

ANTIMICROBIAL PEPTIDES FROM BACTERIAL SOURCES - A REVIEW

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Abstract: Antimicrobial peptides are host defense peptides composed of long chain of amino acids with a lot many properties. The antimicrobial peptides produced by bacteria are collectively called as bacteriocins. Bacteriocins are produced by both Gram positive and Gram negative bacteria and are positively charged or amphiphilic molecules. Even though both Gram negative and Gram positive bacteria produce bacteriocins, those produced by Gram positive Lactic Acid Bacteria are most studied and diverse in nature. They are classified into lantibiotics and Class II peptides. These peptides are having antibacterial, immunomodulatory activities and even antibacterial resistance. They act by forming pores in the cell membrane and thus causing the cell death. Bacteria secrete these peptides as a defense strategy to defend their environment and also able to kill other bacteria. Even though having many beneficial properties and uses, there many challenges in clinical use of these peptides.

Keywords: Antimicrobial peptides, bacteriocin, host defense peptide, cell death, lantibiotics, Class II peptides

1. Introduction

Antimicrobial peptides are oligopeptides which are composed of long chains of amino acids having potential antimicrobial activity. Antimicrobial peptides are also known as “host defense peptides” due to their immunomodulatory activity. These peptides are released from almost all eukaryotic organisms including mammals, insects, amphibians, fishes, plants. Not only eukaryotes but also several prokaryotic microbes also secrete antimicrobial peptides in order to defend their environment niche (Zhang and Gallo, 2016). Various antibiotics are used nowadays to prevent wide range of infections. But the emergence of resistance among antibiotics led to the search of many novel agents that can target the infectious agents. Many preclinical and clinical trials have been going on the field of antimicrobial peptides due to its potential in attacking a range of organisms including bacteria, virus, fungus and parasites. This review is an attempt to put together the antimicrobial peptides that are naturally produced by bacteria and their properties and functions.

2. Antimicrobial Peptides

Antimicrobial peptides are endogenous peptides which are either constitutively expressed or induced thus providing a fast and effective response against pathogens and are considered as a primitive immune defense mechanism in organisms (Reddy *et al.*, 2004). These peptides are classified as anionic antimicrobial peptides, cationic antimicrobial peptides, cationic amphipathic peptides, host defense peptides and α -helical antimicrobial peptides (Bahar and Ren, 2013). The first antimicrobial peptide was discovered in 1939 by Dubos from a soil *Bacillus* strain as an antimicrobial agent that can protect mice from *Pneumococci* infection. Later it was identified as an antimicrobial peptide and was gramicidin (Bahar and Ren, 2013).

Antimicrobial peptides are produced either by ribosomal translation of mRNA or non ribosomal nonpeptide synthesis. The nonribosomal peptide synthesis of antimicrobial peptides mainly occurs in bacteria whereas ribosomal translational synthesis occurs in almost all species including bacteria (Mahlapuu *et al.*, 2016). Antimicrobial peptides are produced *in vitro* by four chemical methods like the culture of industrial microorganisms, genetically modified organisms, enzymatic hydrolysis of proteins and separation from natural sources (Seyfi *et al.*, 2020). Antimicrobial peptides act by disrupting the bacterial cell membrane or by translocating inside the bacterial membrane to a target inside the cytoplasm (Mahlapuu *et al.*, 2016). The mechanisms by which the antimicrobial peptides act include Barrel-Stave, toroidal pore worm hole, carpet and detergent like model (Seyfi *et al.*, 2020). In barrel-stave model antimicrobial peptides form transmembrane channels/pore through which the leakage of cell contents occurs thereby causing the death of the cell (Reddy *et al.*, 2004). In the toroidal pore model the peptides affect the local curvature of the bilayer so that a toroid of high curvature forms there by disrupting the normal segregation of polar and non-polar parts of the membrane (Wimley, 2010). In carpet or detergent model, the peptides form a carpet like coating over the cell membrane and later aggregate to form micelles which causes derangement in the membrane surface of the cell (Reddy *et al.*, 2004; Bahar and Ren, 2013; Seyfi *et al.*, 2020).

3. Naturally derived antimicrobial peptides from bacteria

Antimicrobial peptides are produced from a wide range of species and are having antimicrobial properties. Although having antimicrobial properties, some antimicrobial peptides are produced by the bacteria as a defense against other bacteria; in order to protect their environment. Such antimicrobial peptides isolated from the bacteria are having many properties like antimicrobial

activity, immunomodulatory action, as food preservative etc. (Sablon *et al.*, 2000). One such antimicrobial peptide which is widely distributed in nature and produced by bacteria is Bacteriocin (Pingitore *et al.*, 2007).

Bacteriocins are proteinaceous peptides produced by both Gram positive and Gram negative bacteria and Archea (Sablon *et al.*, 2000; Maroti *et al.*, 2011). They are ribosomally synthesised heat-stable, nontoxic peptides which are susceptible to degradation by proteolytic enzymes present in the gastrointestinal tract (Pingitore *et al.*, 2007). Bacteriocin producing bacteria are immune to their own toxin due to co-expression of immunity proteins. Unlike eukaryotic antimicrobial peptides having a defensive function and protecting the host from invading pathogens, bacteriocins are not a part of defense but are molecules which are attacking other competitive bacteria invading their ecological niche. Bacteriocin promotes and maintains the biodiversity in microbial communities establishment of non transitive or non hierarchical interaction networks. (Maroti *et al.*, 2011). The first antimicrobial peptide from bacteria was discovered in 1925 when Gratia observed the inhibition of *E. coli* Φ by *E. coli* V and was named as colicin (Reeves, 1965). Later the bacteriocins were discovered in 1928 from Lactic Acid Bacteria (Sablon *et al.*, 2000). Bacteriocin production is ubiquitous in nature and is highly potent and specific with narrow spectrum acting at pico to nanomolar concentrations (Hassan *et al.*, 2012). Bacteriocins have a dual mode of action by binding to membrane phospholipids in bacteria and recognizing specific components both of which leads to pore formation and subsequent cell death (Martinez *et al.*, 2016). But it is seen that bacteriocins have antibacterial activity against the closely related bacterial species of the producer (Reeves, 1965; Hassan *et al.*, 2012); however the bacteriocins from Lactic Acid Bacteria have wide range of antibacterial activity, it also attacks unrelated pathogens (Sablon *et al.*, 2000).

3.1 Classification of Bacteriocins

Klaenhammer (1993) classified bacteriocins into four distinct classes which include Class I, Class II, Class III and Class IV. Class I include the bacteriocins like nisin, lactacin 481, lactocin S which are known as lantibiotics. They are named so because lantibiotics are small membrane active peptides containing the unusual amino acids lanthionine, β -methyl lanthionine and dehydrated residues. Class II includes the small heat-stable, non-lanthionine containing membrane active peptides which are again sub classed into Class IIa which are *Listeria active* peptides which includes pediocin PA-1, sakacin A, sakacin P, leucocin A, curvacin A., Class IIb are poration complexes consisting of two Proteinaceous peptides for activity which includes lactococcin G, lactococcin M, lactacin F and Class IIc are thiol activated peptides requiring reduced cysteine residues for activity which include lactococcin B. Martinez *et al.* (2016) gives a modification to Class II as the Class II is sub classed into IIa, IIb, IIc and IId; where Class IIc includes the circular bacteriocins like gassericin A and butyrvibriocin A and finally Class IV consists of miscellaneous peptides like leaderless bacteriocins like lactacin Q, lineal peptides like lactococcin A and bacteriocins not having a dedicated export system but use the general secretory mechanism of the cell like enterocin P and lactococcin 972. Large heat labile proteins like helveticin J, helveticin V-1829, acidophilucin A, lactacins A and B forms the Class III bacteriocins and Class IV consists of the complex bacteriocins which are composed of proteins and required one or more lipid or carbohydrate moiety for their activity like plantaricin S, leuconocin S, lactocin 27, pediocin SJ-1. Cotter *et al.* (2005) proposed a better classification of bacteriocins as two classes- Class I which includes the lanthionine containing lantibiotics and Class II which includes the non-lanthionine containing bacteriocins. Cotter *et al.* (2005) also suggested to move Class III bacteriocins to other class known as bacteriolysins. These bacteriolysins are lytic enzymes than peptides (Perez *et al.*, 2014).

3.2 Sources of Bacteriocins

Bacteriocins are both produced by both Gram negative and Gram positive bacteria; not only the Gram positive and negative bacteria, bacteriocins are also produced by archae (Riley *et al.*, 2002). The first bacteriocin discovered was Colicin and it is produced by the Gram negative bacteria *E. coli*. Apart from *E. coli*, colicin is also produced by other Gram negative bacteria including *Shigella*, *Paracolobactrum*, *Salmonella*, *Aerobacter* and *serratia* sp. (Reeves, 1965). Colicins represent the majority of bacteriocins produced by the Gram negative bacteria and are encoded by colicin genes located on plasmids. Another bacteriocin is pyocin which is produced by *Pseudomonas aeruginosa* and also is closely related to colicin (Riley *et al.*, 2002). Other bacteriocins produced by Gram negative bacteria include Alvecins produced by *Hafnia* Sp., Caratovorocins by *Erwinia* Sp., Arizonacins by *Paracolobactrum arizonae*, Cloacins by *Enterobacter cloacae*, Marcescins by *Serratia* Sp., Pneumocins by *Klebsiella*, Fluocins by *Pseudomonas fluorescens*, Enterococcins by *Enterobacter* Sp. and Pesticins by *Pasteurella* Sp. (Reeves, 1965). The bacteriocins produced by Gram negative bacteria differ from Gram positive bacteria in their lethality and mode of transport. The bacteriocins from Gram positive organisms are diverse in nature (Riley *et al.*, 2002). The major bacteriocin producer in Gram positive world is the Lactic Acid Bacteria which produces diverse number of bacteriocins (Sablon *et al.*, 2000). Some of the bacteriocins produced by Lactic Acid Bacteria include Nisin Z and Q, Enterocin W, Nukacin ISK-1, Munditacin, Leucocin A Lactococcin Q, Lactocyclicin Q, Leucocyclicin Q, Lactacin Q and Z, Weissellicin Y and M, Leucocin Q and N (Perez *et al.*, 2014). Other bacteriocins from Gram positive bacteria include Monoscins from *Listeria monocytogenes*, Megacins by *Bacillus megaterium*, Cerecins by *Bacillus cerecin* and Staphylococcins by *Staphylococcus* Sp. (Reeves, 1965).

3.3 Mode of action of Bacteriocins

Bacteriocins are considered as proteins secreted by bacteria and are significant in medical microbiology particularly in epidemiological studies (Daw and Falkiner, 1996). Bacteriocins from GRAS (Generally Recognised as Safe) Lactic Acid bacteria are mainly used for controlling other pathogens (Montville and Bruno, 1994). Cytoplasmic membrane is the principal target for bacteriocins and the antibacterial activity of many bacteriocins is decreased by reduced positive charge on the surface (Nes and

Holo, 2000). Bacteriocins act by disrupting essential cellular functions like replication, transcription, translation and cell wall biosynthesis. But it is found that the majority of bacteriocins act by forming pores on the membrane surface of susceptible bacteria (Oscariz and Pisabarro, 2001). The most studied member of lantibiotics group of bacteriocin is Nisin which is produced by several strains of *Lactococcus lactis* bacteria (Abee *et al.*, 1995; Oscariz and Pisabarro, 2001). Nisin and other lantibiotics act by interfering with the energy transduction occurring at the cytoplasmic membrane thereby killing the bacterial cells (Hechard and Sahl, 2002). Lantibiotics being positively charged interact with the negatively charged membrane phospholipids favoring the subsequent interaction of bacteriocin's hydrophobic region with the cell membrane (Abee *et al.*, 1995; Oscariz and Pisabarro 2001). The energy for this interaction is obtained from the proton motive force which is the driving force for most of the vital energy demanding processes on the cell membrane (Bruno and Montville, 1993; Montville and Bruno, 1994). The interaction between the hydrophobic part of peptide and bacterial cell membrane results in the generation of pores on the bacterial cell membrane resulting in the efflux of ions, amino acids, ATP leading to proton motive force dissipation and subsequent cell death (Oscariz and Pisabarro, 2001). Earlier it was thought that the nisins act by Barrel-stave model pore formation but later based NMR studies it is found that lantibiotics forms a wedge model pore on the surface of bacterial cell (Hechard and Sahl, 2002). Another mode of action for nisin against bacterial spore was proposed by Montville and Bruno (1994). Nisin stops generation of spores via reaction between the amino acids dehydroalanine and dehydrobutyrine in the peptide with the sulphhydryl groups in the cell membrane. Thus spores are destroyed before the outgrowth of nascent vegetative cells.

Type B lantibiotics like mersacidine and actagardine interfere with the cell wall biosynthesis via mechanism different from that of Type A lantibiotics like nisin. Here the peptides act by inhibiting the incorporation of glucose and D- alanine into the bacterial cell wall membrane thereby inhibiting the biosynthesis of peptidoglycan by inhibiting transglycosylation. Unlike nisin these peptides do not cause any hindrance to DNA, RNA or protein synthesis (Hechard and Sahl, 2002).

Class II bacteriocins act by inducing membrane permeabilisation and thus causing leakage to the bacterial cell membrane (Hechard and Sahl, 2002). The Class IIa bacteriocins are generally termed as Pediocin like antimicrobial peptides (Fimland *et al.*, 2005). These peptides have some specificity towards some particular ions which they conduct across membrane and the specificity differs for each peptides of this class (Oppergard *et al.*, 2007). These specific effluxes across the cell membrane are thought to be the cause of cytotoxic effects resulting in the drop in the intracellular pH and inhibition of enzymatic processes (Todrov, 2009). This ability to differentiate between molecules they transport across membranes indicates that the Class II bacteriocins form sophisticated pores that display specificity with respect to transport of molecules, rather than inducing membrane leakage through a detergent like disruption of membrane (Nissen-Meyer *et al.*, 2010). These bacteriocins also dissipate the proton motive force by disrupting the transmembrane potential and the pH gradient of sensitive cells (Todrov, 2009). It is proposed that the Class IIb peptides form relatively more pores than Class IIa peptides and thus causing membrane permeabilisation (Nes and Holo, 2000).

4. Conclusion

Antimicrobial peptides are important innate host defense peptides having wide range of activities against bacteria, fungus, virus, parasites, anticancer properties and immunomodulatory functions; besides they can also overcome antibacterial resistance (Lei *et al.*, 2019; Seyfi *et al.*, 2020). This varied function of bacteriocins together with its narrow spectrum of activity makes them an important component in the antibacterial therapy, immunity, as food preservative etc. Bacteriocins are very potent as defense peptide and helps in understanding the molecular mechanisms and the physiology behind the stress response in susceptible bacteria (Martinez *et al.*, 2016). Even though these peptides have immense potential as antimicrobial agents in clinical therapy, there are many hurdles hindering the usage of peptides in clinical therapy. High cost of manufacture, potential cytotoxicity in cells, low specificity and lack of robust guidelines are some of the challenges (Bahar and Ren, 2013). Studies are going on this aspect and many of the peptides are under clinical trials now. Being peptides these agents can be synthesised in laboratory and even can be modified with the aid of computational methods. Thus it helps in understanding the mode of action better and can predict their activity. There is scope for further development in this area and in the judicious use of peptides as therapeutics.

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