Journal of King Saud University – Science 31 (2019) 758–767

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Review

Bacteriocin-nanoconjugates as emerging compounds for enhancing antimicrobial activity of bacteriocins

Parveen Kaur Sidhu, Kiran Nehra *

Department of Biotechnology, Deenbandhu Chhotu Ram University of Science & Technology, Murthal 131039, Sonipat, Haryana, India

A R T I C L E   I N F O

Article history:
Received 12 July 2017
Accepted 13 December 2017
Available online 14 December 2017

Keywords:
Antimicrobial spectrum
Bacteriocins
Food preservation
Nano-conjugates
Nanoparticles

A B S T R A C T

Food spoilage which makes food unsuitable for human consumption is one of the major concerns in the food industry. Although various chemical additives are being used commercially for food preservation to prevent spoilage, but these chemically synthesized preservatives cause severe ill-effects on human health. The increasing awareness of health hazards caused due to them among the consumers has led to a demand for availability of chemical-free food in the market. This in turn has created the need to look for new alternatives, which are natural but efficient and do not pose any health issues. In this context, naturally occurring, non-pathogenic lactic acid bacteria, or bacteriocins produced by them have attracted a lot of attention in the scientific community. Bacteriocins are ribosomally synthesized active proteins which show bactericidal mode of action against food spoiling bacteria and are considered to be safe additives for food preservation. However, several hurdles such as sensitivity to proteolytic enzymes, restricted antimicrobial spectrum, high dosage requirement and low yield limit the use of bacteriocins as efficient food preservatives. To combat some of these limitations, more recently bacteriocin-nanoconjugates have emerged as a promising solution. This review focuses on the limitations of bacteriocins in restricting the growth of food-borne pathogens and the potential of nanotechnology in enhancing the antimicrobial activity of bacteriocins.

Contents

1. Introduction

Food safety and stability is a matter of global concern. The past few years have witnessed an escalation in various health issues related to contamination of food by different pathogens. Contamination can be prevented by implementing preservation techniques having the capability of upholding the quality of raw material and of inhibiting the expansion of potential food degrading pathogens.

* Corresponding author.
E-mail address: kirannehra.bt@dcrustm.org (K. Nehra).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2017.12.007

However, food preservation techniques most popularly involve the use of chemical preservatives which cause several side-effects such as: a change in the components of food, diminished nutritive quality, and toxic effect on the human health (Sharma et al., 2006). Therefore, the demand for replacing these chemical compounds with natural preservatives or biopreservatives has gained a huge attention in the past few years (Parada et al., 2007). Contrary to chemical preservatives, biopreservation involves the use of non-pathogenic microorganisms for extending the shelf life of foods (De Martinis et al., 2001). Traditionally, food used to be preserved by Lactic acid bacteria (LABs) which are natural constituents of fermented foods and have the capability to produce desirable changes in texture and flavor of food. They confer their preservative effect by production of various antimicrobial compounds. These compounds include acetic acid, lactic acid, hydrogen peroxide, carbon dioxide and small toxic proteinaceous compounds called bacteriocins (Deegan et al., 2006; Mills et al., 2011; Ghanbari et al., 2013). Bacteriocins are basically protein in nature with molecular mass ranging from 2 kDa to more than 30 kDa. They are secreted extracellularly by bacteria and are gaining much attention due to their GRAS (generally recognized as safe) status and no adverse effect on food. Bacteriocins are believed to be safe for human consumption since they can be degraded by the action of gastrointestinal proteases. Moreover, because they are ribosomally synthesized, their characteristics can be modified to enhance their activity spectrum (Saavedra et al., 2004). They are known to exhibit antimicrobial activity against various Gram positive bacteria including closely related species such as *Bacillus cereus*, *Clostridium botulinum*, *Staphylococcus aureus* and *Listeria monocytogenes.*

Despite several advantages, bacteriocins have certain limitations associated with their use as natural food preservatives, some of which include (i) their restricted antimicrobial spectrum which leads to elimination of only specific closely related food spoiling bacteria (ii) their high dosage requirement to inhibit multidrug resistant bacteria present in water and food (Saravana and Annalakshmi, 2012) (iii) their sensitivity to proteolytic enzymes (Bradshaw, 2003) (iv) their high cost of production and (v) a low yield due to ineffective recovery by purification methods. To overcome these hurdles, scientists are constantly searching for efficient ways to find solutions to some of these limitations. Lately various studies have been conducted to optimize purification and production methods to obtain high yield of bacteriocins (Parada et al., 2007). However, another area which offers high hope in overcoming some of the other limitations is the use of nanotechnology in increasing the stability and shelf life of food, in enhancing the antimicrobial spectrum of bacteriocins, and ensuring safer packaging. Due to their antimicrobial and antifungal properties, nanoparticles have the ability to inhibit growth of numerous micro-organisms. Nanoparticles act by coming in contact with the pathogenic bacterial cell surface and this contact causes various structural changes and harm to the cell, markedly unsettling essential cell functions and at last leading to the bacterial cell death (Zawrah and Sherein, 2011). This potential antimicrobial property of nanoparticles recommends their possible application in preservation of food and food packaging.

In this review we will focus on properties of bacteriocins and their interaction with nanoparticles using different approaches for increasing their efficacy against food spoiling micro-organisms.

2. Bacteriocins

Bacteriocins are proteinaceous compounds produced by various Gram positive and Gram negative bacteria which show antimicrobial activity against related species (De Vuyst and Leroy, 2007). The most commonly available bacteriocin producing bacteria are the lactic acid bacteria. Due to their GRAS status, they are considered as a safe alternative to chemical preservatives. LABs are characterized as cocci or rod shaped and are anaerobic but can withstand and grow in the presence of oxygen (Patil et al., 2010). These microorganisms are found in dairy, meat, and fermented products (Lindgren and Dobrogosz, 1990). Bacteriocins produced by LABs usually have low molecular weight (rarely over 10 kDa); they undergo post-translational modifications and show sensitivity to proteolytic enzymes, especially the proteases, which make them harmless for human consumption (Rodriguez et al., 2000, 2003). Bacteriocins are ribosomally synthesized peptides; and genes for their synthesis are generally arranged in operon clusters and can be located on moving elements such as chromosome in association with transposons or on plasmids (Deegan et al., 2006). Commonly, genes involved in bacteriocin biosynthesis are arranged in three operons: one encoding for structural and immunity genes, second for bacteriocin export genes, and the third for genes involved in regulation of bacteriocin production (Dimov et al., 2005).

The two most commonly occurring forms of bacteriocins are nisin and pediocin. Genetic determinants of nisin are located on bacterial chromosome and genes involved in production and immunity are reported to be present on an operon of size ranging up to 8.5 kb. Nisin operon generally consists of 11 genes and is designated as nisABTCIPRKFEG which includes structural genes, immunity genes, ABC transporter, and regulatory genes (Dimov et al., 2005) (Fig. 1a). Genes involved in biosynthesis of Pediocin PA-1 are located on a plasmid. The operon of size 3.5 kb consists of four genes which encode for structural (pedA), immunity (pedB) and transport proteins (pedC and pedD) (Fig. 1b).

Nisin is a polypeptide with molecular weight of 3354 Daltons and is synthesized as pre-pro-peptide which contains 57 amino acid residues which after post-translational modification generates mature nisin of 34 amino acids (Fig. 2a). Nisin has been reported to be active against Gram-positive bacteria, including *S. aureus* and *L. monocytogenes* which are highly pathogenic food-spoiling microorganisms. However, it has been found to be not very effective against Gram-negative bacteria, yeasts and moulds (Zacharof and Lovitt, 2012). Toxicological studies have confirmed that nisin is not toxic for human consumption, even at levels much higher than those used in foods, but it is restricted to acidic foods due to low stability at neutral pH values (Jeevaratnam et al., 2005). It was approved in 1988 by Food and Drug administration (FDA, 1988) for use at commercial level in cheese, stored soups and pasteurized cheese spreads, which are stored at a chilled temperature (Zacharof and Lovitt, 2012). Pediocin PA-1 polypeptide has a molecular weight of 4646.95 Daltons. It is made up of 44 amino acids and unlike nisin, it does not undergo any post-translational modifications (Fig. 2b). Pediocin PA-1 too holds promising antibacterial activity against a major food spoiling bacteria, *L. monocytogenes* but its activity spectrum is limited as compared to nisin (Benmechernene et al., 2013; Ovchinnikov et al., 2016).

Till date, only these two bacteriocins, nisin and pediocin PA-1 have been licensed for production at commercial level and are marketed as Nisaplin and Alta® 2341 respectively. Thus, a paucity of information on efficient bacteriocins which can be commercialized has created a great impetus in the scientific world for studies involving research leading to the search for other novel and promising bacteriocins which can fight the limitations faced by present-day approved bacteriocins, and can be produced at large scale at a commercial level. However, in addition to the research focus on search for new bacteriocins; to combat some of the limitations of bacteriocins highlighted in the preceding sections, nanotechnology is also being looked upon as an area which can provide a new hope for improving the activity of already commercially approved bacteriocins. Nanoparticles along with bacteriocins

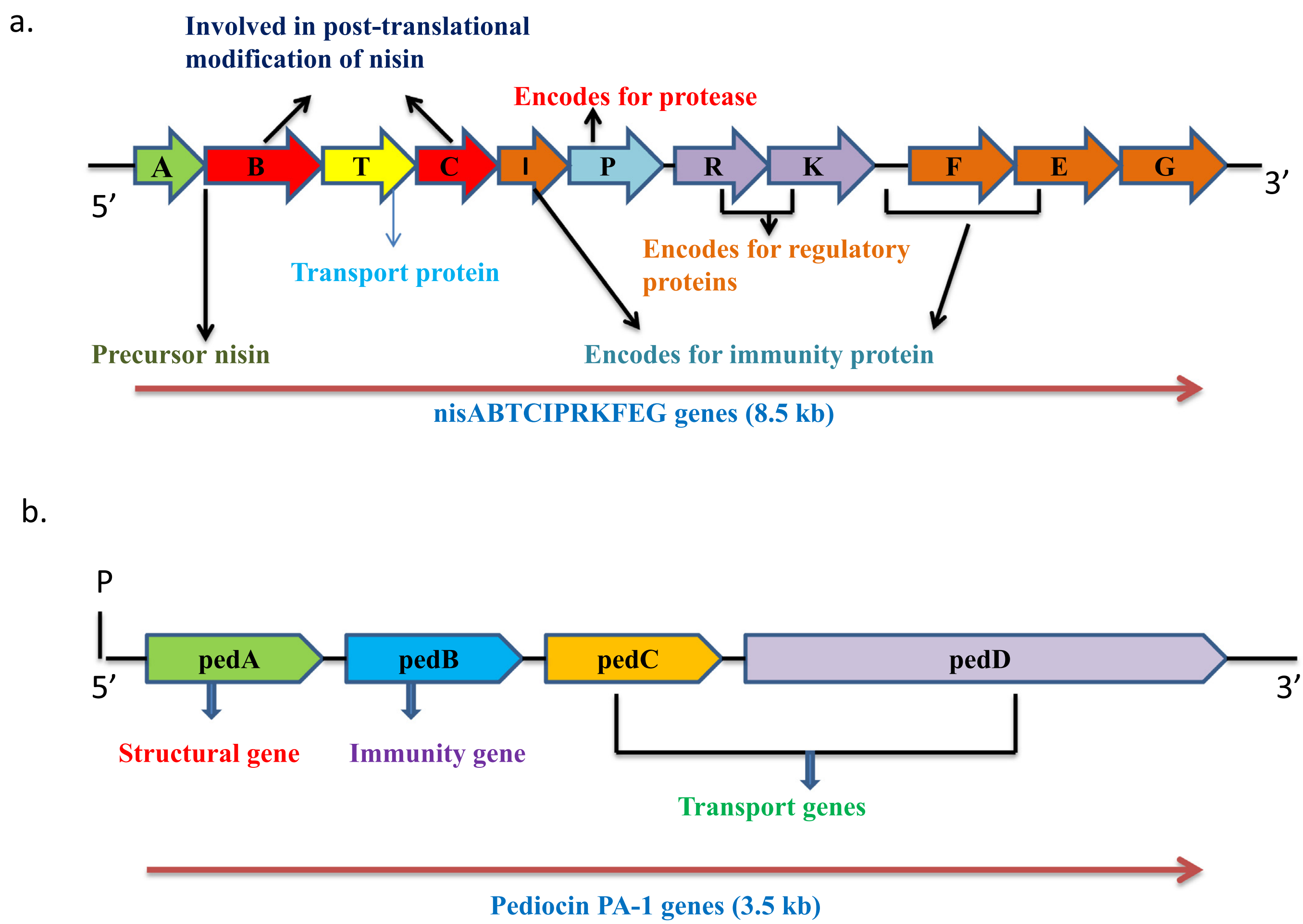

Fig. 1. (a). Genetic constitution of Nisin. (b). Genetic constitution of Pediocin.

can augment the antimicrobial and antifungal activity and can play a major role in food safety and food presservation.

3. Nanoparticles

Nanoparticles (NPs) usually range in dimensions from 1 to 100 nm and have unique properties. With a decrease in the dimension of materials to atomic level, their properties also change (Feynman, 1991). Nanoparticles possess distinctive physicochemical, optical and biological properties which can be manipulated appropriately for desired applications. Since the past decade, they are being widely explored and investigated as potential antimicrobials. The antimicrobial property of nanoparticles is recognized to be an activity of their large surface area in contact with the microorganisms. This interaction over a large surface area contributes in carrying out a broad range of probable antimicrobial activities and finds an immense application in food processing and packaging (Gutierrez et al., 2010).

Among the various potential nanoparticles, silver nanoparticles (AgNPs) are well known for their toxicity towards most of the gram-positive and gram-negative bacteria. The high affinity of silver towards sulphur and phosphorus is assumed to play an important role in contributing to its antimicrobial property (Ravishankar and Jamuna, 2011). The presence of sulfur-containing proteins on bacterial cell membrane leads to the interaction of silver nanoparticles with sulfur-containing amino acids within or outer surface of the cell, which in turn affects bacterial cell permeability. AgNPs, in recent years, have therefore emerged as a potential compound for the production of a novel class of antimicrobials. Similarly, gold nanoparticles (AuNPs) are also being extensively exploited in organisms for their antimicrobial activity because of their biocompatible nature (Daniel and Astruc, 2004; Bhattacharya and Mukherjee, 2008). The potential activity of gold nanoparticles against microbial pathogens depends on the size and shape of the particles. They exert their antibacterial effect by generating holes in the cell wall, leading to the discharge of cell contents and cell death.

Apart from these two major nanoparticles, a few other metal nanoparticles (Zinc oxide and copper oxide) have also been reported by various researchers to exhibit significant antibacterial activity over a large spectrum of microorganisms (Brayner et al., 2006; Buzea and Pacheco, 2007; Jones et al., 2008; Jalal et al., 2010). When the particle size of zinc oxide (ZnO) is brought to nanometer range, it is found to show significant antimicrobial activity. This nano-sized ZnO can easily come in contact with the cell surface and gain entry into the cell and subsequently exhibit distinct bactericidal action which makes it a potential candidate for its use as an antimicrobial agent (Seil and Webster, 2012; Sirelkhatim et al., 2015; Jamdagni et al., 2018). Copper oxide (CuO) also exhibits potential inhibitory properties. A few researchers have synthesized CuO nanoparticles from different sources and have demonstrated their inhibitory activity against various opportunistic pathogens (Rajgovind et al., 2015; Kaur et al., 2016). However, as compared to AgNPs, AuNPs, and ZnO, limited information is available about the antimicrobial activity of CuO nanoparticles. The exact mechanism behind the bactericidal effect of copper

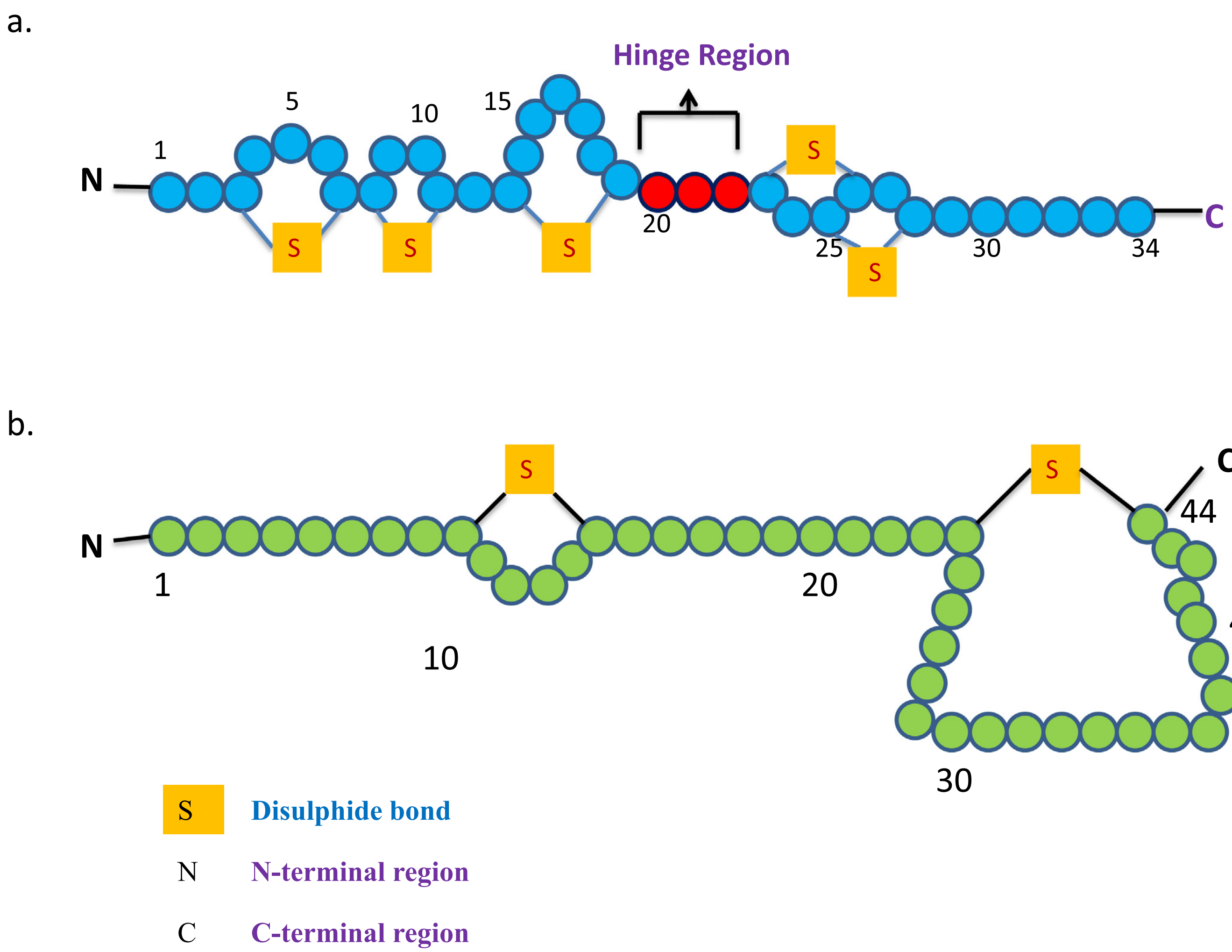

Fig. 2. (a). Primary structure of Nisin. (b). Primary structure of Pediocin.

nanoparticles is not clear, but with some more studies, these nanoparticles too hold a potential to be used as antimicrobials in the food industry.

The potential antimicrobial properties of all these nanoparticles thus provide a means to obtain a desirable increase in the antibacterial spectrum of bacteriocins or in modification of their properties, thus opening up a totally new way to embark upon a wide range of bacterial pathogens.

4. Interaction of bacteriocins with nanoparticles for increased antimicrobial efficacy

In the food industry, bacteriocins have been represented to be potent antimicrobials in hurdle technology, which involves the use of combined treatment to increase the efficacy of food preservation (Cotter et al., 2005). The integration of nanotechnology and biotechnology may be considered as a most recent example of this hurdle technology. Research in the field of nanotechnology in the past two decades has opened doors to unlimited opportunities for solving several problems associated with a wide range of biological products. In the food sector, the interaction between nanoparticles and bacteriocins holds high potential to be beneficial in increasing the antimicrobial spectrum of the latter. The interaction may also lead to a reduction in the requirement of high bacteriocin dosage, and an extension in the shelf life of food. Nano-encapsulations of bacteriocins when used in food preservation protect them from degradation by gastrointestinal enzymes, and can also increase their commercial yield and stability. For formation of nano-encapsulations, different approaches have been used by different scientists to conjugate bacteriocins with nanoparticles (Fahim et al., 2016). Some of the common approaches include (a) encapsulation of bacteriocins in nanoliposomes (b) conjugation of bacteriocins with chitosan nanoparticles and (c) interaction of bacteriocins with metallic nanoparticles.

4.1. Encapsulation of bacteriocins in nanoliposomes

Liposomes are spherical vesicles composed of one or more phospholipid bilayers and are considered as biocompatible and non-toxic (Go'mez-Hens and Ferna'ndez-Romero, 2005). They may vary in size from nanometer to micrometer and have been tested as carriers for therapeutic agents, and bioactive compounds such as bacteriocins. Liposomes are capable of encapsulating both hydrophobic and hydrophilic compounds and are biodegradable in nature. When these are prepared in nano size, nanoliposomes represent a promising vehicle for encapsulation and delivery of several components within the system (Fig. 3a) (Banerjee, 2001; Mugabe et al., 2005; Malheiros et al., 2012a,b). They are known to deliver enzymes, nutrients, vitamins, food additives and antimicrobials (Godwin et al., 2009). Encapsulation by liposomes has shown to protect the encapsulated compounds from environmental and physicochemical alterations (Mozafari et al., 2008a,b).

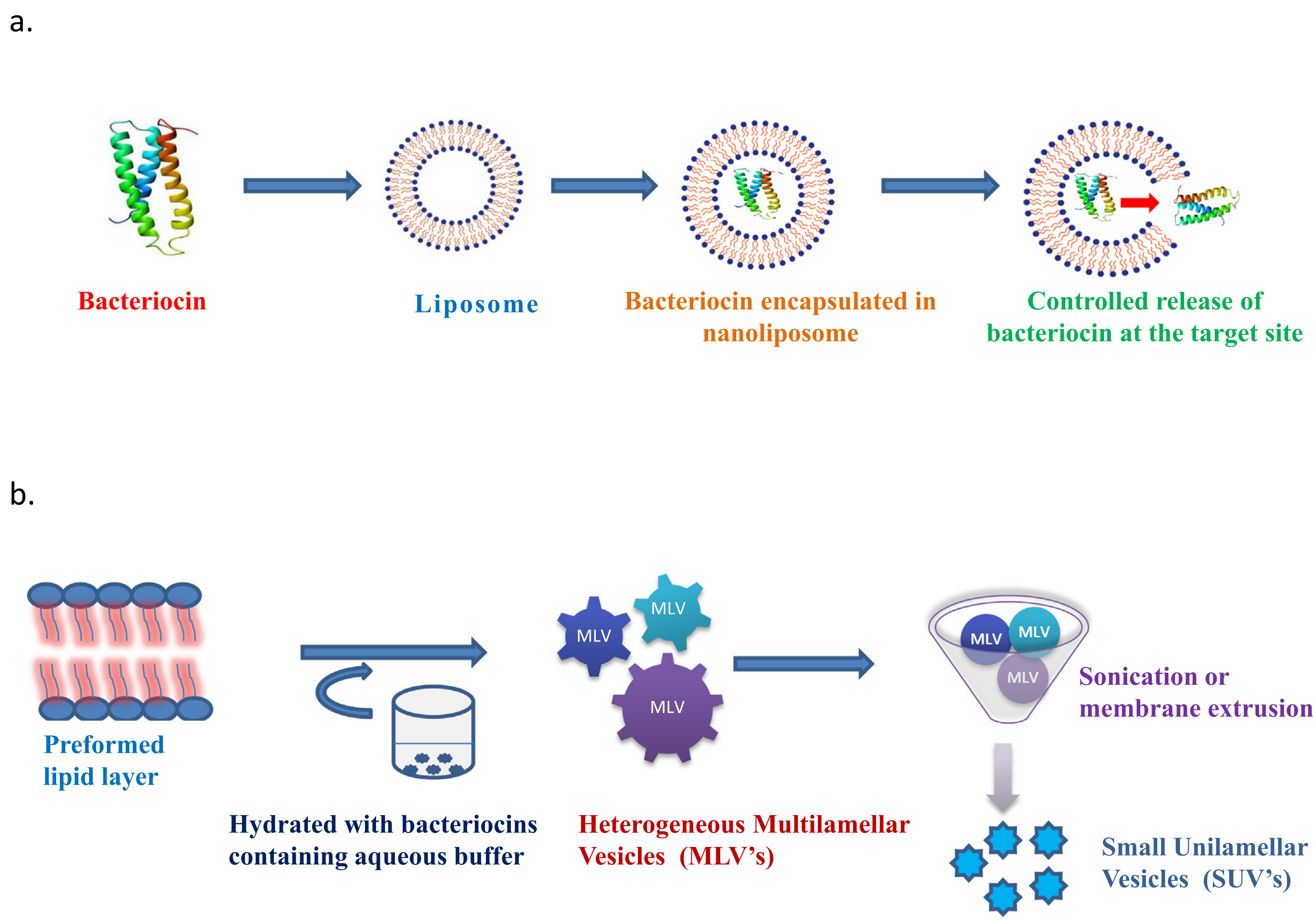

Fig. 3. (a). Schematic representation of encapsulation of bacteriocins in nanoliposomes (b). Diagrammatic representation of thin film hydration method.

Encapsulation of bacteriocins in liposomes has been reported to be achieved by a common method known as thin film hydration method (Fig. 3b). In this method, the chemically synthesized lipid film is hydrated with bacteriocin containing aqueous buffer at a temperature higher than the phase transition temperature of lipids. This results in a disparate population of multilamellar vesicles of about >400 nm in size in which bacteriocins are encapsulated; which are then further processed into homogeneous, small unilamellar vesicles of about 20–80 nm in size by sonication or heating or membrane extrusion (Sharma and Sharma, 1997; Jesorka and Orwar, 2008).

In addition to various advantages of liposome-encapsulated bacteriocins like stability, enhancement and protection from degradation; small doses of these formulations have also been reported to exhibit a better antimicrobial activity in terms of time taken for exerting the antimicrobial effect, and the activity spectrum. For illustration, bacteriocin-like substances produced from *Bacillus licheniformis* P40 contained in phosphatidylcholine nanovesicles have shown to completely inhibit the growth of *Listeria monocytogenes* within the initial 12 min of incubation (Teixeira et al., 2008). A similar study has reported that the nanoliposomes prepared from dipalmitoylphosphatidylcholine/dicetyl phosphate/cholesterol (DPPC:DCP:CHOL) show greater entrapment efficiency for nisin Z. Furthermore, nanoliposomes made from DPPC:DCP:CHOL or from dipalmitoylphosphatidylcholine/stearyl amine/cholesterol have also been shown to hold high stability, which may extend to 14 months at 4 °C, and 12 months at 25 °C, respectively (Colas et al., 2007). Pinilla and Brandelli (2016) co-encapsulated garlic extract with nisin in liposomes, which resulted in enhanced antimicrobial spectrum against various food-spoiling pathogens. In another study, encapsulation of nisin into phosphatidylcholine resulted into promising active packaging material that exhibited strong inhibitory activity against the prevalent food spoiling bacteria, *Listeria monocytogenes,* when incorporated into biopolymer-based films of gelatin or cellulose (Boelter and Brandelli, 2016). Additionally, the encapsulated bacteriocin-like substances have shown to lack any hemolytic activity on human red blood cells, thus signifying their safety as food preservatives (Teixeira et al., 2008). Although nano-encapsulated bacteriocins offer several advantages, but in some studies carried out by researchers, encapsulation of bacteriocins by liposomes has shown to adversely affect the antimicrobial activity of the bacteriocins (Malheiros et al., 2010a, 2012a,b). However, the negative impact on bacteriocin antimicrobial activity can be overcome by selecting suitable phospholipid bacteriocin combinations, avoiding undesirable interactions between liposomes and bacteriocins and obtaining a high purity of starting material including bacteriocins (Were et al., 2003; Malheiros et al., 2010a,b). In order to avoid this negative impact, several studies are underway to optimize the formulations of bacteriocin-loaded liposomes.

4.2. Conjugation with chitosan nanoparticles

Apart from lipid-based nanoparticles, polymeric nanoparticles have also gained much attention due to their immense applications in therapeutics and food safety. Chitosan is a polymer which is biodegradable, biocompatible, bactericidal and non-toxic. In addition to this, chitosan is characterized by its antibacterial,

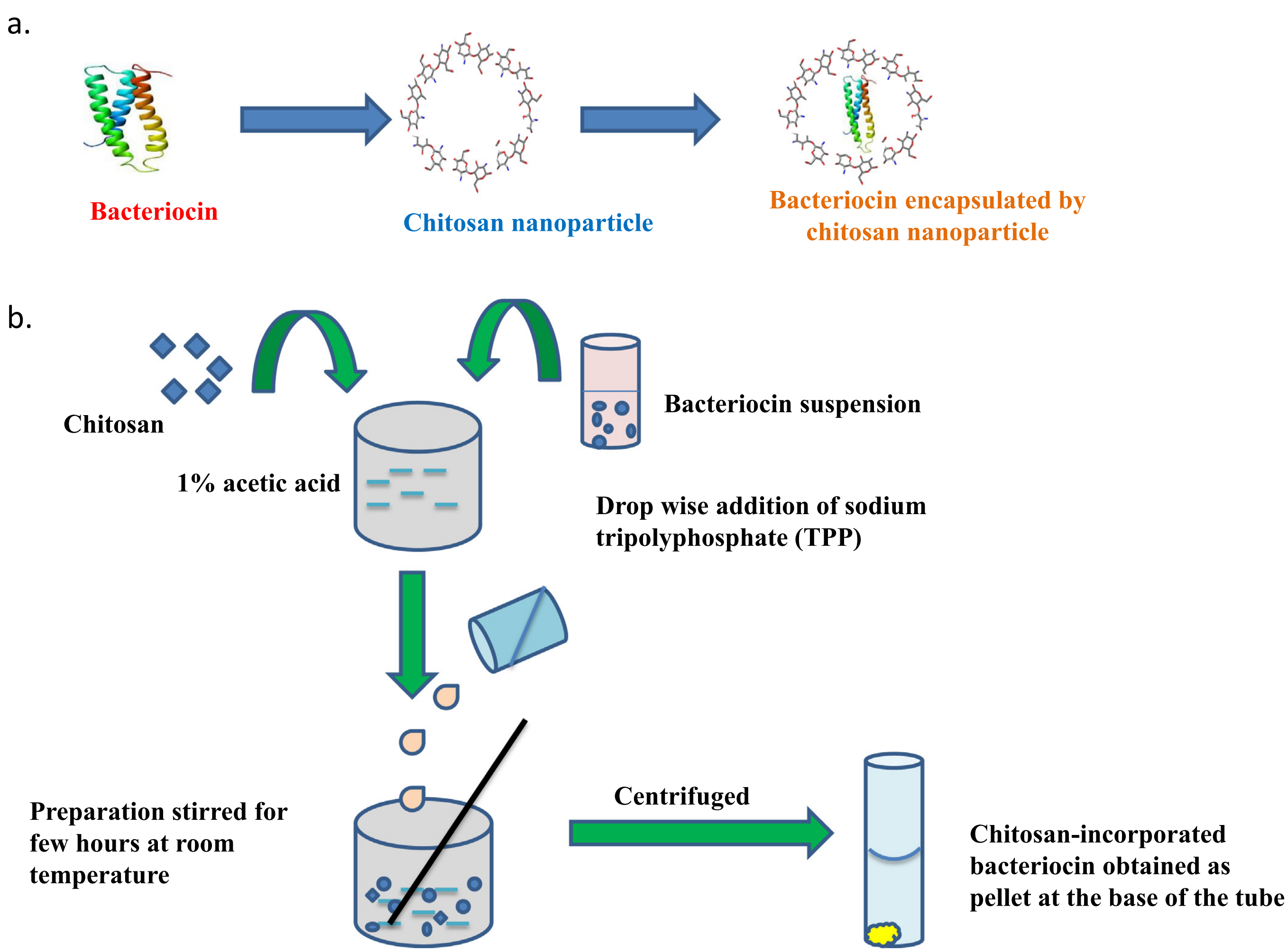

Fig. 4. (a). Diagrammatic representation of bacteriocins encapsulated within chitosan nanoparticles. (b). Diagrammatic representation of ionic gelation method.

antifungal, antiprotozoal, and anticancer activities. Hence, it has been used in the field of medicine and biotechnology for interaction with different compounds (Fig. 4a). Chitosan-bacteriocin conjugation is commonly achieved by ionic gelation method described by Namasivayam et al. (2015) (Fig. 4b). In this method, chitosan and bacteriocin suspension are mixed in 1% acetic acid and are stirred at room temperature with the drop wise addition of sodium tripolyphosphate (TPP). The preparation is centrifuged and the chitosan incorporated bacteriocins are obtained as pellet which can be lyophilized for further use. Regarding its biological activity, the chitosan nanoparticles loaded with nisin have shown to exhibit twofold higher antimicrobial activity than that of nisin alone, when examined against *S. aureus* ATCC 19,117 (Zohri et al., 2010). Also, when the minimum inhibitory concentration (MIC) of nisin-loaded nanoparticles was tested, it was found to be reduced four times than that of the nisin alone (0.5 and 2 mg/ml, respectively) (Zohri et al., 2010). In another study, higher level of antimicrobial activity was exhibited by nisin-loaded chitosan-alginate nanoparticles against *L. monocytogenes* and *S. aureus* in comparison to free nisin (Zohri et al., 2013). A similar study was reported by Namasivayam et al. (2015) in which chitosan nanoconjugate loaded with bacteriocins was tested for its antimicrobial activity against *L. monocytogenes*, which indicated that all the nanoconjugates showed an enhanced antimicrobial activity. The increase in zone of inhibition was more promising in nanoconjugate than in free bacteriocins. In another research, when the antimicrobial effect of chitosan edible film incorporating garlic oil (GO) was compared with a usual food preservative such as potassium sorbate (PS) and bacteriocin nisin (N) at various concentrations against food spoiling bacteria, the result showed more efficacy on combination of both the compounds (Pranoto et al., 2005).

This new class of nano-polymer hybrid thus provides a new dimension to fight bacterial pathogens and could therefore be an efficient weapon against food-borne bacterial pathogens (Sharma et al., 2012; Barnela et al., 2014; Chopra et al., 2014). This suggests the possible use of chitosan-nanoparticle-coated bacteriocins in protecting the food against spoilage caused by pathogenic organisms.

4.3. Interaction of bacteriocins with metallic nanoparticles

At present, the metallic nanoparticles are thoroughly being explored and widely investigated as potential antimicrobials. Among metallic nanoparticles, silver, gold, zinc, and copper have been studied and observed to show potent antimicrobial activity. This may be due to the large surface area of positively charged nanoparticles which allow their binding to the negatively charged bacterial cell membrane. However, silver and gold are more commonly used and investigated nanoparticles for conjugation with bacteriocins as they are expected to have a synergistic effect on the antimicrobial properties.

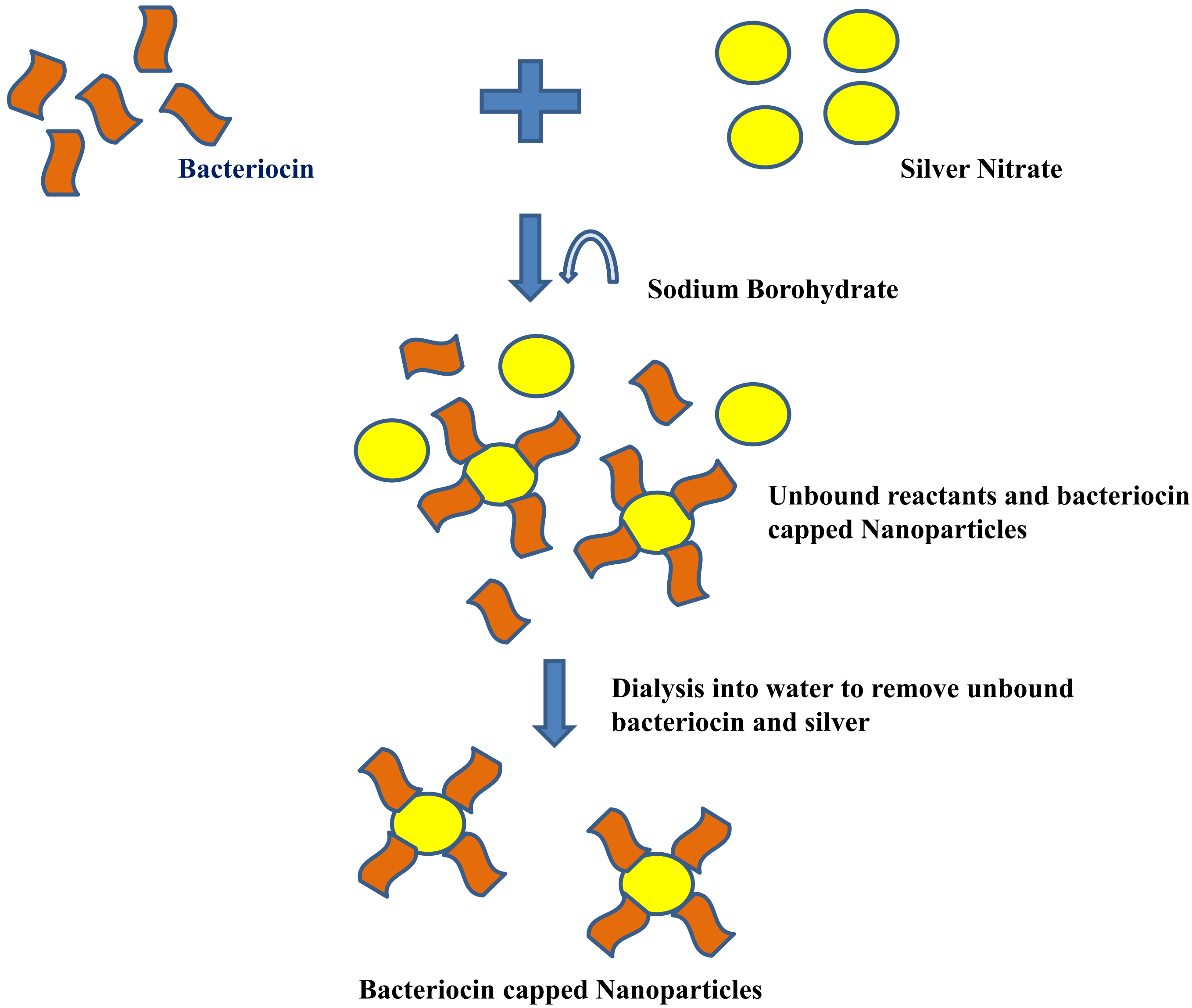

Fig. 5. Schematic representation of green synthesis of bacteriocin capped silver nanoparticles.

More recently, a few studies have reported the development of antibiotic tool using bacteriocins and nanoparticles which are also non-toxic to humans and can be synthesized by a safer method. One of the most commonly used nanoparticles *i.e*., silver has long been known for its antimicrobial activity and for induction of high toxicity to microorganisms. Antimicrobial property of silver nanoparticles can be used to enhance the antimicrobial spectrum of bacteriocins as they themselves have enormous antibacterial potential and hold promise to target food borne pathogenic species (Fayaz et al., 2010; Duncan, 2011). Hence, these have been suggested to be the most suitable contender for conjugation with bacteriocins. Sharma et al. (2012) reported a single-step green method of synthesizing silver nanoparticles (Fig. 5) conjugated with an antibacterial peptide from food-grade lactic acid bacteria. The synthesized enterocin-coated silver nanoparticles (En-SNPs) showed a broad-spectrum inhibition against a number of food-borne pathogenic bacteria without any noticeable toxicity to red blood cells, which reveals that En-SNPs exhibit a much higher antibacterial activity as compared to the chemically synthesized silver-NPs, without the use of hazardous chemicals. In another study conducted by Saravana and Annalakshmi (2012) about 22% increase in antimicrobial activity was recorded with encapsulated nisin than non-encapsulated nisin, as well as the concentration required to inhibit the growth of pathogens was reduced from 125 μg/ml to 62.5 μg/ml. Pandit et al. (2017) synthesized silver nanoparticles from the extract of *Cymbopogon citrates* and conjugated them with nisin to form a nano-bacteriocin conjugate. *In vitro* antimicrobial activity of silver-bioconjugate was evaluated against various food spoilage microorganisms and the results showed the increased antimicrobial potential of nisin after conjugation with silver nanoparticles. Thus, these reports recommend the use of nisin encapsulated silver nanoparticles as a preservative to control the development of food and waterborne pathogens in packed food.

Apart from silver, various other metal nanoparticles have also been used for testing the synergistic effect with bacteriocins for enhancement of antimicrobial spectrum against various harmful pathogens. One of the other contenders is gold nanoparticles. Thirumurugan et al. (2013) reported the combined effect of bacteriocins produced by *L. plantarum* ATM11 along with gold nanoparticles. Combination study was carried out on nisin with gold nanoparticles and free nisin for comparison against food spoiling organisms. Findings revealed that the bacteriocin has a higher

Table 1
General overview of the effect of bacteriocins conjugated with nanoparticles using different approaches.

Nanotechnological approach used	Antimicrobial peptide (Bacteriocin)	Nanoparticle used	Effect of nano-bacteriocin formulation on food spoiling bacteria	Test organisms	References
Encapsulation of bacteriocins in nanoliposomes	Bacteriocin like substances produced from *Bacillus licheniformis* P40	Phosphatidylcholine nanovesicles	Inhibitory activity within few minutes of incubation.	*Listeria monocytogenes*	Teixeira et al. (2008)
	Nisin Z	Dipalmitoylphosphatidylcholine/ dicetylphosphate/cholesterol	Maintains the antimicrobial activity for a longer duration.	*Bacillus subtilis*	Colas et al. (2007)
Conjugation with chitosan nanoparticles	Nisin	Chitosan nanoparticles	Show high antimicrobial activity.	*E. coli*, *S. aureus*	Alishahi (2014)
	Nisin	Chitosan nanoparticles	Demonstrate two- fold higher activity than free nisin.	*S. aureus*	Zohri et al. (2010)
	Nisin	Chitosan-alginate nanoparticles	Maintains higher antimicrobial activity.	*Listeria monocytogenes* *S. aureus*	Zohri et al. (2013)
	Bacteriocin	Chitosan nanoconjugate	Increased antimicrobial activity.	*Listeria monocytogenes*	Namasivayam et al. (2015)
Conjugation of bacteriocins with metallic nanoparticles	Enterocin	Silver nanoparticles	Show broad antimicrobial spectrum against food spoiling bacteria.	Gram positive and Gram negative bacteria	Sharma et al. (2012)
	Nisin	Silver nanoparticles	Increased antimicrobial spectrum.	Gram positive and Gram negative bacteria	Saravana and Annalakshmi (2012)
	Bacteriocins produced by *L. plantarum* ATM11 and commercial nisin	Gold nanoparticles	Bacteriocins possess higher antimicrobial activity.	Food spoiling pathogens	Thirumurugan et al. (2013)

antimicrobial activity against food spoiling organisms when used in combination with gold nanoparticles. Gomashe and Dharmik (2014) conducted a similar study to investigate the bactericidal efficacy of gold nanoparticles (AuNPs) synergistically with the bacteriocin and commercially available peptide nisin on food spoiling microbes. The results revealed that the antibacterial activities increased upon combination of bacteriocin with gold nanoparticles as compared to free nisin. Furthermore, this nanoconjugated bacteriocin has been shown to be safe and non-toxic, as confirmed by biochemical examinations, and histopathological screening tests (Mossallam et al., 2014).

Hence, all the three different approaches can be used to conjugate bacteriocins with nanoparticles to increase the antimicrobial spectrum of the former, which in turn can prove to be an efficient weapon in the fight against food borne pathogens.

5. Bacteriocin-nanoconjugates in food industry

Bacteriocin-nanoconjugates have the potential to find applications in the food and health sector. However, most of the research is limited to food industry as compared to other sectors. The exploitation of bacteriocins in health and pharmaceutical sector is moving at a slow pace as various limitations and challenges are yet to be addressed. Lately, researchers are basically focusing on the utilization of bacteriocin-nanoconjugates in the food industry to overcome the limitation of using bacteriocins alone as preservatives.

A number of studies have been conducted to test the compatibility of antimicrobial peptides such as bacteriocins by incorporating them into the food matrix, or in the form of coatings to investigate their role in inhibiting the growth of food spoiling pathogens (Rhim et al., 2013; Meira et al., 2014). However, they have been found to be susceptible to storage conditions, pressure, production process and change in temperature. Apart from this, their activity is also reported to be affected by their undesirable interaction with food particles and by the action of various endogenous enzymes (Sant'Anna et al., 2011; Lopes and Brandelli, 2017). To combat these problems related to the direct addition of bacteriocins in food; as discussed in the previous sections, nanotechnology has been found to offer an alternative to efficiently deliver and protect the antimicrobial peptide from degradation by using various nanotechnological approaches (Brandelli and Taylor, 2015; Fahim et al., 2016). Additionally, various studies have reported the enhanced antimicrobial activity of bacteriocins against spoilage bacteria after conjugation with nanoparticles (Arthur et al., 2014; Mossallam et al., 2014). A few examples of bacteriocin-nanoconjugates have been illustrated in Table 1. Nano-encapsulation of bacteriocins for their use as biopreservatives has been reported to aid in protecting them from degradation by proteolytic enzymes and thus rendering them stable and increasing the shelf life of food. In recent years, two bacteriocins, namely, nisin and pediocin have attracted a great research interest in the food based industry as they have shown to exhibit a positive role in various applications such as in increasing food shelf life and as a promising substitute to the chemical preservatives (Espitia et al., 2013; Bemena et al., 2014). The collaborative use of these potential bacteriocins along with nanotechnology has allowed the development of new antimicrobial packaging for the preservation of food which has not only increased the antimicrobial spectrum of bacteriocins but also enhanced the shelf life of food without causing any alteration of food components (Lopes and Brandelli, 2017). This shows that nanotechnological approaches have distinctive capabilities to tolerate physical and chemical stresses encountered during different steps of food processing.

6. Conclusion

The interface of nanotechnology and biotechnology opens up new vistas to deal with bacterial pathogens, specifically those creating problems, directly or indirectly, for mankind. It has been proven that bacteriocins alone in food are not likely to ensure complete safety; especially in the case of gram negative bacteria. For this, use of bacteriocins combined with other approaches can be beneficial in increasing the food safety and in inhibiting the growth of food-spoiling microorganisms. Antibacterial peptides produced by food-grade bacteria offer great advantages when combined with nanoparticles. However, advanced studies are needed to be carried out to optimize the formulations of bacteriocin-nanoconjugates. Also extensive toxicological and acceptability tests need to be performed before the products are approved for large scale consumption. So, although the science of nano-bacteriocin conjugates is presently in its nascent form, but it holds a great potential to revolutionize the food industry.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

We wish to express our sincere gratitude to University Grant Commission, New Delhi, India, for providing financial support under the Maulana Azad National Fellowship scheme.

References

Alishahi, A., 2014. Antibacterial effect of chitosan nanoparticle loaded with nisin for the prolonged effect. J. Food Saf. 34 (2), 111–118.
Arthur, T.D., Cavera, V.L., Chikindas, M.L., 2014. On bacteriocin delivery systems and potential applications. Future Microbiol. 9, 235–248.
Banerjee, R., 2001. Liposomes: applications in medicine. J. Biomater. Appl. 16, 3–21.
Barnela, M., Kaur, P., Chopra, M., Thakur, R., 2014. Synthesis, characterization of nisin loaded alginate–chitosan–pluronic composite nanoparticles and evaluation against microbes. LWT-Food Sci. Technol. 59 (2), 1093–1099.
Bemena, L.D., Mohamed, L.A., Fernandes, A.M., Lee, B.H., 2014. Applications of bacteriocins in food, livestock health and medicine. Int. J. Curr. Microbiol. Appl. Sci. 3, 924–949.
Benmechernene, Z., Fernandez-No, I., Kihal, M., Bohme, K., Calo-Mata, P., Barros-Velazquez, J., 2013. Recent patents on bacteriocins: food and biomedical applications. Recent Pat. DNA Gene Seq. 7, 66–73.
Bhattacharya, R., Mukherjee, P., 2008. Biological properties of "naked" metal nanoparticles. Adv. Drug Deliv. Rev. 60, 1289–1306.
Bradshaw, J.P., 2003. Cationic antimicrobial peptides. BioDrugs 17, 233–240.
Brandelli, A., Taylor, T.M., 2015. Nanostructured and nanoencapsulated natural antimicrobials for use in food products. In: Taylor, T.M. (Ed.), Handbook of Natural Antimicrobials for Food Safety and Quality. Elsevier, London, pp. 229–257.
Boelter, J.F., Brandelli, A., 2016. Innovative bionanocomposite films of edible proteins containing liposome-encapsulated nisin and halloysite nanoclay. Colloid. Surf. B 145, 740–747.
Buzea, C., Pacheco, I.I., Robbie, K., 2007. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases 2 (4). MR17-MR71.
Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M.F., Fie'vet, F., 2006. Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. 6 (4), 866–870.
Chopra, M., Kaur, P., Bernela, M., Thakur, R., 2014. Surfactant assisted nisin loaded chitosan-carageenan nanocapsule synthesis for controlling food pathogens. Food Control. 37, 158–164.
Colas, J.C., Shi, W., Rao, V.M., Omri, A., Mozafari, M.R., Singh, H., 2007. Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. Micron 38, 841–847.
Cotter, P.D., Hill, C., Ross, R.P., 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. 3, 777–788.
Daniel, M.C., Astruc, D., 2004. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem. Rev. 104, 293–346.
Deegan, L.H., Cotter, P.D., Hill, C., Ross, P., 2006. Bacterlocins: biological tools for bio-preservation and shelf- life extension. Int. Dairy J. 16, 1058–1071.
De Martinis, E.C.P., Públio, M.R.P., Santarosa, P.R., Freitas, F.Z., 2001. Antilisterial activity of lactic acid bacteria isolated from vacuum-packaged Brazilian meat and meat products. Braz. J. Microbiol. 32, 32–37.
De Vuyst, L., Leroy, F., 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. J. Mol. Microbiol. Biotechnol. 13, 194–199.
Dimov, S., Ivanova, P., Harizanova, N., 2005. Genetics of bacteriocins biosynthesis by lactic acid bacteria. Biotechnol. Biotechnol. Equip. 19, 4–10.
Duncan, T.V., 2011. Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. J. Colloid. Interface Sci. 363 (1), 1–24.
Espitia, P.J.P., Soares, N.F.F., Teófilo, R.F., 2013. Physical-mechanical and antimicrobial properties of nanocomposite films with pediocin and ZnO nanoparticles. Carbohydr. Polym. 94, 199–208.
Fahim, H.A., Khairalla, A.S., El-Gemndy, A.O., 2016. Nanotechnology: a valuable strategy to improve bacteriocin formulations. Front. Microbiol. 7, 1385. https://doi.org/10.3389/fmicb.2016.01385.
Fayaz, A.M., Balaji, K., Girilal, M., Yadav, R., Kalaichelvan, P.T., Venketesan, R., 2010. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against Gram-positive and Gram-negative bacteria. Nanomedicine 6, 103–109.
Feynman, R., 1991. There's plenty of room at the bottom. Science 254, 1300–1301.
FDA (U.S. Food & Drug Administration)/Federal Register, 1988. Nisin preparation: affirmation of GRAS status as a direct human food ingredient. 21 CFR Part 184. Fed. Reg. 53, 11247–11251.
Ghanbari, M., Jami, M., Domig, K.J., Kneifel, W., 2013. Seafood biopreservation by lactic acid bacteria – a review. Lwt-Food Sci. Technol. 54, 315–324.
Godwin, H.A., Chopra, K., Bradley, K.A., Cohen, Y., Harthorn, B.H., Hoek, E.M.V., Holden, P., Keller, A., Lenihen, H.S., Nisbet, R.M., Nel, A.E., 2009. The University of California Center for the environmental implications of nanotechnology. Environ. Sci. Technol. 43, 6453–6457.
Gomashe, A.V., Dharmik, P.G., 2014. Synergistic effect of gold nanoparticles and bacteriocin against food blemishing microbes: a novel approach for food packaging material preparation. Global J. Res. Anal. 3 (5), 1–3.
Go'mez-Hens, A., Ferna'ndez-Romero, J.M., 2005. The role of liposomes in analytical processes. Trends Anal. Chem. 24, 9–19.
Gutierrez, F.M., Olive, P.L., Banuelos, A., Orrantia, E., Nino, N., Sanchez, E.M., Ruiz, F., Bach, H., Gay, Y.A., 2010. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanomedicine 6, 681–688.
Jalal, R., Goharshadi, E.K., Abareshi, M., Moosavi, M., Yousefi, A., Nancarrow, P., 2010. ZnO nanofluids: green synthesis, characterization, and antibacterial activity. Mater. Chem. Phys. 121 (1), 198–201.
Jamdagni, P., Khatri, P., Rana, J.S., 2018. Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity. J. King Saud. Univ. Sci. 30, 168–175. https://doi.org/10.1016/j.jksus.2016.10.002.
Jeevaratnam, K., Jamuna, M., Bawa, A.S., 2005. Biological preservation of foods–Bacteriocins of lactic acid bacteria. Indian J. Biotechnol. 4, 446–454.
Jesorka, A., Orwar, O., 2008. Liposomes: technologies and analytical applications. Annu. Rev. Anal. Chem. 1 (1), 801–832.
Jones, N., Ray, B., Ranjit, K.T., Manna, A.C., 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol. Lett. 279 (1), 71–76.
Kaur, P., Thakur, R., Chaudury, A., 2016. Biogenesis of copper nanoparticles using peel extract of Punica granatum and their antimicrobial activity against opportunistic pathogens. Green. Chem. Lett. Rev. 9, 33–38.
Lindgren, S.W., Dobrogosz, W.J., 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. 87, 149–164.
Lopes, N.A., Brandelli, A., 2017. Nanostructures for delivery of natural antimicrobials in food. Rev. Food Sci. Nutr. Crit. https://doi.org/10.1080/10408398.2017.1308915.
Malheiros, P.S., Daroit, D.J., Silveira, N.P., Brandelli, A., 2010a. Effect of nanovesicle-encapsulated nisin on growth of Listeria monocytogenes in milk. Food Microbiol. 27, 175–178.
Malheiros, P.S., Micheletto, Y.M.S., Silveira, N.P., Brandelli, A., 2010b. Development and characterization of phosphatidylcholine nanovesicles containing the antimicrobial peptide nisin. Food Res. Int. 43, 1198–1203.
Malheiros, P.S., Sant'Anna, V., Utpott, M., Brandelli, A., 2012a. Antilisterial activity and stability of nanovesicle-encapsulated antimicrobial peptide P34 in milk. Food Control. 23, 42–47.
Malheiros, P.S., Sant'Anna, V., Barbosa, M.S., Brandelli, A., Franco, B.D.G.M., 2012b. Effect of liposome-encapsulated nisin and bacteriocin-like substance P34 on Listeria monocytogenes growth in Minasfrescal cheese. Int. J. Food Microbiol. 156, 272–277. https://doi.org/10.1016/j.ijfoodmicro.2012.04.004.
Meira, S.M.M., Zehetmeyer, G., Jardim, A.I., Scheibel, J.M., Oliveira, R.V.B., Brandelli, A., 2014. Polypropylene/montmorillonite nanocomposites containing nisin as antimicrobial food packaging. Food Bioprocess Technol. 7, 3349–3357.
Mills, S., Stanton, C., Hill, C., Ross, R.P., 2011. New developments and applications of bacteriocins and peptides in foods. Annu. Rev. Food Sci. Technol. 2, 299–329.
Mossallam, S.F., Amer, E.I., Diab, R.G., 2014. Potentiated anti-microsporidial activity of Lactobacillus acidophilus CH1 bacteriocin using gold nanoparticles. Exp. Parasitol. 144, 14–21.
Mozafari, M.R., Johnson, C., Hatziantoniou, S., Demetzos, C., 2008a. Nanoliposomes and their applications in food nanotechnology. J. Liposome Res. 18 (4), 309–327.
Mozafari, M.R., Khosravi-Darani, K., Borazan, G.G., Cui, J., Pardakhty, A., Yurdugul, S., 2008b. Encapsulation of food ingredients using nanoliposome technology. Int. J. Food Prop. 11, 833–844.
Mugabe, C., Azghani, A.O., Omri, A., 2005. Liposome-mediated gentamicin delivery: development and activity against resistant strains of Pseudomonas aeruginosa isolated from cystic fibrosis patients. J. Antimicrob. Chemother. 55, 269–271.
Namasivayam, S.K.R., Samrat, K., Debnath, S., Jayaprakash, C., 2015. Biocompatible chitosan nanoparticles incorporated bacteriocin (CSNps-B) preparation for the controlled release and improved anti-bacterial activity against food borne pathogenic bacteria Listeria monocytogenes. Res. J. Pharm. Biol. Chem. Sci. 6 (5), 625–631.
Ovchinnikov, K.V., Chi, H., Mehmeti, I., Holo, H., Nes, I.F., Diep, D.B., 2016. Novel group of leaderless multipeptide bacteriocins from gram-positive bacteria. Appl. Environ. Microbiol. 82 (17), 5216–5224.
Pandit, R., Rai, M., Santos, C.A., 2017. Enhanced antimicrobial activity of the food-protecting nisin peptide by bioconjugation with silver nanoparticles. Environ. Chem. Lett. 1–10. https://doi.org/10.1007/s10311-017-0626-2.
Parada, J.L., Caron, C.R., Medeiros, A.B.P., Soccol, C.R., 2007. Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. Braz. Arch. Biol. Technol. 50 (3), 521–542.

Patil, M.M., Pal, A., Anand, T., Ramana, K.V., 2010. Isolation and characterization of lactic acid bacteria from curd and cucumber. Indian J. Biotechnol. 9, 166–172.
Pinilla, C.M.B., Brandelli, A., 2016. Antimicrobial activity of nanoliposomes coencapsulating nisin and garlic extract against Gram-positive and Gram-negative bacteria in milk. Innov. Food Sci. Emerg. Technol. 36, 287–293.
Pranoto, Y., Salokhe, V.M., Rakshit, S.K., 2005. Physical and antibacte rial properties of alginate-based edible film incorporated with garlic oil. Food Res. Int. 38, 267–272.
Rajgovind, Sharma G., Gupta Kr, Deepak, Jasuja, N.D., Suresh, Joshi C., 2015. *Pterocarpus marsupium* derived phyto-synthesis of copper oxide nanoparticles and their antimicrobial activities. J. Microb. Biochem. Technol. 7, 140–144.
Ravishankar, R.V., Jamuna, B.A., 2011. Nanoparticles and Their Potential Application as Antimicrobials, Science against Microbial Pathogens: Communicating Current Research and Technological Advances. In: Méndez-Vilas, A. (Ed.), Formatex, Microbiology Series, Spain, 1, pp. 197–209.
Rhim, J.W., Park, H.M., Ha, C.S., 2013. Bio-nanocomposites for food packaging applications. Progr. Polym. Sci. 38, 1629–1652.
Rodriguez, E.G.B., Gaya, P., Nanez, M., Medina, M., 2000. Diversity of bacteriocins produced by Lactic Acid Bacteria isolated from raw milk. Int. Dairy J. 10, 7–15.
Rodriguez, E., Martinez, M.I., Horn, N., Dodd, H.M., 2003. Heterologous production of bacteriocins by Lactic Acid Bacteria. Int. J. Food Microbiol. 80, 101–116.
Saavedra, L., Minahk, C., Holgado, A.P., De, R., Sesma, F., 2004. Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH2-terminal sequence. Antimicrob. Agents Chemother. 48, 778–2781.
Sant'Anna, V., Malheiros, P.S., Brandelli, A., 2011. Liposome encapsulation protects bacteriocin-like substance P34 against inhibition by Maillard reaction products. Food Res. Int. 44, 326–330.
Saravana, K.P., Annalakshmi, A., 2012. Enhancing the antimicrobial activity of nisin by encapsulating on silver nanoparticle synthesized by bacillus sp. Int. J. Pharma. Biol. Arch. 3, 406–410.
Seil, J.T., Webster, T.J., 2012. Antimicrobial applications of nanotechnology: methods and literature. Int. J. Nanomed. 7, 2767–2781.
Sharma, A., Sharma, U.S., 1997. Liposomes in drug delivery: progress and limitations. Int. J. Pharm. 154, 123–140. https://doi.org/10.1016/S0378-5173(97)00135-X.
Sharma, N., Kapoor, G., Neopaney, B., 2006. Characterization of a new bacteriocin produced from a novel isolated strain of Bacillus lentus NG121. Antonie Leeuwenhoek 89, 337–343.
Sharma, T.K., Sapra, M., Chopra, A., Sharma, R., Patil, S.D., Malik, R.K., Navani, N., 2012. Interaction of bacteriocin-capped silver nanoparticles with food pathogens and their antibacterial effect. Int. J. Green Nanotechnol. 4, 93–110.
Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H., Ann, L.C., Bakhori, S.K., Hasan, H., Mohamad, D., 2015. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 7 (3), 219–242.
Teixeira, M.L., dosSantos, J., Silveira, N.P., Brandelli, A., 2008. Phospholipid nanovesicles containing a bacteriocin-like substance for control of Listeria monocytogenes. Innov. Food Sci. Emerg. Technol. 9, 49–53. https://doi.org/10.1016/j.ifset.2007.05.001.
Thirumurugan, A., Ramachandran, S., Shiamala Gowri, A., 2013. Combined effect of bacteriocin with gold nanoparticles against food spoiling bacteria – an approach for food packaging material preparation. Int. Food Res. J. 20 (4), 1909–1912.
Were, L.M., Bruce, B.D., Davidson, P.M., Weiss, J., 2003. Size, stability, and entrapment efficiency of phospholipid nanocapsules containing polypeptide antimicrobials. J. Agric. Food Chem. 51, 8073–8079.
Zacharof, M., Lovitt, R.W., 2012. Bacteriocins produced by lactic acid bacteria: a review article. APCBEE Proc. 2, 50–56.
Zawrah, M.F., Sherein, A.E., 2011. Antimicrobial activities of gold nanoparticles against major foodborne pathogens. Life Sci. 8 (4), 37–44.
Zohri, M., Alavidjeh, M.S., Haririan, I., Ardestani, M.S., Ebrahimi, S.E.S., Sani, H.T., Sadjadi, S.A., 2010. A comparative study between the antibacterial effect of nisin and nisin-loaded chitosan/alginate nanoparticles on the growth of Staphylococcus aureus in raw and pasteurized milk samples. Probiotics Antimicrob. Proteins 2, 258–266.
Zohri, M., Shafiee Alavidjeh, M., Mirdamadi, S.S., Behmadi, H., Hossaini Nasr, S.M., Eshghi Gonbaki, S., Ardestani, M.S., Arabzadeh, A.J., 2013. Nisin−Loaded chitosan/alginate nanoparticles: a hopeful hybrid biopreservative. J. Food Saf. 33, 40–49.