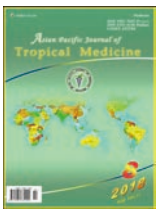

IF: 1.634

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org

doi:



# Potential applications of lactic acid bacteria and bacteriocins in anti-mycobacterial therapy

Anbarasu Sivaraj✉, Revathy Sundar, Radhakrishnan Manikkam, Krupakar Parthasarathy, Uma Rani, Vanaja Kumar

*Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai-600119. Tamil Nadu, India*

ARTICLE INFO

*Article history:*
Received 18 November 2017
Revision 15 March 2018
Accepted 2 April 2018
Available online 1 August 2018

*Keywords:*
Bacteriocin
Lactic acid bacteria
Antimycobacterial peptides
Tuberculosis
Immunomodulation
Hybrid bacteriocin

ABSTRACT

Tuberculosis (TB) is a communicable disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). WHO estimated that 10.4 million new (incident) TB cases worldwide in year 2016. The increased prevalence of drug resistant strains and side effects associated with the current anti-tubercular drugs make the treatment options more complicated. Hence, there are necessities to identify new drug candidates to fight against various sub-populations of *M. tuberculosis* with less or no toxicity/side effects and shorter treatment duration. Bacteriocins produced by lactic acid bacteria (LAB) attract attention of researchers because of its "Generally recognized as safe" status. LAB and its bacteriocins possess an effective antimicrobial activity against various bacteria and fungi. Interestingly bacteriocins such as nisin and lacticin 3147 have shown antimycobacterial activity *in vitro*. As probiotics, LAB plays a vital role in promoting various health benefits including ability to modulate immune response against various infectious diseases. LAB and its metabolic products activate immune system and thereby limiting the *M. tuberculosis* pathogenesis. The protein and peptide engineering techniques paved the ways to obtain hybrid bacteriocin derivatives from the known peptide sequence of existing bacteriocin. In this review, we focus on the antimycobacterial property and immunomodulatory role of LAB and its metabolic products. Techniques for large scale synthesis of potential bacteriocin with multifunctional activity and enhanced stability are also discussed.

## 1. Introduction

Tuberculosis (TB) is known as one of the oldest communicable diseases in human and still a foremost cause of high death in the world. The etiological agent of tuberculosis, *Mycobacterium tuberculosis* (*M. tuberculosis*), which multiplies within macrophages. TB tends to impact more on poorest, migrant communities, young and weak children, immunocompromised people (HIV and aged) and people who have diabetes and cancer. World Health Organization (WHO) has estimated that over 10.4 million people have fallen ill with TB in which around 1.7 million people died in 2016. Further WHO estimates around 600 000 new cases with resistance to rifampicin, of which 490 000 had multiple drug resistant tuberculosis (MDR-TB) (WHO global tuberculosis report-2017). Therefore TB poses serious health problem around the world by way of increase in the rate of MDR- TB, extensive drug resistance (XDR-TB), HIV-TB, paediatric TB and latent TB. The latent tuberculosis infection is asymptomatic and not infectious, but it is at risk of progression to active disease at any point of time. TB treatment requires 6 to 8 months for newly diagnosed patients

✉First and corresponding author: Mr. Sivaraj Anbarasu, Scientist-B, Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai - 600119, India.
Tel: +91 44 24500646
Fax: +91 44 24500646
E-mail: anbuaras18@gmail.com
Foundation project: This work was supported by Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India and Indian Council of Medical Research (ICMR), New Delhi, India (Ref. No: 5/8/5/19/2014-ECD-I).



**How to cite this article:** Sivaraj A, Sundar R, Manikkam R, Parthasarathy K, Rani U, Kumar V. Potential applications of lactic acid bacteria and bacteriocins in anti-mycobacterial therapy-mini review. Asian Pac J Trop Med 2018; 11(8):453-459.

and 18 to 24 months for MDRTB patients. However, the treatment is ineffective for XDR-TB which complicates the treatment options with adverse side effects such as hepato toxicity that discourages both patients and providers.

Antimicrobial peptides such as bacteriocins have many advantages including less immunogenicity, specific affinity to bind on negatively charged prokaryotic cell envelope, and various modes of action[1]. Studies reported that the immunomodulation potential of lactic acid bacteria (LAB) and its metabolites show immune response towards macrophage enhancement by up-regulation and down-regulation of Th1 and Th2 cytokines respectively[2]. Antimicrobial peptides found in most living organisms usually consist of 20 to 60 amino acid residues, which are cationic, amphipathic and have a wide range of activity against microbes[3]. Antimicrobial peptides produced by bacteria are classified into two different types as ribosomally synthesized peptides or bacteriocins and non-ribosomally synthesized peptides which exhibit relatively narrow range of antimicrobial activity and broader antimicrobial activity respectively[4]. Marr *et al*.[5] reported that antimicrobial peptides are mainly bactericidal in nature which induce rapid killing of microbial pathogens and also reveal that an increased concentration is not required to fight against drug resistant strains, as compared to antibiotics. According to Riley and Wertz[6], most of bacteria (> 99%) produce at least one bacteriocin. Bacteriocins derived from LAB, are likely to enter into the pharmacopeia as oral or gastrointestinal antibiotics[7]. There are many reports on LAB producing bacteriocins which show prominent antimicrobial activity against wide range of microbial pathogens and also have strong probiotic potential. Hence, bacteriocin can act either as potent alternative or in synergy with antibiotics to enhance the therapeutic effects and also to decrease the prevalence of resistant strains[8]. Bacteriocins of LAB have all the advantages to be developed as peptide based drugs for multidrug resistant pathogens. Although the advantages of bacteriocins with respect to antimicrobial properties are enormous, the peptide can be hindered by high production costs and potency. Owing to the heterogeneous nature of bacteriocins, unique purification procedures have been considered for each producer strains[9,10].

Recently, the focus has been shifted to immunological functions of LAB with considerable attention on a promising strategy for health-promoting effects[11]. Probiotic LAB has been shown to have the capacity to boost the immunity against infections. According to WHO, the probiotics are described as, "Live microorganisms when administrated in adequate amounts, confer a health benefit on the host"[12]. The proteins secreted and released into the gastrointestinal environment by probiotics might mediate interactions with epithelial cells and immune cells[13]. In this article, research works pertaining to antimycobacterial activity and immunomodulatory property of LAB and its bacteriocins are reviewed. The protein and peptide engineering approaches for the preparation of bacteriocin derivatives with improved activity and stability are also discussed.

## 2. LAB and characteristics of bacteriocins

LAB possess various industrial applications in the dairy industry, pharmaceutical and special dietary applications[14]. LAB produces various compounds including organic acids, diacetyl hydrogen peroxide, bacteriocins, *etc*[15]. They also play a key role in maintaining healthy microbiota and have many benefits including managing diarrhoea, food allergies, inflammatory bowel diseases, gastrointestinal disorders and also possess the potential in the prevention of colon cancer[16-19]. Lactobacilli are known to be highly suitable vehicles for the delivery of compounds to the mucosa homeostasis[20].

Bacteriocins are extracellularly released peptides, which are produced by Gram positive (+) and Gram negative (-) bacterial species. Gram (+) bacteria, particularly LAB, produce bacteriocins in different sizes, structures and inhibitory spectra[21]. Bacteriocins of LAB are categorized into class I, class II, class III based on physicochemical properties. The class I bacteriocins are small peptides (<5 kDa) and also known as lantibiotics (lanthionine containing antibiotics), possess unusual post-translationally modified lanthionine or 3-methyllanthionine[22]. Class II bacteriocins are non-lantibiotics, which are relatively small (<10 kDa), heat stable and have fewer post-translational. They are subdivided into class IIa, class IIb, class IIc and class IId[23]. Class III bacteriocins are large molecular weight (>30 kDa), heat labile proteins. Since this class of bacteriocins are lytic enzymes rather than peptides, it was suggested to be excluded from group of bacteriocins and renamed as bacteriolysins. In contrast to antibiotics, bacteriocins from LAB are believed more natural and safe because of their presence in food items[24]. In recent years, bacteriocins of LAB have potential application in both food and pharmaceutical industries[25]. Nisin, produced from *Lactococcus lactis* subsp. *Lactococcus lactis* is the first bacteriocin that obtained regulatory approval by FDA for use in certain foods in 2005. They are also known for its ability to enhance food safety and increase health benefits[26]. Another bacteriocin, pediocin produced by *Pediococcus pentosaceus* also got approved later for their use in food industry[27].

Typically, bacteriocins form pores on cell wall of target pathogens, especifically in Gram (+) bacteria as they possess high anionic lipid contents in the membrane. The formation of pores in the membrane causes small intracellular components leakage which leads to cell death and the debauchery of the proton motive force[28]. Perez *et al*.[29] reported that the general cationic nature of bacteriocins plays a very important role in their initial interaction with the cell membrane of target strains. The negative charge of bacterial cell membranes and the positive charge of bacteriocin create an electrostatic attraction between them thereby facilitating the interaction of the molecules to the membranes. Due to the cationic nature of bacteriocin, the anionic lipids role in membrane binding has been emphasized. The binding of nisin (class I bacteriocin) to lipid II, which is necessary for bacterial cell-wall synthesis, results in the prevention of proper cell wall synthesis, thereby causing cell death. The nisin-lipid II molecule complex initiates membrane insertion at higher concentrations forming pores in the bacterial cell membrane. Thus, the binding of nisin to lipid II facilitates the preventive action involving cell wall synthesis and membrane pore formation[30,31]. Corr *et al*[32], demonstrated that *Lactobacillus salivarius* UCC118 produced bacteriocin *in vivo*, which protected mice against *Listeria monocytogenes* infection. The possible bactericidal mechanism of nisin on Gram (+) bacterial cell wall including mycobacteria is illustrated (Figure 1).

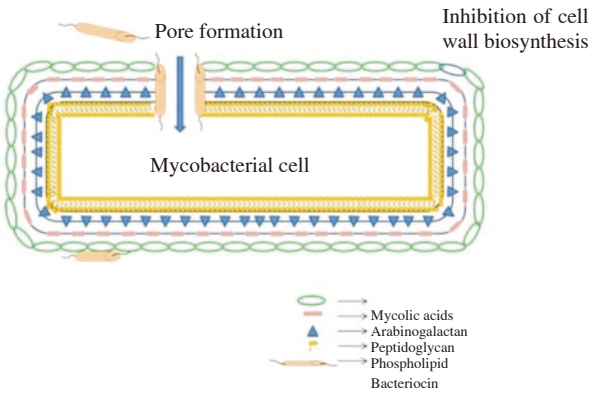


**Figure 1.** Bactericidal mechanism of nisin on cell wall of Gram positive bacteria including *Mycobacteria*.

## 3. Antimycobacterial activity of bacteriocins and LAB

The bacteriocins from LAB have potent activity against various *Mycobacterium* species. The LAB bacteriocin, nisin was tested against *Mycobacterium smegmatis* (*M. smegmatis*) at 10 μg/mL and the results showed that (97.7±2.0)% reduction in internal ATP and leakage of intracellular ATP[33]. Mota-Meira *et al*[34], have shown that nisin A and mutacin B-Ny266 (type A lantibiotics), have ability to kill a broad range of bacteria including *M. smegmatis*. Donaghy *et al*[35], reported that the cell free supernatant of *Lactobacillus paracasei* isolated from cheese has strongly inhibited the growth of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) *in vitro*. On treating sterile milk with this strain, MAP growth was completely undetectable up to 50 d. Bacteriocin of LAB isolated from Boza (Turkish beverage) was tested for antimycobacterial activity. Among the isolates, bacteriocin produced by *Lactobacillus plantarum* (*L. plantarum*) ST194BZ have shown activity against *M. tuberculosis* and growth was repressed up to 69% whereas *Lactobacillus paracasei* ST242BZ, *L. plantarum* ST414BZ and ST664BZ showed 50% of growth repression. In another study, *L. plantarum* ST202Ch, *L. plantarum* ST216Ch, *Lactobacillus sakei* ST153Ch, *Lactobacillus sakei* ST154Ch and *Enterococcus faecium* ST211Ch were isolated from Portuguese fermented meat products and bacteriocins produced from the isolates have significantly reduced the growth of *M. tuberculosis* by 38.3%, 48.6%,16.2%, 16.1% and 21.7% respectively[36,37]. Sosunov *et al*[38], reported that bacteriocin isolated from *Lactobacillus salivarius*, *Streptococcus cricetus* and *Enterococcus faecalis*, shown to have more promising antimycobacterial activity than equal rifampicin concentrations in an *in vitro* model. These bacteriocins were non-toxic for mouse macrophages with activity of >90 MIC at a concentration of 0.1 mg/L. They administered the bacteriocins as a complex with phosphatidylcholine-cardiolipin liposomes in TB infected mice model and have demonstrated its capacity to inhibit intracellular *M. tuberculosis* and to extend the survival of mice. James Carroll *et al*[39], showed that antimycobacterial activity of lacticin 3147 against *Mycobacterium kansasii*, MAP and *M. tuberculosis* H37Ra at $MIC_{90}$ values of 60.0 mg/L, 15.0 mg/L and 7.5 mg/L respectively. Whereas, nisin showed $MIC_{90}$ values of 60 mg/L for *Mycobacterium kansasii* and >60 mg/L for MAP and *M. tuberculosis* H37Ra. Hence, lacticin 3147 found as a more effective antimycobacterial peptide than nisin. Lantibiotics certainly possess sufficient potential for future therapies treating tuberculosis. A study demonstrated that nisin and lacticin 3147 arrest the mycobacterial lipid II moiety and suggest that inherent cell wall modifications do not provide lantibiotic resistance to *Mycobacteria*[40]. Bacteriocins of *Pediococcus pentosaceus* VJ13 exhibited activity against various pathogens including *M. smegmatis*. Zahir *et al*[41], reported that *Aerococcus* sp. ZI1 produces proteinaceous inhibitory substances which showed antagonistic effect against *M. smegmatis*. The process of developing a potential bacteriocin peptide library active against different mycobacteria and its characterization are illustrated in Figure 2. Breifly, the partially purified bacteriocins of LAB are screened for antimycobacterial activity against *M. tuberculosis* H37Rv, MDR *M. tuberculosis* and drug sensitive *M. tuberculosis* using Luciferase reporter phage assay. The active bacteriocin are further subjected to purification by HPLC methods. The lyophilized purified bacteriocins are subjected for anti-TB activity against the *M. tuberculosis* strains. The potential bacteriocin are characterized by LC-MS, peptide mass finger printing. Peptide library is created for each potential bacteriocin showing activity against different mycobacterial strains.

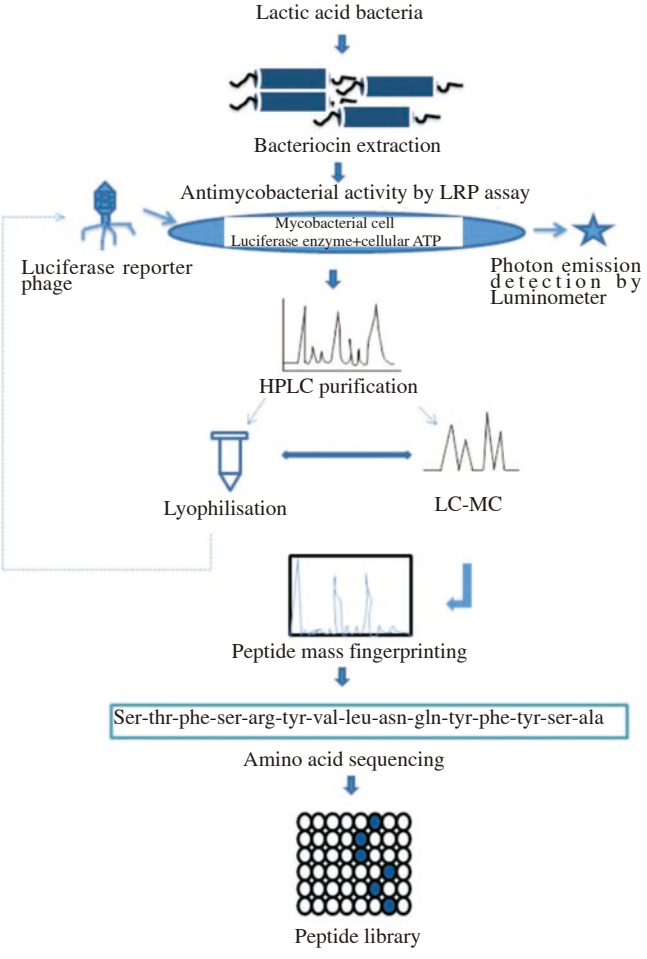


**Figure 2.** Schematic representation of characterization of bacteriocin screening for activity against *M. tuberculosis*, bacteriocin purification and proteomics analysis.

LAB have shown to be a natural effective antimicrobials in food industries that exert inhibitory activity against various microorganisms that cause food spoilage. Studies by Mariam[42,43], have reported that milk fermented with *Lactobacillus* starters has a pronounced antagonistic effect on the *Mycobacterium bovis* (*M. bovis*) BCG and also found undetectable growth of *M. tuberculosis* in the milk by day 7. It is believed that when the *Mycobacterium*-contaminated milk is fermented, the indigenous LAB confer protective effect. The study suggested that selected LAB may have potential applications as antimycobacterial agents. Macuamule *et al*[44], reported that long term fermentation of raw milk with LAB may inactivate *M. bovis* BCG present in milk. It was shown that during fermentation of milk, factors such as non-bacterial and heat-stable components as well as the LAB populations have played a major role in the bactericidal effect against *M. bovis* BCG.

## 4. Immunomodulatory effects of probiotic LAB and their metabolic products

LAB offer attractive opportunities for infectious disease treatment *vis*-à-*vis* their immune modulating capabilities[45]. *M. tuberculosis* replicates within macrophage, thereby inhibiting the maturation of phagosome which is involved in the elimination of *M. tuberculosis*. Autophagy is an immune response which targets bacteria thereby controlling the proliferation of *M. tuberculosis* in macrophages following its infection[46]. Activation of autophagy may also control the inflammation enabling the host immune response against *M. tuberculosis*. Hence, many tuberculosis therapies have been focused on the activation of autophagy with innovative approaches. TB infection itself relatively increases the level of Th2 cytokines and inhibits Th1 cytokines[2]. The interaction of LAB and their products with macrophages and T-cells can lead to the cytokines production[47]. A pilot study conducted by Suarez-Mendez *et al*.[48] for drug resistant therapy by administring IFN- γ as an immune adjuvant. IFN- γ activates autophagy which stimulates the delivery of mycobacteria to lysosomes[49,50]. Kato *et al*[51], demonstrated that male BALB/c mice received intraperitoneal injection of *Lactobacillus casei* (LC 9018) have shown the activation of macrophages and natural killer cells. Some strains of LAB have increased the production of reactive oxygen, nitrogen radicals, monokines of phagocytic cells. Studies demonstrated that the *Lactobacillus acidophilus* derived non-lipopolysaccharide component stimulates the IL-1 and TNF- α production[52,53]. LAB enhance the bactericidal ability of mononuclear phagocytes by increasing autophagy-inducing cytokine such as IFN- γ levels and by reducing IL-4 and IL-13 that is adequate to down-regulate the lung Th2 response, which is known to restrict autophagy[54]. The treatment with probiotic can modulate the immune responses in the lung which enhances the regulatory T cell response in the airway, emphasizing the potential therapeutics[55]. Noverr *et al*.[56], reported that cytokine profiles at the intestinal level and systemically were modulated by orally administered Lactobacilli. LAB can protect airway infection in host animals through an interaction of Peyer's Patches in the gut and enhance respiratory immunity indirectly[57]. LAB probiotics play a key role as immunomodulatory substances and activators of host defence pathway. Increasing evidences suggest that delivered probiotics regulate the immune responses in the respiratory system[58]. The peptidoglycan, polysaccharide, and teichoic acid of LAB cellwall have shown to possess immune-stimulatory properties[59].

Antimicrobial peptide helps in stimulation of innate immune response while reducing associated harmful inflammatory responses[60]. Mitsuma *et al*.[61], reported that pentapeptide (CHWPR) produced by *Bifidobacterium animalis* subsp. *lactis* BB-12 up-regulates the *c–myc* and *IL–6* genes in HL-60 cell line. Herawati *et al*[62], also reported that the bacteriocins isolated from *Lactobacillus acidophilus* were able to improve phagocytosis activity of macrophage. Chen *et al*.[63], showed that live LAB, heat-inactivated LAB or LAB-SCS were able to induce macrophages and show immunopotentiating activities, including the induction of tumour necrosis factor- α , interleukin-6 and NO.

## 5. Improvement of efficiency of bacteriocins and synthesis of hybrid bacteriocins–protein and peptide engineering approach

Several natural antimicrobial peptides which are isolated from natural sources have common characters among their chemical features, which may be linked with their biological activities. Thus, the penetration of the molecule into the target cells can be increased through the modification of molecular structures[64]. Many different processes have been applied to produce antimicrobial peptides in a cost-effective manner through advanced approaches like chemical synthesis, r-DNA technology, cell-free expression systems and transgenic animals or plants. All the processes offer a large production of material required for therapeutic use[65]. Bacteriocins identified with functional activity and sequence have been chemically synthesized in order to increase the scale of production and also to improve the thermal and cleavage stability. Many of the bacteriocins were synthesized by using a Wang resin and by sequentially adding N-Fmoc-protected amino acids by manual or automated synthesis[66]. For instance, Samar Lasta *et al*.[67], have synthesized the bacteriocin J46 by FMOC peptide synthesis. NMR characterization and biophysical studies are carried out for the synthesized peptides to determine the structural confirmation of the peptide in lipid or polar environment for understanding the mechanism of action. The advantages of chemical synthesis of bacteriocins are bulk production, short duration, combinatorial synthesis and peptide back bone engineering for hybrid stable peptides[68,69]. Bacteriocins with engineered functions or increased stability can be produced by combination of chemoenzymatic approach. This integrates the chemical biology (synthesis) followed by molecular biology (r-DNA technology and use of enzymes for modifications) or vice versa. In the first case, the bacteriocins are synthesized by FMOC synthesis followed by enzyme mediated addition of specific functional groups or linkages. Xinya *et al*.[70], described the total synthesis of a circular AS-48 bacteriocin with butelase 1 enzyme by the chemoenzymatic approach. Here the linear AS-48 peptide was synthesized using microwave stepwise synthesis followed by using an Aspargine specific butelase mediated cyclization. The advantage of this approach is that the circular bacteriocin produced, has the ability to withstand pasteurization and this has opened up an arena in the field of food preservation using

bacteriocin. In the second case, the bacteriocins are produced using recombinant DNA technology in *Escherichia coli* or other expression systems followed by the addition of specific functional groups by specific chemical reactions[71].

The synthetic bacteriocins have been shown to be more stable and absence of contaminating proteases than those which are produced from bacterial strains. Many studies have attempted to create bacteriocin variants with enhanced activity[72]. Fimland *et al*[73], have constructed four new hybrid bacteriocins from various pediocin-like bacteriocins by interchanging corresponding modules which are biologically active. All hybrid bacteriocins had significant bactericidal activity. The peptide's hinge region facilitates C-terminal of the bacteriocin insertion into membrane of cell, which leads to cell death through pore formation. James Carroll and Jim O'Mahony[74], have identified numbers of nisin variants with enhanced activity against *Streptococci*, *Staphylococci*, *Clostridium*, *Bacillus* spp, MRSA. Previous study by Carroll *et al*[75], reported that nisin variants such as K22T N20P and M21V have improved antimycobacterial activity against pathogenic mycobacteria.

An improved bacteriocin activity could be obtained by addition of disulphide bridge which results in rigidifying a specific conformation. Moreover, it also enhances the net positive charge of a bacteriocin which promotes the initial electrostatic interaction with the outer cell membrane of target[76,77]. Derksen *et al.*[78], explored the essential of N-terminal disulfide bridge for class IIa bacteriocins activity. The replacements of allylglycine, norvaline, and phenylalanine resulted in retention of leucocin A activity. Oppegard *et al.*[79], synthesized analogues of class IIb bacteriocin such as lactococcin G by replacement of N- and C-terminal residues with D-amino acids. The resulted anologues were less susceptible to exopeptidases without compromising on the activity. Tominaga and Hatakeyama[80], constructed improved version of pediocin PA-1 (Chimera EP) by fusing C-terminal half of pediocin PA-1 and N-terminal half of enterocin A, which showed increased activity against *Leuconostoc lactis*. Authors believed that the design of hybrid bacteriocins with broad spectrum of high specific antibacterial activity through fusing microcins (active on Gram-negative bacteria) and class IIa bacteriocins (Gram-positive bacteria). A novel recombinant hybrid peptide such as Ent35–MccV was designed by combining enterocin CRL35 and microcin V which displayed activity against entero-hemorrhagic *Escherichia coli* and *Lactobacillus monocytogenes*[23,81].

## 6. Conclusions

Due to adverse side effects, long duration and emergence of MDR *M. tuberculosis* and XDR *M. tuberculosis*, the current antimycobacterial drugs still exhibit many barriers for effective treatment to cure the disease. Hence, novel TB drugs from natural sources with non-toxic and shorter treatment durations are needed to target all sub-populations of *M. tuberculosis*. Bacteriocins of LAB exhibit broad spectrum of activity in targeting *M. tuberculosis* that can be developed as a leading molecule for the treatment of tuberculosis. Increasing evidences suggest that enhancement of immune response especially autophagy can control the proliferation of *M. tuberculosis* in macrophages following infection. In this regard, LAB and its metabolites have shown to impact on the immune system thereby enhancing macrophage activation. As LAB is considered "Generally recognized as safe", the LAB can be developed as probiotic supplements for the enhancement of autophagy to kill intracellular pathogens like *M. tuberculosis*. Synthesis and production of large quantity of bacteriocins with increased stability and enhanced activity from an identified peptide sequence of existing bacteriocin are possible with protein and peptide engineering techniques. It is known that multi-drug resistant variants of *M. tuberculosis* have emerged during inadequate tuberculosis treatment. This may be overcome by fusing sequences of two or more known bacteriocins into a new hybrid bacteriocin.

## Conflict of interest statement

The authors declare no conflict of interests.

## Acknowledgments

We are thankful to the management of Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India and Indian Council of Medical Research (ICMR), New Delhi, India (Ref. No: 5/8/5/19/2014-ECD-I) in the form of research grant.

## References

[1] Teng T, Liu J, Wei H. Anti-mycobacterial peptides: from human to phage. *Cell Physiol Biochem* 2015; **35**(2): 452-466.

[2] Ghadimi D, de Vrese M, Heller KJ, Schrezenmeir J. Lactic acid bacteria enhance autophagic ability of mononuclear phagocytes by increasing Th1 autophagy-promoting cytokine (IFN- γ ) and nitric oxide (NO) levels and reducing Th2 autophagy-restraining cytokines (IL-4 and IL-13) in response to *Mycobacterium tuberculosis* antigen. *Int Immunopharmacol* 2010; **10**(6): 694-706.

[3] Silva JP, Appelberg R, Gama FM. Antimicrobial peptides as novel anti-tuberculosis therapeutics. *Biotechnol Adv* 2016; **34**(5): 924-940.

[4] Ge J, Sun Y, Xin X, Wang Y, Pinga W. Purification and partial characterization of a novel bacteriocin synthesized by *Lactobacillus paracasei* HD1-7 isolated from Chinese sauerkraut juice. *Sci Rep* 2016; **6**: 19366.

[5] Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Curr Opin Pharmacol* 2006; **6**(5): 468-472.

[6] Riley MA, Wertz JE. Bacteriocins: evolution, ecology, and application. *Annu Rev Microbiol* 2002; **56**(1): 117-137.

[7] Rossi LM, Rangasamy P, Zhang J, Qui HQ, Wu GY. Research advances in the development of peptides antibiotics. *J Pharm Sci* 2008; **97**(3): 1060-1070.

[8] Arthur TD, Cavera VL, Chikindas ML. On bacteriocin delivery systems and potential applications. *Future Microbiol* 2014; **9**(2): 235-248.

[9] Pingitore E, Salvucci E, Sesma F, Nader-Macías ME. Different strategies for purification of antimicrobial peptides from lactic acid bacteria (LAB). In: A Méndez-Vilas. (ed.) *Communicating current research and educational topics and trends in applied microbiology*. Badajoz: Formatex; 2007, p.

557-568.

[10]Balciunas EM, Castillo Martinez FA, Todorov SD, Franco BDGDM, Converti A, Oliveira RPDS. Novel biotechnological applications of bacteriocins. *Food Control* 2013; **32**(1): 134-142.

[11]Chang C, Wang S, Chiu C, Chen S, Chen Z, Duh P. Effect of lactic acid bacteria isolated from fermented mustard on immunopotentiating activity. *Asian Pac J Trop Biomed* 2015; **5**(4): 281-286.

[12]Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? *Appl Environ Microbiol* 2012; **78**(1): 1-6.

[13]Sánchez B, Bressollier P, Urdaci MC. Exported proteins in probiotic bacteria: adhesion to intestinal surfaces, host immunomodulation and molecular cross-talking with the host. *FEMS Immunol Med Microbiol* 2008; **54**(1): 1-17.

[14]Konings WN, Kok J, Kuipers OP, Poolman B. Lactic acid bacteria: the bug of the new millennium. *Curr Opin Microbiol* 2000; **3**(3): 276-282.

[15]Yusuf MA. Lactic acid bacteria: bacteriocin producer: a mini review. *IOSR J Pharm* 2013; **3**(4): 44-50.

[16]Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**(9): 503-514.

[17]del Carmen S, de Moreno de LeBlanc A, Miyoshi A, Clarissa SR, Azevedo V, LeBlanc JG. Potential application of probiotics in the prevention and treatment of inflammatory bowel diseases. *Ulcers* 2011; **2011**: 1-13.

[18]Zhong L, Zhang X, Covasa M. Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World J Gastroenterol* 2014; **20**(24): 7878-7886.

[19]Borrero J, Chen Y, Dunny GM, Kaznessis YN. Modified lactic acid bacteria detect and inhibit multiresistant Enterococci. *ACS Synth Biol* 2015; **4**(3): 299-306.

[20]Havenith CEG, Seegers JFML, Pouwels PH. Gut associated lactobacilli for oral immunisation. *Food Res Int* 2002; **35**(2-3): 151-163.

[21]Yang SC, Lin CH, Sung CT, Fang JY. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. *Front Microbiol* 2014; **5**(241): 1-10.

[22]McAuliffe O, Ross RP, Hill C. Lantibiotics: structure, biosynthesis and mode of action. *FEMS Microbiol Rev* 2001; **25**(3): 285-308.

[23]Acuna L, Morero R, Bellomio A. Development of wide-spectrum hybrid bacteriocins for food biopreservation. *Food Bioproc Tech* 2011; **4**(6): 1029-1049.

[24]Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. I*nt J Food Microbiol* 2001; **71**(1): 1-20.

[25]Perez RH, Zendo T, Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. *Microb Cell Fact* 2014; **13**(Supplement 1): S3.

[26]Liu S, Han Y, Zhou ZJ. Lactic acid bacteria in traditional fermented Chinese foods. *Food Res Int* 2011; **44**(3): 643-651.

[27]Arthur TD, Cavera VL, Chikindas ML. On bacteriocin delivery systems and potential applications. *Future Microbiol* 2014; **9**(2): 235-248.

[28]Bennik MH, Vanloo B, Brasseur R, Gorris LG, Smid EJ. A novel bacteriocin with a YGNGV motif from vegetable-associated *Enterococcus mundtii*: full characterization and interaction with target organisms. *Biochim Biophys Acta* 1998; **1373**(1): 47-58.

[29]Perez RH, Perez MTM, Elegado FB. Bacteriocins from lactic acid bacteria: a review of biosynthesis, mode of action, fermentative production, uses, and prospects. *Phil Sci Tech* 2015; **8**(2): 61-67.

[30]Breukink E, Wiedemann I, van Kraaij C, Kuipers OP, Sahl HG, de Kruijff B. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. *Science* 1999; **286**(5448): 2361-2364.

[31]Wiedemann I, Breukink E, van Kraaij C, Kuipers OP, Bierbaum G, de Kruijff B, et al. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. *J Biol Chem* 2001; **276**(3): 1772-1779.

[32]Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CGM. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci U S A* 2007; **104**(18): 7617-7621.

[33]Montville TJ, Chung HJ, Chikindas ML, Chen Y. Nisin A depletes intracellular ATP and acts in bactericidal manner against *Mycobacterium smegmatis*. *Lett Appl Microbiol* 1999; **28**(3): 189-193.

[34]Mota-Meira M, LaPointe G, Lacroix C, Lavoie MC. MICs of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against bacterial pathogens. *Antimicrob Agents Chemother* 2000; **44**(1): 24-29.

[35]Donaghy JA, Totton NL, Rowe MT. *The in vitro antagonistic activities of lactic acid bacteria against Mycobacterium avium subsp. Paratuberculosis*. Eighth International Colloquium on Paratuberculosis August 14-17, 2005, Copenhagen, Denmark.

[36]Todorov SD, Dicks LMT. Screening for bacteriocin-producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria comparison of the bacteriocins. *Process Biochem* 2006; **41**(1): 11-19.

[37]Todorov SD, Franco BD, Wiid IJ. *In vitro* study of beneficial properties and safety of lactic acid bacteria isolated from Portuguese fermented meat products. *Benef Microbes* 2014; **5**(3): 351-366.

[38]Sosunov V, Mischenko V, Eruslanov B, Svetoch E, Shakina Y, Stern N, et al. Antymicobacterial activity of bacteriocins and their complexes with liposomes. *J Antimicrob Chemother* 2007; **59**(5): 919-925.

[39]Carroll J, Draper LA, O'Connor PM, Coffey A, Hill C, Ross RP, et al. Comparison of the activities of the lantibiotics nisin and lacticin 3147 against clinically significant mycobacteria. *Int J Antimicrob Agents* 2010; **36**(2): 132-136.

[40]Carroll J, O'Mahony J. Anti-mycobacterial peptides. Made to order with delivery included. *Bioeng Bugs* 2011; **2**(5): 241-246.

[41]Zahir I, Houari A, Iraqui M, Ibnsouda S. *Aerococcus* sp. with an antimycobacterial effect. *Af J Biotechnol* 2011; **10**(83): 19473-19480.

[42]Mariam SH. Interaction between lactic acid bacteria and *Mycobacterium bovis* in Ethiopian fermented milk: insight into the fate of *Mycobacterium bovis*. *Appl Environ Microbiol* 2009; **75**(6): 1790-1792.

[43]Mariam SH. Identification and survival studies of *Mycobacterium tuberculosis* within laboratory-fermented bovine milk. *BMC Res Notes* 2014; **26**(7): 175.

[44]Macuamule CL, Wiid IJ, van Helden PD, Tanner M, Witthuhn RC. Effect of milk fermentation by kefir grains and selected single strains of lactic acid bacteria on the survival of *Mycobacterium bovis* BCG. *Int J Food Microbiol* 2016; **217**: 170-176.

[45]Herich R, Levkut M. Lactic acid bacteria, probiotics and immune system. *Vet Med Czech* 2002; **47**(6): 169-180.

[46]Seto S, Tsujimura K, Horii T, Koide Y. Autophagy adaptor protein p62/SQSTM1 and autophagy-related gene *Atg5* mediate autophagosome formation in response to *Mycobacterium tuberculosis* infection in dendritic cells. *PLoS One* 2013; **8**(12): e86017.

[47]Nüssler AK, Thomson AW. Immunomodulatory agents in the laboratory and clinic. *Parasitology* 1992; **105**(S1): S5-23.

[48]Suárez-Méndez R, García-García I, Fernández-Olivera N, Valdés-Quintana M, Milanés-Virelles MT, Carbonell D, et al. Adjuvant

interferon gamma in patients with drug-resistant pulmonary tuberculosis: a pilot study. *BMC Infect Dis* 2004; **4**: 44.

[49]MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science* 2003; **302**(5645): 654-659.

[50]Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006; **313**(5792): 1438-1441.

[51]Kato I, Yokokura T, Mutai M. Augmentation of mouse natural killer cell activity by *Lactobacillus casei* and its surface antigens. *Microbiol lmmunol* 1984; **28**(2): 209-217.

[52]Balasubramanya NN, Lokesh BR, Ramesh HP, Krishnakantha TP. Effect of lactic microbes on superoxide anion generating ability of peritoneal macrophages and tissue histopathology of murines. *Indian J Dairy Biosci* 1995; **6**: 28-33.

[53]Rangavajhyala N, Shahani KM, Sridevi G, Srikumaran S. Nonlipopolysaccharide component(s) of *Lactobacillus acidophilus* stimulate(s) the production of interleukin-1 alpha and tumor necrosis factor-alpha by murine macrophages. *Nutr Cancer* 1997; **28**(2): 130-134.

[54]Ghadimi D, de Vrese M, Heller KJ, Schrezenmeir J. Lactic acid bacteria enhance autophagic ability of mononuclear phagocytes by increasing Th1 autophagy-promoting cytokine (IFN- γ ) and nitric oxide (NO) levels and reducing Th2 autophagy-restraining cytokines (IL-4 and IL-13) in response to *Mycobacterium tuberculosis* antigen. *Int Immunopharmacol* 2010; **10**(6): 694-706.

[55]Isolauri E, Salminen S, Ouwehand AC. Microbial-gut interactions in health and disease. Probiotics. *Best Pract Res Clin Gastroenterol* 2004; **18**(2): 299-313.

[56]Noverr MC, Huffnagle GB. The "microflora hypothesis" of allergic diseases. *Clin Exp Allergy* 2005; **35**(12): 1511-1520.

[57]Izumo T, Maekawa T, Ida M, Noguchi A, Kitagawa Y, Shibata H, et al. Effect of intranasal administration of *Lactobacillus pentosus* S-PT84 on influenza virus infection in mice. *Int Immunopharmacol* 2010; **10**(9): 1101-1106.

[58]Mortaz E, Adcock IM, Folkerts G, Barnes PJ, Paul Vos A, Garssen J. Probiotics in the management of lung diseases. *Mediators Inflamm* 2013; doi: http://dx.doi.org/10.1155/2013/751068.

[59]Takahashi T, Oka T, Iwana H, Kuwata T, Yamamoto Y. Immune response of mice to orally administered lactic acid bacteria. *Biosci Biotechnol Biochem* 1993; **57**(9): 1557-1560.

[60]Brown KL, Hancock RE. Cationic host defense (antimicrobial) peptides. *Curr Opin Immunol* 2006; **18**(1): 24-30.

[61]Mitsuma T, Odajima H, Momiyama Z, Watanabe K, Masuguchi M, Sekine T, et al. Enhancement of gene expression by a peptide p(CHWPR) produced by *Bifidobacterium lactis* BB-12. *Microbiol Immunol* 2008; **52**(3): 144-155.

[62]Herawati I, Diki H, Prima NF. Effect of lactic acid filtrate and bacteriocins of *Lactobacillus acidophillus* on hagocytosis activity of macrophages cell againts enteropathogenic *Escherichia coli* (EPEC). *Microbiol Indones* 2014; **8**(4): 183-190.

[63]Chang CK, Wang SC, Chiu CK, Chen SY, Chen ZT, Duh PD. Effect of lactic acid bacteria isolated from fermented mustard on immunopotentiating activity. *Asian Pac J Trop Biomed* 2015; **5**(4): 281-286.

[64]Maria-Neto S, de Almeida KC, Macedo ML, Franco OL. Understanding bacterial resistance to antimicrobial peptides: from the surface to deep inside. *Biochim Biophys Acta* 2015; **1848**(11): 3078-3088.

[65]Li JWH, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? *Science* 2009; **325**(5937): 161-165.

[66]Ferchichi M, Fathallah M, Mansuelle P, Rochat H, Sabatier JM, Manai M, et al. Chemical synthesis, molecular modeling, and antimicrobial activity of a novel bacteriocin, MMFII. *Biochem Biophys Res Commun* 2001; **289**(1): 13-18.

[67]Lasta S, Fajloun Z, Darbon H, Mansuelle P, Andreotti N, Sabatier JM, et al. Chemical synthesis and characterization of J46 peptide, an atypical class IIa bacteriocin from *Lactococcus lactis* subsp. *cremoris* J46 Strain. *J Antibiot (Tokyo)* 2008; **61**(2): 89-93.

[68]Brimble MA, Edwards PJ, Harris PW, Norris GE, Patchett ML, Wright TH, et al. Synthesis of the antimicrobial S-linked glycopeptide, glycocin F. *Chemistry* 2015; **21**(9): 3556-3561.

[69]Escano J, Smith L. Multipronged approach for engineering novel peptide analogues of existing lantibiotics. *Expert Opin Drug Discov* 2015; **10**(8): 857-870.

[70]Hemu X, Qiu Y, Nguyen GK, Tam JP. Total synthesis of circular bacteriocins by butelase 1. *J Am Chem Soc* 2016; **138**(22): 6968-6971.

[71]Slootweg JC, Peters N, Quarles van Ufford HL, Breukink E, Liskamp RM, Rijkers DT. Semi-synthesis of biologically active nisin hybrids composed of the native lanthionine ABC-fragment and a cross-stapled synthetic DE-fragment. *Bioorg Med Chem* 2014; **22**(19): 5345-5353.

[72]Liu W, Hansen JN. Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. *Appl Environ Microbiol* 1990; **56**(8): 2551-2558.

[73]Fimland G, Blingsmo OR, Sletten K, Jung G, Nes IF, Nissen-Meyer J. New biologically active hybrid bacteriocins constructed by combining regions from various pediocin-like bacteriocins: the C-terminal region is important for determining specificity. *Appl Environ Microbiol* 1996; **62**(9): 3313-3318.

[74]Carroll J, O'Mahony J. Anti-mycobacterial peptides. Made to order with delivery included. *Bioeng Bugs* 2011; **2**(5): 241-246.

[75]Carroll J, Draper LA, O'Connor PM, Coffey A, Hill C, Ross RP, et al. Comparison of the activities of the lantibiotics nisin and lacticin 3147 against clinically significant mycobacteria. *Int J Antimicrob Agents* 2010; **36**(2): 132-136.

[76]Fimland G, Johnsen L, Axelsson L, Brurberg MB, Nes IF, Eijsink VG, et al. A C-terminal disulfide bridge in pediocin-like bacteriocins renders bacteriocin activity less temperature dependent and is a major determinant of the antimicrobial spectrum. *J Bacteriol* 2000; **182**(9): 2643-2648.

[77]Lohans CT, Vederas JC. Development of class IIa bacteriocins as therapeutic agents. *Int J Microbiol* 2012; **2012**: 386410.

[78]Derksen DJ, Boudreau MA, Vederas JC. Hydrophobic interactions as substitutes for a conserved disulfide linkage in the type IIa bacteriocins, leucocin A and pediocin PA-1. *Chem Bio Chem* 2008; **9**(12): 1898-1901.

[79]Oppegard C, Rogne P, Kristiansen PE, Nissen-Meyer J. Structure analysis of the two-peptide bacteriocin lactococcin G by introducing D-amino acid residues. *Microbiology* 2010; **156**(6): 1883-1889.

[80]Tominaga T, Hatakeyama Y. Development of innovative pediocin PA-1 by DNA shuffling among class IIa bacteriocins. *Appl Environ Microbiol* 2007; **73**(16): 5292-5299.

[81]Acuña L, Picariello G, Sesma F, Morero RD, Bellomio A. A new hybrid bacteriocin, Ent35–MccV, displays antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria. *FEBS Open Bio* 2012; **2**: 12-19.