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Review Article

A Novel Bacteriocins Inhibited More Number of Pathogens: A Review

Mojess Vadlapudi, Mary Sandeepa G*

Department of Biotechnology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India

ABSTRACT

Bacteriocins are a diverse group of bacterial antimicrobial peptides. Bacteriocins containing a string of amino acids, hence these are called as a proteins, these are produced by wide range of probiotics. Bacteriocins having the ability to inhibit the growth of disease causing microorganisms. Bacteriocins destruct the plasma membrane potential through leakage of cellular contents (ions, ATP), and formation of a pore in the plasma membrane. Regarding to bactibase website from 1992 to until now 442 bacteriocins were isolated, purified and characterized, in these 442 only few bacteriocins having the ability to show antagonistic activity on high number of disease causing microorganisms. In this review mainly focus on which bacteriocins are produced from which bacterial species and what are the methods were used to purify that bacteriocins. Many researchers were used ammonium sulphate precipitation method for purification of bacteriocins, in this review several methods were discussed for purification of a novel bacteriocins. For example variacin it is a bacteriocin, it was isolated from the *Micrococcus* variants, it have the ability to show antagonistic activity on 26 disease causing microbes that are *Clostridium botulinum*, *Bacillus cereus*, *Enterococcus faecalis*, etc. In this review we discussing about only seven bacteriocins about their purification methods, produced organisms and inhibitory activity.

Keywords: Variacin; Subpeptin JM4-B; Thuricin-S; Divergicin M35; Bacteriocin

INTRODUCTION

Bacteriocins are antimicrobial peptides, proteins or complex proteins produced by different microbial species. Enterocin TW21 is a novel bacteriocin, isolated first time from the *Enterococcus faecium* D081821 strain, to confirm a novel bacteriocin before need to study detailed about structure of proteins, why because of amino acids are present in proteins like a strings and also find out bacteriocin was encoded with which gene [1]. Listeriosis caused by *Listeria monocytogenes* is a bacterial infection with a High Case-Fatality Rate (HCFT) of approximately 20% to 30%, it is occurs mainly in elderly, neonates, immune compromised patients, this gram-positive intracellular bacterium is widespread in the natural environment, *E. faecium* bacteriocins shows antagonistic activity against *Listeria monocytogenes* hence in this reviews provides information about how to purify and characterize the *Enterococcus faecium* bacteriocins then these are useful for antagonistic activity of against *Listeria monocytogenes* [2].

Lactococci producing the broad-spectrum antimicrobial peptide lacticin 3147 fail to confer protection against *Listeria monocytogenes* infection in a mouse model, despite the efficacy of the bacteriocin against this pathogen *in vitro*. Diverse groups of probiotics are produced bacteriocins abundantly, bacteriocins are ribosomally synthesised (protein synthesis occurs in ribosomes) antimicrobial peptides produced by mainly probiotics. The majority of probiotics is use today includes species of Lactic Acid Bacteria (LAB), bacteriocins are acts as antimicrobial compounds or inhibit pathogenic microbial growth. Bacteriocins function as signaling peptides, either signaling other bacteria through quorum sensing and bacteria across talk within microbial communities [3]. Bacteriocin carnocin U149 was isolated from *Carnobacterium* species, carnocin U149 has a bactericidal mode of action, it was shown to be heat tolerant and stable between PH^2 and 8 [4]. Bovicin HJ 50 is a novel lantibiotic produced by *Streptococcus bovis* HJ50, bovicin HJ50 was active against *Lactobacillus curvatus* LTH1174, *Bacillus subtilis* AS1.1087, *Bacillus megaterium* AS1.941, *M. flavus*

Correspondence to: Sandeepa MG, Department of Biotechnology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India; E-mail: deepavsu2013@gmail.com

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NCIB8166, *Leuconostoc dextranicum* 181 and *Leuconostoc mesenteroides* AS1.2. MS analysis of bovicin HJ50 reduced with DTT indicated that bovicin HJ50 contains a disulfide bridge. However, when assayed in the presence of DTT, the titer of bovicin HJ50 against *M. flavus* NCIB8166 was neither decreased nor increase. Bovicin HJ50 has two thioester bridges and a disulfide bridge, this would give a peptide with a calculated molecular mass of 3429.96 Da [5]. A novel bacteriocin, lacticin Z, produced by *Lactococcus lactis* QU 14 isolated from a horse's intestinal tract was identified. Lacticin Z is a novel bacteriocin was produced by *Lactococcus lactis* QU isolated from a horse's intestinal tract. Lacticin Z was active against *Bacillus subtilis* JCM 1465, *Bacillus circulons* JCM 2504, *Bacillus coagulans* JCM 2257, *Lactobacillus plantarum* ATCC 14917, *Lactobacillus* brevis JCM 1059, *Streptococcus bovis* JCM 5802, *Escherichia coli* JM109, lacticin Z is a newly identified lactococcal bacteriocin with some unusual characteristics, such as absence of a leader sequence and alkali stability, lacticin Z can be an advantage in alternative or complementary use with other LAB bacteriocins. Some bacteriocins, class, producer organisms and bacteriocin acts against which pathogenic microoraganisms was given below Table 1.

Table 1: Bacteriocins from in the year of 2000 onwards, this literature was collected by bactibase database website.

S. No	Bacteriocin	Producer organism and year	Class
1	Lacticin 3147 A2	*Lactococcus lactis* IFPL105 2000	Lantibiotic
2	Coagulin A	*Bacillus coagulans* I4 2000	Lantibiotic
3	Enterocin 1071A	*Enterococcus faecalis* 2000	Lantibiotic
4	Plantaricin 1.25 beta	*Lactobacillus plantarum* 2000	Lantibiotic
5	Bioticin B	*Clostridium botulinum* 213B 2000	Lantibiotic
6	Microcin H47	2001	Lantibiotic
7	Lactococcin MMFII (novel)	*Lactococcus lactis* MMFII, 2001	IIa
8	Ruminococcin A	*Ruminococcus gnavus* 2001	Lantibiotic
9	Serracin-P	2002	
10	Mundticin KS	*Enterococcus mundtii* NFRI 2002	Lantibiotic
11	Nalocin C8	*Halobacterium* AS7092 2003	Lantibiotic
12	Streptin	*Strepococcus pyogens* 2003	Lantibiotic
13	Plantaracin 423	*Saccharomyces cervisiae* 2003	
14	Lactacin -F LafX	*Lactobacillus johnsonii* NCC 2004	IIa
15	Enterocin CRL35	NH2-terminal sequence 2004	Lantibiotic
16	Haloduracin hal alpha	A two-component lantibiotic 2006	Lantibiotic
17	Salivaricin A3	*Streptococci* 2006	
18	Divergin A	*Carnobacterium divergens* 2006	Lantibiotic
19	Curvalicin-28a, 28 b and c	*Lactococcus curvatus* CWB1-B28 2009	Lantibiotic
20	Hominicin	*Streptococcus hominis* MBBL 2010	Lantibiotic

In the above given Figure 1 total five flavonoids were isolated in the year of 2000, *plantarum* 1.25 beta bacteriocin was isolated from *Lactobacillus plantarum*, enterocin 1071A was isolated from the *Enterococcus* BFF 1071, bioticin B was isolated from the *Clostridium botulinum* strain 213B, lacticin 3147A2 was isolated from the *Lactococcus lactis* IFPL 105, coagulin A was isolated from the *Bacillus coagulans*. In the year of 2001 three bacteriocins were isolated, ruminococcin A was isolated from *Ruminococcus*

gnavus, *Lactococcin* MMFII (novel bacteriocin) was isolated from the *Lactococcus lactis* and microcin H47. In the year of 2002 two bacteriocins were isolated, mundticin was isolated from *Enterococcus mundticin* NFRI 7393 and another bacteriocin is serracin P. In the year of 2003 three bacteriocins were isolated, halocin C8 was isolated from the *Halobacterium* strain as7092, plantaricin 423 was isolated from the *Saccharoyces cervisiae*, streptin was isolated from the *Streptococcus pyogens*. In the year of 2004 two bacteriocins were isolated, lacticin F (laf X) and lacticin F (laf A) were isolated from the *Lactobacillus johnsonii*, and another one is enterocin CRL35. In the year of 2006 three bacteriocins were isolated, divergin A was isolated from the *Carnobacterium divergens*, salivaricin A3 was isolated from the human saliva and another bacterions are haloduracinn hal alpha and haloduracin hal beta were isolated. In the year of 2009 curvalicin-28a was isolated from the *Lactobacillus curvatus*. In the year of 2010 hominicin was isolated from the *Staphylococcus hominis* MBBL.

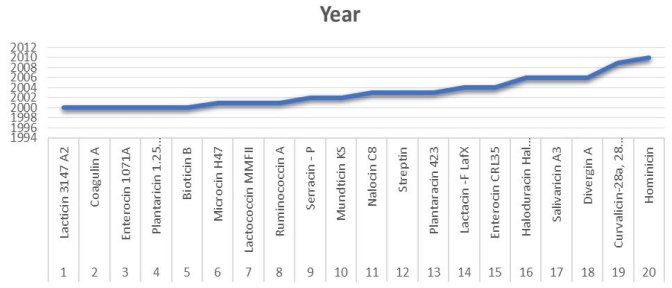

Figure 1: Bacteriocins are isolated and purified and characterized from 2000 to 2010.

LITERATURE REVIEW

Methods for purification of novel bacteriocins

From 1992 to until now bacteriocins were purified and produced from 442 microbial strains, only few bacteriocins were able to inhibit the growth of disease causing microorganisms. That bacteriocins are as follows (bactibase data base website). Subpeptin JM4-B bacteriocin was produced by *Bacillus subtilis* JM4, these bacteriocin have the ability to inhibit the growth of 13 microorganisms are as follows. *Salmonella*, *Bacillus cereus*, *Bacillus megaterium*, *Lactobacillus viridescens*, *Micrococcus flavus*, *Corynebacterium glutamicum*, *Enterobacter cloacae*, *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Pediococcus acidolacti Spirillum cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa*. Subpeptin JM4-B bacteriocin was purified by following methods, ammonium sulphate precipitation, sequential SP-sepharose fast flow, sephadex G-25 and C18 reverse phase chromatography [6]. Thuricin-s bacteriocin was produced from the *Bacillus thuringenesis*, it having the ability to inhibit 12 microorganisms growth, 12 microorganisms are as fallows, *Listeria monocytogenes*, *Bacillus subtilis*, *Enterobacter cloacae*, *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Pediococcus acidolactici*, *Streptococcus* thermophilus, *Spirillum cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa*. Thuricin-s purified through high performance liquid chromatography, molecular mass determined by mass spectrometry ESI-TOF-MS, is 31.37.61 Da [7]. Mutacin-H-29B bacteriocin was produced from the *Streptococcus mutans*, it having the ability to inhibit the growth of 15 microorganisms are as follows, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus*, *Peptostreptococcus micros*, *Pediococcus acidilactici*, *Clostridium sporogenes*, *Corynebacterium diptheriae*, *Actinomyces*, *Garnerella vaginelis*, *Propionibacterium acnes*. *Listeria monocytogenes*, *Mycobacterium smegmatis*. This mutacin S was purified by the hydrophobic chromatography from a liquid preparation consisting of cheese whey permeate (6% w/v) supplemented with yeast extract (2%) and $CaCo_3$ (1%), molecular mass of purified peptide was evaluated at 3246.08+0.1 Da by MALDI-TOF MS [8]. Butyrivibriocin OR79 bacteriocin was produced from the *Butyrivibrio fibrisolvens*, it having the ability to inhibit the growth of 5 microorganisms, are as fallows, *Butyrivibrio fibrisolvens*, *Butyrivibrio crossotus*, *Clostridium clostridime*, *Lachnospira multiparus*. *Ruminococcus flavefaciens*. Butyrivibriocin OR79 bacteriocin was purified by ammonium sulphate and acidic precipitation, reverse phase chromatography, and high-resolution gel filtration [9]. Variacin bacteriocin was produced from *Micrococcus* variants, it having the ability to inhibit the growth of 26 microorganisms are as fallows, *Lactobacillus helveticur*, *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus delbrueckii*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus*, *Lactococcus lactis*, *Micrococcus varians*, *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria innocua*, *Listeria monocytogenes*, *Clostridium spp.*, *Staphylococcus aureus*, *Staphylococcus carnosus*, *Staphylococcus saprophyticus*, *Staphylococcus* sp-*Bacillus subtilis*, *Bacillus cereus*, *Bacillus pumilis*. Variacin bacteriocin purification process, elution was done with 50% acetonitrile, 0.1% trifluoroacetic acid [10]. A novel divergicin 750 was produced from the *Carnobacterium* divergens, it having the ability show antimicrobial activity on four pathogens are as fallows, *Carnobacterium*, *Enterococcus*, *Listeria monocytogenes*, *Clostridium perfrigens*. Divergicin 750 was purified by ammonium sulphate precipitation and sequential S-sepharose, hydrophobic interaction and reversed phase chromatography, peptide sequence, 3447.7 agreed well with that obtained by mass spectrometry [11]. Divergicin M35 bacteriocin was produced by *Carnobacterium* divergens, it shows antimicrobial activity on nine microorganisms are as follows, *Listeria monocytogens*, *Listeria seeigeri*, *Listeria welshimeri*, *Listeria gyari*, *Listeria murrayi*, *Listeria ivanovii*, *Listeria innocua*, *Carnobacterium divergens*, *Carnobacterium piscicola* (Table 2). Divergicin M35 bacteriocin was purified by C18-Sep-pack column and reverse pahse-high pressure liquid chromatography, divergicin M35 had a molecular mass of 4518.75 Da as determined by mass spectrometry (Table 3). The amino acid sequence of divergicin M35 was found to consist of 43 amino acids with four cysteine residues [12]. Plantaricin K bacteriocin produced by *Lactobacillus plantarum*, it shows antimicrobial activity on ten microorganisms are as fallows, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus sakei*, *Lactobacillus viridescens*, *Pediococcus pentosaceus*, *Carnobacterium piscicola*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus curvatus* [13].

Table 2: Bacteriocins produced from different microorganisms.

Bacteriocin	Purification methods	Shows antagonistic activity on different microbes
Subpeptin JM4-B (WU SM, Sun, et al.)	Ammonium sulphate precipitation, sequential SP-sepharose fast flow, sephadex G-25 and C18 reverse-phase chromatography.	*Salmonella, Bacillus cereus, Bacillus megaterium, Lactobacillus viridescens, Micrococcus flavus, Corynebacterium glutamicum, Enterobacter cloacae, Lactobacillus acidophilus, Lactobacillus lactis, Pediococcus acidolacti- Spirillum cholerae, Shigella flexneri, Pseudomonas aeruginosa.*
Thuricin-S (Sonia Chehimi, et al.)	Purified through high performance liquid chromatography, molecular mass determined by mass spectrometry ESI-TOF-MS, is 31.37.61 Da.	*Listeria monocytogenes, Bacillus subtilis, Enterobacter cloacae, Lactobacillus acidophilus, Lactobacillus lactis, Pediococcus acidolactici, Streptococcus thermophilus, Spirillum cholerae, Shigella flexneri, Pseudomonas aeruginosa.*
Mutacin-H-29B (C. Nicolas, H. Morency, et al.)	Hydrophobic chromatography from a liquid preparation consisting of cheese whey permeate (6% w/v) supplemented with yeast extract (2%) and $CaCo_3$ (1%).	*Micrococcus luteus, Staphylococcus aureus, Streptococcus, Peptostreptococcus micros, pedio coccus acidilactici, Clostridium sporogenes, Corynebacterium diptheriae, Actinomyces viscosus, Garnerella vaginelis, Propionibacterium acnes. Listeria monocytogenes, Mycobacterium smegmatis.*
Butyrivibriocin OR79 (M.L. Kalmokoff, et al.)	Ammonium sulphate and acidic precipitation, reverse-phase chromatography, and high-resolution gel filtration.	*Butyrivibrio fibrisolvens, Butyrivibrio crossotus, Clostridium clostridime, Lachnospira multiparus, Ruminococcus flavefaciens.*
Variacin (D. Pridmore, et al.)	Variacin bacteriocin purification process, elution was done with 50% acetonitrile, 0.1% trifluoroacetic acid.	*Lactobacillus helveticur, Lactobacillus bulgaricus, Lactobacillus lactis, Lactobacillus delbrueckii, Lactobacillus sakei, Lactobacillus curvatus, Leuconostoc mesenteroides, Streptococcus thermophilus, Lactococcus lactis, Micrococcus varians, Enterococcus faecalis, Enterococcus faecium, Listeria innocua, Listeria monocytogenes, Clostridium sp, Staphylococcus aureus, Staphylococcus carnosus, Staphylococcus saprophyticus, Staphylococcus sp-Bacillus subtilis, Bacillus cereus, Bacillus pumilis.*
Divergicin 750 (Askild Hoick, et al.)	Divergicin 750 was purified by ammonium sulphate precipitation and sequential S-sepharose, hydrophobic interaction and reversed phase chromatography.	*Carnobacterium, Enterococcus, Listeria monocytogenes, Clostridium perfrigens.*
Divergicin M35	Divergicin M35 bacteriocin was purified by C_{18}-Sep-pack column and reverse pahse-high pressure liquid chromatography.	*Listeria monocytogens, Listeria seeigeri, Listeria welshimeri, Listeria gyari, Listeria murrayi, Listeria ivanovii, Listeria innocua, Carnobacterium divergens, Carnobacterium piscicola.*

Table 3: Bacteriocins produced from different microorganisms.

Bacteriocins	Anti-microbial activity on	Year	Producer organism
Variacin	26 microorganisms	1996	*Micrococcus varians*
Mutacin-H-29B	15 microorganisms	2006	*Streptococcus mutans*
Subpeptin JM4-B	13 microorganisms	2005	*Bacillus subtilis*
Thuricin-S	12 microorganisms	2007	*Bacillus thuringenesis*

Divergicin M35	08 microorganisms	2004	*Carnobacterium divergens*
Butyrivibriocin OR79	05 microorganisms	1999	*Butyrivibrio fibrisolvens*
Divergicin 750	04 microorganisms	1996	*Carnobacterium divergens*

Methods for structural characterization of bacteriocins

Literature was collected from bactibase website structural characterization. Different bacteriocins were structurally characterized by different methods as follows. Cinnamycin bacteriocin produced from the *Streptoverticilium griseoverticulatum*. This bacteriocin structurally characterized by 2DDE NMR spectroscopy, it having the ability to show antiquorum sensing activity on herpes simplex virus. Actagardine it is characterized by through 1AJI NMR spectroscopy, produced from the *Actinoplanes liguriae* (gram-positive), it acts against on Streptococci, *Streptococcus pyogens*, it is considered as a lantiobiotic class. Mersacidin bacteriocin was characterized by 1QOW NMR spectroscopy, 1MQX NMR spectroscopy, 1MQY NMR spectroscopy, IMQ2 NMR spectroscopy. Mersacidin bacteriocin was produced from the *Bacillus* species (strain-HIL-Y85), it is considered as a lantibiotic type B class. Epidermin bacteriocin resolved by IG5Q NMR spectroscopy, it is considered as a lantibiotic class. Nukacin Isk bacteriocin structure resolved by 5Z5Q NMR, 5Z5R NMR spectroscopy, it was produced by *Staphylococcus warneri*, it considered as a lantibiotic class. It acts against on *Bacillus subtilis* JCM 1465 and *Lactobacillus* sakei JCM 1157. Plantaricin bacteriocin was structurally characterized by 2KHG NMR spectroscopy it was produced by *Lactobacillus plantarum* (gram-positive bacteria) [14].

DISCUSSION

Variacin bacateriocin it shows antimicrobial activity on 26 microorganisms, it shows activity against gram-positive food spoilage bacteria [15]. *Lactobacilli delbrueckii* is usually considered as nonpathogenic, but although it has been described as the causative microorganism of urinary tract infection in elderly women and male adult (Table 4). Botulism is a neuroparalytic disease in humans and animals resulting from the actions of neurotoxins produced by *Clostridium botulinum* and rare strains of *Clostridium butyricum* and *Clostridium baratii*. Botulinum-producing organisms are dispersed widely throughout the world in soils and sediments and the intestine of animals [16]. The pathogenicity of *B. cereus*, whether intestinal or non-intestinal, is intimately associated with tissue-destructive/reactive exoenzyme production, a 17-year-old neutropenic patient with acute lymphoblastic leukemia developed *B. cereus* bacteremia traced to drinking lukewarm tea prior to her bacteremic episode [17], *Bacillus cereus* growth inhibited by variacin bacteriocins [18]. *Enterococcus* faecalis is a gram-positive bacterium that can cause a variety of nosocomial infections of which urinary tract infections are the most common [19]. *Staphylococcus saprophyticus* is a gram-positive, coagulase-negative, non-hemolytic coccus that is a common cause of uncomplicated Urinary Tract Infections (UTIs), particularly in young sexually active females. *S. saprophyticus* is the second most common cause of community-acquired urinary tract infections, after *Escherichia coli* [20].

Table 4: Bacteriocins inhibited microorganisms.

Growth inhibited by bacteriocins	Causing diseases	Bacteriocin
Lactobacillus delbruecki	Urinary tract Infection in elderly women and male adult.	Variacin
Clostridium botulinum	Neurotoxins were produced.	Variacin
Bacillus cereus	Tissue destruction, acute lymphoblastic leukemia.	Variacin
Enterococcus faecalis	Urinary tract infections.	Variacin
Staphylococcus saprophytic	Urinary tract infections in young sexually active females.	Variacin
Listeric monocytogenes	Invasive diseases CNS infections bacterial meningitis.	Divergicin M 35
L. Monocytogenes *L. Ivaanovii*	Gastroenteritis bacteremia in males. Hepatic carcinoma in males.	Divergicin M 35

Listeria monocytogenes is a gram-positive, facultative intracellular bacterium that causes invasive diseases in humans and animals, especially Central Nervous System (CNS) infections. *Listeria monocytogenes* usually ranks as the third or fourth most common cause of bacterial meningitis in North America and Western Europe. Two species of *Listeria* are pathogenic; *L. monocytogenes* infects humans and animals, and *L. ivanovii* has been considered to infect ruminants only. We report *L. ivanovii*–associated gastroenteritis and bacteremia in a man.

CONCLUSION

This isolate was indistinguishable from prototypic ruminant strains. *L. ivanovii* is thus an enteric opportunistic human pathogen, it causes bacteremia in males and hepatic carcinoma in females.

Meningitis was caused by *Micrococcus luteus*. *Staphylococcus aureus* is one of the most frequent worldwide causes of morbidity and mortality due to an infectious agent. This pathogen can cause a wide variety of diseases, ranging from moderately severe skin infections to fatal pneumonia and sepsis of the 200 *Clostridium* spp. known to exist, approximately 30 have been associated with human disease. *Clostridium sporogenes* bacteremia secondary to lower limb cellulitis and osteomyelitis in an immunocompetent patient.

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