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Biosynthesis and Potential Applications of Bacteriocins

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Bacteriocins are proteinaceous compounds, which are ribosomally synthesized antimicrobial peptides produced by both Gram-positive and Gram-negative bacteria. They are phenomenologically analogous to yeast and paramecium killing factors, and are structurally, functionally, and ecologically diverse. Bacteriocins differ from the traditional antibiotics in one critical way. They have a relatively narrow killing spectrum and are toxic only to bacteria closely related to the producing strains. The bacterial membranes are the target for bacteriocins activity. Bacteriocins can be classified into several groups in which classes I and II are well studied. Bacteriocins have been used as biotechnological tools for therapeutic and commercial applications due to their specific modes of actions. Applications of bacteriocins include treatment of infectious diseases of both humans and plants and as preservatives in foods, pharmaceuticals, cosmetics and various biomedical products.

Key words: Lactic acid bacteria (LAB), Probiotics. Bacteriocins, Biosynthesis, Applications.

Probiotics are defined as "live microbial food supplements or components of bacteria which have been shown to have beneficial effects on human health"[1]. The probiotic bacteria usually produce several useful compounds such as bacteriocins, exopolysaccharides, short chain-fatty acids, free amino acids, bioactive peptides, vitamins, digestive enzymes, immunomodulatory compounds and oligosaccharides (Fig. 1). Probiotic bacteria modulate the gut flora by the production of various compounds such as organic acids, hydrogen peroxide, carbon dioxide, cyclic dipeptides, fatty acids and proteinaceous toxins[2,3].

The most extensively studied and widely used probiotic bacteria are the lactic acid bacteria (LAB), particularly the *Lactobacillus*, *Bifidobacterium* species and *Bacillus* spp.(such as *Bacillus coagulans*, *B. subtilis* and *B. licheniformis)*, are "generally regarded as safe" (GRAS) bacteria[4]. *Bacillus coagulans* (previously known as *Lactobacillus sporogenes*) is preferred to other probiotic strains mainly for its ability to form terminal endospores, which can easily survive in the harsh climate of the stomach and L (+) lactic acid, which easily metabolized[5]. It reduces intestinal absorption of cholesterol by retarding the secretion of bile salts in the gut. It also helps in strengthening the immune system by probiotic activities and by killing pathogens through the production of a bacteriocin, called coagulin[6]. Bacteriocins are defined as proteinaceous antibacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides produced by both Gram-positive and Gram-negative bacteria[7]. Bacteriocins differ from the traditional antibiotics in one critical way: they have a relatively narrow killing spectrum. Therefore they are toxic only to bacteria closely related to the producing strain. Accordingly, they can be

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considered "designer drugs" that target specific bacterial pathogens. Bacteriocins can not only inhibit the growth of microorganisms (prokaryotic cells) but also eukaryotic cells. For example, neocarzinostatin produced by *Streptomyces neocarzinostaticus* was shown to inhibit both bacteria and various mouse ascitic tumor cells[8]. *Escherichia coli* and other *Enterobacteriaceae* family members are the few examples of Gram negative bacteria and *lactic acid bacteria*, *Bacillus* species belong to Gram positive bacteria that produce bacteriocins. Lactic acid bacteria (LAB) occur naturally in several raw materials like milk, meat and flour used to produce foods[9]. LAB is used as natural or selected starters in food fermentations in which they perform acidification due to production of lactic and acetic acids flavours. They protect food from spoilage and pathogenic microorganisms by producing organic acids, hydrogen peroxide, diacethyl, antifungal compounds such as fatty acids or phenyl lactic acid and or bacteriocins. Bacteriocins produced by various bacteria have generated attention among researchers for the last few years due to their potential medical, pharmaceutical, biomedical and food applications due to their specific modes of action.

Classification of Bacteriocins

Bacteriocins produced by lactic acid bacteria were subdivided into four distinct classes (Table.1) based on the genetic and biochemical resemblances.

Lantibiotics (Class I)

Lantibiotics are small, ribosomally synthesized membrane-active peptides (<5 kDa) containing high proportion of unusual amino acids, including thioether amino acids lanthionine, β-methyl-lanthionine, and the dehydrated residues like dehydroalanine and dehydrobutyrine. The presence of these residues was first identified in nisin. Lacticin 481, Lactocin S, Sublancin 168, Subtilin, Streptococcin A-FF22, Plantaricin W, Staphylococcin C55, Lacticin, Actagardine and Mersacidin are the few other examples of Class I bacteriocins. Based on the peptide structure and mode of action, Class I bacteriocins have been further classified into two types. Type A bacteriocins (Nisin, Subtilin, Streptococcin A-FF22) are linear peptides with membrane disrupting mode of action. Type B bacteriocins (actagardine and mersacidin) are circular peptides with inhibitory activities[10]. Based on similarities in the size, net charge and sequence of the leaders, the group *IA* lantibiotics can be further classified into two main groups, i.e. class *IAI* (Nisin, Subtilin,) class *IAII* (Lacticin 481, Streptococcin A-FF22) and class *IAIII* (Plantaricin W, Staphylococcin C55 and Lacticin). The lactocin S N-terminal extension displays no homology with the class IAI or class IAII leader peptides and may therefore represent a new class[11].

Small, Heat-Stable, Non-Lanthionine Containing, Membrane-Active Peptides (Class II)

Class II bacteriocins are ribosomally synthesized as inactive prepeptides that are modified by post-translational cleavage of the N-terminal leader peptide generally at a double glycine(Gly-Gly$^{-2/-1}$) site to release mature cationic peptides that are amphipathic and thermostable. These are less than 10 kDa in size. This site being not restricted to class II bacteriocins is also present in some lantibiotics[12]. The mature bacteriocins are predicted to form amphiphilic helices with varying amounts of hydrophobicity, β-sheet structure, and moderate (100 °C) to high (121°C) heat stability; e.g. Pediocin PA-1, Lactococcin A, B, and M, Leucocin A, Sakacin A (= curvacin A), Sakacin P, and Lactacin F. Protein engineering of lactococcin B indicated that its cysteine residue was not necessary for activity.

Subgroups within the class II bacteriocins

Listeria-Active Peptides (Class II a)

The Class IIa bacteriocins are often described as listericidal, small (<10kDa), heat-stable, unmodified peptides of 37 (Leucocin A and Mesentericin Y105) to 48 (Carnobacteriocin B2 and Enterocin SE-K4) amino acids and having a net positive charge, with pI values ranging from 8 to 10. Sequence alignment of class IIa bacteriocins reveals that they consist of a highly conserved hydrophilic and charged N-terminal part harboring the consensus sequence YGNGV(X)C(X)4C(X)V(X)4A (X denotes any amino acid) and a more variable hydrophobic and/or amphiphilic C-terminal part. Based on amino acid sequence alignments, further division of the class IIa bacteriocins into three or four subgroups have been suggested. Examples are Leucocin A, Sakacin A, Sakacin P, Mesentericin Y105 [13-16].

Table 1. Bacteriocins and their classification

Bacteriocin Class	Characteristics	Type	Group	Example	Producer Organism	Ref.
Class I	Lantibiotics containing high proportion of	A	AI	Nisin	*Lactococcus lactis*	[20]
	unusual amino acids lanthionine, â-			Subtilin	*Bacillus subtilis*	[21]
	lanthionine and dehydrated		Epidermin	*Staphylococcus epidermidis*	[22]	
	residues like dehydroalanine		AII	StreptococcinA-FF22	*Streptococcus pyogenes*	[23]
	and dehydrobutyrine			Mutacin II	*Streptococcus mutans*	[24,25]
	with molecular masses of <5 kDa.	AIII	Plantaricin W	*Lactobacillus plantarum*	[26]	
				Staphylococcin C55	*Staphylococcus aureus*	[27]
				Lacticin	*Lactococcus lactis*	[28]
		B	-	Mersacidin	*Bacillus sp. HIL Y-85*	[29]
				Actagardine	*Actinoplanes liguriae*	[30]
Class II	Non modified heat-stable bacteriocins	A	-	Pediocin PA-1	*Pediococcus acidilactici PAC1.0*	[31]
	containingpeptides with			Mesentericin Y105	*Leuconostoc mesenteroides*	[32]
	molecular masses of <10 kDa.	B	-	Leucocin A-UAL187	*Leuconostoc gelidum UAL 187*	[33]
				Lactacin F	*Lactobacillus johnsonii*	[34]
				Thermophilin 13	*Streptococcus thermophilus*	[35]
				Plantaricin S	*Lactobacillus plantarum*	[36]
			-	Plantaricin EF	*Lactobacillus plantarum*	[37]
		C		Enterocin P	*Enterococcus faecium T136*	[38]
				Divergicin A	*Carnobacterium divergens*	[39]
			-	Acidocin B	*Lactobacillus acidophilus*	[40]
				Listeriocin743A	*Listeria innocua*	[41]
		D	-	Enterocin I	*Enterococcus faecium*	[42]
Class III	Protein bacteriocins (Heat labile) .			Helveticin J	*Lactobacillus heleveticus*	[43]
	with molecular masses of >30 kDa			Helveticin V-1829	*Lactobacillus helveticus*	[44]
		-	-	Caseicin	*Lactobacillus casei*	[45]
				Acidophilicin A	*Lactobacillus acidophilus*	[46]
Class IV	Large high molecular weight bacteriocins containing an undefined mixture of proteins, lipids, and carbohydrates.	-	-	Cepacin	*Psueudomonas cepacia*	[45]

Poration Complexes Consisting of Two Proteinaceous Peptides Class (II b)

These bacteriocins are cystibiotics that contain one disulfide bridge in the N-terminal half of the molecule. Many of these bacteriocins (including class IIa) contain a high degree of sequence identity in the N-terminal amino acid residues whereas the C-terminal amino acids are relatively diverse. The Class IIb bacteriocins form pores in the membranes of target cells and disrupt their proton gradient of target cells. Their activity is dependent on the complementary activity of two different proteinaceous peptides. Examples include Lactococcin G, Lactococcin M, Lactacin F and two-component peptide systems found in the operon located in the Plantaricin A gene cluster[17].

Small, Heat-stable, and Non-modified Bacteriocins Translated with *sec*-dependent Leaders

These bacteriocins are cystibiotics but they lack the YGNGVXC motif of class IIa and IIb bacteriocins and the disulfide bridge spans the N- and C-sections of the molecule. Their genetic arrangement is unusual because their structural and immunity genes are encoded on opposite strands of the DNA and in the opposite orientation to one another. Carnobacteriocin A is a regulated bacteriocin with genes for secretion, induction and regulation; whereas enterocin B contains only two genes in 12 kb of contiguous chromosomal DNA, suggesting that export of this bacteriocin might be achieved through the secretion proteins of enterocin A that is also encoded on the

Table 2. Mechanistic activity of different bacteriocins

Bacteriocin Class	Type	Group	Example	Mechanism of inhibition	Reference
Class I	A	AI	Nisin Subtilin Pep5 Epidermin	Destroy the integrity of the cytoplasm membrane via the formation of membrane channels.	[62]
		AII	StreptococciA-FF22 Mutacin II		
		AIII	Plantaricin W Staphylococcin C55 Lacticin		
	B	-	Mersacidin Actagardine	Form a tight complex with peptidoglycan precursor undecaprenyl diphosphoryl-N-acetylmuramic acid-(pentapeptide) - N-acetyl glucosamine, known as lipid II.	[29]
Class II	A	-	Pediocin PA-1 Mesentericin Y105	Formation of hydrophilic pore.	[17]
	B	-	Lactacin F Thermophilin 13 Plantaricin S	Formation of amphiphilic β- helices, which enables the peptides to interact with and permeabilize the target cell membrane.	[63]
	C	-	Enterocin P Propionicin T1 Listeriocin743A	Form a pore that specifically conductspotassium ions.	[38]

chromosome. The class IIc bacteriocins have a wide range of effects on membrane permeability, cell wall formation and pheromone actions of target cells. Examples include carnobacteriocin A, enterocin B, divergicin A and acidocin B.

Peptides Containing One (thiolbiotics) or No Cysteine Residues Class (II d)

This is a small group of bacteriocins that do not contain the YGNGVXC motif and contain only one or no cysteine residues. (Lactococcins A and B are included in this group but lactococcin B is the only bacteriocin that has been characterized as a thiolbiotic. Site directed mutagenesis of the cysteine residue resulted in an active peptide unless the replacement was with a positively charged amino acid.

Large Heat-Labile Proteins (Class III)

These bacteriocins are greater than 30 kDa in size. This class comprised the complex bacteriocins, composed of protein plus one or more chemical moieties (lipid, carbohydrate) required for activity. Examples are Helveticin J, Helveticin V, Acidophilicin A, Plantaricin S, Leuconocin S, Plantaricin S and T, Pediocin SJ-1. A fourth class, proposed by Klaenhammer[7] is rather questionable. The existence of this fourth class was supported by the observation that some bacteriocin activities were destroyed by glycolytic and lipolytic enzymes. However, such bacteriocins have not yet been characterized adequately at the biochemical level and the recognition of this class therefore seems to be premature.

Large High Molecular Weight Bacteriocins (Class IV)

These bacteriocins consist of an undefined mixture of proteins, lipids, and carbohydrates. However, this group has yet to be confirmed by purification and biochemical characterization. Some of the rare exceptions are Cepaciacin from *Pseudomonas cepacia* 5779 and a novel antibacterial substance produced by a *Lactobacillus delbrueckii* that have been studied. Cepaciacin 5779 is a complex molecule consisting of several protein subunits and a carbon part and a protein-carbohydrate ratio of 3:1[18]. These bacteriocins are thermolabile, stable at narrow range of pH and decompose under the effect of proteases thus a representating of a new type of the bacteriocin-like substance[19].

Genetics and Biosynthesis of Bacteriocins

Bacteriocins are polypeptides which are synthesized ribosomally. The genes responsible for bacteriocin production and immunity are usually arranged in the operon clusters[47, 48, 49]. For linear unmodified bacteriocins, which have the, carnobacteriocins, plantaricins, and sakacins, it appears that specific inducing peptides or peptide pheromones trigger the synthesis of bacteriocins that are usually located on the same gene cluster. Bacteriocin gene clusters can be located on the

Table 3. Bacteriocins and their applications

Bacteriocin	Application	Reference
Nisin	Used as food preservative because it is a natural, toxicologically safe and broad spectrum antibacterial activityPrevented the growth of *Listeria monocytogenes*	[64], [68]
Thuricin	Treat Clostridium difficile-associated disease.	[64]
Consept™, a liquid Nisin	Traditional iodophor in prevention of new intramammary infections	[64]
Lysostaphin	Used to treat staphylococcal mastitis	[65]
Subtilosin	Shown to have potent spermicidal activity	[66]
	pediocin PA-1, divergicin 35antilisterial	[70]
piscicolin 126	Relieved Listeria infection in various tissues	[71]
Abp118	Showed good antilisterial activity	[72]
Lacticin 3147	Inhibits the growth of S.aureus, MRSA, and vancomycin-resistant strains of Enterococcus faecalis	[74]
mutacin 1140	Active against tooth decay bacteria	[75]
EP2512430 A2	Formulation have been developed for treating body odours	-
Patent application number: 20100048476	The present invention relates to a composition for preventing and treating acne	-

chromosome, as in the case of mersacidin[50] and subtilin[51] and plasmids, as in the case of divergicin A [39] and sakacin A[52], or transposons, as in the case of nisin[53] and lacticin 481[54]. The lantibiotic biosynthesis operons generally contain genes coding for the prepeptide are the structural gene (LanA), two genes LanB and LanC (or in some cases only one gene, LanM), with no sequence similarity to other known genes encode enzymes involved in the formation of lanthionine and methyl lanthionine required for modification reactions (LanB,C/LanM), a gene encoding a serine proteinase which is responsible for the removal of the leader sequence of the lantibiotic prepeptide (LanP), a gene (LanT) encoding what appears to be a membrane associated ABC (ATP-binding cassette) transporter that transport proteins involved in peptide translocation, two genes LanK and LanR encoding two component regulatory proteins that transmit an extracellular signal and thereby inducing lantibiotic production. (LanR, K), and immunity genes (LanI, LanFEG) encoding proteins that protect the producer from the producer lantibiotic, the abbreviation lan refers to

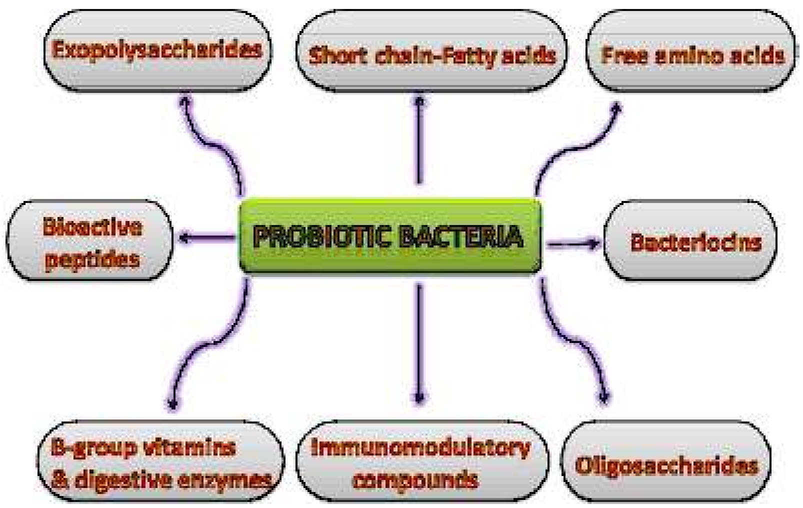

Fig. 1. Overview of compounds produced by probiotic bacteria

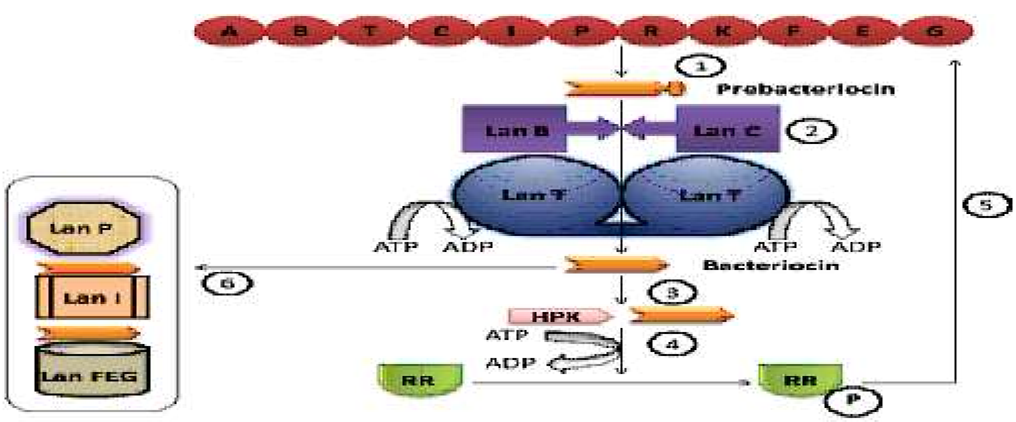

Fig. 2. Schematic diagram of the biosynthesis of lantibiotics (1) Formation of prebacteriocin; (2) The prebacteriocin is then modified by LanB and LanC, translocated through a dedicated ABC-transporter LanT and processed by LanP, resulting in the release of matured bacteriocin; (3) Histidine protein kinase (HPK) senses the presence of bacteriocin and autophosphorylates; (4) The phosphoryl group (P) is subsequently transferred to the response regulator (RR); (5) RR activates transcription of the regulated genes; and (6) Producer immunity mediated by immunity proteins, LanI, and dedicated ABC-transport proteins, LanFEG.

homologous genes of different lantibiotic gene clusters.

Biosynthetic Pathway

Most bacteriocins are synthesized as a biologically inactive prepeptide carrying an N-terminal leader peptide that is attached to the C-terminal propeptide. For lantibiotics, the serine, threonine, and cysteine residues in their propeptide parts undergo extensive post-translational modification to form Lan/MeLan. The biosynthetic pathway of lantibiotics follows a general scheme as shown in (Fig. 2): formation of prepeptide, modification reactions, proteolytic cleavage of the leader peptide, and the translocation of the modified prepeptide or mature propeptide across the cytoplasmic membrane. Cleavage of the leader peptide may take place prior to, during, or after export from the cell. Based on the biosynthetic pathway, 2 categories of genetic organization of lantibiotics, groups I and II, can be identified. This classification scheme has nothing to do with the above classification scheme that divides lantibiotics into type A and type B lantibiotics, since group I and II lantibiotics can be either type A or type B lantibiotics. For example, lacticin 481, which belongs to group II according to this genetic organizational scheme, is a type A lantibiotic. In the production of the group I lantibiotics, as in the case of nisin, epidermin, subtilin, and Pep5, the dehydration reaction is presumably catalyzed by the LanB enzyme, while LanC is involved in the thioether formation. The modified prepeptide is processed by a serine protease LanP and Tran located through the ABC-transporter LanT. In contrast, lantibiotics of group II, as in the case of cytolysin, lacticin 481, and mersacidin, are very likely modified by a single LanM enzyme[55,56], and processing takes place concomitantly with transport by LanT(P). Lactocin S is the exception to this classification. It is modified by a single LanM enzyme and processing takes place prior to export and may therefore represent a new group[56]. Class II bacteriocins are synthesized as a prepeptide containing a conserved N-terminal leader and a characteristic double-glycine proteolytic processing site, with the exception of class IIc bacteriocins, which are produced with a typical N-terminal signal pathway[40, 57].

Unlike the lantibiotics, class II bacteriocins do not undergo extensive post-translational modification. Following the formation of prepeptide, the prepeptide is processed to remove the leader peptide concomitant with export from the cell through a dedicated ABC-transporter and its accessory protein. The biosynthetic pathway of class II bacteriocins is shown in (Fig. 3).

Post-Translational Modification, Activation and Transportation

Ingram first proposed a 2-step post-translational modification reaction of a pre-lantibiotic leading to formation of Lan/MeLan. Initially, the hydroxyl amino acids, serine and

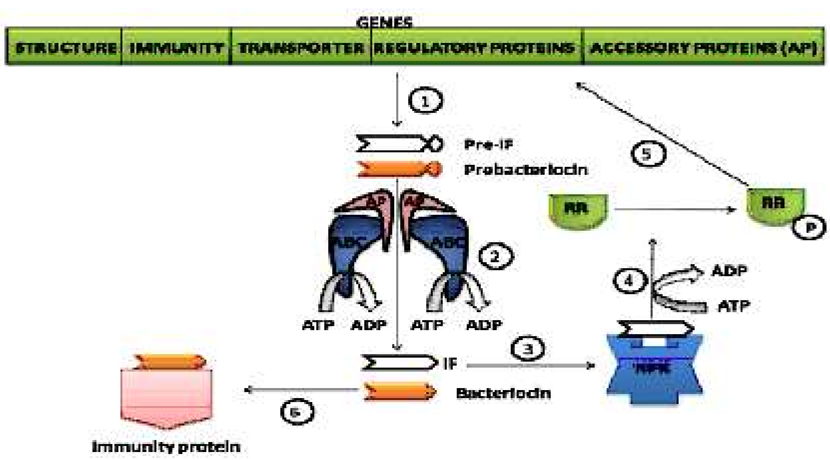

Fig. 3. A schematic diagram of the biosynthesis of class II bacteriocins (1) Formation of prebacteriocin and prepeptide of induction factor (IF); (2) The prebacteriocin and pre-IF are processed and translocated by the ABC-transporter, resulting in the release of mature bacteriocin and IF; (3) Histidine protein kinase (HPK) senses the presence of IF and autophosphorylates; (4) The phosphoryl group (P) is subsequently transferred to the response regulator (RR); (5) RR activates transcription of the regulated genes; and (6) Producer immunity

threonine, are dehydrated to yield 2, 3-didehydroalanine and 2, 3-didehydrobutyrine, respectively[57, 58]. Some dehydrated amino acids do not contain cysteine residues and remain as such in the mature peptide; others undergo an intramolecular Michael addition reaction that involves the thiol groups of neighboring cysteine residues and the double bonds of the didehydroamino acids, resulting in the formation of thioether bridges. Following the modification reactions, the modified pre-lantibiotics undergo proteolytic processing to release the leader peptide that leads to activation of the lantibiotic. For group I lantibiotics, the leader peptide is removed by a serine protease, LanP, and, depending on the location of LanP, this can take place before or after the peptide is exported from the producing cell via a dedicated ABC-transporter, LanT. For example, the proteases LanP of epicidin 280 and Pep5[13] are located intracellularly so that proteolytic processing takes place within the cell. In contrast, the proteases of nisin and epidermin[59], which are located extracellularly, activate the lantibiotics only after export by the ABC-transporter. The ABC-transporter contains 500-600 amino acids and is characterized by 2 membrane-associated domains. The N-terminal domain consists of 6 membrane-spanning helices that can recognize the substrate and form its pathway across the membrane, while the cytoplasm C-terminal domain contains 2 ATP-binding domains with the conserved ATP-binding or Walker motif. ATP hydrolysis, which likely occurs at the ATP-binding domains, provides energy for the export process.

The LanB and LanC enzymes, together with LanT transporter, probably form a multimeric membrane-associated complex. For group II lantibiotics, which possess a conserved double-glycine cleavage site, proteolytic processing takes place concomitantly with export through a hybrid ABC-transporter. This unique ABC-transporter possesses an N-terminal protease domain of approximately 150 amino acid residues that cleaves the double-glycine leader .This is exemplified in (Fig. 4). Substantial similarities exist between the leader peptides of class IIa and b and those of group II lantibiotics. Both contain the characteristic double-glycine cleavage sites. The conservation of the cleavage site strongly suggests that the mechanism of processing and translocation of class IIa and b bacteriocins is very similar to that of the group II lantibiotics. Class IIc bacteriocins are processed by a signal peptidase during translocation across the cytoplasmic membrane.

Regulation of Bacteriocin Biosynthesis

The biosynthesis of lantibiotics and non lantibiotics is usually regulated through well-known 2-component regulatory systems. These regulatory systems consist of 2 signal-producing proteins, a membrane-bound histidine protein kinase (HPK), and a cytoplasmic response regulator (RR). In this signal transduction pathway, HPK autophosphorylates the conserved histidine residue in its intracellular domain when it senses a certain concentration of bacteriocin in the environment[60]. The phosphoryl group is subsequently transferred to the conserved aspartic acid residue on the RR receiver domain and the resulting intramolecular change triggers the response regulator to activate the transcription of

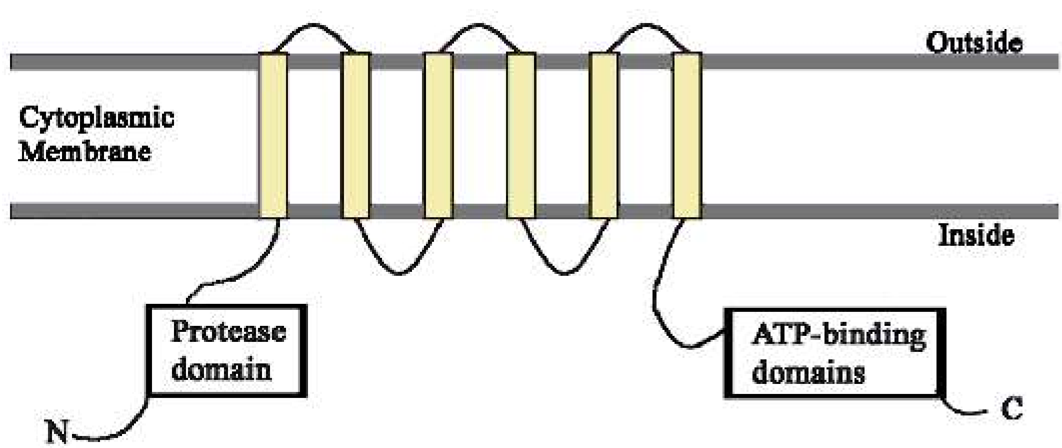

Fig. 4. ABC-transporter with an N-terminal protease domain

the regulated genes. These regulated genes include the structural gene, the export genes, the immunity genes, and in some cases, the regulatory genes themselves[61]. For nisin and subtilin, the bacteriocin molecule itself apparently acts as an external signal to auto regulates its own biosynthesis via signal transduction. In contrast, most class II bacteriocins produce a bacteriocin-like peptide with no antimicrobial activity and use it as an induction factor (IF) to activate the transcription of the regulated genes.

The IF is a small, heat-stable, cationic and hydrophobic peptide that is first synthesized as prepeptide with a double-glycine leader sequence. A dedicated ABC-transporter specifically cleaves the leader peptide of IF concomitant with export of the mature peptide from the cell. The secreted IF acts as an external signal that triggers transcription of the genes involved in bacteriocin production.

Mode of Bacteriocin Action

Bacteriocins inhibit the growth of target organisms in different mechanisms. Many bacteriocins act by destroying the membrane integrity of the target organism called cell lysis and few bacteriocins are bacteriostatic. For example, the class I bacteriocin nisin and some of the class II bacteriocins have been shown to be membrane-active peptides that destroy the integrity of the cytoplasmic membrane via the formation of membrane channels (Table 2). They alter the membrane permeability and therefore cause leakage of low molecular mass metabolites or dissipate the proton motive force, thereby inhibiting energy production and biosynthesis of proteins or nucleic. Most bacteriocins produced by lactic acid bacteria display a bactericidal effect on the sensitive cells, all or not resulting in cell lysis. On the other hand, other bacteriocins, such as lactocin 27) leucocin A and leuconocin S have been reported to act bacteriostatically. However, the designation of lethal versus static effect can be dependent upon aspects of the assay system, including the number of arbitrary units, the buffer or broth, the purity of the inhibitor, and the indicator species and cell density used. The class IAI lantibiotic nisin was shown to form ion-permeable channels in the cytoplasmic membrane of susceptible cells, resulting in an increase in the membrane permeability, disturbing the membrane potential and causing an efflux of ATP, amino acids, and essential ions such as potassium and magnesium. Ultimately, the biosynthesis of macromolecules and energy production are inhibited resulting in cell death. Nisin does not require a membrane receptor but requires an energized membrane for its activity, which appeared to be dependent on the phospholipid composition of the membrane. Lactococcin A can alter the permeability of the *L. lact is* cytoplasmic membrane leading to the loss of proton motive force and leakage of intracellular ions and constituents. LcnA acts in a voltage independent manner on intact cells or membrane vesicles, but not on liposomes suggesting that a specific membrane receptor is required for LcnA recognition and action. Analogously, the antimicrobial activity of Las5 was not dependent on an energized membrane, but required a trypsin-sensitive protein receptor to elicit bactericidal action on protoplasted cells. The voltage independent activity of lactococcin B, similar to thiol-activated toxins, was proposed to be dependent on the reduced state of its unique cysteine residue on position 24. Recently, it was shown by means of protein engineering that the Cys-24 residue was not necessary for activity of lactococcin B. Lactococcin G is a novel lactococcal class IIB bacteriocin whose activity depends on the action of two peptides. The combination of *a* and *b* peptide dissipated the membrane potential, induced a dramatic decrease in the cellular ATP level, and resulted in a rapid efflux of potassium. The class IIA pediocins PA-1/AcH and JD were reported to exhibit their bactericidal action at the cytoplasmic membrane and to cause a collapse of the pH gradient and proton motive force. Furthermore, a leakage of K+, UV adsorbing materials, permeability to ONPG, and in some cases cell lysis, although not attributed to the primary pediocin AcH action were observed. Pediocin PA-1 was shown to dissipate the proton motive force and inhibit the amino acid transport in sensitive cells. The mechanism of action of the class III bacteriocins remains to be elucidate[7]. Some bacteriocins show antimicrobial activity through their enzymatic activities. For example, colicin E2 shows DNase activity, colicin E3 shows RNase activity and megacin A-216 shows phospholipase activity against the target organism.

Applications

Food, pharmaceutical and medical

industries have been using bacteriocins as a wide range of applications. Bacteriocins have antifungal activities. Food preservative, nisin is a natural, toxicologically safe and broad spectrum antibacterial activity[64]. Thuricin from Bacillus thuringiensis DPC 6431 was used to treat Clostridium difficile-associated disease. Consept™, a liquid Nisin formulation was commercialized as a pre and postmilking dip, and was shown to be equivalent to traditional iodophor in prevention of new intra-mammary infections. Recombinant Lysostaphin was used to treat staphylococcal mastitis in an experimental challenge study, and was compared to traditional antibiotics[65]. Bacteriocins that are active against vaginal pathogens are also reported as having spermicidal activity. Subtilosin produced by B. amyloliquefaciens, was shown to have potent spermicidal activity[66]. The infections caused by contaminated biomedical implant devices have been reported[67]. Nisin, adsorbed to silanized surfaces, prevented the growth of Listeria monocytogenes[68]. Lactobacillus spp. form part of the normal bacterial flora in the vagina and ensure a reduced risk of bacterial vaginosis and urinary tract infections[69]. In vitro studies reported on antilisterial bacteriocins including pediocin PA-1, divergicin 35, and nisin, while only a few were done in vivo[70]. When injected intravenously into the tail vein of BALB/c mice, piscicolin 126 relieved Listeria infection in various tissues[71]. Abp118, a bacteriocin produced by Lactobacillus salivarius UCC118, also showed good antilisterial activity in infected mice[72]. Pseudomonas aeruginosa is the main causative agent of nosocomial pneumonia in cystic fibrosis (CF) patients[73]. Lacticin 3147, a two-peptide lantibiotic produced by Lactococcus lactis subsp. lactis, inhibits the growth of S. aureus, MRSA, and vancomycin-resistant strains of Enterococcus faecalis[74]. A strain of Streptococcus mutans that produces mutacin 1140 is active against tooth decay bacteria[75]. Recently, the formulation have been developed for treating body odours using a bacteriocin (EP2512430 A2). The present invention relates to novel use of bacteriocin derived from Enterococcus faecalis SL-5. More particularly, the present invention relates to a composition for preventing and treating acne (Patent application number: 20100048476).

CONCLUSIONS

The bacteriocins show a broad spectrum of inhibition to some extent; however it is inactive against fungal strains. Bacteriocins are synthesized in less amounts, therefore huge amounts of bacteriocins are required. The bacteriocins amounts can be increased by genetic manipulations of the bacteriocin producing organisms. For genetic manipulation, one should characterize and understand the molecular mechanisms, structure-function relationships, and mechanisms of action of bacteriocins. Chemical modifications of the bacteriocins are also required to improve their activities and properties. Such techniques could be utilized to improve the stability and production of bacteriocins, so that they may be more applicable in medical, pharmaceutical, biomedical and food products.

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