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REVIEW

Antimicrobial peptides as natural bio-preservative to enhance the shelf-life of food

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Abstract Antimicrobial peptides (AMPs) are diverse group of natural proteins present in animals, plants, insects and bacteria. These peptides are responsible for defense of host from pathogenic organisms. Chemical, enzymatic and recombinant techniques are used for the synthesis of antimicrobial peptides. These peptides have been found to be an alternative to the chemical preservatives. Currently, nisin is the only antimicrobial peptide, which is widely utilized in the preservation of food. Antimicrobial peptides can be used alone or in combination with other antimicrobial, essential oils and polymeric nanoparticles to enhance the shelf-life of food. This review presents an overview on different types of antimicrobial peptides, purification techniques, mode of action and application in food preservation.

Keywords Antimicrobial peptides · Bio-preservation · Food preservation · Nisin

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Introduction

Generally, food industry depends on chemicals for the preservation of foodstuff and to increase the shelf life of food. The use of chemical preservatives such as nitrites and sulfur dioxide may cause adverse effects on human health and also on the nutrition level of food. Due to the traditional food preservation practices, the safety and standard quality of food is inadequate for the consumers, because of the excess use of chemical preservatives, bacteria have developed resistance (Saeed et al. 2009). Hence, there is a pressing need to search new natural preservatives for the preservation of food. The major benefit of using antimicrobial peptides is that it preserves the food without changing its quality and it is not harmful (Wang et al. 2016). Considering the problem of food-spoilage, food products can be preserved by using microbes and their antimicrobial products as bio-preservatives, which improve the shelf-life of food and enhance the food safety (Galvez et al. 2014; Song et al. 2014).

Alternatively, antimicrobial peptides are also referred to as cationic host defense peptides (Abraham et al. 2014, Bala and Kumar 2014), anionic peptides/proteins (Harris et al. 2009), cationic antimicrobial peptides, and α-helical antimicrobial peptide (Riedl et al. 2011). Antimicrobial peptides may be synthesized ribosomally or non-ribosomally. In eukaryotes and prokaryotes, antimicrobial peptides are synthesized in ribosome, and hence known as ribosomally synthesized peptides. Non-ribosomal peptides are synthesized in the cytosol of fungi or bacteria (Riberio and Carrasco 2014; Rea et al. 2011). For example, lipopeptide is non ribosomally synthesized peptide, which possess broad spectrum activity against multidrug resistant microorganisms (Pasupleti et al. 2012; De Zoysa et al. 2015).

The major problem in the food industry is contamination of food by pathogens, such as *Salmonella* spp., *Shigella* spp.,

Micrococcus spp., *Enterococcus faecalis, Bacillus licheniformis*, *Escherichia coli*, *Listeria monocytogenes, Staphylococcus aureus, Campylobacter jejuni, Yersinia enterocolitica, Vibrio parahemolyticus, Escherichia coli* 0157:H7, and *Clostridium botulinum,* etc. Many researchers pointed out that antimicrobial peptides demonstrated activity against several food-borne pathogens, and therefore, can help in the food preservation (Elayaraja et al. 2014; Hintz et al. 2015; Kraszewska et al. 2016). Antimicrobial products such as antimicrobial proteins or peptides secreted from animals, plants and bacteria are employed for food preservation. Fermentation is one of the best example of food preservation, which involves the growth of the microorganisms. Among these, lactic acid bacteria are the most prominent microorganisms in the process of the fermentation, which produces organic acids, other metabolites and antimicrobial proteins known as antimicrobial peptides (Abdelbasset et al. 2008). Lactic acid bacteria have been used as a bio-preservative for fermented food, because of production of lactic acid, hydrogen peroxide and small amount of peptides, which inhibit the growth of microorganisms. United States Food and Drug Administration (USFDA) approved lactic acid bacteria as a safe agent for the preservation of fermented food (Meza et al. 2015; Upendra et al. 2016). Now a days, *Bacilli* are used in preservation of food as they secrete antimicrobial peptides (Sumi et al. 2015). This type of biopreservation does not alter the flavor, maintain the texture and aroma, without changing the physical, chemical and biological properties of the food. The experiments conducted on the laboratory animals demonstrated that antimicrobial peptides are generally non-toxic to the humans. Moreover, these are thermoresistant, and even after pasteurization they showed excellent antimicrobial activity against both Gram positive and Gram negative bacteria (Galvez et al. 2014; Zhao et al. 2015; Upendra et al. 2016).

In vertebrates, antimicrobial peptides act as the first line of defense because they play role in inhibition of the primitive stage of destruction (Bagley 2014). Usually, they are composed of 10–50 amino-acid residues arranged in different groups depending upon the amino acid composition, size and conformation (Barany and Merrifield 1980; Strempel et al. 2015). In general, when an antimicrobial peptide is folded in certain membrane or membrane- like environment, one part of the antimicrobial peptide is positively charged, which consist of lysine and arginine residues, while the other side contains a proportion of hydrophobic residues (Bagley 2014).

History of antimicrobial peptide

Alexander Fleming in the late 1920s recognized lysozyme and contemplated it as the first antimicrobial peptide (Fleming 1922). Unfortunately, the exact mode of action of lysozyme was not known to the researchers (Vocadlo et al. 2001). Later, its mode of action i.e. the enzymatic destruction of the bacterial cell wall was determined by Salton (1958). Antimicrobial peptides were first documented in prokaryotic cells. Hotchkiss and Dubos (1940) isolated an antimicrobial substance from *Bacillus brevis* and named as gramicidin, which showed in vitro and in vivo activity against many Gram-positive bacteria. Later, it was reported that Gramicidin is effective against infected wounds of guinea-pig and since then it is being used as a therapeutic agent (Gause and Brazhnikova 1944). In 1941, antimicrobial peptide Tyrocidine was reported with activity against both Gram-positive and Gram-negative bacteria (Zaffiri et al. 2012).

In 1942, the antimicrobial peptide like substance was isolated from the endosperm of wheat (*Triticum aestivum*) which demonstrated antimicrobial activity against various phytopathogens such as *Pseudomonas solanacearum, Xanthomonas compestris* (Fernandez et al. 1972). Later on, it was named as purothionin (Mak and Jones 1976; Ohtani et al. 1977). First time in 1956, antimicrobial peptide defensin was isolated from the leukocyte of rabbit (Ohtani et al. 1977). The antimicrobial protein lactoferrin was obtained from milk (Groves et al. 1965; Stephens and Marshall 1962). Later on in 1970s and 1980s, many authors reported isolation of antimicrobial peptides from the human leukocytes (Ganz et al. 1985). In 1987, antimicrobial magainins were isolated from the African clawed frog *Xenopus laevis*. In 1990, the first anionic antimicrobial peptide was isolated from *Xenopus laevis* (Brogden et al. 1997).

Sources of antimicrobial peptides

Antimicrobial peptides occur in both prokaryotes (Table 1) (e.g. bacteria) and eukaryotes (e.g. fungi, plants and animals) (Bagley 2014; Berglund et al. 2015).

Peptides from prokaryotes

Antimicrobial peptides from microorganisms

Antimicrobial peptides originated from the bacteria recognized as peptides from prokaryotes. Hiolbiotics, colicin, lantibiotic and microcin are the various examples of prokaryotic peptides (Bagley 2014).

Bacteriocin is produced by bacteria such as lactic acid bacteria, *Bacillus*, *Staphylococcus*, *Streptomyces* and *Streptoverticillium* spp. Nisin (Fig. 1), pediocin PA-1/AcH are few examples of antimicrobial peptides isolated from microorganisms.

Table 1 Sources of antimicrobial peptides

Sr. no	Source of antimicrobial peptides	Name of antimicrobial peptides
1	Insects	Cecropin A, ceratotoxin, stomoxyn, spinigerenin, thanatin, heliomicin, sapecin, defensin A, smD1, gallerimycin, termicin, royalisin, drosomycin, drosocin, metchnikowin, apidaecin IA,abaecin, formaecin, lebocin, pyrrhocoricintin, attacins, coleoptericin, diptericin (Song and Zheng 2015)
2	Amphibians	Magainins (Oyinloye et al. 2015).
3	Plants	Plant defensins, cyclotides, 2S albumin, lipid transfer proteins, hevein-like proteins knotins, snakins (Hintz et al. 2015)
4	Milk	β-lactoglobulin, αs2-casein, β-casein (Fadaei 2012)
5	Bacteria	Lacticin 3147, Nisin, Lactococcin B, Leucocin A, Enterocin A, Pediocin A,Pediocin F, Pediocin PA-1,Mesentericin Y105, Pediocin AcH, Acidophilin, Acidolin, Lactacin B, Lactacin F, Lactobacillin, Lactobrevin, Reuterin, Bulgarin, Plantaricin SIK-83, Plantaricin A, Plantaricin B, Lactolin, Lactolin 27, Helveticin J, Lactobin A, Sonorensin (Chopra et al. 2014)

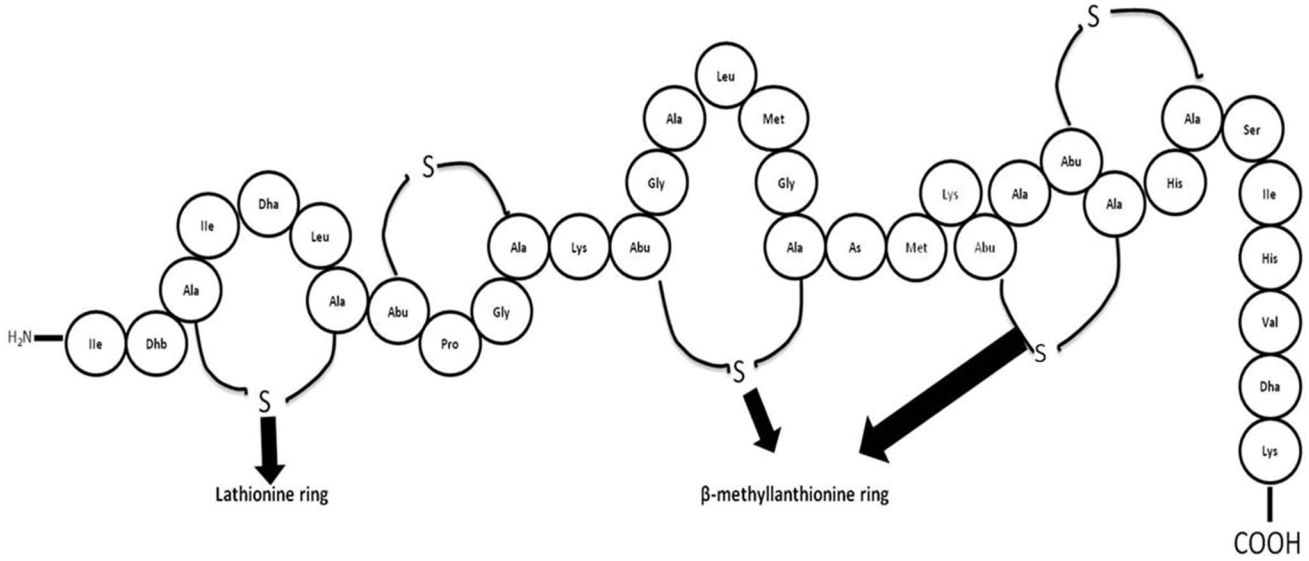

Fig. 1 Structure of nisin

Eukaryotic peptides

Antimicrobial peptides from animals

These include the peptides obtained from mammals, amphibians and fish, etc. Antimicrobial peptides from mammals are secreted in the mucosal epithelial cells and paneth cells. Mammalian leukocyte is rich source of antimicrobial peptides which helps in preventing the bacterial infection. These antimicrobial peptides are cationic in nature (Strempel et al. 2015). Pleurocidin and protamine are two types of antimicrobial peptides isolated from fish which showed activity against *L. monocytogens* and other food-spoilage organisms and hence, could be used in food preservation. Moreover, magainins (Fig. 2) derived from amphibians, show broad-spectrum activity against Gram-positive and Gram-negative bacteria. It is generally used for the preservation of meat and cheese (Tiwari et al. 2009).

Antimicrobial peptides from insects

Insects produce maximum number of antimicrobial peptides which demonstrated antimicrobial activity against *Micrococcus luteus, Aerococcus viridians, Bacillus megaterium, Bacillus subtilis, Bacillus thuringiensis,* and *Staphylococcus aureus*. Sarco toxin IIA, hymenoptaecin, attacin, diptericin and coleoptericin are examples of antimicrobial peptides produced by insects (Song and Zheng 2015; Wang et al. 2016).

Antimicrobial peptides from plants

Plant antimicrobial peptides are cysteine-rich with molecular weight in the range of 2–9 kDa. They can be isolated

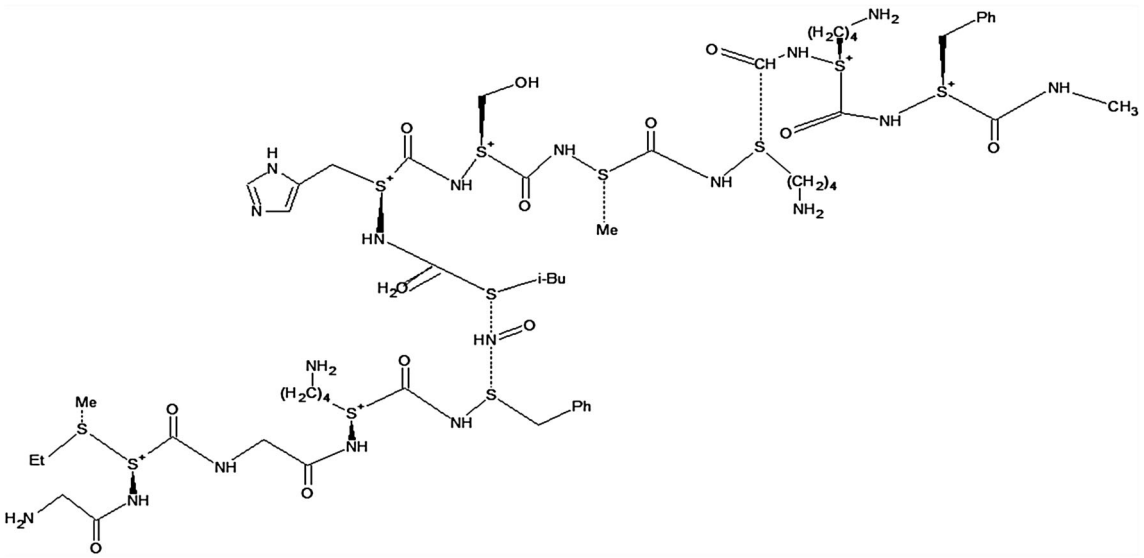

Fig. 2 Structure of magainins

from various plant parts such as tubers, leaves, pods, seeds and flowers. These peptides exhibit broad antibacterial activity against *L. monocytogens, S.* Typhimurium and *E. coli* O157:H7, and therefore, can be used in food preservation. Lipid transfer protein 2, Snakin1, Kalata B1, thionin and potato defensins are examples of plant antimicrobial peptides (Hintz et al. 2015).

Antimicrobial peptides from milk

Lactoferricin, kappacin and k-casecidin are examples of antimicrobial peptides derived from milk proteins. They possess remarkable antibacterial activity and hence, used as food preservatives (Fadaei 2012).

Types of antimicrobial peptides

Antimicrobial peptides from eukaryotes are divided into two types: cationic peptides and anionic peptide. Examples of cationic and anionic peptides have been provided in Table 2.

Cationic peptides

More than 1000 cationic peptides are known, which exhibit positive charge and hence, termed as cationic antimicrobial peptides (CAPs) (Strempel et al. 2015).

1. Defensins

Defensins are cysteine-rich cationic peptides isolated from plants, mammals and insects. They act as natural antibiotic and show antibacterial, antifungal and antiviral activity. There are three types of defensins known from human beings namely, human neutrophile peptide 1 (Fig. 3), 2 and 3 (HNP1, 2 and 3). Also, in human β defensisns (hBD) are produced by certain epithelial cells, which possess antimicrobial activity.

Plant defensins contain 45–54 amino acid residues in its structure. From the structural study, it was confirmed that plant defensins are characterized by 8 cysteine and 2 glycine at position 13 and 34 with an aromatic acid and glutamic acid in its structure. Plant defensins do not show antibacterial activity but possess antifungal activity against plants and human pathogens (Oyinloye et al. 2015).

2. Cathelicidins

These cationic peptides are mostly found in the mammals. There are about 30 cathelicidins reported from mammals. The cathelicidin (LL-37) present in the human body is known for its activity against bacteria, fungi and viruses (Oyinloye et al. 2015).

3. Cecropins

Cecropins were first identified in cecropia moth. It comprises of 35–37 amino acid residues with the molecular weight of 3–4 kDa. It shows antibacterial, antifungal, antiprotozoal activity (Bagley 2014).

4. Histone derived and amino acid enriched peptides

These are two types of antimicrobial peptides, which ensure antimicrobial activity by the disruption of microbial cells.

(i) Histone derived peptides

Buforin and parasin are two examples of histone derived peptides, which are generally obtained from amphibians.

Table 2 Examples of few cationic and anionic peptides

Sr. no	Names of antimicrobial peptides	Molecular formula	Single amino acid code sequence
1	Magainins 1	$C_{11}H_{177}N_{29}O_{28}S$ 2466.9	GIGKFLHSAKKFGKAFVGEIMNS
2	Defensins HNP-1 human	$C_{150}H_{222}N_{44}O_{38}S_6$ 3442.03	ACYCRIPACIAGERRYGTCIYQGRLWAFCC
3	Cathelicidins LL37	$C_{205}H_{340}N_{60}O_{35}$ 4493.37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
4	Cecrocin B	$C_{176}H_{302}N_{52}O_{41}S$ 3834.66	YIQHNFUJWYYSEC-MQAAYMCRSA-N
5	Maximin H5	$C_{99}H_{160}N_{20}O_{29}$ 2022.38	ILGPVLGLVSDTLDDVLGIL
6	Dermcidin1L	$C_{210}H_{359}N_{57}O_{71}$ 4818.5	SSLLEKGLDGAKKAVGGLGKLGKDAVED

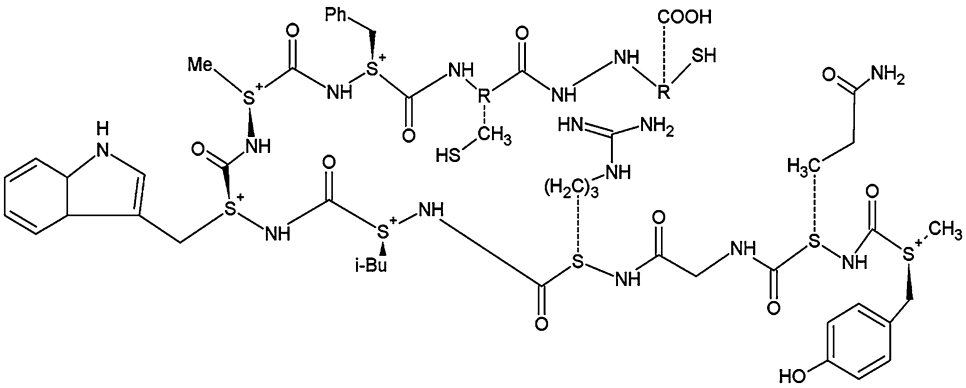

Fig. 3 Structure of human neutrophile peptide 1 (HNP1)

Histone derived peptides are highly active against fungi and bacteria (Bagley 2014; Oyinloye et al. 2015; Wang et al. 2011).

(ii) Amino acid enriched peptides

These peptides are rich in amino acids, such as proline and glycine. These are generally found in mammals and insects with strong antimicrobial activity (Bagley 2014; Oyinloye et al. 2015).

Cationic peptides are further classified into three groups depending on their structures: α-helical peptides, β-sheet peptides and extended peptides.

- α-helical peptides

α-helical peptides are characterized by α helical conformation with a slight bending at its central position (Peravali et al. 2013). These peptides in an aqueous environment, lose structure but regain its structure when kept in a hydrophobic solvent. Magainin, cecropin and pexiganan are known examples of α-helical peptides (Bala and Kumar 2014).

- β-sheet peptides

This class of peptides has an antiparallel β-sheet. They are generally stabilized by the disulphide bridges and responsible for the stability of the peptides. α-, β-defensins and protegrin are examples of β-sheet antimicrobial peptides (Bala and Kumar 2014).

- Extended peptides

Extended peptides are without secondary structures because they consist of proline and glycine residues. Hydrogen bond and Vander Waal forces are responsible for interaction of extended peptides; for example, antimicrobial peptide histatin present in human saliva (Bagley 2014).

Anionic peptides

Anionic peptides are very small with molecular weight ranging from 721.6 to 823.8 Da. It possesses antimicrobial activity against Gram-positive as well as Gram-negative bacteria (Bagley 2014, Cruz et al. 2014)

. These peptides are rich in glutamic and aspartic acid. Maximin-H5 and Dermcidin are two examples, which are derived from human sweat. The drawback of anionic peptide is that it requires positively charged ion for its activity e.g. Zn^{2+} (Bagley 2014).

Mode of action of antimicrobial peptides

The mechanism or mode of action of antimicrobial peptide mainly depends on the interaction of peptides with the cell membrane and its composition. Generally, antimicrobial peptides interact with the membrane by the electrostatic interaction (Guilhelmelli et al. 2013). Antimicrobial peptides have membrane permeabilizing action. They can enter into the membrane resulting into its disruption (Bolintineanuand Kaznessism 2011). For understanding the mechanism of antimicrobial peptides, various models have been proposed which occur when peptides attach the cell membrane of bacteria and it completely disrupts the cell membrane integrity (Berglund et al. 2015). Carpet model, toroidal pore model, brave straw model and aggregate model are well-studied models of antimicrobial peptides (Strempel et al. 2015; Zhao et al. 2015).

Carpet model

Carpet model, also known as a detergent model, was proposed by Gazil et al. (1996). This model is the most described model of membrane destabilization, in which peptides assemble and accumulate in a parallel manner around the membrane of microorganisms. Therefore, it is known as carpet model. When the threshold concentration level of peptide reaches to maximum, these molecules penetrate into lipid membrane of bacteria. In the year 1999, according to Shai-Matsuzaki-Huang, in "carpet" model globular bilayer destabilization occurs which marks the membrane disruption of microorganisms (Conde et al. 2012; Berglund et al. 2015).

Toroidal pore model

This model is also known as wormhole hypothesis (Pelegrini et al. 2011) which was described by Ludtke et al. (1996). It is the second most characterized and studied peptide after the carpet model. Antimicrobial peptides get assembled on a bacterial membrane by its hydrophilic region of the peptide and polar region of phospholipid layer. When the peptides interact with the charged hydrophobic cell membrane of bacteria, it transforms into α helical structure. At the initial stage, helices remain parallel to the bacterial membrane but when the concentration level of peptides reach the maximum level, all the peptides change their orientation in such a way that they are perpendicular to the membrane. After the development of sufficient stress by antimicrobial peptides, thinning and destabilization of the membrane occurs, which result into the disruption of membrane integrity (Carneiro et al. 2015).

Barrel-stave model

In this model, antimicrobial peptides are assembled around the bacterial membrane in the form of a bundle. The positive end of the peptide interacts with the hydrophobic chain of the fatty acid present in the phospholipids layer of bacterial membrane. The initial step involved in this model is the binding of peptides with the membrane like a monomer. Aggregation of peptides resulted into the membrane disintegration, which leads to the formation of pores. Finally, bacterial cell death takes place (Bahar and Ren 2013; Song and Zheng 2015).

Aggregate model

In this model, peptide attachment occurs due to the electrostatic interaction between hydrophilic region of the peptide and the phospholipids layer of membrane. These aggregates are arranged vertically on the membrane and form sphere-like structure. The aggregates contain water molecule with discharge of fluid resulting into membrane disruption (Song and Zheng 2015).

Many antimicrobial peptides do not have ability to permeate the cell membrane of the bacteria and such mode of action is known as non membrane permeabilizing mechanism. Many antimicrobial peptides bind with the intracellular targets such as cell wall, nucleic acids, and disturbs the protein modification mechanisms. Antimicrobial peptide interacts with the peptidoglycan layer or lipopolysaccharides leading to the formation of pores. Some of the bacteriocin interact with the lipid II and inhibit the cell wall synthesis, forms transmembrane process and affects the efflux pump and loss of ions is observed (Fig. 4). Various researchers investigated that few antimicrobial peptides enter inside the cell without the disruption of cell membrane and cell wall and targets the nucleic acids such as DNA, RNA (Zhao et al. 2015; Scocchi et al. 2016). Certain antimicrobial peptides inhibit the DNA synthesis, do not form the septum of the membrane. It also affects the enzyme activity at the time of replication and protein synthesis. Antimicrobial peptides also block the process of translation and transcription. Protein modification process is also affected because of the antimicrobial peptides (Guilhelmelli et al. 2013; Song et al. 2014; Zhao et al. 2015) (Fig. 4).

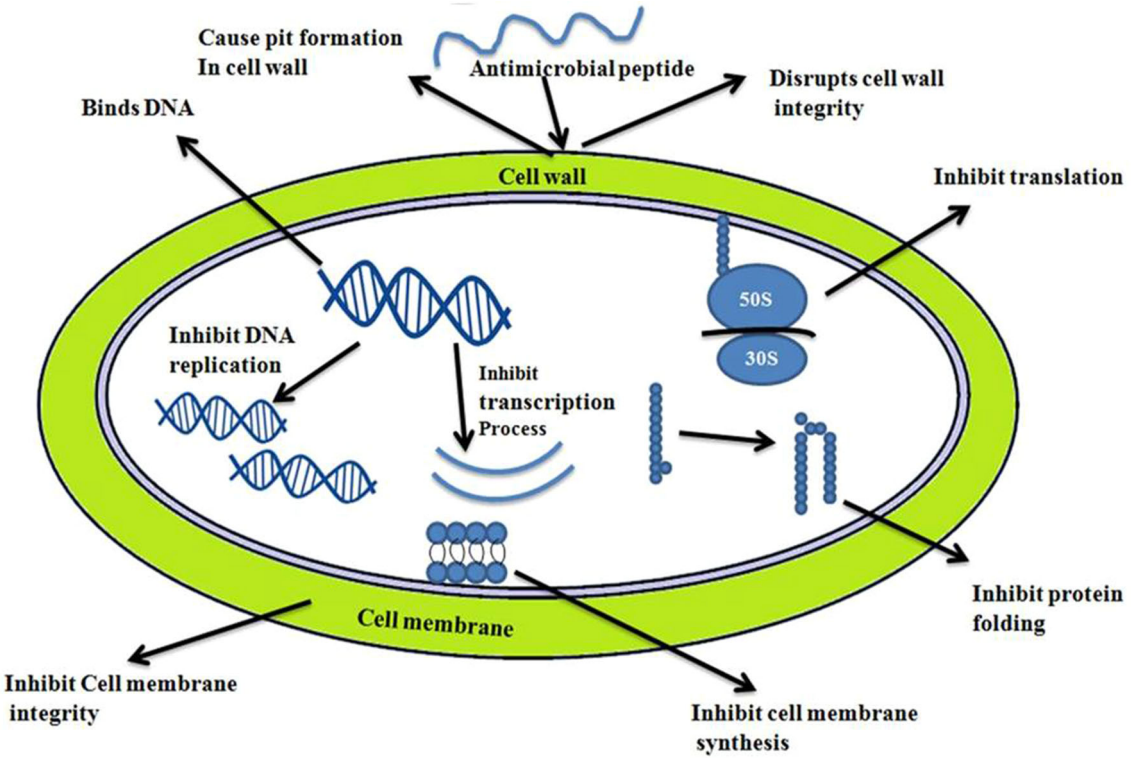

Fig. 4 Mode of action of antimicrobial peptides

Methods of peptide synthesis

Due to ever increasing need of peptides, there is a necessity of synthesizing new peptides, which can be used in the clinical, biochemical and pharmacological research (Colins et al. 2012). Chemical, physical and biological methods have been developed for the synthesis of peptides.

Chemical method of synthesis

There are two types of chemical methods used for the synthesis of peptides: (i) classical method (traditional method, and (ii) solid phase synthesis.

In classical method or traditional method of peptide synthesis, many steps are involved at the time of synthesis of peptide. Even after synthesis of antimicrobial peptides, various purification steps like extraction, precipitation and chromatography are required. It is tedious, time consuming and difficult to perform (Etchegaray and Machini 2013).

Solid phase synthesis (SPS) is a simple method, in which peptide synthesis takes place in an abundant amount. A solid support is used at the time of peptide synthesis which is known as resin. It has a functional group called linker, which helps in the synthesis of peptides. The generalized steps used for the synthesis of peptides are acylation of an amino acid, N-terminal removal (deprotection), complex peptide-linker-resin formation (coupling) and cleavage of complex peptide linker resin (cleavage and total deprotection) (Etchegaray and Machini 2013).

Among the above two chemical methods of peptide synthesis, SPS technique is better as it is faster and its automation is easy to perform. This technique is also beneficial as unnatural amino acids can be incorporated in the reaction which helps in the peptide synthesis. The only drawback of chemical method of synthesis is that it is an expensive method.

Enzymatic method of synthesis

In this method, enzyme can be in free or in immobilized state. This method is generally used for the synthesis of a very short peptide (So et al. 1998). Protease enzymes such as chymotrypsin, papain, alcalase, pronase, penicillin acylase and lipase are mostly used in the enzymatic method of peptide synthesis (Chen et al. 2010). Key advantages of the enzymatic reaction are mild conditions of the reaction and high region specificity of the enzyme (Chen et al. 2010).

Recombinant method of synthesis

In this method of synthesis, cloning and gene expression in microorganisms are two important events, which results in the peptide production. Several peptides are synthesized by this method. Generally, *E. coli* is used as a host organism

Table 3 Different purification techniques of antimicrobial peptides

Sr. no	Purification technique	Description of method
1	Ammonium sulphate precipitation	Salting out of protein is performed by using ammonium sulphate precipitation. Percent of ammonium sulphate precipitation varies with antimicrobial peptides. It is used for the partial purification of peptides
2	Ion exchange chromatography	Depends on the charge of peptide and surface of resisn.Cationic or ionic resins are used as per the charge of the antimicrobial peptides. High strength exchanger is required for large scale purification
3	Affinity chromatography	Depends on the interaction between protein or peptide and a specific ligand present in chromatography matrix. It is not so much routinely used for the purification as the other chromatographic techniques
4	Size exclusion chromatography	Depends on the molecular weight of peptides. Generally used after the ammonium salt precipitation. Purification efficiency of antimicrobial peptides is somehow lower
5	High performance liquid chromatography and Reverse phase HPLC	This is the method of choice, complete purification of antimicrobial peptides. Silica based column is used. Automatic sampling devices, gradient automated pumps. It is highly recommended method of purification of peptides
6	Acetone precipitation	It is used for the salting out of protein and partial purification of antimicrobial peptides
7	Thin layer chromatography	Silica plates are used for determining the purity of the antimicrobial peptides. It is easy to perform, less costly, small quantity of sample is required
8	Polyacrylamide gel electrophoresis (PAGE)	Two gels are required for the running the samples stacking gel and resolving gel. It is used for determining the purity and the molecular weight of the peptide
9	UV–Visible spectrophotometry (UV–Vis spectroscopy)	Used for the qualitative purification of antimicrobial peptides, spectra of crude extract is compared with the standard curve of antimicrobial peptide

for the synthesis of peptides (Sewald and Jakubke 2002; Wang et al. 2011).

Among all the above methods for antimicrobial peptides synthesis, recombination technique is better as compared to chemical and enzymatic methods. This method is a good option for the discovery of new peptides, which will certainly help in the preservation of food.

Methods for purification of antimicrobial peptides

Bacteriocins are often ribosomally synthesized, extracellularly released low molecular weight peptides usually with 30–60 amino acids, which shows bactericidal effect on other closely related bacteria (Meza et al. 2015; Settanni and Corsetti 2008). Bacteriocins are cationic in nature, as they are rich in lysyl and arginyl residues. More research has been carried out on the bacteriocin produced by Gram-positive bacteria especially from lactic acid bacteria (Meza et al. 2015). Ammonium sulphate precipitation, ion exchange chromatography, size exclusion chromatography, affinity chromatography, capillary electrophoresis and phase high-performance liquid chromatography are various techniques used for the purification of antimicrobial peptides (Galvez et al. 2014). Common methods used for determining antimicrobial peptide is depicted in Fig. 4 and comparison of all the techniques is given in Table 3. The generalized method used for the purification of bacteriocin is given in Fig. 5. Purification technique should be easy and simple with minimum processing. For large scale purification of antimicrobial peptides, at least two types of chromatographic techniques are used in combination such as ion exchange chromatography and gel permeation followed by HPLC (Espitia et al. 2012).

Ammonium sulphate precipitation

Ammonium precipitation method is used for the isolation of many antimicrobial peptides including bacteriocins. This technique is used for salting out of proteins or peptides. The crude extract of peptide is first centrifuged then cell-free supernatant is cooled at 20 min at 10,000 rpm and 4 °C. The pellet of antimicrobial peptides is dissolved in distilled water or buffer solution and later membrane dialysed. This step is followed by gel filtration for purification of the peptides (Mohanty et al. 2016).

Acetone precipitation method

Like ammonium sulphate precipitation, acetone precipitation is used for the salting out of protein or peptides. The crude extract of the bacteriocin is centrifuged to get the cell free extract. The supernatent is seperated and the acetone is added in that cell free filtrate, then it is allowed to cooled and kept overnight in the freeze at 4 °C, which is followed by the centrifugation at 10,000 rpm. The obtained pellet is dissolved in deionized water and can be purified by using

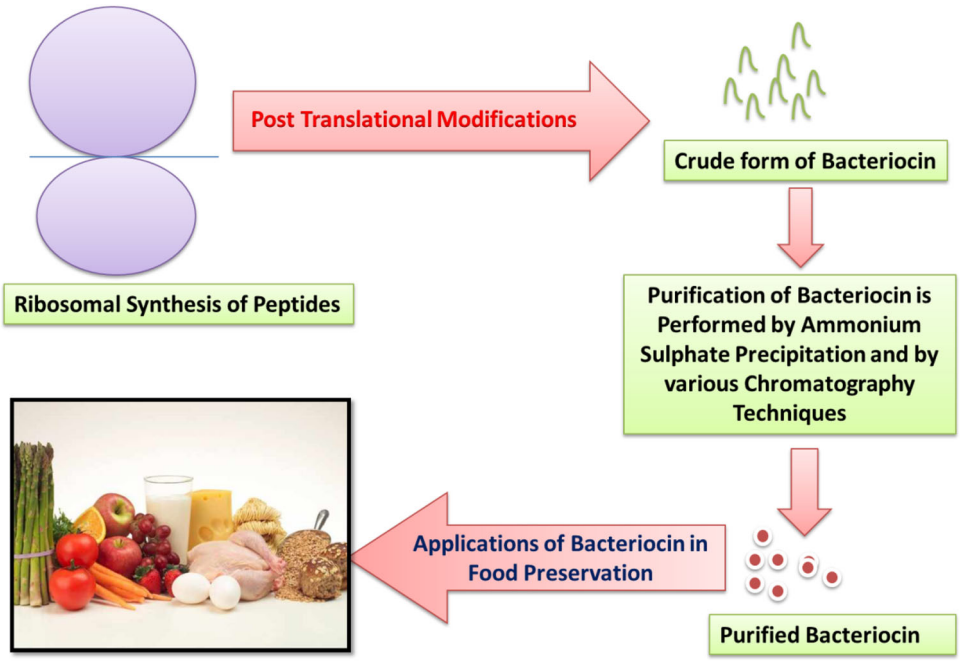

Fig. 5 Method for bacteriocin production

other chromatographic techniques (Biswas and Banerjee 2016).

Ion exchange chromatography (IEC)

IEC is a separation technique, which depends on the interaction of the charge present on the surface of peptides and resin. It may be cationic or anionic exchange depending on charge of the peptide. For large scale purification of peptides high flow rate and strength exchanger are required, which can increase the efficiency of purification. The most commonly used salt is sodium chloride (Colins et al. 2012).

Affinity chromatography

Affinity chromatography depends on the interaction between proteins or peptide and a specific ligand present in chromatography matrix. Peptide of interest bound to ligand and unbounded material is washed away. The bound protein can be obtained by providing suitable conditions such as changing pH, ionic strength or polarity. This method is employed for the purification of peptides but unfortunately, it provides partial purification (Colins et al. 2012).

Size exclusion chromatography

It is also known as gel permeation or gel filtration method, which depends on the molecular weight of protein or peptides. Molecule separation of this technique depends on the size exclusion. Gel permeation technique is generally used for desalting of ions so, it is employed after the salt precipitation. In this method, resin acts as stationary phase; whereas, buffer is used as a mobile phase. Salt is present in the buffer solution which is responsible for the separation of proteins and beads (Colins et al. 2012). The drawback of this technique is that the capacity of column used for size exclusion chromatography is less and the flow rate is slow. Usually for the purification of antimicrobial peptides, Sephadex G-100 and 0.02 M sodium acetate buffer are used. Column elution is also conducted by using the same buffer. The collected fraction of the eluted sample is then tested by using SDS-PAGE to determine the molecular weight of the desired antimicrobial peptide (Jabeen and Khanum 2014; Mohanty et al. 2016).

Capillary electrophoresis

It is a versatile method used for the purification of peptides. It relies on the migration of the charges present in the peptides as per the charge present in the solution and also on the charge of electric field (Colins et al. 2012).

High performance liquid chromatography (HPLC)

HPLC is considered as one of the most important methods, which is used for isolation and purification of peptides irrespective of their source and its quantity (Colins et al. 2012). In this method, column and high pumping systems are utilized for the purification of peptides. The charge present on the surface of stationary column and antimicrobial peptides are opposite. Two solutions, such as 0.1 %

trifluroacetic acid and 80 % acetonitrile are used for maintaining the charge, pH of the column and for the proper purification of peptides. Automatic and high pumping system is the major advantage of this technique, so the manual errors which take place by other methods can be minimized. Different flow rate and solvents can be used as per the purification of the desired peptide (Mohanty et al. 2016; Sankar et al. 2012).

Reversed-phase high performance liquid chromatography (RP-HPLC)

RP-HPLC is a well-known tool for the purification of the antimicrobial peptides. For the purification of peptides, the lypohilized peptide is dissolved in water and fractionated under reverse HPLC and passed through RP-C18 column. The eluted sample is collected at various times interval. Its absorbance is noted and purity or maximum content of peptides can be detected (Sankar et al. 2012).

The stability and purity of peptide can be efficiently determined by reverse phase HPLC and hence, it is widely employed for large scale purification of peptides. In HPLC and Reverse phase HPLC, automated controller pumps are used. Handling device is also automated to enhance efficiency. Ion exchange chromatography and ammonium sulphate precipitation yield the partial purification of the peptides (Mohanty et al. 2016).

Thin layer chromatography

Thin Layer Chromatography or High Performance Thin Liquid Chromatography (HP TLC) is is recently used by the researchers for the purification of the peptides. HP TLC method have improved the purification of peptides. It has several advantages such as it is easy to perform, cheaper, small quantitiy of sample is required. It is performed on 40 % silica gel. Then the sample is loaded on the silica plate and it is subjected to different organic solvent system. Then separated bands or spots appeared on the plates and it is observed under UV-trans illuminator. The eluted spot of antimicrobial peptide is generally tested for its antimicrobial activity (Biswas and Banerjee 2016).

Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel electrophoresis is one of the techniques used for determining the purity and the molecular weight. The purified antimicrobial peptide is subjected to tricine sodium do decyl sulphate polyacrylamide electrophoreis. Two gels are used for performing the electrophoresis, one is (5 %) stacking solution and another is separating gel (12 %). Polypeptide standard solution is used as the standard for estimating the molecular weight of the peptide. At the time of electrophoresis, two different voltages are used. When the samples are in stacking gel, the voltage is 30 V; whereas when the samples are in resolution gel the voltage is 150 V (Zhao et al. 2015; Upendra et al. 2016).

UV–Visible spectrophotometry (UV–Vis spectroscopy)

The qualitative purification of antimicrobial peptide is determined with the help of UV–Vis spectrophotometry. It was investigated by the researchers that for determining the purity of bacteriocin, the UV Vis spectra ranges from 200 to 400 nm. After comparing the UV–Vis spectra of standard along with purified crude extract, purity of the crude extract can be estimated (Song et al. 2014; Zhao et al. 2015; Upendra et al. 2016). Currently, Upendra et al. (2016) reported the purity of crude extract of nisin by comparing the UV–Vis spectra of the standard nisin. It was found that pure nisin shows UV–Vis absorbance at 280–320 nm.

Matrix-assisted laser desorption/ionization (MALDI)

MALDI is supposed to be the potential method to analyse the molecular mass of the purified peptides. It can be used to estimate the purity of the antimicrobial peptides. For the sample analysis, very small quantity of the sample is required, which is mixed with the suitable amount of the matrix. It is allowed to mix properly and then placed on the metal slide. The metal plate containing sample is irradiated with UV light (Nitrogen laser light). The matrix absorbs the laser energy and it get ionized. The matrix contains several ionized matrix ions and transfers proton to the antimicrobial peptide molecule and after that it forms the charged ions. It produces the spectra showing the molecular mass of the sample (Song et al. 2014).

Application of peptides in food preservation

For increasing the shelf-life of food, various antimicrobial agents such as peptides and bacteriocins synthesized by Gram-positive and Gram-negative bacteria are used as bio-preservative for cheese, yogurts and portuguese fermented meat, etc.

Nisin as a natural food preservative

Dairy products

Preservation of cheese and yogurt

Nisin is commercially known as nisaplin^{TM} and sold in its lyophilized state (Mills et al. 2011). It is used as a natural

preservative of cheese, which is generally contaminated with *Lactobacillus* spp, *L. monocytogens,* and *S. aureus.* These bacteria are responsible for the flavor change and results into gas production (Upendra et al. 2016).

Block cheese, slice, spreads, sauces and dips are different forms of processed cheese. All these products are very much prone to the bacterial contamination, which is generally responsible for food-spoilage. Usually, processed food product possesses a high pH, and very low redox potential and hence, it is suitable for the growth of *Clostridium* spp. (D'Amato and Sinigaglia 2010; Suganthi et al. 2012). Therefore, in order to increase the shelf-life of cheese nisin is used. Interestingly, Zohri et al. (2013) found that nisin loaded chitosan nanoparticles can be used for the preservation of cheese. Other antimicrobial peptides used in the biopreservation of dairy products are Pediocin ACH. These peptides are used in milk and in the preparation of cheese. These peptides demonstrated antimicrobial activity against *L. monocytogens*, *E. coli* and *S. aureus*. Enterocin As-48 is used in the preservation of milk and Manchego cheese (Galvez et al. 2014).

Nisin can be used as a preservative to increase the shelf-life of yogurt. It inhibits the food-spoilage and maintain the flavor and aroma of yogurt (D'Amato and Sinigaglia 2010). Combination of nisin with other antimicrobials such as thymol (Solomakos et al. 2008) and lysozyme (Mangalassary et al. 2007) is very efficacious. Solid lipid nanoparticles incorporated with nisin possess antibacterial activity against food-borne pathogens (Prombutara et al. 2012).

Canned vegetables and juice

Nisin is used in the canned food products in order to prevent the spoilage from thermophilic microorganisms. It is estimated that utilization of nisin in the canned vegetable at room temperature can be used to increase its shelf-life (Suganthi et al. 2012). Microbes, including *Clostridium* spp. *Clostridium thermosacchrolyticum* and *Geobacillus stearothermophilus* produce thermophilic spores. Nisin prevents thermophilic spore-forming micoorganisms, which are responsible for the food-spoilage and can also be used in canned peas, carrots, potatoes, baby corn, etc. (D'Amato and Sinigaglia 2010; Galvez et al. 2014; Upendra et al. 2016). In addition, nisin is also used as an additive in the storage of fruit and vegetable juice, such as tomato juice and mint extract (Galvez et al. 2014).

Alcoholic beverages

Nisin can also be used for the growth inhibition of acid tolerant microorganisms, which cause spoilage of alcoholic beverages. It prevents the growth of *Lactobacillus*, *Leuconostoc* and *Pediococcus,* which results into the spoilage of beer and wine (Bezares et al. 2007). In brewing industries, nisin is used as an additive in the fermenters. It also enhances shelf-life of beer (Suganthi et al. 2012).

Meat products

Nisin can be used as a preservative for the processing and enhancing the shelf-life of the meat products. Nisin is not much effective when used on the surface of meat products, which might be due to poor absorption and solubility. Research revealed that nisin is very effective when used in combination with small quantities of nitrite (Suganthi et al. 2012). There along with nisin, few studies have reported that other bacteriocin such as Pediocin PA-1, Enterocin AS-48, Enterocins A and B, sakacin, leucocin A can also be used in the biopreservation of meat products. Pediocin was found to be better incase of biopreservation of meat products. While in rest of the preservation products nisin is the most dominant (Galvez et al. 2014).

Fish preservation

Generally, Gram-negative bacteria cause deterioration of fish and fish- products. In vacuum packed fish products, certain Gram-positive bacteria viz., *Clostridium* spp. and *L. monocytogens* are responsible for the spoilage of fish and sea food. Nisin can be used for the preservation of fish and the other sea products (Suganthi et al. 2012; Upendra et al. 2016). The combination of nisin along with MicroGARD® serve as the best solution for the preservation of fish and it prevents the growth of aerobic microorganisms (Galvez et al. 2014).

Other antimicrobial peptides in food preservation

Pediocin PA-1is a commercially available bacteriocin, which is being marketed as Alta™ 2341. It is used in some countries as a food additive to inhibit the growth of *L. monocytogens,* which causes spoilage of meat (Settanni and Corsetti 2008). Enterocin AS-48 is an antimicrobial peptide used for the preservation of cider, fruit and vegetable juice. Another preservative, Enterocin CCM4231 is used for the preservation of Soya milk (Settanni and Corsetti 2008).

Bovine and activated lactoferrin (ALF) present in milk has characteristic iron binding ability, which is approved in USA, as a safe preservative of meat and beef products. In Turkey, defensin is widely used for the preservation of chicken. Pleurocidin peptide isolated from mucus membrane of *Pleuronectes americanus* has potential activity against food-spoilage organisms such as *Vibrio parahemolyticus, L. monocytogenes, E. coli* O157:H7, *Saccharomyces cerevisiae,* and *Penicillium expansum* (Tiwari

et al. 2009). Pediocin AcH peptide is generally recognized as a safe food preservative and used for the preservation of dairy products. Similarly, reutrin is used for the preservation of milk. Another important antimicrobial peptide variacin is produced by *Kokuria varians* which is utilized in vanilla chilled dessert chocolate mousse. Warnericin RB4 peptide isolated from *Staphylococcus warneri* is utilized in the acidic soft-drinks. Moreover, two types of plantaricins- Plantaricin P and plantaricin S are used in the preservation of green olives (Galvez et al. 2014; Upendra et al. 2016).

Promises of antimicrobial peptides

Although, the new technologies have been developed, the food preservation is still an important issue, as improper food preservation results in the health problems (Balciunas et al. 2013).

There are several benefits of using bacteriocin (nisin) in food preservation, which is synthesized by lactic acid bacteria (Galvez et al. 2014):

(i) It is approved as safe in food consumption because bacteriocins are sensitive to the proteases in the gastrointestinal tract and also active against food-borne pathogens (Elayaraja et al. 2014).
(ii) It is not poisonous to eukaryotic cells.
(iii) It possesses broad-spectrum activity against food-spoiling microorganisms.
(iv) It is pH and heat resistant.
(v) It shows minimum inactivation when exposed to the protease enzymes.

Recent research demonstrated that due to many advantages of antimicrobial peptides over chemical preservatives, these can surely enhance the storage life of food products by preventing the growth of food-spoilage organisms. Moreover, antimicrobial peptides are eco-friendly. Addition of bio-preservatives helps in preventing economic loss of food-spoilage (Galvez et al. 2014).

Conclusions and future perspectives

Food-spoilage by various microorganisms is responsible for food-borne diseases. Due to the excessive use of antibiotics, bacteria have developed resistance to them. Hence, biopreservation is the only option to inhibit the growth of food-spoilage organisms. Except nisin, no other peptide is approved internationally for safe food preservation. Although, antimicrobial peptides are promising for the food preservation, still research needs to be carried out in order to address food-spoilage problem. There is pressing need to develop novel antimicrobial peptides by modifying the primitive structure of the peptide by various methods of peptide synthesis for increasing the shelf-life of food products in the food industry.

Biological preservation of food products is the eco-friendly approach for inhibition of contamination of food to some extent. Moreover, there is a need to enhance the quality of existing antimicrobial peptides and to search for new ones. Post-translational modifications in the bacteriocin producing bacteria will help to understand the genes, which codes for bacteriocin. After knowing the gene of interest responsible for the synthesis of antimicrobial peptides, it can be transferred into another bacterium by the conjugation method, which will definitely enhance the production of antimicrobial peptides for the preservation of food. By fusing two antimicrobial peptides the efficacy of antimicrobial peptides can be increased, resulting in management of food contamination. Moreover, recombinant PCR technique will be useful in combining two genes responsible for the synthesis of novel antimicrobial peptide which will be of superior in quality as compared to the individual antimicrobial peptide. It will serve as an alternative for inhibition of food-borne pathogens.

Computational knowledge regarding the structure of antimicrobial peptides may prove to be a novel tool for the synthesis of new antimicrobial peptide, preservation of food and also for better understanding of the structure of antimicrobial peptides. There are databases available online, which helps in understanding, structure of antimicrobial peptides and will be very effective in the preservation of food. The chemical method of the peptide synthesis is beneficial in case analysis of novel peptides are taken into consideration. As in case of naturally occurring peptides we insert the additional or remove the unavailable amino acids but it can be added in chemical method and it will be helpful in the discovery of novel peptides. The efficiency of antimicrobial peptides can be enhanced, if it will be used in combination with antibiotics. It will also help to tackle the problem of microbial resistance to antibiotics. The food-spoilage can be effectively minimized if antimicrobial peptides are used along with nanoparticles. Coating of antimicrobial peptides along with metal nanoparticles will be useful in solving the problem of food contamination.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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