactoba-cillus plantarum*	Used for clinical aspects	(Johansson et al., 1993)
Lactobacillus sake	Gram positive, facultative anaerobe, rod shape		lacticin 3147 and lacticin 481	Red meat and fish	Class I bacteriocin, 3,7 kDa, active between pH of 4,5 and 7,5	*Lactoba-cillus, Leuconostoc, Pediococcus*	Food industry, veterinary medicine and in treatment of human diseases	(Guinane et al., 2005).
Lactococcus lactis subsp. Lactis ATCC 11454	Spherical or ovoid cells, gram positive, non-motile		Nisin	Dairy ferment-ed product	Class I lantibiotic, 3,5 kDa, 34 amino-acids, commercially available	*Listeria monocyt-ogenes, Bacillus cereus* & *Staphyl-ococcus aureus.*	Used in preservation of dairy products e.g. cheese, yogurt and effective against mastitis causing pathogens	(Broadbent et al., 1989)

Lactococcus lactis	Gram positive, ovoid or spherical, occur in pairs or short chain, gram +ve		Lacticin 3147	Milk, cheese, egg etc	Class I two-component lantibiotic, 4,2 kDa, heat-stable, active under acid and physiologi-cal pH	*Clostridium sp. Listeria monocyt-ogenes, Staphylo-coccus aureus, Enterococcus faecalis, Propionib-acterium acne, Streptococcus mutans,*	Antimicrobial activity against food born pathogen and food spoilage bacteria	(McAuliffe et al., 1998).
Leuconostoc *gelidum*	Gram positive, ovoid cocci, without cytochrome		Leucocin A	Isolated from plant matter, kefir etc	Class II bacteriocin, 3,9 kDa, 37 amino-acids, stable at low pH values, even after heating (100°C for 20 min)	*Lactobacillus, Enterococcu-s faecalis, Listeria monocyt-ogenes.*	Control of listeria in vacuum and modified atmosphere stored meat products	(Saucier et al., 2005)
Lactobacillus rhamnosus	Rod shaped appear in chain, Short gram positive strain		Rhamnosin	Grape peel	1427 Da - 602.6 Da, broad range of activity and inhibit MRB	Gram-positive bacteria, *Listeria monocyt-ogenes, Staphylo-coccus aureus*	Inhibition of mold and psychrotr-ophes in cottage cheese	(Makhal et al., 2015)
Pediococcus acidilactici	Gram positive, cocci, in pairs or tetrad form		Pediocin F	Found in plants and milk.	Class II bacteriocin, 4,5 kDa, sensitive to proteolytic enzymes.	*E. coli* Other Gram positive bacteria	Application in food, avoid food spoilage	(Schöbitz et al., 2006).
Pediococcus pentosaceous	Non-motile, gram positive, spherical, gram positive		Pediocin A	soy sauce mash or moromi	Class II bacteriocin, 2,7 kDa, sensitive to proteolytic enzymes and heat stable.	*Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Staphyloc-occus, Enterococcus*	Active against food borne pathogen	(Cintas et al., 1998)

ACTIVITY OF BACTERIOCIN IN ANTIMICROBIAL COATING FILMS AND NANOTECHNOLOGY

The standard of food quality is being increased over time, along with the concern to reduce waste in the environment, which encourages the research of edible coating having antimicrobial additives. The most broadly investigated biopolymers i.e. 'Hydrocolloids' (proteins and polysaccharides) used in edible coatings and films applied to cheese. These biopolymers are used to modify the atmosphere of surrounding of foods, and make a barrier between the food and the environment, without changing organoleptic and nutritional properties it helps to improve the safety, quality, and functionality of food products (Han, 2003; Valdés et al., 2017). Using bacteriocin in coating films inhibits the growth of unwanted microorganisms like nisin/chitosan combination gives effective antlisterial action (Guo et al., 2014; Muriel-Galet et al., 2012) when applied on the covered surface of the food. Stability against proteolytic enzymes is provided, by immobilizing bacteriocin via covalent linkage in the packaging system (Bali et al., 2014; Al-Mathkhury et al., 2011).

Besides this, through nanoliposomes, nanoemulsions, nanoparticles, and nanofibers we can obtain the bacteriocin nano-capsules which have possible applications in the food industry and medical field. Nisin, pedocin and sublitosin including in nanoemulsions have been tested in combination with carvacrol, curcumin, and cymene against *L. monocytogen, E. coli, S. typhimurium* and *Candida lusitaniae* (Zhang et al., 2014; Ndoti-Nembe et al., 2013). In deep wounds infected with *S. aureus* can be treated with nanofibers with ethylene oxide and poly (D, L-lactic acid) including nisin or plantaricin (Dicks et al., 2011; Brand et al., 2013). In dairy products, to increase the shelf life the bacteriocins and/or bacteriocin-producing LABs are incorporated in coatings and films. The food packed with coatings and films containing antimicrobial metabolites synthesized by LABs are responsible for the inhibition of pathogenic microorganisms (CaoHoang et al., 2010; da Silva Malheiros et al., 2010; Aguayo et al., 2016) or having a viable count of LAB in the film. Without bacteriocin coating the dairy products spoiled immediately as compared with bacteriocin application on films and coatings. Bacteriocins along with antimicrobial films and coating are used in dairy products which are responsible for the inhibition of pathogenic microbes as described in the form of flow sheet diagram given below in Fig. 2.

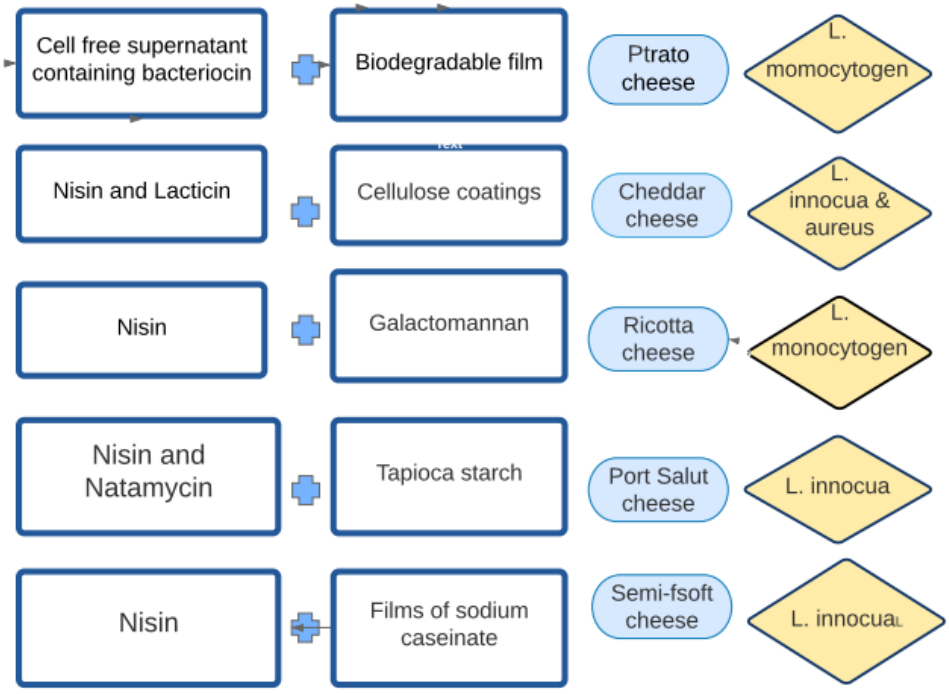


Figure 2: Combination of bacteriocins with Antimcrobial films and coating, inhibiting pathogens (Silva et al., 2018).

On the other hand, via using 'Nanotechnology' the bacteriocin nano-capsules are utilized in food and have medical applications as well. In the Fig. 3, a combination of nano-capsules and bacteriocins are mentioned which are responsible for inhibiting pathogens.

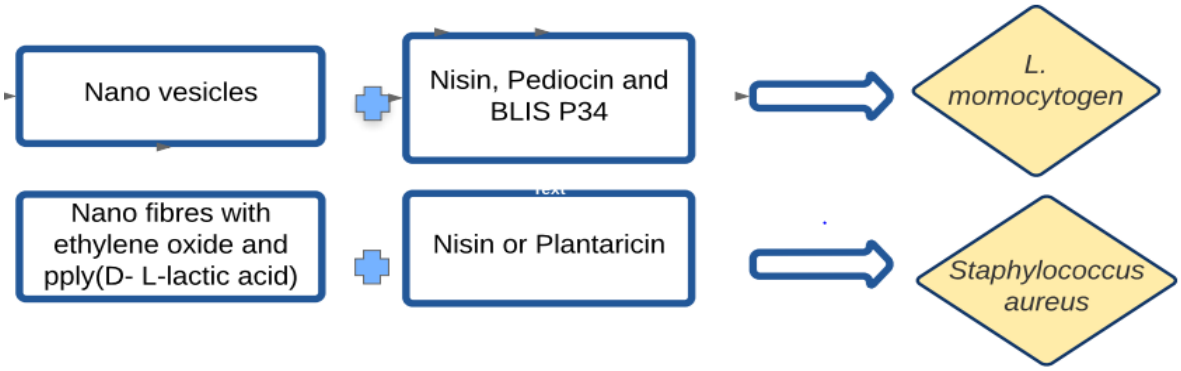


Figure 3: Combination of Nano capsules with bacteriocin, inhibiting pathogens (Silva et al., 2018).

CLINICAL APPLICATIONS

The first and foremost requirement of practical utilization of Bacteriocins is in hospital settings where appearance of Multi-drug Resistant pathogens is most evident and many nosocomial infections due to such MDR strains are becoming a global threat to human health. With the alarming condition of bacterial resistance, alternative of anitibiotic was required for the betterment of human health. Number of bacteriocin is used as probiotic in replacement of antibiotic which play crucial role in boosting our defense mechanism (Immunity System). The notable resistant pathogens are *Staphylococcus aureus, Enterococci, Pneumococci, A.baumannii, Citrobacter freundii, E.coli, Klebsiella pneumoniae* and *Proteus spp*. The most important ones Vancomycin-Resistant- *Enterococci* and Multi- Drug-Resistant *Staphlococcus aureus* are recently been found to be sensitive against lacticin 3147 and Nisin A. Both stated bacteriocins are also important in inhibiting liver, spleen and kidneys-pathogens. Other infectious and resistant strains of micro-organisms have also been found to be controlled by bacteriocins NAI-107, microbisporicin and Mutacin B-Ny266 (Ahmad et al., 2017).

Toxicity profile of Bacteriocins

Bacteriocins are generally considered as safe alternatives of conventional antibiotics in literature and very few studies depict their consequent side-effects. No Doubt they are promising candidates to be used as substitute for antibiotics but here we will highlight finding of few studies that were meant for predicting the therapeutic efficacy and toxicity associated with certain Bacteriocins. The highly cationic nature of bacteriocins cannot be neglected as far as their safety in animal and human is concerned as they can equally be interactive with cell-membrane's phosphatidylethanolamine (PE) component as Teichoic Acids of target-Bacterial cell-walls. PE in mammalian cells serves as substrate for inflammatory responses mediator Phosphholipase-A2. The confiscation of PE by cinnamycin and duramycin might be the cause of Immune Modulation reactions via indirect inactivation of PL-A2. Another potential problem speculated with Binding of bacteriocins with PE is their deposition inside the cell-membrane causing change in membrane's bio-physical properties and altered ion-channels (Dicks et al., 2018). Cancer cells being negatively charged are also the best target for bacteriocin preferential attack. Such verdicts have been obtained from studies on head and neck squamous-cell-carcinoma-cell lines where Nisin was found to effectively perform apoptosis and cell-cycle arrest of cancerous cells selectively without implicating any toxicity on human consumption (Dicks et al., 2018).The preclinical invivo and in-vitro trial findings have showed novel-circular bacteriocin AS-48 non-toxic as no loss of cell-viability at therapeutic concentration was found (Cebrián et al., 2019).

The in-vitro cytoxicity, of Peptide-p34 was tested in different eukaryotic cells and was found to be toxic to VERO cells (Vaucher et al., 2010). The crude-supernatant of AMP- LR14 from *Lactobacillus plantarum* Strain LR/14 showed delayed life cycle of fruit fly at 10mg/ml conc. While at 15 m-g/ml conc. exerted a strong larvicidal consequence. Ingestion of said peptide by fruit flies also resulted in profound loss in weight and size (Gupta et al., 2014). Sometime bacreriocinogenic strain from Gram-positive group of Bacteria act as virulence Factors. A two peptide enterococcal Lantibiotic named Cytolysin has been observed for its broad spectrum of activity which it exert not only against Gram-positive bacteria but it is also lethal against eukaryotic cell-lines such as Human-Bovine, Horse Erythroytes, Retinal-cells and also causes acute terminal outcomes in Human Infections. It is believed that its anti-eukaryotic activities might have been selectively adapted for Eukaryotic cell-predation in soil and water ecologies. Also, another non-lanthionine-containing peptide secreted by some group A streptococci named Haemolysin, and Streptolysin S, are found to cause invasive infections (Cotter et al., 2005).Keeping in view these findings practical utilization of bacteriocins require accurate and safe clinical trials and approval from regulatory authorities.

Bacteriocin used as Probiotic for treatment

Lactobacilli which are a bacteriocin producing organism, present naturally in the human gut as natural micro flora can be used as a probiotic to influence the gut ecology. Hence in most stomach problems, we are advised to take yogurt for the good working of our natural micro flora to combat harmful bacteria. The gut micro-organisms provide health benefits including inactivation of carcinogenic compound's potential, activate our defense mechanism, and responsible for the reduction of serum cholesterol. Bacteriocin helps to compete with potentially pathogenic gut micro flora and also enhances the ability of colonization organisms. Hence, FAO/WHO (2002) recommended on the careful evaluation of probiotics in food. As probiotics, are live microorganisms that, when applied in the adequate quantity, have a health benefit on the host (Sanders, 2008; Schrezenmeir and De Vrese, 2001). In the pharmaceutical shops of Pakistan most common probiotics used are GUTCARE, BIFLORA®, HIFLORA^{TM}, ECOTEC^{TM}, AMYBACT® and LAKTY^{K}.

Intestinal Infection

Several bacteriocin are used as a probiotic in the replacement of antibiotics which plays a vital role in boosting our defense mechanism (Immunity System) (López-Cuellar et al., 2016). In the Table 2 names of some bacteriocins are mentioned which are used as probiotics as alternative of antibiotics for the treatment of Gastro Intestinal Tract (GIT) infections.

Table 2: Comparison of common antibiotics and bacteriocins for antimicrobial potential.

Antibiotic used for treatment	Alternative Bacteriocin Used As (Probiotic)	Producing strain	Bioactivity	Potential application	References
Carbapenems	Plantaricin 423	*Enterococcus mundtii*	Antimicrobial agent	GIT infections	(Kumarasamy et al., 2010) (Dreyer, 2018; Van Zyl, 2018)
Cyclic lipopeptide	Piscicolin 126	*Carnobacterium piscicola*	Antimicrobial agent	GIT infections	(Coates et al., 2011) (Ingham *et al*., 2003)
Metronidazole	Pediocin PA-1	*Pediococcus acidilactici*	Antimicrobial agent	GIT infections	(Persky and Brandt, 2000) (Cintas et al., 1998; Dabour et al.,2009)
Penecillin	Peptide ST4SA	*Enterococcus mundtii*	Antimicrobial agent	GIT infections	(Tan and Tatsumura, 2015) (Knoetze et al., 2008; Dreyer, 2018;Van Zyl, 2018

CONCLUSION

This review highlights the antimicrobial activity of bacteriocin and its utilization in the field of both pharmaceutical and food products. Owing to abundant diversity and successful incorporation make it an ideal candidate for researchers. With the growing problems of antibiotic resistance, food shortage and increasing Multi-Drug Resistance (MDB) in the 21st century encourages the researcher to study about the natural product such that bacteriocin which shows promising applications in the food industry in term of increasing shelf life of food and preserve quality. LAB plays a vital role in the application of bacteriocin as they are generally regarded as safe (GRAS) and approved by the FDA. Bacteriocins have inhibitory action against food borne pathogens like *L. monocytogenes*, but they are not antibiotics. Bacteriocin is different from antibiotics in its synthesis and mode of action. Furthermore, unlike antibiotic resistance, bacteriocin resistance is not usually determined genetically and organisms which show resistance to antibiotics are not cross-resistant generally with bacteriocins. Therefore, bacteriocin could be considered as potential alternative to antibiotic. In clinical aspects bacteriocin used as a probiotic which show antimicrobial activity not only in bacteria but has effective action against virus and fungal infection. The bacteriocin showcases hidden potential which motivates the scientist for new inventions and continuing the search for more novel bacteriocin with dedicated and promising applications.

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