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Bacteriocins against Foodborne Pathogens (Review)

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Abstract—Bacteriocins are peptides or proteins synthesized by the bacteria on ribosomes and have the ability to inhibit or even kill bacteria other than the producing strain. Both Gram-positive bacteria (GPB), as well as Gram-negative bacteria, produce bacteriocins. However, GPB, mostly lactic acid bacteria, produce the vast majority of bacteriocins. Natural food preservation strategies have gained importance as the market for minimally processed and ready-to-eat food items has grown. Among various natural antimicrobial compounds, research interest in bacteriocins has been increased in the recent years. Bacteriocins being safe and effectively tolerated by the human gastrointestinal tract are proposed as a better natural alternative compound among the other natural and commonly used chemical food preservatives. Several studies documenting potential applications of bacteriocins in food products such as dairy, meat and meat products, fish, and beverages have been documented. Nisin is one of the bacteriocins which has gained regulatory approval for usage in foods. The review summarizes classification of bacteriocins, their mode of action and proposed application in food preservation and safety.

Keywords: bacteriocins, foodborne pathogens, lactic acid bacteria, Gram-positive bacteria, Gram-negative bacteria

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Bacteriocins, ribosomally synthesized natural peptides, are formed by the bacteria in a highly competitive polymicrobial environment. They are closely related to the strain that produces them [1]. These peptides hinder similar or closely related bacterial strains from growing, by interacting with and killing cells containing unique surface receptors [2]. Bacteriocins formed by Gram-positive (GPB) and Gram-negative (GNB) bacteria have antimicrobial activity, that may aid in the elimination of other closely related bacterial species [3]. While both GPB and GNB produce bacteriocins, the majority of bacteriocins used today for food preservation are the result of lactic acid bacteria's (LAB) secondary metabolism. These peptides differ in structure, biosynthesis mechanism, action, self-immunity, and gene regulation [2].

This review highlights about the bacteriocins and their effects against the various foodborne pathogens (FBP). Various areas of bacteriocins are covered including classification, biosynthesis, and mode of action, usage as well as their antimicrobial properties. These factors are important because bacteriocins may be a viable alternative to antibiotics, thus assisting in the fight against antibiotic resistance. In addition, issue of bacteriocins resistance is also discussed which is important to be addressed for the conventional use of bacteriocins in the near future.

General classification of bacteriocins. The continuous finding and characterization of bacteriocins along with the study of their structures, amino acid sequence, action, and biosynthesis process, has been followed by the constant changes in the classification of the bacteriocins [4]. Various classification systems were considered by several researchers on the basis of factors like size, molecular composition, and structure of these compounds [1]. Few authors classified bacteriocins on the basis of different factors like physical properties or chemical structure, stability, size of the molecules, organism involved in their production, and their mechanism of action [5]. In addition, on the basis of the cell wall type, bacteriocins are classified into two major groups: bacteriocins produced by GPB (BGPB) and GNB (BGNB) [2]. A list of bacteriocins produced by bacteria has been listed in Table 1.

BGPB. Bacteriocins can be classified into 3 or 4 groups depending upon size, composition as well as structure (Fig. 1). Class I or the lantibiotics are the small membrane-active peptides (less than 5 kDa) that have been modified post-transcriptionally forming residues of unusual amino acids like dehydrated amino acids, lanthionine, or b-methyl lanthionine [4]. The amino acids present form multiple ring structures providing rigidity to the bacteriocin structure and also structural stability to heat, pH as well as resistance against the actions of proteases [2]. The two major

Table 1. Classification of bacteriocins

Bacteriocin (type/subclass)		Producer micro-organism	Sensitive bacteria	Mechanisms of the action	Potential application(s)	Ref.
GPB						
Class I (lantibiotics)						
Class Ia	Nisin	*Lactococcus lactis*	*Staphylococcus aureus, Enterococcus*	Inhibition of cell wall synthesis	Food biopreservative	[69]
	Epilancin 15X	*Staphylococcus epidermidis*	*Staphylococcus aureus, Staphylococcus agalactiae*		Therapeutics	[70]
	Microbisporicin	*Microbispora cornallina*	*Streptococcus* spp, *Neisseria* spp., *Clostidium* spp		Therapeutics	[71], [72]
	Gallidermin	*Staphylococcus gallinarum*	*Staphylococcus aureus, Streptococcus epidermidis*		Therapeutics	[73]
Class Ib	Mersacidin	*Bacillus* spp.	*Streptococcus* spp.	Inhibition of cell wall synthesis	Synthetic biology	[5]
	Actagardin	*Actinoplanes* sp.	*Streptococcus* spp., *Staphylococcus* spp.		Therapeutics	[74], [2]
	Lacticin	*Lactococcus lactis*	*Lactococcus lactis, Listeria monocytogenes, Bacillus subtilis*	Pore formation	Food biopreservative	[75]
Class II (non-lantibiotics)						
Class IIa	Leucocin	*Leuconostoc gelidum*	*Listeria monocytogenes,*	Pore formation	Cleaning applications	[76], [77]
	Pediocin PA-1	*Pediococcus acidilactici*	*Listeria monocytogenes, Staphylococcus aureus, Pseudomonas* spp., *Escherichia coli*		Food biopreservative	[78]
	Acidocin A	*Lactobacillus acidophilus*	*Bacillus cereus, Staphylococcus aureus*		Food biopreservative	[79]
Class IIb	Lactococcin G	*Lactococcus lactis*	*Lactococcus lactis, Streptococcus pneumoniae*	Pore formation	Therapeutics	[80]
	Lactococcin Q	*Lactococcus lactis* QU4	*Lactococcus lactis*		Therapeutics	[81]
	Plantaricin	*Lactobacillus plantarum*	*Listeria monocytogenes, Staphylococcus aureus*		Therapeutics	[82]

Table 1. (Contd.)

Bacteriocin (type/subclass)		Producer micro-organism	Sensitive bacteria	Mechanisms of the action	Potential application(s)	Ref.
Class IIc	Lactococcin A	*Lactococcus lactis*	*Lactococcus lactis*	Pore formation	Food biopreservative	[83]
	Divergicin A	*Carnobacterium divergens*	*Listeria monocytogenes*		Food biopreservative	[84]
	Acidocin B	*Lactobacillus acidophilus* M4	*Listeria* spp., *Clostridium* spp.		Therapeutics	[85]
Class III (bacteriolysins)						
	Zoocin A	*Streptococcus equi* ssp. *zooepidemicus*	*Streptococcus* spp.	Cell wall disruption	Therapeutics	[86]
	Lysostaphin	*Staphylococcus simulans*	*Staphylococcus, Enterococcus*		Therapeutics	[87], [88]
	Helveticin	*Lactobacillus crispatus*	*Staphylococcus aureus, Staphylococcus saprophyticus, Enterobacter cloacae*		Food biopreservaive	[89]
	Plantaricin S	*Lactobacillus plantarum*	*Enterococcus faecalis*		Food biopreservative	[90]
GNB						
Colicins	Klebicins	*Klebsiella* spp.	*Escherichia, Pseudomonas, Salmonella*	Pore formation/ inhibition of nucleic acid synthesis	Therapeutics	[91]
	Pyocins	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*		Therapeutics	[92]
Microcins	E492	*Klebsiella pneumoniae*	*Escherichia coli, Salmonella enteritidis*	Inhibit nucleic acid synthesis	Food biopreservative	[93]
	J25	*Esherechia coli*	*Escherichia coli, Salmonella enterica*		Therapeutics	[94]
	L	*Esherechia coli*	*Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa*		Therapeutics	[95]

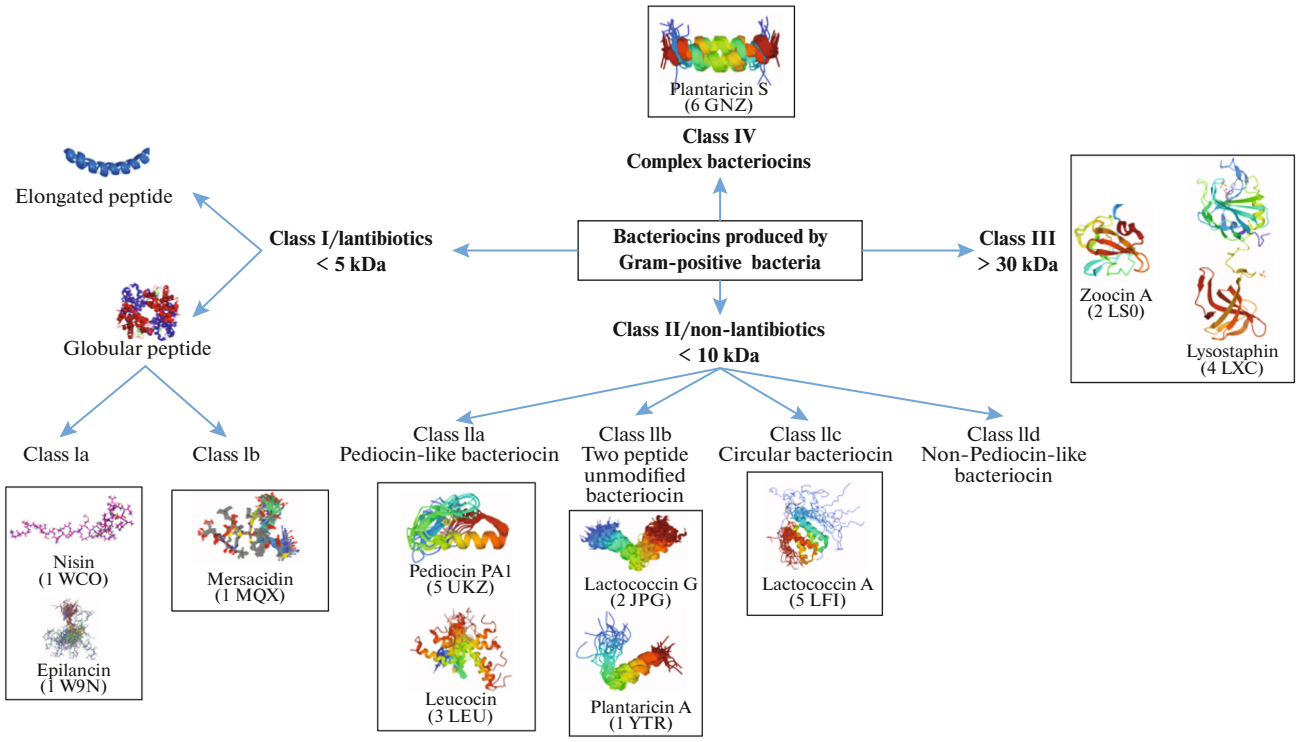

Fig. 1. Bacteriocins produced by GPB. GPB produce 4 classes of bacteriocins on the basis of molecular weight. These are further classified on the basis of their weight and mode of action. Numbers indicate reference ID in PDB.

subclasses of lantibiotics are positively charged elongated peptides and negatively charged globular peptides. The positively charged peptides act by forming pores in the bacterial membranes whereas the negatively charged peptides work by inhibiting particular enzymes that are essential to the bacteria being attacked [1]. Class Ia lantibiotics such as nisin, epilancin 15X, and microbisporicin, produced by *Lactococcus lactis*, *Staphylococcus epidermidis* 15X154, and *Microbispora* spp., respectively, act by inhibiting the cell wall synthesis, as N-terminal domain of bacteriocin binds to lipid II, a peptidoglycan precursor, and the C-terminal domain, involved in formation of pores, which in turn affects the membrane potential. Class Ib lantibiotics includes mersacidin, produced by *Bacillus* sp. strain HIL Y-85.54728, and actagardin produced by *Actinoplanes* sp. Both of them have a compact globular tertiary structure [2]. Mersacidin and other type b lantibiotics bind with lipid II molecules, inhibiting cell wall biosynthesis [5]. This property makes mersacidin a potent inhibitor of GPB. The calcium ions increase the *in vivo* activity of this bacteriocin [6].

The class II bacteriocins (or non-lantibiotics) include heterogeneous set of peptides that unlike class I consist of standard amino acid residues connected by disulphide bridges or cyclized at the N- and C-termini [4]. This class includes small (<10 kDa), heat-stable, post-translationally unmodified bacteriocins [2]. There are no unusual amino acids in this class of bacteriocins. Furthermore, post-translational modifications such as bisulfide bridge formation is found only among few members like pediocin PA-1, and pediocin AcH formed by various strains of *Pediococcus acidilactici*. Peptides of this class are also heat-stable, and function by destabilizing as well as permeabilizing bacterial membranes, when treated cells they can also result in pore formation in the membrane [1]. The majority of lantibiotics, as well as some bacteriocins belonging to class II, inhibits peptidoglycan synthesis when they bind to lipid II of bacterial cell envelope [7].

The four subclasses of class II bacteriocins include: class IIa, also known as pediocin-like bacteriocins, class IIb or two-peptide unmodified bacteriocins, class IIc or circular bacteriocins, and class IId or unmodified, linear, non-pediocin-like bacteriocins [8]. Leucocin A, acidocin A, and pedocin PA-1 are some of the examples of class IIa bacteriocins. Because of their anti-listerial activity, these bacteriocins are also identified anti-listerial bacteriocins. They have linear structure with bisulfide bridges [1]. The effect of pediocins, produced by *Pediococcus* spps. was prominent than that revealed for nisin when tested with FBPs like *Listeria monocytogenes*, and *Staphylococcus aureus* as well as GNB like *Pseudomonas* spps., and *Escherichia coli*. This bacteriocin is highly stable in aqueous solution, has a broad pH range, and is heat- and freeze-resistant. These characteristics make

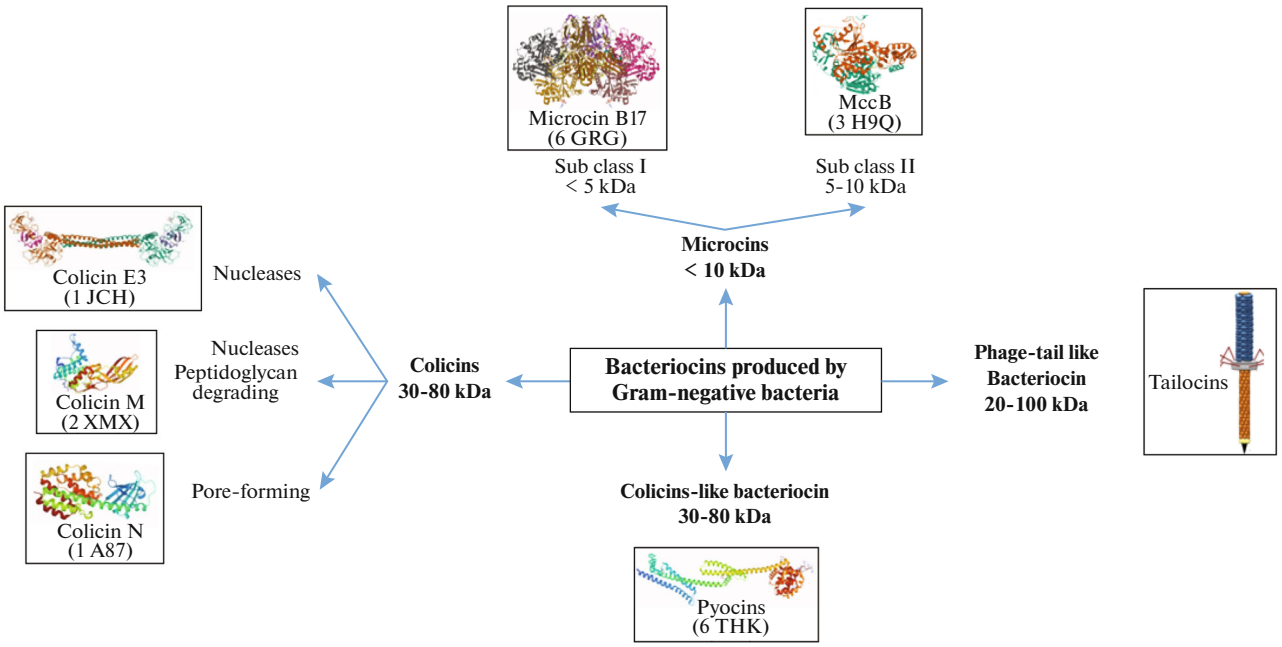

Fig. 2. Bacteriocins produced by GNB. GNB produce 4 classes of bacteriocins based on their molecular weight. These are further classified on the basis of their weight and mode of action. Numbers indicate reference ID in PDB.

it a favorable option to be used in dairy products [7]. Bacteriocins of class IIb consist of two-peptide bacteriocins. Lactococcin G (*L. lactis* strains LMG2081 and BGBM50), lactococcin Q (*L. lactis* QU4), and plantaricin NC8 (*Lactobacillus plantarum* ZJ316) are examples of class IIb. Subclass IIc includes bacteriocins like lactococcin A (*L. lactis*), divergicin A (*Carnobacterium divergens (Lactobacillus divergens))* or acidocin B (*Lactobacillus acidophilus* M4). These bacteriocins contain one (cystibiotics) or two (thiolbiotics) Cys residues in structure [1]. Class IIc bacteriocins are a diverse class of antimicrobial peptides. The post-translational modifications of precursor proteins of class IIc result in the formation of circular backbone, after the covalent linking of both N- and C-termini [4]. The circular backbone of the bacteriocins provides more stability. This subclass of bacteriocins causes pore formation by acting on the plasma membrane of complex cells. Pore formation results in ion depletion, which affects potential gradient and eventually leads to cell destruction [9]. Class IId bacteriocins contain single peptide bacteriocins which are linear, not modified, and different from pediocin-like bacteriocins. Including other bacteriocins apart from the remaining subclasses, makes them a heterogeneous group of antimicrobial peptides from variety of strains [4].

Class III or bacteriolysins are greater than 30 kDa in size. These antimicrobial peptides are susceptible to heat [6]. This class includes bacteriocins like helveticin J (IIIb), zoocin A, and lysotaphin (IIIa). Their antibacterial effect is related to the enzymatic activity, like in the case of endopeptidase, which causes cell wall disruption [1]. The proteins belonging to this class exhibit domain-type structures where different domains are responsible for different functions like translocation, binding to specific receptors as well as lethal activity [4]. This class of bacteriocins has different mechanisms of action as compared to those revealed in classes I and II; also, this subclass is not generally included in the antimicrobial peptides (AMP) family [10].

Class IV includes complex proteins composed of either lipid components or carbohydrates [4]. Bacteriocins like plantaricin S or leuconocin S of class IV act by affecting the bacterial cell membrane. They are found to be sensitive to glycolytic or lipolytic enzymes [1]. Class IV and even sometimes class III bacteriocins are excluded from the classification by some of the researchers since their molecular weight is high, and also, they do not contain 10–70 amino acid chains in total like the first groups do [10].

BGNB. BGNB possess a narrow range of antimicrobial activity in comparison with BGPB. Most of the bacteriocins are isolated from *E. coli* and *Pseudomonas* or *Klebsiella* strains. Colicins, colicin-like, phage-tail-like bacteriocins, and microcins are the four major classes of BGNB [1] (Fig. 2).

Colicins are the high molecular weight (30–80 kDa) bacteriocins, which are heat- as well as protease-sensitive compounds. During stress, *E. coli* strains with a single colicinogenic plasmid develop maximum of these bacteriocins. While producing colicins, there is a simultaneous production of a lysis protein, which is actually harmful to the producers [11] According to

some researchers, based on the mode of action, colicins have 2 subclasses**.** The mode of action of colicins like colicins A, B, E1, Ia, Ib, K and 5 is either resulting in the pore formation in the cell wall of bacteria or damaging the nucleic acid structures which are alike action of DNases, RNases or tRNases [1]. Colicins are classified into 3 classes based on mode of action: nucleases, peptidoglycan degrading ones, and pore-forming colicins. The receptors participating in the transportation of nutrients such as vitamin B12 (cobalamine receptor BtuB), nucleoside-specific receptor Tsx, hydroxamate siderophores (siderophore receptor FhuA), catecholate siderophores (receptors FepA, Cir, and Fiu) are all involved in the absorption of colicin by target cells. On the other hand, porin proteins are used by certain colicins to regulate the passive diffusion of compounds like sugars, amino acids, and phosphates through the external membrane [2].

Colicin-like bacteriocins are produced by other GNB and are similar to colicins in structure, size, and function [2]. This class includes bacteriocins like klebicins, and S-pyocins produced by *Klebsiella* spp. and *P. aeruginosa,* respectively [12]. The pore-formation or nuclease activity can be the factors responsible for the antimicrobial action [1].

Microcins are the small peptides with lower molecular mass (<10 kDa), produced mostly by *E. coli* under stress, specifically under poor nutrient conditions [11]. These highly stable peptides are generally resistant to proteases, extreme pH as well as different temperatures, and they also play a role in competitive interactions amongst the Enterobacteriaceae members [2]. The microcins are encoded by the clusters of genes located in plasmids or sometimes carried by the chromosomes [11]. Microcins are further classified in 2 subclasses. Subclass I includes peptides like microcins B17, C7-C51, D93, and J25 which undergo post-translational modification and have molecular weight less than 5 kDa [1]. They work by inhibiting the activity of enzymes including RNA polymerase, DNA gyrase I and II, which are the key bacterial enzymes. They can also hinder the respiratory chain system [2]. Subclass II contains higher molecular weight (5–10 kDa) plasmid proteins, which are either minimally modified or unmodified. Examples of this subclass are microcins E492, MccV, MccL, H47, Mcc24, I47, and G47 [1]. They act by forming pores in the target cells' cell membranes, hence damaging the cells [2]. Microcins interact with different cells and act via different ways like membrane disruption (microcin E492), inhibition of functions of essential enzymes such as ATP synthase complex, RNA polymerase, DNA gyrase, and aspartyl-tRNA synthetase which are observed in case of microcins M, H47, microcin J25, microcin B17, and microcin C, respectively [1].

The size of phage tail-like bacteriocins is between 20–100 kDa, and they contain 8–14 various polypeptide subunits, thus, the structures resemble the bacteriophage tail modules [2]. These bacteriocins act by causing perforation in the membrane of bacterial cells and hence killing the cell. Bacteriocins produced by *P. aeruginosa* are widely studied [1]. Some of the researchers have divided this class into 2 groups: R- and F-type tailocins. R-type tailocins form a long shell encircled tube, where receptor-binding proteins (RBP) are present at one end of the tube, whereas F-type does not have a shell. The mode of action of this class is predicted to involve shell compression, permeation of the cell wall till nucleus, causing channel or pore formation, which affects the membrane potential of the target cell [2].

BIOSYNTHESIS OF BACTERIOCINS

The genetic factors that code for bacteriocin synthesis are organized in operons (either one or two) with different constituents that can be found on plasmids, conjugative transposable elements, or host chromosome [13]. In mersacidin and subtilin, the group of genes is found on chromosomes, whereas they are present on plasmids in case of sakacin A and divergicin A, and on transposons in case of nisin and lacticin 481. The particular peptides or peptide pheromones acting as inducers are commonly present on the same gene cluster in case of linear unmodified bacteriocins including plantaricins, sakacins, and carnobacteriocins which promote bacteriocin synthesis. Bacteriocins are generally made from inactive pre-peptides, lacking biological or biochemical activity. The pre-peptide contains one leader peptide, also known as N-terminal linked to pro-peptide or C-terminal peptide [14].

Since the development of bacteriocins is regarded as an adaptive response, it is influenced by a variety of environmental aspects. The regulation mechanism is carried out by a signal transduction system that consists of 3 components: an inductor peptide (IP) acting as signal, a sensor histidine protein kinase, and a response regulator protein. IPs are small cationic molecules that serve as a signal for regulation carried out by quorum sensing, which regulates bacteriocin biosynthesis. The induction method can be explained using one of two models. According to one model, IP is formed in lesser amounts at a constant rate, and, thus, accumulates over time as the cell grows. The expression of the genes in the bacteriocin gene cluster increases as the levels of IP upsurges causing the induction. The second model suggests that IP is produced at a lower level than that needed for self-induction, and that its development increases temporarily as a result of various environmental factors. As the level rises above the threshold, IP initiates its own synthesis [3].

Biosynthesis of BGPB. Generally, the bacteriocins produced in the form of inactive pre-peptides, which are also known as pre-bacteriocin leader peptide and pro-peptide at 2 termini: N- and C-, respectively. The biologically inactive pre-peptide is transformed into active peptide form after undergoing step-by-step pro-

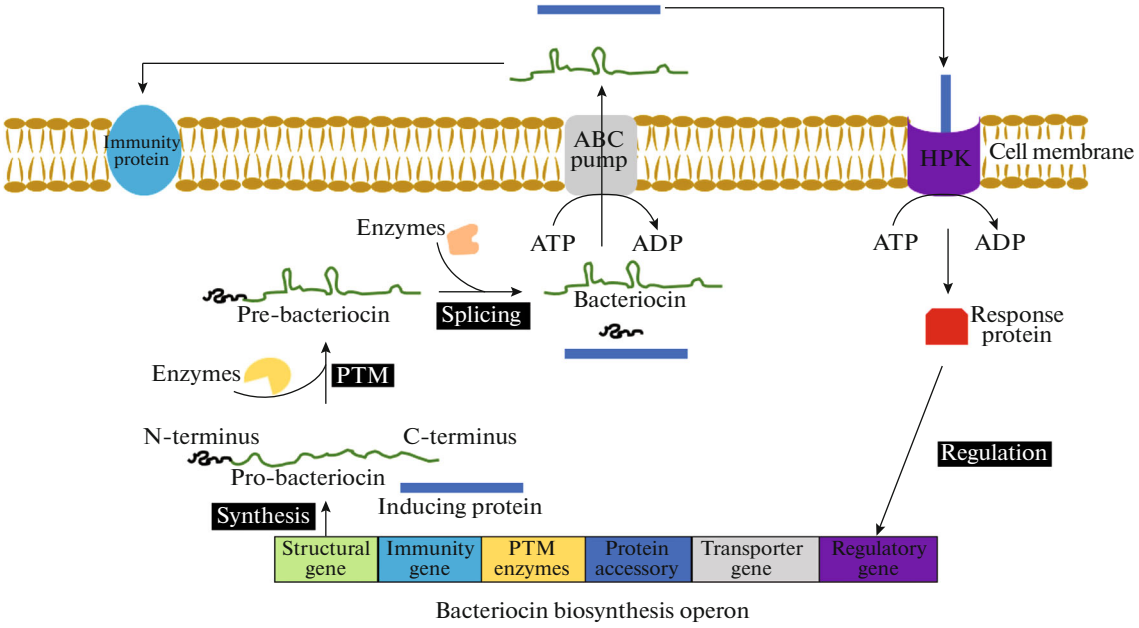

Fig. 3. Biosynthesis of bacteriocins by GPB. The inactive form of bacteriocin is produced by the bacteriocin operon with leader peptide at the N-terminus. The bacteriocin undergoes post-translational modification and splicing to form a mature bacteriocin. Alternatively, splicing might occur during the transportation process. The inducing protein is synthesized, transported out of the cell along with the bacteriocin, and binds His protein kinase receptor to activate the response protein and regulate the bacteriocin biosynthesis. The bacteriocin synthesized binds to immunity protein which protects self-killing by the bacteriocin.

cesses of pre-bacteriocin synthesis, precise modification, late splicing of the leader peptide, and pro-peptide translocation through the cell membrane (Fig. 3) [3]. In case of BGPB, the genetic materials often associated with the transferable elements are carried by plasmids or sometimes even chromosomes. Various genes are involved in the formation of class I BGPB (e.g. nisin), which are organized into groups with genes responsible for structure, regulation, modification, transportation as well as self-immunity [1]. Class I BGPB or lantibiotics encoded by structural gene *lanA*, functional genes *lanB*, *lanC*, *lanM*, *lanD,* and *lanJ*, gene of ABC-type peptide translocators *lanT*, proteolytic processing gene *lanP*, immunity genes *lanI, FEG*, and regulation genes *lanR, lanK, lanO, lanX*, lie together within close vicinity [13]. The structural gene encodes an inactive bacteriocin with a double Gly- or peptide signal-type leader sequence at the N-terminus. Two preserved Gly are found at the C-terminal in case of leader peptide of double Gly type. The ABC transporters recognize this, and the leader sequence is processed, followed by the secretion of active bacteriocin into the extracellular medium [3]. In case of class II BGPB, maturation commonly occurs along with the transport, and there is no involvement of any specialized post-translational genes [1].

In lantibiotics, the modifications of amino acids occur in the cell in a very short interval, where the leader peptide is cut off probably at the late stage of processing, however, in class II bacteriocins the leader peptide is cut off during the transportation via cytoplasmic membrane. Though the leader peptide is cleaved off later in order to form biologically active form, it has various important roles. The leader peptide can protect the producer strain from the effect of its own bacteriocin because the bacteriocin is inactive until it is secreted [1]. This leader peptide also maintains the peptide in the biologically inactive form during maturation, serves as a recognition sequence for modification enzymes and/or transport, supports pro-peptide domain in order to maintain suitable conformation required for enzyme-substrate interaction [13]. Some class I BGPB's pro-peptides undergo modifications post-transcriptionally before being transported to the extracellular space via the ABC transporter. Serine protease acts on the pro-peptide as it enters the extracellular space, slicing away the leader peptide and hence releasing mature lantibiotic. In certain cases, a single proteolytic enzyme from the ABC transporters family transports the pro-peptide while also cleaving the leader peptide [1].

Class II bacteriocins generally require minimum of 4 genes: genes for bacteriocin pre-peptide, immunity protein, ABC-type transport protein, and membrane-bound accessory protein required for export [13]. The immunity gene encodes small proteins containing from 51 to 154 amino acids that guard the producer strain from the bacteriocin effect and, thus, provides immunity to the producer [13]. A sec-dependent N-terminal leader peptide is present in some class II BGPBs and needed for the transportation through the general secretory sec-pathway. In class II BGPB, subclasses

IIa and IIb can utilize specific ABC transporter maturation proteins and secretion proteins (AMS) to transport and cut off the leader peptide simultaneously. Since pre-peptide structure contains sec-signal peptide, the transportation might carry out via through the sec-dependent pathway too [1]. In case of circular bacteriocins, biosynthesis occurs in 3 steps, which includes cutting off the leader sequence, circularization, and exportation to the extracellular space. However, the specific mechanism involved in case of these bacteriocins is still not clear. The leader peptides of circular bacteriocins contain 2–48 amino acid residues and the removal of the leader sequence is assumed to be the initial step of biosynthesis [15]. The majority of studies have concluded that for processes like cleavage of leader peptide, the circularization or even the secretion, the leader peptides do not serve as a signal for recognition. The leader peptide might be needed only as the critical checkpoint of the biosynthesis like in garvicin ML [16].

Biosynthesis of BGNB. Depending on the producing organisms, the biosynthetic pathway of the bacteriocins might vary (Fig. 4) [1]. *E. coli* produces antimicrobial proteins called colicins, which destroy other *E. coli* strains during secretion. Colicins act on inner membrane leading to pores formation, hence inhibiting the synthesis of cell wall as well as causing nucleic acids degradation [17]. The colicin operon can be induced by different stress factors like poor nutrition, less oxygen content, DNA damage, and stationary growth phase. The synthesis of colicins occurs without post-translational modification. All colicins have 3 domains with various functions like an N-terminal domain responsible for translocation, a central receptor-binding domain, and a C-terminal domain having cytotoxic effect. These domains together aid colicins in their mechanism of action [1]. The central receptor-binding domain aids colicins in identifying target cells by facilitating the initial step of binding to a receptor protein present on the surface of the cell. Then, through the translocation domain present in N-terminal, colicins cross the outer membrane, and finally the cytotoxic domain promotes either formation of pores or enzymatic degradation, causing target cell disruption. Colicins utilize nutrient transporters present in the outer membrane, inner membrane and periplasmic proteins for their transportation [17].

Like colicins, the production of microcins is influenced by the stress factors. Conditions like poor nutrition, and stationary phase of bacterial growth generally leads to overproduction of microcins [1]. However, different from colicin, the microcins synthesized by Enterobacteriaceae have antibacterial and antitumorigenic properties, rather than affecting the producer strain [8]. The genetic materials involved in their production are arranged either on plasmid or in chromosome. In subclass I microcins, the genes are organized into groups including structural genes, genes for export, genes for self-immunity and genes for post-translational modification. Microcins, like most BGPB, are synthesized using pre-peptides, where the leader peptide has to be cut off to produce active bacteriocin. Although the leader peptide must be removed, it is necessary for intracellular microcin stabilization, chaperone folding process, and molecule recognition by the export method. In the case of subclass I microcins, the leader peptide helps in identification of enzymes involved in modification of peptide post-translationally. During transfer of microcin, the leader peptide is cleaved, which is most likely mediated by the export system. The export of microcins is usually carried out by the proteins of ABC transporters family [1].

The gene clusters for pyocin development in *P. aeruginosa* are only found on the chromosome, and these are of 3 different types. The chromosome-encoded pyocins include S-type pyocins are colicin-like proteins, whereas R-type and F-type pyocins resemble phage tail-like proteins [18]. The F-type has an elastic and non-contractile structure; however, R-type possesses rigid and contractile structure. Since pyocins contain killing peptide and immunity peptide bound tightly together, they can be induced by mutation of DNA and can be inactivated or killed by single hit on the target cells [1].

General mode of action. The common mode of action of most of the bacteriocins involves the release of intracellular components, and dissipation of protein motive force (PMF), which ultimately leads to rapid loss of cell viability and cell death [19]. Bacteriocins inhibit the bacterial growth by facilitating formation of pores on surface of the cell or interfering with the cell wall synthesis process. The pore formation is known as one of the most common mechanisms of the bacteriocin responsible for bactericidal action [20].

Antimicrobial mechanism of BGPB. General mode of action of BGPB entails the formation of pore, and modification of enzyme activity, or quorum sensing [21]. BGPB usually disrupt membrane integrity of the bacteria, hence killing the cell (Fig. 5) [1]. Lantibiotics affect and inhibit the synthesis of cell wall, when they interact with lipid II enzyme as the receptor. The peptidoglycan subunits are transported from the cytoplasm to the cell wall by this enzyme [22]. Lantibiotics like nisinA, and lacticin 3147 bind to lipid II component, and lead to formation of pore, causing leakage of intracellular components. Generally the bacteriocins of class II, involve mannose-phosphotransferase system (Man-PTS), which acts as a receptor [23]. Bacteriocins are highly cationic, like class I bacteriocin lacticin which binds to phospholipid bilayer, a negatively charged membrane. The interaction takes place between bacteriocin's hydrophobic end and the membrane generating unspecific ionic channels leading to the pore formation. This in turn leads to leakage of ions, ATP, and small proteins [21]. Type A lantibiotic nisin is the most commonly studied GPB, which acts

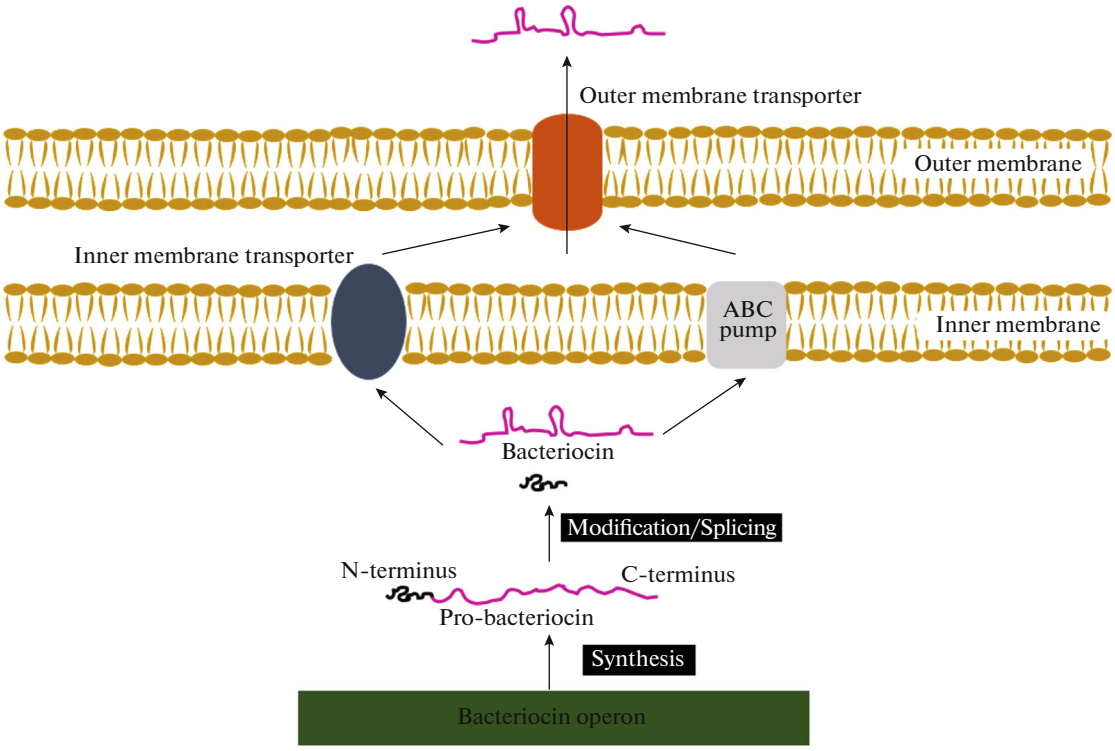

Fig. 4. Biosynthesis of bacteriocins by GNB. The inactive form of bacteriocin is produced by the bacteriocin operon with leader peptide at the N-terminus. The bacteriocin undergoes modification/splicing to form a mature bacteriocin which is either secreted to the periplasm by a porin transporter or via ABC pump. With the help of outer membrane transporter, the mature bacteriocin is secreted out of the cell.

by blocking lipid II activity and inserts into the bacterial membrane leading to pore formation, hence disrupting the membrane potential leading to bacterial death [22]. The bacteriocin was discovered to function on the cytoplasmic membrane permeability, causing electrolyte efflux and the dissipation of the PMF killing the sensitive bacteria. Subclass Ia of BGPBs like epidermin and gallidermin also have similar mechanism of action as nisin. In lacticin 3147, there are two-peptide lantibiotics: LtnA1 and LtnA2. Initially, the LtnA1 interacting with lipid II, followed by the complex lipid II-LtnA1 formed recruiting LtnA2. The LtnA2 then entered the membrane, and led to pore formation [1]. The effect of CHQS, a bacteriocin from *Enterococcus faecalis* TG2, as well as its mode of action against *Listeria ivanovii*, was investigated in a study. It was found that the bacteriocin acts on the permeability of cytoplasmic membrane causing electrolytes efflux like of potassium along with PMF depletion, hence killing the sensitive bacteria. Depending on the dose of the bacteriocin, there is release of macromolecules including ATP, proteins, and also nucleic acids [19]. Lantibiotics belonging to type B inhibit the enzyme modulation in the target bacteria, like mersadicin which affects the cell wall synthesis of the bacteria. Class II bacteriocins cause leakage of different ions when there is pore formation on the surface of target cell's membrane, causing depletion of PMF [21]. Bacteriocins of class II, such as pediocin PA-1, lactococcin A, and sakacin P attack the Man-PTS containing enzyme EII which is carbohydrate-specific protein complex. This enzyme is composed of 3 different proteins including AB, C, and D that act as targets for the bacteriocins. When the bacteriocin interacts with this system, it leads to receptor opening permanently, hence leading to continuous and uncontrolled efflux of the intracellular molecules [1]. Class III bacteriocins contain catalytic domain and target recognition site at N- and C-terminals, respectively. These bacteriocins catalyze the hydrolysis of cell wall, hence causing the lysis of the sensitive cells [21]. A study was done to purify, characterize, and determine antimicrobial as well as anti-biofilm potential of the bacteriocin from *Bacillus subtilis* GAS101. The bacteriocin isolated was able effectively to inhibit the growth of *S. epidermidis*, as well as *E. coli*, both of them being indicator organisms. It demonstrated a wide range of antimicrobial and anti-biofilm effects even in wide temperature range from 30 to 121°C [1]. Plantaricins, bacteriocins produced by *L. plantarum* were studied for their characteristics, as well as their effect against *B. cereus*. The bacteriocin was found to affect the permeability of the cell membrane, causing pore formation and potassium leakage. This degrades the membrane integrity, cell morphology, and also genes expression responsible for

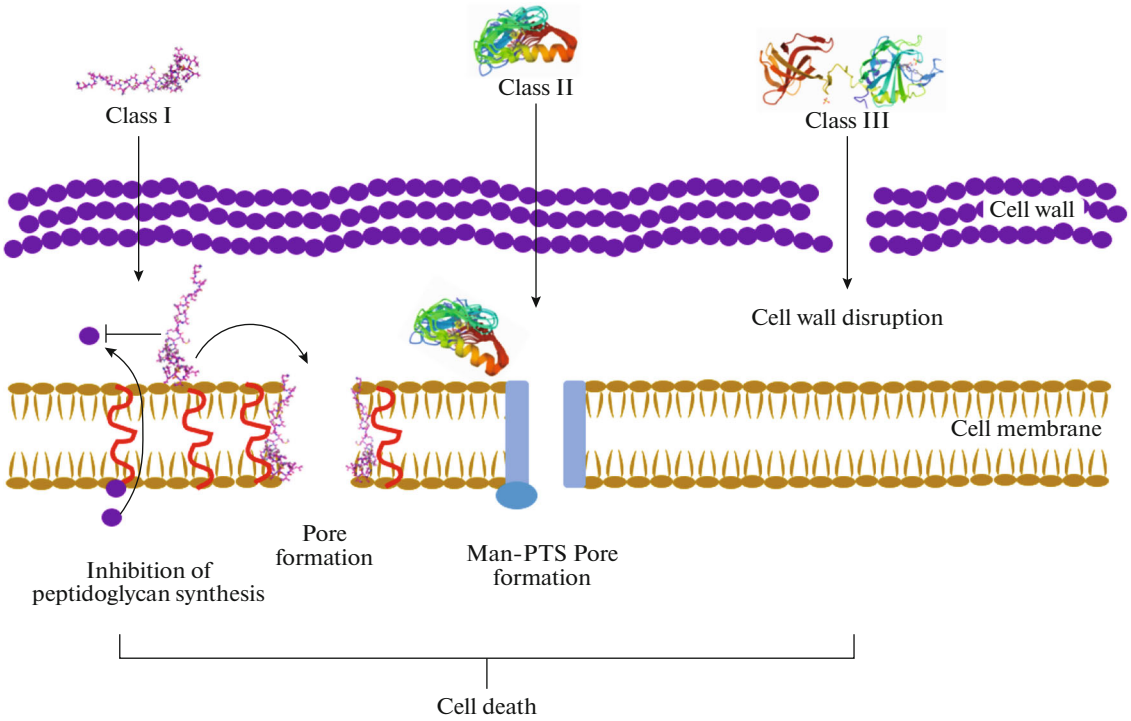

Fig. 5. Mode of action of BGPB. Class I bacteriocins inhibit peptidoglycan synthesis as well as promote pore formation. Class II bacteriocins mediate pore formation through Man-PTS system. Class III bacteriocins disrupt the cell wall of the bacteria.

production of cytotoxin, peptidoglycan, and also the cell division, hereby inhibiting *B. cereus* growth [24].

Antimicrobial mechanism of BNPB. BNPB like microcins act on the essential bacterial enzymes including RNA polymerase, DNA gyrase, or Asp-tRNA synthetase (Fig. 6) [23]. Microcins demonstrate antimicrobial activity by 2 different processes; microcins H, M and E492 act on intracellular membrane of the target cell leading to pores formation, whereas microcin B17, J25 and C directly act on the enzymes present within the cells. In order to have antimicrobial effect, the microcins have to enter the target cell of the sensitive strain and act by utilizing particular receptors located on the external membrane. They also use receptors involved in iron absorption as well as porins of external membrane [1]. Colicins, which are formed by *E. coli*, destroy other non-host *E. coli* by acting on the intracellular membrane leading to pores formation, affecting the synthesis of the cell wall as well as destroying internal cell components such as DNA and RNA. Some colicins have to pass through the external membrane of the target cells while others must pass across the internal membrane. This depends on the colicins' mode of cytotoxicity [17]. *P. aeruginosa* pyocins S1, S2, and S3 act via DNase activity leading to target cell death. Pyocin S4 has tRNase activity, whereas pyocin S5 is a pore-forming toxin [12]. Bacteriocins of phage-tail like type inhibit the competing bacteria by binding to a target cell via receptor-binding proteins. Once they bind to the target, they cause rapid death. Though the bactericidal spectrum is very narrow, they have demonstrated the bactericidal activity against various species [25]. In case of phage tail-like bacteriocin e.g. R-type pyocins of *P. aeruginosa*, the pyocin interacts with cell surface lipopolysaccharide by tail fiber RBPs, which affect the membrane permeability, hence affecting the potential gradient and respiration, eventually killing the cell. When R pyocin from *P. aeruginosa* acts on *Neisseria gonorrhoeae*, it affects the oxygen uptake as well as macromolecular synthesis of the target cells [12]. The bactericidal effect of F-type bacteriocins has not been studied well, however, it may involve disruption of membrane gradients as well [25].

Self-immunity mechanism. The bacteriocins are highly toxic in nature; therefore, the bacteria producing these peptides develop specialized mechanism to protect themselves. This mechanism is generally mediated by immunity proteins [26]. These proteins have a high level of specificity to the bacteriocins and are resistant to them [21]. In case of lipid II-targeting BGPB, ABC transport and specific self-immunity proteins are important factors in immunity. ABC system removes the BGPB which are bound to the membrane, hence protecting the producer strain [1]. The immune mechanism in case of class I lantibiotics like nisin, involves the isolation of the bacteriocins via Nisl protein on the bacterial cell membrane as well as removal of the bacteriocins from the cells by NisFEG, an ABC transporter. In case of class IIa bacteriocin

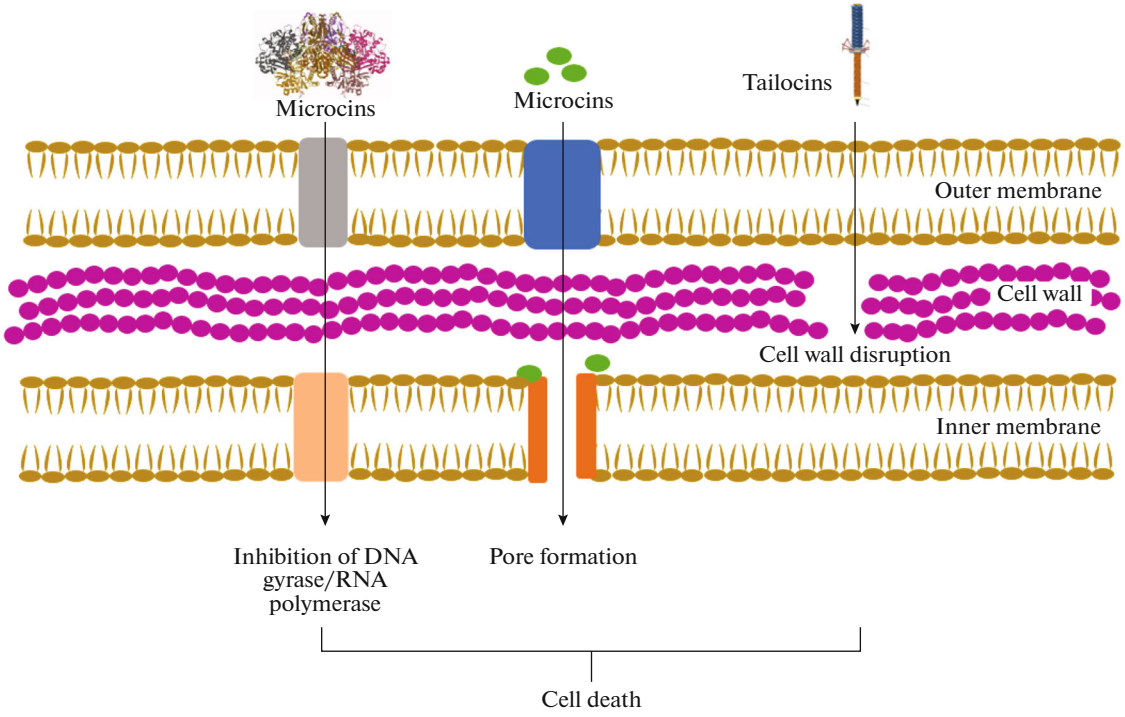

Fig. 6. Mode of action of BGNB. Few microcins enter the cell and inhibit the cellular processes such as inhibition of DNA gyrase or RNA polymerase. While others mediate pore formation on the inner membrane of the bacteria. Tailocins mediate cell death by disrupting the cell wall.

like pediocin-like bacteriocins and class IIc like lactococcins A and B, the immunity proteins act directly on the receptor of the bacteriocin [26]. The self-immunity mechanism in mersacidin involves binding of immunity protein to the bacteriocin present on the membrane of bacteria, which is then removed via ABC transporter from the cells. However, in lactococcin A, a Man-PTS targeting BGPB, the specific proteins bind to the bacteriocin, which in turn prevents it to affect the function of Man-PTS [1].

The self-immunity mechanism in case of subclass IIb of BGPB is not well understood [26]. In microcin C, particularly the proteins functioning as transport proteins as well as specific serine peptidase play vital role in immune mechanism. The serine peptidase deactivates the bacteriocin and also provides resistance to the bacteriocin in non-producer bacteria. In class II microcins, the immunity proteins bind with the microcin, hence avoid its interaction with the membrane [1]. Immunity protein, encoded by the same plasmid as colicin, is produced by all *E. coli* strains that manufacture colicin. Immunity protein defends the producers from produced toxins, but not from the effects of other colicins. These immunity proteins act by binding colicin's catalytic site or blocking the access to the target site [27]. Enzymatic colicins' immunity proteins bind to the catalytic domain or C-terminal, and then either block the active site or the substrate binding site of the enzyme. In case of colicins that act by pore formation, self-immunity proteins are located in the internal part of bacterial membrane. These proteins act together with the cognate colicin, hence preventing the formation of a channel in the producer strain's internal membrane. R- and F-type pyocins, which are phage tail-like bacteriocins, lack a particular receptor found in sensitive cells, ensuring self-immunity in these pyocins [1]. The binding proteins, export proteins and degrading enzymes are encoded by immunity genes. Since these genes are usually found in the same operons, they are easier to produce and transfer from the cell, which prevents toxic AMP levels from increasing. Immune proteins are encoded by genes present together with the genes encoding maturation, transport, and activation proteins [28].

Bacteriocins and antibiotics. In the current scenario, the increasing rate of development of antibiotic resistance is affecting the role of antibiotics [29]. However, bacteriocins are showing a promising future in the development of antibiotics against various pathogens. Bacteriocins are stable and show significant effect against other bacteria including antibiotic-resistant strains [30]. Because of their high specificity and multi-antibiotic resistant properties, clinically bacteriocins are used as viable antibiotics against pathogenic bacteria [21] Having properties like potency, low toxicity, both broad and narrow activity spectra, *in situ* production by probiotics, ability to

undergo bioengineering process, the bacteriocins are possible antibiotic alternatives [30]. Bacteriocins formed by LAB might be able to contribute better in the production of antibiotics than others. Since LAB are the bacteriocins commonly utilized in some of the steps of food processing as well as preservation and fermentation, their antimicrobial properties and safety have already been widely recognized [31]. LAB produce nisin and other bacteriocins that are beneficial to human beings and health, and also in food production.

When a study was done using carcinoma cell lines, particularly of head and squamous cell, nisin induced apoptosis in the preferential manner as well as stoppage of cell cycle, without causing any toxicity when consumed by human [6]. The bacteriocins have low oral toxicity; therefore, many of them are used in food industry. Many bacteriocins have broad antimicrobial activity, such as thuricin CD, a sactibiotic developed by *Bacillus thuringiensis* that has antimicrobial activity comparable to metronidazole and vancomycin. Furthermore, antibiotics can severely affect the commensal microbiota composition, while thuricin CD has no such effect [30]. Nukacin ISK-1 is a novel bacteriocin which is developed by *Staphylococcus warneri*. This bacteriocin has been found to be effective in the treatment of biofilms formed by methicillin-resistant *S. aureus* (MRSA). Vancomycin-resistant enterococci (VRE) as well as MRSA were found to be inhibited by the lantibiotic NAI-107 made by *Microbispora sp*. strain ATCC-PTA-5024. *Bacillus clausii* strain GM17 produced Bac-GM17 has a bactericidal effect against various GNB well as GPB. It can also inhibit the growth of fungus, *Candida tropicalis* [29]. Lantibiotics like nisin, actagardine, epidermin, gallidermin, mutacin B-Ny266, Pep5, planosporicin, and lacticin 3147, as well as their genetically modified derivatives, showed significant *in vitro* activity on pathogens like *Staphylococcus pneumoniae*, staphylococci, MRSA, VRE, and *Clostridium difficile* [30]. Nisin A along with its naturally occurring form nisin Z can act against the microbial agents that lead to food poisoning and spoilage [32]. Since they can work on both narrow spectrum or related species and wide spectrum or unrelated species of the generating strain, like in case of *Campylobacter jejuni* and *E. coli*, LAB bacteriocins have a wide range of spectra. The eukaryotic cells produce antimicrobial peptides having lower activities by 10^2 to 10^3-folds, however, LAB can inhibit or even kill the bacteria which are active at concentration of nanomolar region [33]. Lantibiotics were found to prevent the growth of enterococci and/or staphylococci both inside and outside of the bladder tube or catheter. When given intravenously, nisin was found to be better in activity as compared to antibiotic vancomycin against *S. pneumoniae*. When nisin F was administered in the respiratory tract of the rats, it inhibited the growth of *S. aureus* [30]. The efficacy of only durancin 61A as well as its combination with other bacteriocins like reuterin, nisin, pediocin PA-1, microcin J25 as well as antibiotics like vancomycin or tetracyclin against resistant clinical pathogens was studied. Durancin 61A, a glycosylated bacteriocin formed by *Enterococcus durans* 61A, was found to be effective when tested on clinically drug resistant species - *Enterococcus faecium* VRE, and MRSA, and *C. difficile*. Inhibition of multi-resistant clinical pathogens was also demonstrated when it was combined with other bacteriocins and antibiotics [34]. When studied in animal model, colicin E1 was able to prevent diarrhea in pig, but dose was insufficient for elimination of pathogen *E. coli*. Bacteriocin pyocin-S2 provided protection against from lethal *P. aeruginosa* infection in *Galleria mellonella* larvae as well as murine lung infections when given 1 h after the infection [35]. When experimentation was done in mice, *Lactobacillus casei* str. LAFTI L26, which produces bacteriocin, was able significantly to inhibit both enterohaemorrhagic *E. coli* as well as *L. monocytogenes*.

Bacteriocins are more amendable to bioengineering as compared to classical antibiotics [30]. Bacteriocins have the capability to not only serve as antibiotic substitutes, but also to act as antibiotic synergists. In some cases, they may also act as antagonists. Lacticin 3147 when used with polymyxin, acts synergistically to inhibit *E. coli* and *Cronobacter* spp. Lacticin Q combined with nisin avoids inactivation of nisin at alkaline pH value. When nisin and chloramphenicol were used together, rather than inhibiting the growth of MRSA, they antagonized each other [29].

Use of bacteriocins against FBP. Foodborne diseases affect millions of people worldwide, where diarrheal diseases caused by FBP are the primary causes of illness and deaths in the developing countries, causing deaths of approximately 1.9 million people annually at the global level [36]. In any food industry, the crucial factors to be considered include raw materials' quality, their properties, and the safety and stability of products while maintaining the shelf-life [21]. However, different factors like raw materials, various processing as well as storage conditions, distribution, and consumption processes offer the favorable settings for food poisoning, pathogenic, or spoilage bacteria to proliferate [36]. Bacteriocins remain stable in the food products, since they possess stability against heat, pH as well as food-associated enzymes. They have minimal effects on the gut microbiota, and are effective against FBP and spoilage microorganisms. These factors make bacteriocins a potential option as bio-preservatives, safe for human consumption [37]. The food industries use food preservatives including different chemical preservatives to avoid growth of FBP, prevent food spoilage and preserve the food for longer duration [21]. Bacteriocins utilized as preservatives can reduce the need for other chemical preservatives, heat treatments, or any other types of physical treatments [36].

Bio-preservation. Food preservation has become an important issue, since FBP can easily affect the preserved as well as fresh food items at various temperature ranges [36]. Frequent consumption of the products with chemical preservatives can affect the gut microflora containing either healthy or pathogenic bacteria, causing conditions like breathing difficulties, obesity and in some case even cancer [21]. Bacteriocins are considered as a natural food preservative in order to avoid FBP in different types of food products because they show antimicrobial effect against both pathogenic bacteria and bacteria causing spoilage of foods [38]. Bacteriocins are safer choice for human consumption because of inhibitory effect against FBP, and also because of the proteinaceous nature. They are thought to be acted upon by protease and destructed in the gastrointestinal tract after consumption. Bacteriocins are quickly deactivated by digestive enzymes, hence the bacterial microflora in the intestinal tract remains intact [36]. Bacteriocins fulfill the requirements which are important for any bio-preservatives to be used commercially, therefore, they are being considered as a potent preservative in the food industry. Bacteriocins are non-toxic, safe for human consumption, economical for the industrial purpose, effective even at relatively low concentrations, stable while storing and maintain intestinal microflora. They are resistant against enzymes present in food, have wide range of antibacterial spectrum against food spoilage bacteria and heat stability. They are effective at wide range of pH as well as salt concentration, do not affect the product in which they are added, do not have medicinal use, and are accepted by recognized authorities. Also, it is necessary for them to be approved as food additives or GRAS substance by FDA [5]. Due to frequent use, some of the microorganisms have developed resistance against the commonly used preservatives, which is creating havoc in food industry. Therefore, naturally produced antimicrobial agents like bacteriocins can be used as preservatives [21]. Bacteriocins have a lower chance of causing resistance because their mode of action involves disrupting the stability of membrane, and their fragments do not interfere with target cells. Therefore, bacteriocins can be considered as one of the solutions for the rapid development of the microbial resistance against antibiotics [39].

Bacteriocins produced by LAB and used for bio-preservation. LAB are rod- and coccus-shaped bacteria that are Gram-positive, immobile, and do not form spores. Various species like *L. monocytogenes*, *E. faecalis*, *S. aureus*, and *S. typhimurium* are all inhibited by LAB isolates from food and animal sources [21]. LAB's bacteriocins are more potent as bio-preservatives than other bacteriocins because since many years they have been used for production of fermented foods, also U.S. Food and Drug Administration has provided most of them with GRAS status with safety approval [39]. The LAB bacteriocins are thermostable, have tolerance for wider range of pH, and possess proteolytic activity, which makes them favorable to be used as preservative in various food products. Having these properties, bacteriocins can resist heat as well as various pH levels of food under storage conditions [36]. The LAB-derived bacteriocins are highly stable, have no taste as well as odor, and are effective against FBP as well as spoilage-causing bacteria, therefore, are emerging as a potential option as food preservatives [40]. Till date nisin which has GRAS status approved by FDA is the most common bacteriocin used as a food preservative in a commercial level [38]. LAB-derived bacteriocins are usually used in fresh meat as well as bovine and poultry remain. Nisin was found to be more effective in decreasing the number of coliforms, *E. coli*, and aerobic bacteria, when combined with lactic acid (1.5%, 25°C) than when used alone. *L. monocytogenes* growth was reduced when bio-protective cultures of species including *Lactobacillus fermentum* ACA-DC179, *E. faecium* PCD71, and a combination of various bacteriocins of subclass IIa were used in meat products [39]. Among various bacteriocins produced by LAB, nisin is considered as safest food preservative and is used by around 45 countries commercially in the food industry [36]. Nisin prevents the growth of GPB, *Bacillus* spores as well as *Clostridium* spp. Nisin is used as a bio-preservative in brewery products, milk and milk products like mayonnaise, processed cheese, and other processed foods like soups, meats, tomatoes and canned vegetables in more than 50 nations. *P. acidilactici* produces bacteriocin pediocin PA-1/AcH which has potency to be used as bio-preservative commercially in meat fermentation and dried sausages [39]. In USA, nisin is added in canned products in order to prevent the growth of *Clostridium botulinum*. Nisin is a healthy preservative to use in milk and milk products, fruits and vegetables, and meats, because it prevents the growth of pathogenic bacteria and bacteria causing spoilage [21]. In Spain, when pediocin PA-1 (*P. acidilactici* MCH14) was incorporated in the dried and fermented sausages, it was able to inhibit both pathogens, *L. monocytogenes* as well as *Clostridium perfringens*. Similarly, when the pediocin PA-1/AcH producing *P. pentosaceus* BCC3772 strain was included in the Thai's traditional pork sausage's fermentation process it showed antilisterial property keeping the sensory properties of the food intact [39].

Bacteriocins against FBP. *L. monocytogenes*. The most common FBP, *L. monocytogenes*, are responsible for the transmission of foodborne diseases. In order to reduce occurrence of *L. monocytogenes,* using non-pathogenic LAB for the biopreservation is a mild and natural method. *Lactococcus lactis* produces nisin which is known to possess antilisterial activity in food. However, U.S. Food and Drug Administration has limited it's use to 15 ppm in meat [41]. Nisin has demonstrated inhibitory activity against *Listeria* in culture media as well as in various food items. In vitro

tests have confirmed the inhibitory effects of pediocin PA-1 (*P. acidilactici* UL5), on different strains of *L. monocytogenes* [42]. The activity of bacteriocins such as enterocin FH99, nisin, and pediocin 34, upon *L. monocytogenes* ATCC 53135 was tested individually and in combination. Comparatively the antilisterial effects of the bacteriocins were found to be better when used in combination rather than individually. In sequence, nisin, pediocin 34, and enterocin FH99 were found to exert effective inhibitory action against the pathogen with nisin being the most effective one among them [43]. A research involved *Pediococcus pentosaceus* DT016, a bacteriocinogenic LAB previously isolated from lettuce was used as a pediocin producer, and its bio-preservative effect against *L. monocytogenes* was observed. Even under different conditions including normal microbiota, both physical and chemical surrounding, *P. pentosaceus* DT016 generated bacteriocins in every vegetables studied. In comparison, pathogen counts in all vegetables were lower when treated with *P. pentosaceus* DT016 than when water-chlorine solution was added in vegetables. In addition, the number of *L. monocytogenes* increased during the storage time (4°C) in the samples containing water-chlorine solution, while they decreased for 7 days in the samples treated with pediocin, then marginally increased later. However, washing with pediocin caused significant decrease in pathogen load than the other washings [44]. Moreover, a study revealed that, gassericin A, produced by *Lactobacillus gasseri* LA39 when tested against *S. aureus* as well as *L. monocytogenes*, demonstrated maximum antimicrobial effect [45].

***Clostridium* spps.** Nisin was utilized to prevent *Clostridium spp.* producing gas from causing late blowing in cheese [7]. In order to avoid *Clostridium* spores growth in cheese as well as pasteurized cheese spreads, nisin may be a viable alternative to nitrate [46]. A single-peptide lantibiotic, lacticin 481, is found be active against *Clostridium tyrobutyricum*, and *L. monocytogenes* [7]. The antibacterial effect of *E. faecium* LCW 44 was found to be broad against gram-positive bacteria of the genera *Listeria, Staphylococcus, Lactobacillus*, and *Clostridium*, but not against GNB [47]. Effect of nisin, vancomycin, and metronidazole on *C. difficile* was evaluated using minimal inhibitory concentrations (MIC) technique. Nisin was found to be more active with MIC90 of 0.256 mg/dL as compared to other agents. It also had strong bactericidal activity as compared to the rest; therefore, nisin can be a potent agent in managing diarrhea, with *C. difficile* as a causative agent [46]. The minimum concentrations of nisin required to inhibit the growth of vegetative cells of *C. tyrobutyricum*, *C. perfringens*, *C. butyricum*, *C. sporogenes*, were 4.8, 0.75, 0.17, and 38.4 g/mL, respectively. Since the spores of *C. tyrobutyricum* are thermostable and can undergo germination in ripening cheese, they cause degrades hard cheese by late blowing process. *L. lactis* subsp. *lactis* DPC3147 produces lacticin 3147, which was able fully to inhibit 4–5 log spores/mL along the course of 24 h at a concentration of 45 g/mL. Enterocin AS-48, a cyclic bacteriocin formed by *E. faecalis* A-48-32 is effective against different species of *Bacillus* and *Clostridium*. *P. pentosaceus* L and S produce 2 different bacteriocins – pentocin L and pentocin S, respectively, which possess inhibitory effect against vegetative strains of both *Clostridium* as well as *Bacillus* [48]. A research was conducted to show that a bacteriocin-producing *Streptococcus hyointestinalis* B19 strain in combination with lytic bacteriophages (A3 and P4) had antimicrobial activity against *C. perfringens*. The bacteriocin generated demonstrated potential inhibition against 4 *C. perfringens* strains and all of the *L. monocytogenes* strains studied. When isolated, bacteriocin and phages were used in combination, synergistic effect was observed as compared to when used individually. *C. perfringens* population significantly lowered down during the probiotic test with *S. hyointestinalis* B19 strain [49]. The FDA approved the usage of nisin in USA as an additive with anti-botulism property in canned foods in order to prevent *C. botulinum* shortly after FAO/WHO accepted it as a safer alternative as food bio-preservative. Bacteriocins of various classes, including class I—nisin, class IIa—pediocins, class IIb—plantaricins, some belonging to class IIc—different enterocins, and class IV—duracin demonstrated inhibitory effect upon *Clostridiodes* and *Clostridium* spp. Last bacteria were found to be inhibited when plantaricins-producing strains of LAB were applied, also the normal functional properties as well as gut homeostasis were maintained [46]. Micrococcin P1, a bacteriocin developed by the food-grade strain of *Staphylococcus equorum*, was tested in soft cheese for anti-listerial ability. *L. monocytogenes* growth was significantly reduced, however, the effect observed depended on the contamination level, and also after 10–16 days of maturation regrowth of viable *Listeria* was seen [7].

***Staphylococcus* spps.** Staphylococci mostly live as natural flora on human skin and mucous membranes, however, some of them are also opportunistic organisms. *S. aureus* from Micrococcaceae family is the most common and serious bacterial pathogen among them. This pathogen produces various toxins including staphylococcal enterotoxins responsible for many diseases along with food-borne diseases. With the development, various *S. aureus* have acquired multidrug-resistant property which is potential risk to humankind since they can easily enter the human's food chain. Therefore, it is necessary to find the effective method of biopreservation as well as antimicrobial drugs against *S. aureus* as well as the resistant strains [50]. Nisin demonstrated strong effect against the FBP—*S. aureus, and L. monocytogenes* present in dairy products [7]. Nisin has wide spectrum of antimicrobial effect against various organisms like *Clostridium* spp., *Bacillus cereus, S. aureus*, *L. monocytogenes*, also the LAB species. Nisin acts by causing hindrance in

the cell wall synthesis and formation of pore in the membrane of the bacterial cell [51]. The antibacterial activity of crude along with the fractionated extracts of 36 LAB isolates was tested, and cell-free supernatant (CFS) showed a more efficient inhibitory effect against *S. aureus* than cell wall and intracellular extracts. This might have been because of the presence of antimicrobial products like bacteriocin, lactic acid hydrogen peroxide and also externally secreted bacteriocin-like substances. The *L. plantarum* USM8613 CFS as well as extract of cell wall demonstrated dominant inhibition upon *S. aureus*, where the antimicrobial compounds of CFS led to formation of membrane pores, causing leakage of cell substances, hence causing massive inflow of compounds present in the extracellular matrix inside the cell resulting in swelling of cells [52].

***Escherichia* spps.** *Escherichia* is an opportunistic pathogen; however, some serotypes and clones of this genus are known for role in intestinal and extra-intestinal diseases. Among its species, *E. coli* is the common one responsible for various infections including mild gastroenteritis to most severe cases of hemolytic uremic syndrome [53]. Pathogens and spoilage bacteria like *E. coli*, *Salmonella* spp, and *L. monocytogenes* are usually found in fruits and vegetables. Maintaining proper sanitation by washing them can decrease the load but does not eliminate it completely [54]. Different strains of LAB produce fatty acids like palmitic, stearic, lauric, capric, and cis-vaccenic acids which exert antibacterial effect on *E. coli*, and *S. aureus* by disrupting as well as disintegrating pathogen's cell membrane [52]. A research was performed for the evaluation of biopreservation potential possessed by LAB isolates from Ready-To-Eat seafood over *Listeria* spp. and *E. coli*. Several strains of *Carnobacterium* and *Leuconostoc* showed broad antimicrobial effect by total growth inhibition of *Listeria* spp. and medium effect against *E. coli* (medium antimicrobial activity from 3 to 6 log CFU reduction). There are only few studies describing activities of LAB against GNB, thus, these strains including *E. coli* can be promising alternatives for [53]. Among various bacteriocins produced by LAB, nisin is well known for its biopreservative property. However, many studies have shown that combining nisin with other preservatives is the most efficient way to preserve as well as extend the shelf life of foods. When melons were washed with a solution of nisin of 25 g/mL concentration, 1% of hydrogen peroxide, 1% of sodium lactate, and 0.5% of citric acid, the population of *E. coli* O157:H7 which was 5.27 log CFU/cm^2 reduced to less than 1 log CFU/cm^2. Nisin-producing strains were used to test the effect on pathogens, *L. monocytogenes* and *E. coli,* in 2 types of cheese, Feta and Camembert. In *L. monocytogenes*, the effect of nisin was better, however, *E. coli* O157:H7 was able to survive through the manufacturing process and was found in the cheese having nisin-producing strain after 75 days of storage with a much higher population than the initial inoculum [7].

Bacteriocins in active packaging. Active packaging refers to packaging method where not only the quality and freshness of the product is maintained, but there is interaction between the package, product as well as the environment. This interaction helps in either maintenance of sensory properties, or extension of the food's shelf life, or preservation and enhancement of the product safety. As consumers have become more concerned about the consistency, shelf life as well as safety of food products, this method is becoming more important. Among different forms of active packaging, antimicrobial packaging is a promising and interesting form [55]. Microbial food spoilage and food poisoning, leading to food-borne infections are the major concerns in food industries. Therefore, it is necessary to screen carefully every step starting from production to distribution of the food products. Among these steps, food packaging also plays a vital role in processing, preservation as well as quality maintenance. Since last decade, the addition of bacteriocins into the packaging films as a method of biopreservation has been an active research, since it not only prevents the microbial growth on direct contact but also maintains quality, storage life and the food safety [56]. In most of the cases, incorporating bacteriocins directly in the food products leads to adsorption of bacteriocins in the food matrices which are easily degradable, hence leading to loss of antibacterial activity. Hence, using them in packaging films or coating can help to improve activity making them stable in the food systems [7].

In last few years, antimicrobial active packaging has gathered a lot of interest leading to its further development to make it more effective, efficient, and long-lasting as well as eco-friendly by improving the activity of added antimicrobial substances [57]. Prior to the introduction of antimicrobial packages, most industries attempted to extend the storage life of food items by dipping the food entirely in antimicrobial solution or spraying foods with antimicrobials, which could be neutralized on contact, diluted to levels lower than the standard concentration, or even disperse rapidly through the food matrix, rendering this process ineffective. Antimicrobial packaging, which includes bacteriocins as a packaging material, greatly reduces the pathogen growth and development, helps to preserve food quality and safety, and extends storage life, which are taken care of even during the transport, storage, and distribution processes [55]. The packaging coatings and films contain a thin layer of biopolymers that communicate with the food's ambient atmosphere, creating a blockade between food and environment that improves the food's consistency, safety, and organoleptic properties [7]. Colicins, alveicins, cloacins, tailocins are produced by GNB however bacteriocins produced by GPB are preferred more for the incorporation in food items. Since the preparations from the latter do not contain lipopolysaccharides or

endotoxins which may lead to health hazards on consumption, their strains can be directly added without extensive purification [57]. Nisin (*L. lactis*) is studied extensively for the biopreservation following 2 different methods – applying directly in food or using in antimicrobial packages. Nisin demonstrates poor effect in alkaline as well as neutral pH, high temperatures and is less effective against GNB. Therefore, these conditions have to be considered for while using nisin as food biopreservative. Bacteriocins produced by *Bacillus* spp also have found to be safe for their incorporation in food and agriculture industry [55]. Generally, fresh cheeses contain high amount of caseins, lipids, and water, making them highly perishable. This in turn, deteriorates the quality of cheese as well as supports the growth of pathogenic microorganisms, hence increasing the risk of foodborne infection. Thus, using bacteriocins-coated films can help to maintain product's safety as well as increasing storage period. Various studies demonstrated decrease in the levels of pathogens like *L. monocytogenes*, *Listeria innocua* and *S. aureus*, while incorporating nisin with other compounds like sodium caseinate, lacticin, galactomannan, natamycin [7]. Bacteriocin formed by *L. casei* in the probiotic drink Yakult was used in a study to create an antimicrobial packaging device. With the help of agar diffusion assay, the result was tested upon *S. aureus* and *E. coli,* where the bacteriocin adsorbed on the packages diffused in the medium, inhibiting both species [56]. The efficacy of active packaging films comprising partially purified antibacterial peptide (ppABP) in food preservation was investigated. *B. licheniformis* Me1 developed ppABP utilizing cellulose films, and low-density polyethylene (LDPE) by 2 processes - soaking and spreading coat. It was found that LDPE films released the peptide immediately upon the contact with water whereas in cellulose films, the coated peptide released gradually, and both activated films had antibacterial effect against the pathogens leading to reduced growth rate of the target microorganism [55].

Application of bacteriocins in food products. Bacteriocins incorporation in the food products requires pre-determination of their effectiveness in the food systems. Certain criteria like being approved as GRAS, having broad spectrum of inhibitory effect, high specific activity, heat and pH stability, beneficial effects like maintaining safety, quality as well as freshness of food, optimal solubility and stability in the food systems, need to be fulfilled before adding them in the food [39]. There are 3 popular approaches to utilize bacteriocins for food biopreservation: inoculating the food with bacteria which produce bacteriocin, adding purified or semi-purified bacteriocins for preservation process, and adding a product that has previously undergone fermentation process with a strain producing bacteriocin as a part of ingredients in the food processing [58]. Different technological strategies of bacteriocin application in food have also been described in following ways - directly adding bacteriocins in food or immersing in a peptide-containing solution, bacteriocin adhesion in active packaging like cellulose edible films, polyethylene-type plastic films, and incorporating LAB cultures and bacteriocin preparation in antimicrobial coatings [39].

Direct inoculation of bacteria. In this process, the inoculum is either built up or there is direct addition of bacteria in the food products. The cultures have to be metabolically active bacterial cells which are able to disseminate in the food substrate actively and start producing the bacteriocins [59]. Being the safety for humans LAB and their metabolites have become preferred option for food preservation either in form of growth extracts or the purified/semi-purified forms or fermentation [58]. Bacterial strains producing bacteriocins may be used as the key starting cultures in case of food fermentation, assisting fermentation process, or acting as an assisting culture in conjunction with bacteriocin-resistant starter strains. They can also be used as cultures in non-fermented foods because in order to enhance preservation, since they are safe to consume and help in maintenance of food quality [59]. Generally, nisin is used in dairy factories since it is effective in controlling different pathogens like *L. monocytogenes* and *S. aureus*. It is commonly used in cheese as well as pasteurized cheese spreads to replace nitrate which helps in inhibition of the growth of clostridia spores. Either the live bacteria or bacteriocins itself can be added to the dairy products which will enhance the safety, storage period and product quality. When the direct inoculation of LAB bacteria is done in dairy products like yogurt, cheese, there is continuous release of bacteriocins throughout the maturation and storage phase as well [7].

Adding purified bacteriocins. The antimicrobial agent found in the distilled preparation, and not the crude bacteriocin fermentate, is referred to as purified bacteriocins [58]. Adding completely purified or semi-purified and concentrated forms of bacteriocins in food products is more effective than using direct inoculum of the bacteriocinogenic cultures. However, bacteriocin effectiveness is determined by factors such as ability to get adsorbed in food materials, dissolve properly, it's dispersal over the food matrix, and enzyme interaction. As a result, if bacteriocin lacks any of these properties, its efficacy can be limited [7]. In 1960s, purified form of nisin from *L. lactis* subspp. *lactis* was the earliest bacteriocin to be recognized as a food bio-preservative by FAO/WHO. Purified form of plantarin 163 which is naturally secreted by *L. plantarum* 163, was able to remain constant even at high temperatures and acidic pH, it was sensitive to protease activity. It demonstrated broad field of antimicrobial action when tested with closely related bacteria. These properties make plantarin 163 a suitable option for food preservation [21]. When semi-purified form of lacticin 481 was added to fresh cheese followed by storage at refrigeration temperatures, caused reduction of

L. monocytogenes by 3 life cycles in the time interval of 3–7 days, while the non-purified form showed mild bacteriostatic properties. When used in skimmed milk and yoghurt, a distilled enterocin CCM 4231 demonstrated similar inhibitory effect against *L. monocytogenes* and *S. aureus* but the inhibitory effect against *S. aureus* present in yoghurt was less effective after 24 h [7]. BacTN635, a semipurified bacteriocin formed by *L. plantarum* TN635 isolated from meat, had an inhibitory effect on the proliferation of spoilage bacteria as well as the foodborne pathogen *L. monocytogenes* present in chicken breast and beef. This ultimately helps to extend the storage life of refrigerated meat and meat products. BacFL31 is produced by *E. faecium* FL 31 present in meat products, and it inhibits both *Salmonella typhmurium* and *L. monocytogenes* [39]. *L. lactis* forms lactococcin BZ, an antibacterial compound with wide spectrum activity against both GPB and GNB. Application of semi-purified lactococcin BZ in skimmed and full fat milk reduced the count of *L. monocytogenes* to the level which cannot be detected when stored at 4°C, and 20°C. Through the entire period of storage the inhibitory effect was constant [7]. Reutericyclin, produced by *Lactobacillus reuteri*, is the first low-molecular-weight extremely hydrophobic compound that acts and inhibits the cytoplasmic membrane enzymes of the target organisms like *S. aureus*, *L. innocua*, and *E. faecium*. Reutericyclin can be used in food products in a distilled form by fermenting with a strain producing reutericyclin, or it can also be added as inactive cells lacking metabolic activity where composition of food itself aids the bacteriocin's dissemination within the cells [21]. Though numerous studies and advances have been done in the application of bacteriocin in the food industry, usage of both distilled and semi-distilled bacteriocins remains limited. One of the reasons for this might be high cost of the bacteriocin isolation and purification [7]. Even though numerous studies have been done on the effect of various bacteriocins of LAB, many countries still allow using only the purified form of nisin [21].

Addition of fermented product containing producer of bacteriocins. LAB are the commonly used bacteria in the fermentation process, where lactic acid as well as bacteriocins are the products formed by bacteria outside the cell. The ability to produce bacteriocin is the important characteristic for the LAB incorporated in the food fermentation as the starter cultures since bacteriocins are responsible for producer cells' competitiveness [60]. When LAB is allowed to grown on the complex substrate, there is the formation of fermentate of crude bacteriocin which contains other components too [58]. Apart from direct inoculum, and purified/semi-purified form, bacteriocins can be added in the dairy products as a part of fermentation process. Several studies have been conducted that demonstrate the efficient use of bacteriocins and LAB producing bacteriocins in dairy products such as milk, yoghurt, and cheese leading to successful control of pathogens [7]. The common bacteriocin used in food industry includes nisin, however, application in the meat products might not be effective because of the low solubility, probability of enzymatic destruction, and also lack of effective inhibition of spoilage as well as foodborne pathogens [39]. In bakery item, sourdough is a vital ingredient used for acidification, leavening, flavor compounds production, and biopreservation of bread. The taste and nutrient contents of sourdough bakery products are determined by LAB, which are the principal bacteria incorporated in sourdoughs. An enhanced amount of sourdough increased the heat sensitivity of *B. subtilis* spores. Bacteriocin-producing strain *L. plantarum* VTT E-78076 as well as *P. pentosaceus* VTT E-90390 inhibited the growth of rope-forming strains including *B. subtilis* along with *B. licheniformis* in white bread. For more than 15 days, heated cultures of *Leuconostoc mesenteroides* A27, along with *Leuconostoc plantarum* E5 inhibited the *B. subtilis* G1 spores from growing on bread slices. Inhibitory effect was observed in the growth of *Bacillus* and other pathogens on fermentation carried out by LAB to pH 4.0 or less [61]. Bacteriocins can enhance the fermentation process, hence accelerating cheese ripening and also improving the flavor. Since the bacteriocinogenic LAB when added in yogurts and cheese produce bacteriocins regularly throughout the maturation and storage phase, they can be a potential agent to be added as main culture or the helper one in the fermentation. However, there may be compatibility issues between the strain producing bacteriocin and other cultures involved in dairy product fermentation process [7]. In case of meat and meat products, pediocin produced by *P. acidilactici* can be considered for the application since it demonstrates antimicrobial activity against *Listeria* spp. For biopreservation, pediocin PA-1/AcH formed by *P. acidilactici* can be incorporated commercially in dried sausages and fermented meat products. The antilisterial effect of pediocin PA-1/AcH produced by *P. pentosaceus* BCC3772 was demonstrated during the fermentation phase of pork sausages while preserving the product's sensory characteristics. Having such properties, this strain can be added as a starter culture in various fermented foods like sauerkraut, sausages, and cheese. Pediocin and nisin when used together reduced the population of *Lactobacillus sakei* in vacuum-packed sliced ham [39].

Resistance to bacteriocins in food borne pathogens. The bacteriocins exert antimicrobial effect upon interaction with bacterial membrane, thus, development of bacteriocins resistance involves the modification of membrane structure, fluidity, and charge which will ultimately affect the bacteriocin activity [2]. Bacteriocin resistance might occur via gene mutations or horizontal gene transfer leading to alteration in the cell wall and membrane, various receptors as well as vital systems. Hence, cross resistance might occur when bacteriocins are used in combination with antibiotics,

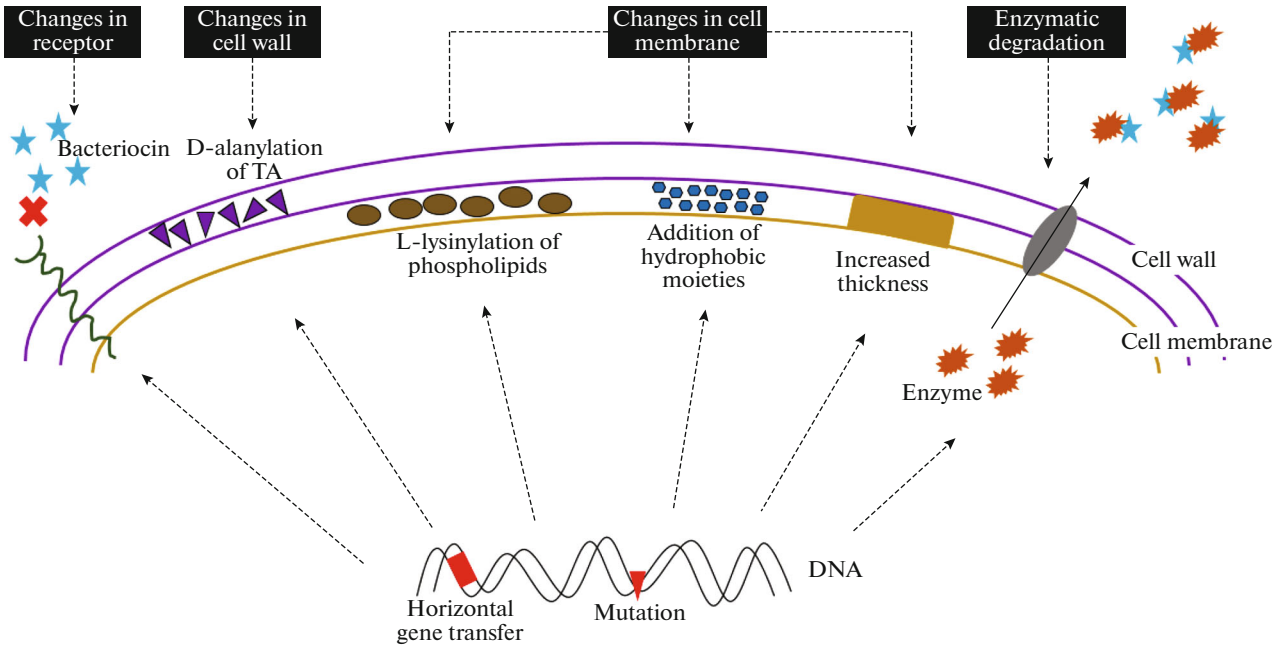

Fig. 7. Resistance of pathogens to bacteriocins. Bacteriocin resistance can be achieved by mutation or horizontal transfer of gene. This can lead to changes in the receptor recognition for bacteriocin. Modification of the cell wall, cell membrane can hinder the action of bacteriocins. Enzymes secreted by the pathogen can degrade the bacteriocins, thereby gaining resistance.

leading to production of multidrug resistance variants [62]. Different modifications might be responsible for the development of bacteriocin resistance like change in the membrane receptors, teichoic acids located in the cell wall undergoing process of D-alanylation present in the cell wall, phospholipids present in the membrane of cell undergoing L-lysinylation, and change in the fatty acids present in the cell membrane (Fig. 7) [63]. Involvement of two-component system VirR/VirS which is important in *L. monocytogenes* virulence was observed in case of nisin resistance in *L. monocytogenes*. This component involves the modification of the membrane with addition of hydrophobic component in the cell surface of bacteria, which affects bacteriocins. Increased thickness and rigidity of the bacterial membrane, have also found to be one of the cause for nisin resistance. In some of the nisin-resistant GPB, there was production of nisinase, a nisin-degrading enzyme [1]. Different processes may be involved in bacteriocin development, such as imitating the producer strains' natural defense immune mechanism, where strains that do not produce any bacteriocins may have homologous genes to the genes responsible for self-immunity found in strains that produce bacteria. Bacteriocin degradation via enzymes released by attacked bacterial species might also lead to bacteriocin resistance. Enzyme nisinase is released by pathogens like *Paenibacillus polymyxa* and *B. cereus* which leads to nisin degradation. Any kind of modification in cell wall's surface charge due to gene mutation, binding process of bacteriocin is affected hence providing resistance to *Listeria* spp against nisin and pediocin [62]. Down-regulation of gene expression of Man-PTS in *L. lactis*, and *L. monocytogenes* led to the growth of tolerance in subclass II bacteriocins, involving a change in sugar metabolism from mannose or glucose to galactose. Microcin resistance was caused by a number of factors, including degradation of microcin, changes in microcins' targets present inside the cell, and degradation of efflux pump activity. The resistance to microcin in *Bacillus anthracis* was initiated by the serine protease MccF, which cleaves an amide bond between Asp at C-terminal and the nucleotide portion of active microcin C, affecting inhibitory effect on tRNA synthetase. The development of resistance towards colicins in some of the *E. coli* strains was because of alteration in colicin receptors and/or the intracellular targets, whereas some strains involved over-production of siderophore leading to competition with colicins present at the corresponding outer membrane receptors [1].

A multi-hit combination of various types of bacteriocins as well as bioengineering techniques might help to overcome bacteriocin resistance since the process provides variants with multiple functions. Therefore, bioengineered variants of nisin are more effective against species like MRSA, VRE, and *C. difficile* [62]. Compared to antibiotics, bacteriocins are synthesized ribosomally; therefore, bioengineering at particular residues of amino acids can be carried out to make bacteriocin more effective against the pathogens [63].

Drawbacks of bacteriocins. Though bacteriocins have many advantages to be used for antimicrobial

effect, there are some disadvantages like susceptibility to the proteolytic enzymes as well as eventual toxicity in mammalian cells [1]. Since bacteriocins are active even in low concentrations, they are less stable within the living organism, which makes them prone to the activity of proteolytic enzymes. There are very limited clinical application of bacteriocins which can also be considered as a drawback [62]. They have low molecular weight, i.e. hardly over 10kDA, undergo posttranslational modification, and are simply degraded in the gastrointestinal tract of mammals by the protease [32].

Susceptibility to proteolytic enzymes. One of the major drawbacks of using bacteriocins as medicine is its reduced stability upon the interaction with the proteolytic enzymes in the intestine or human tissues [2]. After ingestion of foods containing bacteriocins, the ability of bacteriocins can be changed due to their adherence to food's components, degradation by enzymes present in the gastrointestinal tract, change in pH, and contact with the gut microbiota [62]. The oral administration of bacteriocins has to undergo through several barriers which eventually affect the stability as well as their activity. Proteolytic enzymes like pepsin, trypsin, and chymotrypsin present in the stomach and the small intestine quickly inactivate or even degrade the bacteriocins, mainly class II known to be comparatively more sensitive. Pediocin PA-1 was found to be stable in the stomach but eventually degraded on exposure in small intestine [31]. Another study was performed regarding the proteinaceous nature of substances involved in inhibition, by treating isolated CFS from LAB, and *Bifidobacterium* strains derived from the oregano honey containing variety of enzymes. Different proteolytic enzymes like lipase, trypsin, α-amylase, and pepsin were included in the study. It was concluded that the tested strains were susceptible, and the zone of inhibition was not present when the enzymatic treatment of CFS was done which indicated that substances of protein origin like bacteriocin-like substance were responsible for the zone of inhibition [54]. In case of class I lantibiotic, extensive modifications occurring post- translationally in propeptide regions like Thre, Ser and Cys residues lead to incorporation of multiple thioether rings in the structure of bacteriocin, which is believed to provide thermal stability, prevention from proteolytic degradation as well as antibiotic activity [5]. When the analogs of lactococcin G were integrated with amino acids having D-configuration, it demonstrated reduced sensitivity towards exopeptidases with intact activity. Nano-encapsulation, for example, not only protects bacteriocins from degradation by protease and unintended communications with rest of the food materials, but also improves their effectiveness against a variety of pathogens, including multi-resistant bacteria. When consumed orally, the bacteriocins are directly exposed to the enzymatic degradation and various pH in gastrointestinal tract. Bacteriocins have reduced half-life compared to the antibiotic equivalents since they are sensitive to proteases [2]. Proper delivery system like parenteral delivery can be developed to overcome proteolytic digestion in the gastrointestinal tract can overcome this inactivation problem [62].

Toxicity for mammalian cells. Bacteriocins are currently thought to be safe for human use, but enterococcal cytolysin has shown toxicity beyond the bacteriocins' MIC for preventing food spoilage and pathogenic bacteria development. The toxic effects depend on the bioavailability and absorption of bacteriocin after intake [31]. The alteration of intestinal microbiota by pure bacteriocins and probiotics is not always beneficial to the humankind. Bacteriocins might interact with the beneficial gut microbiota present and lead to their negative imbalance, hence causing numerous local and systemic diseases [62].

A study was conducted using the vero cell lineages to highlight cytotoxic effect related to purified bacteriocin, which indicated that there is more than 70% of viability at the MIC in comparison to the untreated cells. The concentrated antimicrobial extract obtained from *B. subtilis* demonstrated 6% cytotoxic effect when tested on caco-2 cells. Using different preparations, varying exposure time, and other factors, studies have found that bacteriocins have no cytotoxic effect on the mammalian cells [20]. The level of toxicity of different bacteriocins (nisin A, bacST4SA, plantaricin 423) with host endothelial as well as epithelial cells (caco2 and HUVEC) was investigated. The viability of cells was not significantly affected while treating with 25 and 50 μM of the bacteriocins mentioned; however, treatment with 100 μM compounds resulted in a slight reduction in cell viability. Nisin A was found to be slightly more cytotoxic (41%) in comparison with plantaricin 423 (21%), and bacST4SA (12%). Also, the bacteriocins were found to be more lethal to HUVEC cells as compared to caco-2 cells [64]. On daily administration of 0.825 mg/kg of nisin for straight 21 days, a study in mice revealed potential signs of toxicity, including changes in cells and tissues of the spleen, liver as well as skin. This may also be because the industrial nisin used in this analysis had a high salt concentration. Many other studies performed in mice indicated no signs of toxicity like on administration of pediocin N6 and lactocin 160 [31].

There are very limited data on the bacteriocin cytotoxicity on human beings especially when exposure is present in the long-term scenario, which highlights the need to cover this area which will further determine the antimicrobial potential and safety of the bacteriocin [1].

Factors affecting bacteriocin production. There are various influencing factors on the production of bacteriocin like growth phase of bacteria, composition of growth medium, culture conditions as well as exogenous factors. Factors including carbon and nitrogen sources, as well as growth factors, have a significant impact on LAB growth and bacteriocin development

[65]. Bacteriocin production by LAB depends on the media composition like the ratio of carbon and nitrogen and glucose being the preferable choice of carbon [66]. It was found that the growth of *L. mesenteroides* L124, and *Lactobacillus curvatus* L442 was greatly affected by the carbon (glucose) amount, and source of nitrogen. Apart from carbon and nitrogen, NaCl and ethanol, also are known to stabilize the production of bacteriocin. However, the effect of NaCl and ethanol varied (stimulated or inhibited bacteriocin production). Sugars, vitamins along with nitrogen can be added to prepare enrichment media, but the proper amount must be considered since an overabundance can inhibit bacterial growth as well as bacteriocin production. *L. lactis* and *S. pyogenes* utilize glucose preferentially for the production of nisin Z and streptococcin SAFF22, where glucose leads to high growth rates, rapid consumption of substrate, and extensive product formation. Yeast and beef extracts, peptone, malt sprouts, and soybean are the various sources of nitrogen which are utilized in production of bacteriocin. Bacteria respond according to the source of nitrogen, some are not able to use organic nitrogen source [67]. The best conditions for the LAB growth and the bacteriocins production were determined. Two different culture media (MRS and BHI) at various pH (4.5, 5.5, 6.2, 7.4, and 8.5) were used, incubating bacteria at 20, 37 and 44°C. Among the LAB used, bacteriocinogenic *L. curvatus, E. faecium*, and *Lactobacillus paracasei* subspp. *paracasei* demonstrated proper growth in both the media at given temperatures, whereas *Streptococcus thermophilus* was unable to grow at temperature of 20°C. The ideal range of pH to support bacteriocins growth lies between 6.2–8.5 at the temperature of 37°C during the growth in MRS media [68]. A resting cell system was established with 20 h of cell incubation and 37°C, and the role of exogenous factors like pyruvic acid, amino acids, α-ketoglutaric acid, glycerol on Lac-B23 was investigated. Pyruvic acid and glycerol at optimal concentrations of 1 and 3%, respectively, increased the rate of production of bacteriocin. Also, amino acids Cys and Gly were able to enhance the production of bacteriocin whereas Glu, Tyr, and Ala showed no effect [65]. The optimal temperature required for the production of bacteriocin varies depending on the situation. Slow rate of growth was observed at less temperature due to more energy release by *Lactobacillus amylovorus* DCE 471 during amylovorin L471 formation. However, sakacin P was produced at higher rate at low temperature due to temperature-dependent rate limiting steps. The optimum pH for the production of bacteriocins varies from pH 5.5 to 6.0 and even less than 5.0 depending on the property of the bacterial strains used. Being facultative anaerobic microorganisms, LAB are usually affected by the oxidative stress [67].

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With the increase in the antimicrobial resistance among the FBP the need for novel alternative antimicrobials is increasing. Bacteriocins having potential antimicrobial activity are not only natural but projected to be safe for human consumption. However, further investigations are required to optimize the operating conditions to overcome obstacles, such as bacteriocin ineffectiveness in food due to enzymatic degradation, adhesion of bacteriocins in food materials, decreased dissolving capacity, and/or irregular dispersal of bacteriocins in food products. In addition, the effect of bacteriocins on the expression of virulence factors or development of persisters needs further investigation. Overall form the data available, bacteriocins can be the potential agent to be used instead of antibiotics which can assist in food preservation and safety without development of antimicrobial resistance.

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COMPLIANCE WITH ETHICAL STANDARDS

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AUTHOR CONTRIBUTIONS

AK, RB prepared the first draft. SD conceptualized the idea and provided critical inputs. AK and SD finalized the manuscript. All the authors read and approved the manuscript. AK equal contribution author.

REFERENCES

1. Simons, A., Alhanout, K., and Duval, R.E., *Microorganisms*, 2020, vol. 8, no. 5, p. 639.
2. Zimina, M., Babich, O., Prosekov, A., Sukhikh, S., Ivanova, S., Shevchenko, M., et al., *Antibiotics*, 2020, vol. 9, no. 9, pp. 1–21.

3. Lafuente-Rincon, D.F., Chavez, T.E.V., and De la Fuente-Salcido, N.M., *Afr. J. Microbiol. Res.*, 2016, vol. 10, no. 45, pp. 1873–1879.
4. *Prokaryotic Antimicrobial Peptides: From Genes to Apllication*, Drider, D. and Rebuffat, S., Eds., Springer, 2011, pp. 29–53.
5. Johnson, E.M., Jung, D.Y.G., Jin, D.Y.Y., Jayabalan, D.R., Yang, D.S.H., et al., *Crit. Rev. Food Sci. Nutr.,* 2018, vol. 58, no. 16, pp. 2743–2767.
6. Ahmad, V., Khan, M.S., Jamal, Q.M.S., Alzohairy, M.A., Al Karaawi, M.A., and Siddiqui, M.U., *Int. J. Antimicrob. Agents*, 2017, vol. 49, no. 1, pp. 1–11.
7. Silva, C C.G., Silva, S.P.M., and Ribeiro, S.C., *Front. Microbiol.*, 2018, vol. 9, no. 9, p. 594.
8. Kumariya, R., Garsa, A.K., Rajput, Y.S., Sood, S.K., Akhtar, N., and Patel, S., *Microb. Pathog.*, 2019, vol. 128, pp. 171–177.
9. *Prokaryotic Antimicrobial Peptides: From Genes to Applications*, Drider, D. and Rebuffat, S., Eds., Springer, 2011, pp. 213–236.
10. Ibrahim, O.O., *EC Microbiol.*, 2019, vol. 7, no. 15.7, pp. 591–608.
11. *Prokaryotic Antimicrobial Peptides: From Genes to Application*, Drider, D. and Rebuffat, S., Eds., Springer, 2011, pp. 55–72.
12. Brown, C. L., Smith, K., McCaughey, L., and Walker, D., *Biochem. Soc. Trans.*, 2012, vol. 40, no. 6, pp. 1549–1552.
13. *Research and Applications in Bacteriocins*, Rile, M.A. and Gillor, O., Eds., Taylor and Francis, 2006.
14. Kodali, V.P., Lingala, V.K., Karlapudi, A.P., Indira, M., Venkateswarulu, T C., and Babu, D.J., *J. Pure Appl. Microbiol.*, 2006, vol. 7, no. 4, pp. 2933–2945.
15. Gabrielsen, C., Brede, D. A., Nes, I.F., and Diep, D.B., *Appl. Environ. Microbiol.*, 2013, vol. 80, no. 22, pp. 6854–6862.
16. Perez, R.H., Zendo, T., and Sonomoto, K., *Front. Microbiol.*, 2018, vol. 9, pp. 2085.
17. Jin, X., Kightlinger, W., Kwon, Y., and Hong, S.H., *Synth. Biol.*, 2018, vol. 3, no. 1, pp. 1–11.
18. Liu, J., Chen, P., Zheng, C., and Huang, Y.P., *Appl. Environ. Microbiol.*, 2013, vol. 79, no. 18, pp. 5593–5600.
19. Cao, S., Du, R., Zhao, F., Xiao, H., Han, Y., and Zhou, Z., *Food Control*, 2019, vol. 96, pp. 470–478.
20. Sharma, G., Dang, S., Gupta, S., and Gabrani, R., *Med. Princ. Pract.*, 2018, vol. 27, no. 2, pp. 186–192.
21. Bharti, V., Mehta, A., Singh, S., Jain, N., Ahirwal, L., and Mehta, S., *Int. J. Pharm. Pharm. Sci.*, 2015, vol. 7, no. 9, pp. 20–29.
22. Quereda, J J., Meza-Torres, J., Cossart, P., and Pizarro-Cerdá, J., *Gut Microbes*, 2017, vol. 8, no. 4, pp. 384–391.
23. Mathur, H., Fallico, V., O'Connor, P.M., Rea, M.C., Cotter, P.D., Hill, C., and Ross, R.P., *Front. Microbiol.*, 2017, vol. 8, pp. 1–14.
24. Du, H., Yang, J., Lu, X., Lu, Z., Bie, X., Zhao, H., et al., *J. Agric. Food Chem.*, 2018, vol. 66, no. 18, pp. 4716–4724.
25. Scholl, D., *Annu. Rev. Virol.*, 2017, vol. 4, pp. 453–467.
26. Kjos, M., Snipen, L., Salehian, Z., Nes, I.F., and Diep, D.B., *J. Bacteriol.*, 2010, vol. 192, no. 8, pp. 2068–2076.
27. Jakes, K.S. and Cramer, W.A., *Annu. Rev. Genet.*, 2012, vol. 46, pp. 209–236.
28. Smits, S.H.J., Schmitt, L., and Beis, K., *FEBS Lett.*, 2020, vol. 594, no. 23, pp 3920–3942.
29. Cavera, V.L., Arthur, T.D., Kashtanov, D., and Chikindas, M.L., *Int. J. Antimicrob. Agents*, 2015, vol. 46, no. 5, pp. 494–501.
30. Cotter, P.D., Ross, R.P., and Hill, C., *Nat. Rev. Microbiol.*, 2012, vol. 11, no. 2, pp. 95–105.
31. Soltani, S., Hammami, R., Cotter, P. D., Rebuffat, S., Said, L. B., Gaudreau, H., et al., *FEMS Microbiol. Rev.*, 2021, vol. 45, no. 1, pp. 1–24.
32. Zacharof, M.P. and Lovitt, R.W., *APCBEE Procedia*, 2012, vol. 2, pp. 50–56.
33. Drider, D., Bendali, F., Naghmouchi, K., and Chikindas, M.L., *Probiotics Antimicrob. Proteins*, 2016, vol. 8, no. 4, pp. 177–182.
34. Hanchi, H., Hammami, R., Gingras, H., Kourda, R., Bergeron, M. G., Ben Hamida, J., et al., *Future Microbiol.*, 2017, vol. 12, no. 3, pp. 205–212.
35. Behrens, H.M., Six, A., Walker, D., and Kleanthous, C., *Emerg. Top. Life Sci.*, 2017, vol. 1, no. 1, pp. 65–74.
36. Abamecha, A., *Indo Am. J. Pharm. Res.*, 2017, vol. 7, p. 01.
37. O'Connor, P.M., Kuniyoshi, T.M., Oliveira, R.P., Hill, C., Ross, R.P., and Cotter, P.D., *Curr. Opin. Biotechnol.*, 2020, vol. 61, pp. 160–167.
38. Chikindas, M.L., Weeks, R., Drider, D., Chistyakov, V.A., and Dicks, L.M., *Curr. Opin. Biotechnol.*, 2018, vol. 49, pp. 23–28.
39. Da Costa, R.J., Voloski, F.L.S., Mondadori, R.G., Duval, E.H., and Fiorentini, A.M., *J. Food Qual.*, 2019, vol. 2019, article ID 4726510.
40. Nishie, M., Nagao, J., and Sonomoto, K., *Biocontrol Sci.*, 2012, vol. 17, no. 1, pp. 1–16.
41. Necidová, L., Mrňousová, B., Haruštiaková, D., Bursová, Š., Janštová, B., and Golian, J., *LWT—Food Sci. Technol.*, 2019, vol. 116, article ID 108459.
42. Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-Silleras, B., and Redondo-Del-Río, M.P., *Antibiotics* (Basel), 2019, vol. 8, no. 4, p. 208.
43. Kaur, G., Singh, T.P., and Malik, R.K., *Braz. J. Microbiol.*, 2013, vol. 44, no. 1, pp. 63–71.
44. Ramos, B., Brandão, T.R.S., Teixeira, P., and Silva, C.L.M., *Food Microbiol.*, 2020, vol. 85, article ID 103282.
45. Hathout, A.S. and Aly, S.E., *J. Am. Sci.*, 2010, vol. 6, no. 12, pp. 889–898.
46. Todorov, S.D., Kang, H.J., Ivanova, I.V., and Holzapfel, W.H., *Front. Bioeng. Biotechnol.*, 2020, vol. 8, pp. 1–16.
47. Abanoz, H.S. and Kunduhoglu, B., *Korean J. Food Sci. Anim. Resour.*, 2018, vol. 38, no. 5, pp. 1064–1079.
48. Egan, K., Field, D., Rea, M.C., Ross, R.P., Hill, C., and Cotter, P.D., *Front. Microbiol.*, 2016, vol. 7, p. 461.
49. Heo, S., Kim, M.G., Kwon, M., Lee, H.S., and Kim, G.B., *Korean J. Food Sci. Anim. Resour.*, 2018, vol. 38, no. 1, pp. 88–98.
50. Jiang, H., Zou, J., Cheng, H., Fang, J., and Huang, G., *BioMed Res. Int.*, 2017, vol. 2017, article ID 7657190.
51. Delesa, D.A., *Int. J. Adv. Res. Biol. Sci.*, 2017, vol. 4, no. 12, pp. 178–190.
52. Yong, C.C., Yin, K.B., Sasidharan, S., Piyawattanametha, W., Kim, S.H., Khemthongcharoen, N., et al., *Ann. Microbiol.*, 2015, vol. 65, no. 2, p. 1037.

53. Stupar, J., Holøymoen, I.G., Hoel, S., Lerfall, J., Rustad, T., and Jakobsen, A. N., *Foods*, 2021, vol. 10, no. 2, p. 271.
54. Voidarou, C., Alexopoulos, A., Tsinas, A., Rozos, G., Tzora, A., Skoufos, I., et al., *Appl. Sci.*, 2020, vol. 10, no. 20, pp. 1–18.
55. Nithya, V., Murthy, P. S., and Halami, P. M., *J. Appl. Microbiol.*, 2013, vol. 115, no. 2, pp. 475–483.
56. Damania, P., Patel, R., Shaw, R., Kataria, R. P., and Wadia, A., *Microbiol. Res.*, 2016, vol. 7, no. 1, p. 6622.
57. Becerril, R., Nerin, C., and Silva, F., *Molecules*, 202, vol. 25, no. 5, p. 1134.
58. *Encyclopedia of Food Microbiology*, Batt, C. and Patel, P., Eds., Elsevier, 2014, pp. 180–186.
59. *Food Biopreservation. Springer Briefs in Food, Health, and Nutrition*, Gálvez, A., López, R.L., Pulido, R.P., and Burgos, M.J.G., Eds., Springer, 2014.
60. Mora-Villalobos, J.A., Montero-Zamora, J., Barboza, N., Rojas-Garbanzo, C., Usaga, J., Redondo-Solano, et al., *Fermentation*, 2020, vol. 6 no. 1, pp. 1–21.
61. *Advances in Applied Biotechnology*, Petre, M., Ed., IntechOpen, 2012. https://doi.org/10.5772/30692
62. Meade, E., Slattery, M. A., and Garvey, M., *Antibiotics*, 2020, vol. 9, no. 1, pp. 32.
63. Garsa, A. K., Kumariya, R., Sood, S. K., Kumar, A., and Kapila, S., *Probiotics Antimicrob. Proteins*, 2014, vol. 6, no. 1, pp. 47–58.
64. Dreyer, L., Smith, C., Deane, S.M., Dicks, L.M.T., and van Staden, A.D., *Sci. Rep.*, 2019, vol. 9, no. 11481, pp. 1–11.
65. Yi, H., Han, X., Yang, Y., Liu, W., Liu, H., Zhang, Y., et al., *Int. J. Mol. Sci.*, 2013, vol. 14, no. 12, pp. 24355–24365.
66. Pérez, A.R., González, A.E., Agrasar, T.A., and Guerra, P.N., *Curr. Biochem. Eng.*, 2013, vol. 1, no. 1, pp. 9–24.
67. Abbasiliasi, S., Tan, J.S., Ibrahim, T.A. T., Bashokouh, F., Ramakrishnan, N.R., Mustafa, S., and Ariff, A.B., *RSC Adv.*, 2017, vol. 7, no. 47, pp. 29395–29420.
68. Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, S., et al., *AMB Express*, 2018, vol. 8, no. 1, p. 10.
69. Hsu, S.T., Breukink, E., Tischenko, E., Lutters, M. A., de Kruijff, B., Kaptein, R., et al., *Nat. Struct. Mol. Biol.*, 2004, vol. 11, no. 10, pp. 963–967.
70. Ekkelenkamp, M.B., Hanssen, M., Danny Hsu, S.T., de Jong, A., Milatovic, D., Verhoef, J., et al., *FEBS Lett.*, 2005, vol. 579, no. 9, pp. 1917–1922.
71. Hsu, S.T., Breukink, E., Bierbaum, G., Sahl, H.G., de Kruijff, B., Kaptein, R., et al., *J. Biol. Chem.*, 2003, vol. 278, no. 15, pp. 13110–13117.
72. Castiglione, F., Lazzarini, A., Carrano, L., Corti, E., Ciciliato, I., Gastaldo, L., et al., *Chem. Biol.*, 2008, vol. 15, no. 1, pp. 22–31.
73. Saising, J., Dube, L., Ziebandt, A.K., Voravuthikunchai, S.P., Nega, M., and Götz, F., *Antimicrob. Agents Chemother.*, 2012, vol. 56, no. 11, pp. 5804–5810.
74. Vériest, L., Aretz, W., Bonnefoy, A., Ehlers, E., Kurz, M., Markus, A., et al., *J. Antibiot.* (Tokyo), 1999, vol. 52, no. 8, pp. 730–741.
75. McAuliffe, O., Ryan, M.P., Ross, R.P., Hill, C., Breeuwer, P., and Abee, T., *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 2, pp. 439–445.
76. Fregeau Gallagher, N.L., Sailer, M., Niemczura, W.P., Nakashima, T.T., Stiles, M.E., and Vederas, J.C., *Biochemistry*, 1997, vol. 36, no. 49, pp. 15062–15072.
77. Balay, D.R., Dangeti, R.V., Kaur, K., and McMullen, L.M., *Int. J. Food Microbiol.*, 2017, vol. 255, pp. 25–31.
78. Bédard, F., Hammami, R., Zirah, S., Rebuffat, S., Fliss, I., and Biron, E., *Sci. Rep.*, 2018, vol. 8, no. 1, pp. 1–13.
79. Abeer Mohammed, A.B., Al-Saman, M.A., and Tayel, A.A., *J. Basic Microbiol.*, 2017, vol. 57, no. 9, pp. 744–751.
80. Rogne, P., Fimland, G., Nissen-Meyer, J., and Kristiansen, P.E., *Biochim. Biophys. Acta*, 2008, vol. 1784, no. 3, pp. 543–554.
81. Zendo, T., Koga, S., Shigeri, Y., Nakayama, J., and Sonomoto, K., *Appl. Environ. Microbiol.*, 2006, vol. 72, no. 5, pp. 3383–3389.
82. Kristiansen, P.E., Fimland, G., Mantzilas, D., and Nissen-Meyer, J., *J. Biol. Chem.*, 2005, vol. 280, no. 24, pp. 22945–22950.
83. Kristiansen, P.E., Persson, C., Fuochi, V., Pedersen, A., Karlsson, G.B., Nissen-Meyer, J., et al., *Biochemistry*, 2016, vol. 55, no. 45, pp. 6250–6257.
84. Benabbou, R., Subirade, M., Desbiens, M., and Fliss, I., *Front. Microbiol.*, 2018, vol. 9, p. 2824.
85. Acedo, J.Z., van Belkum, M.J., Lohans, C.T., McKay, R.T., Miskolzie, M., and Vederas, J.C., *Appl. Environ. Microbiol.*, 2015, vol. 81, no. 8, pp. 2910–2918.
86. Akesson, M., Dufour, M., Sloan, G.L., and Simmonds, R.S., *FEMS Microbiol. Lett.*, 2007, vol. 270, no. 1, pp. 155–161.
87. Climo, M.W., Patron, R.L., Goldstein, B.P., and Archer, G.L., *Antimicrob. Agents Chemother.*, 1998, vol. 42, no. 6, pp. 1355–1360.
88. Sabala, I., Jagielska, E., Bardelang, P.T., Czapinska, H., Dahms, S.O., Sharpe, J.A., et al., *FEBS J.*, 2014, vol. 281, no. 18, pp. 4112–4122.
89. Sun, Z., Wang, X., Zhang, X., Wu, H., Zou, Y., Li, P., et al., *J. Ind. Microbiol. Biotechnol.*, 2018, vol. 45, no. 3, pp. 213–227.
90. Ekblad, B. and Kristiansen, P.E., *Sci. Rep.*, 2019, vol. 9, no. 1, pp. 1–10.
91. Denkovskienė, E., Paškevičius, Š., Misiūnas, A., Stočkūnaitė, B., Starkevič, U., Vitkauskienė, A., et al., *Sci. Rep.*, 2019, vol. 9, no. 1, pp. 1–11.
92. Dingemans, J., Ghequire, M.G., Craggs, M., De Mot, R., and Cornelis, P., *MicrobiologyOpen*, 2016, vol. 5, no. 3, pp. 413–423.
93. Thomas, X., Destoumieux-Garzón, D., Peduzzi, J., Afonso, C., Blond, A., Birlirakis, N., et al., *J. Biol. Chem.*, 2004, vol. 279, no. 27, pp. 28233–28242.
94. Soudy, R., Wang, L., and Kaur, K., *Bioorg. Med. Chem.*, 2012, vol. 20, no. 5, pp. 1794–1800.
95. Pons, A.M., Delalande, F., Duarte, M., Benoit, S., Lanneluc, I., Sablé, S., et al., *Antimicrob. Agents Chemother.*, 2004, vol. 48, no. 2, pp. 505–513.