JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

LANTIBIOTICS OF MILK ISOLATES: A SHORT REVIEW ON CHARACTERIZATION AND POTENTIAL APPLICATIONS

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https://doi.org/10.55251/jmbfs.3702

ARTICLE INFO

Received 11. 9. 2020
Revised 14. 9. 2021
Accepted 21. 9. 2021
Published 1. 2. 2022

Regular article

OPEN ACCESS

ABSTRACT

Milk, due to its high nutritional content, is an excellent medium for supporting growth of diverse group of microorganisms, many of which produce beneficial compounds like bacteriocins. Class I bacteriocins, called lantibiotics, are ribosomally synthesized, post-translationally modified peptides containing unusual amino acids, such as dehydrated and lanthionine residues with antibacterial activities. Bacterial strains isolated from milk and dairy products produce a range of lantibiotics which can employed for development of food preservatives, flavor enhancers and as alternate treatment strategies for multi drug resistant bacterial pathogens. The diverse category of lantibiotics from milk isolates include well characterized prototypes like nisin to newer peptides yet to be studied. In this review, details of most prominent lantibiotics obtained from milk isolates have been presented with special focus on applications of these lantibiotics in therapeutics and food.

Keywords: Lantibiotics, milk, bacterial strains, antimicrobials, multi drug resistant pathogens

INTRODUCTION

The diverse microbial populations in milk apart from contributing to the desirable traits of various milk-derived products produce a range of antimicrobial compounds that confer preservative action. Ribosomally synthesized antibacterial peptides (bacteriocins) produced by inherent microflora form an important class of bioactive compounds in milk **(Leroy and De Vyust, 2010)**. These compounds have gained considerable interest in the recent past owing to their inhibitory action against a wide range of pathogenic microorganisms **(Farkas-Himsley 1980; Sahl and Bierbaum, 2008)**. Amongst the various classes of bacteriocins, the Class I bacteriocins, called lantibiotics, have been the focus of many biomedical research groups due their ability to aggressively destroy target cells and multiple modes of action **(Cotter *et al.*, 2005b; Cavera *et al.*, 2015)**. Lantibiotics are small (< 5kDa, 19-38 amino acids) heat-stable bacteriocins which are synthesized by post-translational modifications to include the unusual thioether amino acids lanthionine (Lan) and methyllanthionine (MeLan) **(McAuliffe *et al.*, 2001; Cotter *et al.*, 2005a; Dufour *et al.*, 2007).** Since the discovery of the prototype lantibiotic, Nisin in 1928 **(Rogers and Whittier, 1928)**, more than 10 different kinds of lantibiotics have been characterized till date. These peptides are largely produced by Gram positive bacteria which include species like *Lactobacillus, Leuconostoc* and *Enterococcus* that are constitute the dominant microflora of milk and other dairy products. Several lantibiotics with potential applications in as antimicrobials in therapeutics, food industry and agriculture have also been reported to be produced by many strains of phylum *Actinobacteria* **(Gomes *et al.*, 2017)**. Also, a lantibiotic called pseudomycoicidin has been heterologously produced in *E.coli* **(Basi-Chipalu *et al.*, 2015)**

The major aspect that distinguishes lantibiotics from other lanthionine containing peptides is their antimicrobial action, hence the name lantibiotic (*Lan*thionine containing an*tibiotics*). In recent times, overuse of antibiotics has led to the emergence of multidrug resistant bacteria, thereby prioritizing research on the search for alternative treatment strategies. Moreover, antibiotics present several drawbacks such as inhibition of normal microbiota and diverse side effects (Iannitti and Palmieri, 2010). In this aspect, lantibiotics with their unique structural make up and distinctly different mechanisms of action constitute an emerging class of natural products which are promising alternatives to currently used antibiotics **(Sahl and Bierbaum, 2008; Cavera *et al.*, 2015)**. Lantibiotics are generally inhibitory to Gram positive bacteria, many of which are precarious human pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin intermediate *S. aureus* (VISA), vancomycin resistant enterococci (VRE), *Streptococcus pneumoniae* and *Clostridium difficile*, amongst others **(Cotter *et al.*, 2013)**. This feature makes them suitable for use in human and veterinary medicine and also in the pharmaceutical industry **(Dischinger *et al.*, 2014)**.

Milk forms an important component of diet of majority of the population globally, particularly in the Indian subcontinent. The natural nutrient rich composition of milk makes it an excellent medium to support survival of microorganisms which include lantibiotic producing bacterial strains also. Study of lantibiotics from these bacterial strains will provide an important insight in the use of milk and dairy products as effective mediums for multistrain probiotic cultures and of milk derived lantibiotics in therapeutic applications. The objective of this study is to provide collated details of important lantibiotics from bacterial strains isolated from milk and the applications of these lantibiotics in food industry and therapeutics.

GENERAL PROPERTIES OF LANTIBIOTICS

Till date, over a 100 different types of lantibiotics have been discovered and characterized and have been exhaustively reviewed **(McAuliffe *et al.*, 2001; Wiley and van der Donk, 2007; Basi- Chipalu, 2016)** Based on their biosynthetic pathways **(Willey and van der Donk, 2007)**, lantibiotics are classified in 4 classes: class I lantibiotics are those modified by two separate enzymes, a LanB (dehydratase) and LanC (cyclase); class II are modified by a single LanM enzyme with both dehydratase and cyclase activity **(Siezen *et al.*, 1996)**. Class III and class IV are designated as lanthipeptides as they have no or weak antimicrobial activities and instead they perform morphogenetic activities and signaling functions for the producer cells **(Willey and Gaskell, 2011)**. The prominent lantibiotics in milk isolates mostly belong to Class I category and few of them belong to Class II.

Lantibiotics are synthesized as inactive prepeptides which are later converted to active peptides by extensive post translational modifications. Structurally, the most distinguishing feature of lantibiotics is presence of a high proportion of unusual amino acids, including the thioether amino acids Lan and MeLan and a number of dehydrated amino acids, such as the K,L-unsaturated amino acids Dha and Dhb, which are formed by sequence-specific dehydration of serine and threonine respectively during post translational modifications. Interaction of double bond in Dha and the thiol (-SH) group of a neighbouring cysteine residue results in formation of thioether Lan and MeLan. Hence presence of these intramolecular bridges makes lantibiotics polycyclic structures containing a number of Lan rings **(Ingram, 1969)**.

Lantibiotics are inhibitory to Gram-positive bacteria but are ineffective against Gram-negative bacteria, probably due to the outer membrane present in Gram negative bacteria that prevents entry of lantibiotic into the cell **(Castiglione *et al.*, 2008)**. Although the bactericidal action is by different mechanisms **(Asaduzzaman and Sonomoto, 2009)** the primary mode of action of lantibiotics is based on either pore formation and/or inhibition of peptidoglycan synthesis **(Brötz *et al.*, 1998)**. Destruction of target cells by pore formation has been well studied in Nisin **(Wiedemann *et al.*, 2001)** and lacticin 3147 b and other lantibiotics by gallidermin and epidermin **(Bonelli *et al.*, 2006)**. The insertion of lantibiotic on to the membrane of the target cells is mediated by lipid II in the cell membrane **(van Heusden *et al.*, 2002)**, which also stabilizes the resulting pores **(Breukink *et al.*, 1999; Wiedemann *et al.*, 2001)**. Following pore formation, cytoplamic contents and ions leak out of the cells thereby leading to collapse of the bacterial cell wall and death. Lan rings in lantibiotics such as nisin, mersacidin and planosporicin also bind to lipid II molecule in the cell wall and displace it. This causes accumulation of peptidoglycan precussors inside the cell leading to inhibition of peptidoglycan syhthesis **(Hasper *et al.*, 2006; Castiglione *et al.*, 2007)**. It has been also observed that the lantibiotic nukacin ISK-1is bacteriostatic rather than bactericidal, which is brought about by the reduction in the width of the cell wall leading to incomplete formation of the septum during cell division and thus inhibiting growth **(Assaduzzaman and Sonomoto, 2009)**.

LANTIBIOTIC PRODUCING BACTERIAL ISOLATES IN MILK

Milk in healthy animals is sterile but becomes colonized thereafter by microorganisms from a variety of sources such as animal skin, water, air, milking equipment, soil and other sources **(Vacheyrou *et al.*, 2011)**. Lactic acid bacteria (LAB), a group of bacteria which ferment lactose to lactate, make up the dominant population in milk from various animals and human. The most common LAB genera in milk include *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Enterococcus.* Apart from LAB, bacterial strains of other genera such as *Staphylococcus*, *Micrococcus*, Microbacterium, Coliforms and *Bacillus* are commonly found **(Quigly *et. al.*, 2012)**. Many of these strains from raw milk produce are capable of producing putative bacteriocin-like compounds which have been shown to be active against human pathogens such as *L. monocytogenes, Staph. aureus, C. tyrobutyricum, C. sporogenes, Ent. faecalis, Ent. faecium* and *Ent. durans* **(Alegría *et al.*, 2010; Ortolani *et al.*, 2010; Perin *et al.*, 2012)**. A consolidated list of lantibiotic producing milk isolates have been given in Table 1. Amongst the lactic acid bacteria isolated from mik and dairy products, *Lactococcus lactics* produces two important lantibiotics nisin and lacticin **(Piard *et al.*, 1993; Ryan *et al.*, 1996; Rodriguez *et al.*, 2000)**. Nisin from *L. lactis* isolated from milk has been shown to be inhibitory against *L. monocytogenes* as well as other pathogens including *E. coli* and *Staphylococcus spp*. **(Bravo *et al.*, 2009; Alegría *et al.*, 2010;Ortolani *et al.*, 2010; Cosentino *et al.*, 2012; Perin *et al.*, 2012)**. *Lactobacillus plantarum* isolated from milk and milk products such as cheese and kefir has been reported to produce plantaricin C and plantaricin W **(Turner *et al.*, 1999; Holo *et al.*, 2001;Todorov, 2008)**. Other lactic acid bacteria producing lantibiotics include *Enterococcus feacalis* **(Booth *et al.*, 1996; Nes *et al.*, 2014)**, *Streptococcus thermophilus* **(Gul *et al.*, 2012)** and *Streptococcus macedonicus* **(Georgalaki *et al.*, 2002; Georgalaki *et al.*, 2013)** which are inhibitory against many pathogenic microorganisms and microbes causing food spoilage. As research advances, discovery of new strains adds on to the already existing list of lantibiotic producing lactic acid bacteria. One such strain, *Streptococcus bovis* HJ50 has been isolated from milk and the lantibiotic produced by the strain has been named bovicin HJ50 **(Xiao *et al.*, 2004)**.

Table 1 Lantibiotic producing bacterial strains isolated from milk and dairy products

Bacterial strain	Lantibiotic	Reference
Lactococcus lactis	NisinA	Gross & Morell (1971)
	Nisin Z	de Vos (1993)
	Lacticin 3147	Ryan *et al*. (1996)
	Lacticin 481	Piard *et al*.(1993)
Lactobacillus plantarum	Plantaricin C	Turner *et al*.(1999)
	Plantaricin W	Holo *et al*. (2001)
Enterococcus feacalis	Cytolysin	Booth *et al*.(1996)
Streptococcus macedonicus	Macedocin	Georgalaki *et al*.(2002)
Streptococcus bovis	Bovicin HJ50	Xiao *et al*.(2004)
Non aureus Staphylococci (NAS)	Type 1 lantibiotics; nisin homologues	Carson *et al*.(2017)

Apart from lactic acid bacteria, lantibiotic production has also been reported in few strains of non- *aureus* Staphylococci (NAS) isolated from bovine milk. In a recent study by **Carson *et al.*, (2017)** presence of bacteriocin clusters in whole genome of 441 NAS bovine milk isolates was determined by genome mining tools, BLAST, and comparison of genomes of closely related NAS isolates. Type 1 lanthipeptides producing gene clusters were identified in *S. capitis, S. epidermidis and S. equorum* by comparison with known lantibiotic gene clusters **(Carson *et al.*, 2017)**. This indicates that using genome mapping tools and BLAST searches, new novel bacterial strains and lantibiotics can be identified more rapidly and accurately. Almost all the lantibiotics characterized till date are produced by Gram positive bacterial strains **(Nes and Tagg, 1996)** and this holds true for lantibiotics of milk isolates. There are no reports of lantibiotic produced by Gram negative species in milk and dairy products.

PROMINENT LANTIBIOTICS OF MILK AND DAIRY ISOLATES

Lantibiotics from strains isolated from milk are particularly interesting, considering that most of the inherent bacterial strains in milk and milk derived products have GRAS (Generally Regarded As Safe) status. In this section, a brief overview of the structure, characterization, genetics and mode of action of prominent lantibiotics produced by bacterial strains isolated from milk and dairy products have been discussed. A snapshot of properties of these lantibiotics has been given in Table 2.

Nisin

Nisin was accidentally discovered in 1928 in fermented milk cultures and commercially marketed in England in 1953 by Aplin & Barrett as an antimicrobial agent **(Rogers and Whittier, 1928; Delves-Broughton *et al.*, 1996)**. Initially called as 'Group N (streptococci) inhibitory substance' in 1947, it is the only bacteriocin that has been approved by the World Health Organization for use as a food preservative since 1969. Specifically, in 1983 it was added to the positive list of food additives as E234 in Europe in 1983 and in 1988 it received FDA approval as a GRAS (generally recognised as safe) substance **(De Vuyst and Vandamme, 1994; Cotter *et al.*, 2005b; Sobrino-López and Martín-Belloso, 2008)**. At present it is available as a commercial formulation called Nisaplin manufactured by Danisco and is widely used in food industry as a natural biopreservative for different types of foods **(de Arauz *et al.*, 2009)**. Nisin is naturally produced by food-grade strains of *Lactococcus lactis* subsp. *lactis* and non-food *Streptococcus uberis*. Till date, 8 natural variants (A, Z, F, Q, U, U2, and H &P) and around 10 bioengineered variants of nisin have been elucidated **(Shin *et al.*, 2017)**. These variants differ not only in amino acid composition but also in solubility and diffusion characteristics **(Lubelski *et al.*, 2008; de Aruaz *et al.*, 2009)**. Of these variants, nisin A, Z, F and Q belong to *L. lactics* isolated from milk and dairy products.

Structure of nisin was elucidated in 1971 and it is a single peptide composed of 34 amino acid residues, with a molecular mass of 3.5 kDa. Nisin belongs to the class-I bacteriocins (type A lantibiotics). It is heat stable and particularly active at low pH **(Liu and Hansen, 1990)**. It was also reported that the ring structure in nisin (ring A in nisin A and ring C in nisin Z) was important for its biological activity i.e. inhibition of bacterial growth and inhibition of spore germination **(McAuliffe *et al.*, 2001)**. Opening of the ring structures by hydrolytic cleavage or replacement of rings by disulphide bonds lead to loss of activity **(Rollema *et al.*, 1996; van Kraaji *et al.*, 2000)**.

Nisin displays a broad spectrum of activity against different Gram-positive bacteria and inhibits outgrowth of spores of bacilli and clostridia primarily through pore formation in the target cell wall **(McAuliffe *et al.*, 2001)**. This attribute makes is an excellent biopreservative and even as a therapeutic agent in pharmaceutical, veterinary and health care products **(de Arauz *et al.*, 2009)**. Examples include the inhibition of *Bacillus* spp., *Clostridium* spp. and *Staphylococcus aureus* including MRSA (methycillin resistant *Stap. aureus*) and Enterococci including VRE (vancomycin resistant enterococci) **(Field *et al.*, 2015)**. The theraupeutic potential of nisin also extends to other clinical frontiers such as oral health, cancer and mastitis where studies have shown positive outcomes with nisin **(Shin *et al.*, 2017; Małaczewska and Kaczorek-Łukowska, 2021)**.

Despite proven effects, several limitations still hinder the use of nisin in various applications. Its low solubility at neutral pH, its protease sensitivity, sensitivity and inhibition by nisin non- resistance strains in milk and milk products prevents its widespread applications **(Bhatti *et al.*, 2004; Sobrino-López and Martín-Belloso, 2008)**. Also, resistance towards nisin by the target bacteria may compromise its efficacy. Resistance to nisin often appears after direct exposure to a low level of lantibiotic or as part of an adaptive response to another stress and is in tandem with changes in membrane charge and fluidity, cell wall thickness and charge, and combinations thereof **(Cotter *et al.*, 2005b)**. The use of bioengineered variants can be a solution to overcome these shortcomings.

Table 2 Properties of prominent lantibiotics from milk isolates

Lantibiotic	Size	Molecular weight	Mode of action	Application
Nisin	34 amino acids	3.5 kDa	Pore formation in target cells	• Food preservative • Biomedical applications
Lacticin 3147	59 amino acids	6.1 kDa	Pore formation in target cells	• Treatment of mastitis • Cheese processing
Lacticin 418	27 amino acids	2.9 kDa	Inhibition of peptidoglycan synthesis of target cells	• Antilisterial biopreservative for dairy products • Flavor enhancer in cheese
Macedocin	26 amino acids	2.8 kDa	Inhibition of peptidoglycan synthesis of target cells	• Biopreservative
Plantaricin C	27 amino acids	Not available	Pore formation in target cells	Not available
Cytolysin	Not available	Not available	Hemolytic	Not available
Bovicin HJ50	58 amino acids	3.4 kDa	Cell membrane permeabilisation	Not available

Lacticin 3147

Lacticin 3147 is a two-component lantibiotic produced by natural dairy isolates such as *L. lactis* subsp. *lactis* DPC 3147 and *L. lactis* IFPL 105 **(Guinane *et al.*, 2005)**. It is very heat stable, broad-spectrum lantibiotic that is active over a wide pH range against several Gram-positive bacteria, including food spoilers (e.g. *Clostridium* spp.), food pathogens (e.g. *L. monocytogenes, Staph. aureus*, and *Bacillus cereus*) and clinical pathogens (e.g. vancomycin resistant Enterococci) **(Leroy and De Vuyst, 2010)**. Lacticin 3147 consists of two peptide chains *viz.* Ltn A1, 30 amino acids, 3.3 kDa and LtnA2, 29 amino acids, 2.8 kDa. Post translationally, Serine to D-alanine conversion has been reported in both peptides of lacticin 3147 and it has been postulated that the broad antimicrobial inhibitory spectrum of lacticin 3147 is due to these D-alanine residues **(Ryan *et al.*, 1999)**.
Like nisin, lacticin 3147 also destroys target cells by formation of transmembrane pores and this is enhanced when target cells are energised. The pores formed by lacticin 3147 were shown to be selective for ions and not larger compounds such as ATP. Loss of ions via pores results in immediate dissipation of the energy and hydrolysis of internal ATP leading to cell death **(McAuliffe *et al.*, 2001)**. The broad range of antibacterial activity and lytic effects on related genera makes lacticin 3147 an excellent candidate for development of commercial products for various food related applications. Studies have shown that lacticin 3147 effectively inhibits growth of *Listeria monocytogenes* on cheese surface and hence can be developed as a bioprotective strain for the control of *L. monocytogenes*, a common dairy contaminant **(O'Sullivan *et al.*, 2006)**. The high heat stability enhances its use in spray dried formulations and a lacticin 3147-based powder has successfully been evaluated as an antilisterial biopreservative for dairy products **(Morgan *et al.*, 2001; Guinane *et al.*, 2005)**. Lacticin 3147 has also been studied in the treatment of bacterial mastitis and staphylococcal and enterococcal infections including VRE **(Lawton *et al.*, 2007; Piper *et al.*, 2009)**.

Lacticin 481

Lacticin 481 is produced by several strains of *Lactococcus lactis* namely *L. lactis* CNRZ481 and *L. lactis* ADRIA85LO30 **(Piard *et al.*, 1993; van den Hooven *et al.*, 1996)**. Unlike nisin and lacticin 3147, which are broad-spectrum bacteriocins, lacticin 481 is a medium-spectrum bacteriocin that is mainly active towards clostridia and LAB **(Guinane *et al.*, 2005)**. This lantibiotic is made up of 27 amino acids and is 2.9 kDa in size and belongs to class II lantibiotic group. Structurally, Lacticin 481 is quite distinct and contains the unusual α,β-unsaturated amino acid dehydrobutyrine and the uncommon thioether-bridging residues lanthionine and 3-methyllanthionine, giving it an unusual bridging pattern **(van den Hooven *et al.*, 1996)**. The primary mode of action of lacticin 481 is by inhibition of peptidoglycan synthesis of target cells **(Wiley and van der Donk, 2007)**. In fact, lacticin 481 is not very effective on pathogenic bacteria but can inactivate pathogens in combination with physicals methods such as high-pressure treatments **(Rodriguez *et al.*, 2005)**. The primary application of this lantibiotic is in dairy industry where lacticin 481-producing strains have been applied to prevent the growth of detrimental bacteria, such as *C. tyrobutyricum* **(Thuault *et al.*, 1991)** and non-starter LAB **(O'Sullivan *et al.*, 2003)** in dairy products. Also, it has been seen that lacticin 481-mediated lysis of certain *Lactobacillus* strains in cheese led to an increase flavor formation through the release of aminopeptidases **(Garde *et al.*, 2007)** and esterases **(Ávila *et al.*, 2007)**.

Macedocin

Another member of the lacticin 481 group of lantibiotics is macedocin which is produced by *Streptococcus macedonicus* ACA-DC 198 **(Georgalaki *et al.*, 2002; Georgalaki *et al.*, 2010)**. *Streptococcus macedonicus* is a dairy streptococcus isolated from Greek Kasseri cheese **(De Vuyst and Tsakalidou, 2008)**. Macedocin is composed of 26 amino acids with a molecular mass of 2.8 kDa. It is active at pH values between pH 4 and 9 and it is relatively heat stable. Slow inactivation of this lantibiotic occurs in the presence of rennet **(Georgalaki *et al.*, 2002)**. The antagonistic activities of macedocin suggest potential to combat spoilage and late loss in hard and semi-hard cheeses. Several macedocin producing strains displayed antibacterial activity towards C. *tyrobutyricum* and *Propionibacterium freudenreichii* subsp. *shermanii* **(Georgalaki *et al.*, 2000; Lombardi *et al.*, 2004)**. Apart from that, macedocin also inhibits food spoilage bacteria such as *Bacillus*, pathogenic strains such as *Listeria* and a broad spectrum of LAB, suggesting a role for macedocin-producing strains as food starter cultures **(De Vuyst and Tsakalidou, 2008)**. Biotherapeutic applications have not been reported for macedocin so far.

Plantaricins

Plantaricins are another group of lantibiotics that are produced by *Lactobacillus plantarum* , a common milk isolate **(Todorov, 2009)**. Although they remain as the least characterized of the milk lantibiotics, plantaricin C and plantaricin W have been studied in certain aspects **(Turner *et al.*, 1999; Holo *et al.*, 2001)**. Plantaricin C is produced by *L. plantarum* LL441 isolated from Cabrales cheese **(Gonzalez *et al.*, 1994)**. It was identified as a lantibiotic by Turner *et al*. in 1999, who also elucidated the structure of this lantibiotic. Plantaricin C is a 27 amino acid peptide with a linear N- terminal end and a globular C-terminus, making it structurally similar to lacticin 481**(Turner *et al.*, 1999)**. Like nisin, plantaricin C inactivates bacterial strains by formation of pores in the cytoplasmic membrane leading to the dissipation of the proton motive force and the release of intracellular molecules (i.e., glutamate and ATP) in sensitive cells **(Gonzalez *et al.*, 1996)**. However, it has been seen that plantaricin C also has an effect on the peptidoglycan layer in *Lactobacillus fermentum* in electron microscopy studies. In this aspect, plantaricin C resembles the mersacidin group of lantibiotics, which are known to inhibit peptidoglycan synthesis.
Plantaricin W is produced by the strain *Lactocbacillus plantarum* LMG 2379 **(Holo *et al.*, 2001)** and is a two component peptide. Although the structure this lantibiotic has not been determined, models predict the structure to have a central lanthionine with two overlapping thioether bridges close to C-terminus, making the structure similar to Type A lantibiotics like nisin **(Holo *et al.*, 2001)**. Both of the peptides have intrinsic antimicrobial properties but do not work independently and require the complimentary action of their partner peptide **(Ryan *et al.*, 1999; Holo *et al.*, 2001)**. Considering the structural aspects, the mode of action of plantaricin W appears to be similar to nisin i.e. it probably inhibits bacteria by pore formation. Little is known about the applications of plantaricins, thereby making them good candidates for further research and development.

Cytolysin

Enterococcus faecalis, a culture prevalent in dairy products produces cytolysin which is the only lantibiotic-type enterocin currently known **(Booth *et al.*, 1996)**. Cytolysin is a two-peptide bacteriocin and both structural subunits contain lanthionine residues **(Booth *et al.*, 1996)**. Presence of two linear peptides makes it structurally different to other linear lantibiotics, such as nisins A and Z that consist of only one linear peptide. It is also different to the smaller and globular lantibiotics produced by *Streptomycetes* **(de Vos *et al.*, 1995; Sahl *et al.*, 1995)**. Cytolysin is haemolytic and it is active against eukaryotic cells (erythrocytes) and Gram-positive bacteria **(Gilmore *et al.*, 1994; Booth *et al.*, 1996)**.

Bovicin HJ50

One of the latest lantibiotic discovered from milk isolate is bovicin HJ50. This lantibiotic is produced by *Streptococcus bovis* HJ50 isolated from raw milk **(Xiao *et al.*, 2004)**. Structurally, it is a 58 amino acid cationic peptide consisting of an N-terminal leader sequence of 25 amino acid and a C-terminal propeptide domain of 33 amino acid. It has two thioether bridges and a disulfide bridge with two modified threonine residues. The molecular mass was determined to be 3.4 kDa.

Like most lantibiotics produced by lactic acid bacteria, bovicin HJ50 showed a narrow range of inhibiting activity and was inhibitory to only Gram positive bacterial strains. In inhibitory action was seen against *Lactobacillus curvatus* LTH1174, *Bacillus subtilis* AS1.1087, *Bacillus megaterium* AS1.941, *M. flavus* NCIB8166, *Leuconostoc dextranicum* 181 and *Leuconostoc mesenteroides* AS1.2, but it did not inhibit *Listeria monocytogenes.* The inhibitory action of bovicin HJ50 could be due to cell membrane permeabilisation of target strains. Applications of this new lantibiotic are yet to be studied.

GENOMIC ASPECTS OF LANTIBIOTICS FROM MILK ISOLATES

Genes that are involved in lantibiotic biosynthesis are arranged in clusters, on transposable elements (e.g. nisin), on host chromosome or on plasmids (e.g. lacticin 481) **(Chatterjee *et al.*, 2005)**. Till date, the amino acid sequences and gene clusters of several lantibiotics have been characterized and it has been observed that there is significant heterogeneity in the propeptide compositions and in the order and orientation of their gene clusters **(Seizen *et al.*, 1996)**. At the genetic level lantibiotic gene cluster typically comprises of a structural gene (*lan*A) and other genes that code for proteins responsible for posttranslational modification of the prepeptide (*lan*B and *lan*C, or *lan*M), proteolytic processing (*lan*P), transport (*lan*T), self immunity of the producer (*lan*I and *lan*EFG), and regulation of biosynthesis (*lan*R, *lan*K, and *lan*Q) **(Guder *et al.*, 2000; McAuliffe *et al.*, 2001)**. Propeptide compositions and the order and orientation of gene clusters vary considerably amongst the lantibiotics described till **(Siezen *et al.*, 1996)**. Also, not all lantibiotic producer strains differ in the complement of *lan* genes, which indicates considerable variety of posttranslational modifications made to the final peptide and differences in mechanisms of processing, immunity and regulation of the different lantibiotics **(Bierbaum *et al.*, 1996**). The knowledge about the genomic make up of lantibiotics is very important for bioengineering these peptides in order to improve their antibacterial action resistant pathogens. GenBank accession numbers of prominent lantibiotics from milk isolates has been given in Table 3.

Table 3 Genetic sequences of prominent lantibiotics from milk isolates

Lantibiotic	GenBank Accession Number	Reference
Nisin A	HM219853	Parapouli *et al.*, 2013
Nisin Z	X61144	Mulders *et al.*, 1991
Lacticin 3147	AE001272	Ryan *et al.*,1996
Lacticin 481	WP_032489363	Sahl *et al.*, 1995
Plantaricin W	AY007251	Holo *et al.*, 2001
Macedocin	DQ835394	Papadelli *et al.*, 2007
Bovicin HJ50	AY173079	Xiao *et al.*,2004

APPLICATIONS OF LANTIBIOTICS OBTAINED FROM MILK ISOLATES

Lantibiotics have unique structural chemistry and post translational modifications, which confer many desirable properties such as broad spectrum of antibacterial activity, small size, low molecular weight, thermostability and resistance to most proteolytic enzymes. This makes them excellent candidates for development of a vast array of applications in areas of food and therapeutics. Over the years, numerous studies have been conducted to define new areas of applications of lantibiotics produced by strains commonly found in milk. The advantage of milk-derived antibiotics lies in the fact that most of the strains are GRAS (generally regarded as safe) thereby addressing the safety aspects of these molecules. Also, since they are derived from milk microbes, milk can be employed as delivery system for use of lantibiotics either in food applications or as medicines.

Food applications

The broad spectrum antimicrobial activity of milk-isolate lantibiotics particularly against food borne pathogens has been useful in studying the use of these peptides as food preservatives. Since its discovery in 1928, nisin has been used for decades in the food industry and is the only FDA approved commercially produced lantibiotic. It is sold in more than 40 countries and was added to the positive list of food additives by the EU as additive number E234 (EEC 1983). Owing to its GRAS status, it has been used as an effective and safe food preservative in processed dairy products, canned fruits and vegetables **(Delves-Broughton, 1990)**. Application of nisin or nisin-producing starter cultures has been well documented in cheese products to control spoilage bacteria and food-borne pathogens **(Thomas and Delves-Broughton, 2001; Sobrino-López and Martín-Belloso, 2008)**. Inhibition of *Clostridium tyrobutyricum* by nisin producing *L. lactis* strains in hard and semi-hard cheeses prevents excess gas formation and formation of defective cheese due to late blowing. It has been also used to control *Listeria* sp. in whey and cheese **(Samelis *et al.*, 2003)**.

Apart from nisin, other lantibiotics of milk isolates have been evaluated for their preservative actions. Lacticin 3147-producing strain can work as biopreservatives for the control of *L. monocytogenes* in cheese, **(O'Sullivan *et al.*, 2006)** or to inhibit clostridia that causes late loss (Martínez-Cuesta et al. 2010). Lacticin 3147-producing dairy isolates can be employed as starters in cheese making **(Coakley *et al.*, 1997; O'Sullivan *et al.*, 2003)**. Since it is heat stable, a spray dried lacticin 3147-based powder formulation has successfully used as an antilisterial biopreservative for dairy products **(Morgan *et al.*, 2001; Guinane *et al.*, 2005)**. Lacticin 481-producing *L. lactis* strains have been used as flavor enhancers in cheese processing **(Garde *et al.*, 2007; Ávila *et al.*, 2007)**. Macedocin, produced by S. *macedonicus* is active against many food pathogens including *Bacillus, Clostridium* and *Listeria*, suggesting the potential of use of macedocin-producing strains as food starter cultures **(De Vuyst and Tsakalidou, 2008)**. The biopreservative aspects of other milk lantibiotics that inhibit food pathogens can be further explored for food preservation.

Biotherapeutic applications of lantibiotics

Indiscriminate use of antibiotics has led to the emergence of multi drug resistant bacterial pathogens and this mandates the discovery of new antimicrobials. In this aspects lantibiotics offer great potential as antibiotic alternatives since their structure and chemical makeup is unique and unusual and most of these peptides have been studied to have broad spectrum activities against many Gram positive pathogens including methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin intermediate *S. aureus* (VISA), vancomycin resistant enterococci (VRE), *Streptococcus pneumoniae* and *Clostridium difficile*, amongst others **(Cotter *et al.*, 2013)**. There are several reports on the in vitro potency of lantibiotics against nosocomial pathogens **(Piper *et al.*, 2009; Field *et al.*, 2015)**. Recently, techniques such as LC-UV-MS dereplication coupled with bioautography have been used to identify new strains that produce lantibiotics such as microbisporicin which has commercial potential against antibiotic resistant strains **(Carrano *et al.*, 2015)**.

Biomedical applications of nisin have reviewed in details recently **(Shin *et al.*, 2016)**. Nisin by itself or in combination with conventional antibiotics, such as vancomycin or ciprofloxacin has been shown to be effective against MRSA strains in several studies **(Brumfitt *et al.*, 2002; Dosler and Gerceker, 2011; Singh et al., 2013)**. In another study it was seen that nisin exhibited bactericidal effects against a diverse panel of Gram-positive bacteria, including MRSA, VRE and *S. pneumoniae* **(Severina *et al.*, 1998)**. In addition, a nisin producing L. lactis strain was shown to reduce the intestinal colonization of VRE in a mouse infection model **(Millette *et al.*, 2008)**. Nisin is also effective against biofilm formation on medical devices **(Okuda *et al.*, 2013)** and formation of biofilm by MRSA Xen 31 strain **(Ahire and Dicks, 2015)**. Since it is highly effective against Staphylococcus species, nisin also has the potential as a veterinary bactericidal agent particularly in the treatment of mastitis caused by Staphylococcus in dairy cows **(Cao *et al.*, 2007; Wu *et al.*, 2007)**.

Apart from its efficacy against pathogens causing infectious diseases, nisin has several applications in oral health as an anticariogenic agent **(Tong *et al.*, 2010)** and an anti-biofilm lantibiotic **(Shin *et al.*, 2015)**. The cytotoxic and tumerogenic properties of nisin specifically pertaining to skin cancer have also been studied and it was found that nisin inhibited tumerogenesis in *in vivo* models **(Joo *et al.*, 2012)**. Lacticin 3147 is the other lantibiotic of milk isolate that has been shown to have biotherapeutic potential. It has been studies in the treatment of bacterial mastitis and infection caused by *Staphylococcus* and enterococci including vancomycin resistant enterococci (VRE) and skin conditions like acne **(Galvin *et al.*, 1999; Lawton *et al.*, 2007)**. This indicates to the vast area of research still unexplored in the field of lantibiotics in biomedical applications.

CONCLUSIONS AND FUTURE PROSPECTIVES

Milk forms an important part of the diet of most of the population globally and is the chief source of nutrients for people of developing nations. In this aspect beneficial milk derived compounds are especially important as they can be incorporated easily into the dietary regime. As the list of lantibiotics produced by milk isolates continues to grow, it is evident that this group of antimicrobials is diverse in terms of their structure, genetics and mode of action. This provides scope for the discovery of new structural elements and functionalities and consequently, new applications, be it in the area of food preservation or as antimicrobials targeted towards more resistant pathogens. Genetic engineering and protein engineering can be exploited in the construction of novel lantibiotic variants with desirable traits. Database mining and PCR analysis of genomic DNA of milk isolates could be employed in discovery of new lantibiotics and extend the range of applications and development of alternatives for antimicrobial drugs.

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