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Research Article

ANTIMICROBIAL PEPTIDES FORM MICROBES - REVIEW

T. SIVAKUMAR

PG and Research Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Krishnapuram, Kanchepuram -631 51, Tamil Nadu, India

Corresponding Author: *shiva.fungi@gmail.com*

Abstract

Antibiotics are components or substance that kills or inhibits the growth of microbes produced naturally by microbes or chemically synthesized. In the last few years many cationic peptides have been isolated from a wide range of animal, plant, and bacterial species. These compounds comprise a diverse class of molecules used in host defense by plant, insect, fishes, crustaceans, amphibians, birds, mammals, and humans.

Keywords: Antibiotics, antimicrobial substances, pathogenic bacteria, peptide antibiotics

Introduction

At the end of the 20th century, world wide rapid increase in pathogenic bacteria which are resistant to available antibiotics. This alarming situation has its origin in the excessive and often inappropriate use of antibiotics in human and animal health care for the treatment and prevention of infections (Yeaman *et al*., 2003). To overcome this, antimicrobial peptides (AMPs) have come as a potential antibiotic due to their killing ability against a wide spectrum of bacterial species, including drug-resistant strains. These peptides because of their small sizes (11 to 39 amino acids) and antimicrobial potencies have therapeutic potential in the treatment of infection in humans (Hancok, 1997).

Production of antimicrobial substances seems to be a general phenomenon for most bacteria. A microbial defense system is produced, including broad spectrum classical antibiotics, metabolic by-products such as organic acids, and lytic agents such as lysozyme. In addition, several types of protein exotoxins and bacteriocins, which are biologically active peptide moieties with bactericidal mode of action were described (Riley and Wertz 2002; Yeaman and Yount 2003). They can be divided into three major classes of which class I and II are quite heat-stable. Class I contains modified bacteriocins, so-called lantibiotics, that are found among many different Gram-positive bacteria but have yet to be found in Gram-negative bacteria. They are divided into two subclasses (a) the linear and cationic peptide and (b) the globular peptides; the latter normally are hydrophobic but not cationic. The second major class (II) contains small heat-stable bacteriocins that lack post translational modifications as found in lantibiotics, and are presently clustered into atleast two groups: pediocin-like bacteriocins and two-peptide bacteriocins (Diep *et al.,* 2002). A third class (III) of

bacteriocins has been also defined. They are normally larger in size and are easily subjected to heat inactivation (Klaenhammer *et al.,* 1993).

Hundreds of peptide antibiotics have been described in the past half-century (Honcock *et al.,* 1995). These falls into two classes, non ribosomally synthesized peptides, such as the gramicidins, polymyxins, bacitracins, glycopeptides, etc., and ribosomally synthesized peptides. Nonribosomally synthesized peptides can be described as peptides elaborated in bacteria, fungi and actinomycete that contain two or more moieties derived from amino acids (Kleinkauf *et al.,* 1988). Antimicrobial, ribosomally synthesized, cationic peptides have been recognized only recently as an important part of innate immunity (Bevins, 1994).

Cationic antimicrobial peptides are a structurally diverse group of molecules that are found virtually in all eukaryotes examined to date. In addition to their proven role in killing a wide variety of potential pathogens including Gram-positive and Gram-negative bacteria, fungi and viruses, they are also multifunctional modulators of innate immunity (Scott and Hancock, 2000). They are known to interact with the outer membrane via the self-promoted uptake pathway permitting good activity against Gram negative bacteria (Hancock *et al*., 1995; Hancock and Chapple, 1999). However, examination of these peptides has shown general trends but little sequence homology, and this suggests that each peptide has evolved to act optimally in the environment in which it is produced against local microorganisms.

The antimicrobial peptides are found in animal tissues exposed to microbes or cell types that are involved in host defense. Epithelial surfaces secrete antimicrobial peptides from both barrier epithelia and glandular structure (Zasloff, 1987; Diamond *et al.,* 1991; Ouellette and Selsted, 1996; Jones and Bevins., 1992). Phagocytic cells contain several types of storage organelles for microbicidal substances and digestive enzymes.

Almost all antimicrobial peptides are amphipathic. The simplest antimicrobial peptide structures whose mechanism of action has been investigated are either α-helices or β-hairpins. Both types of peptides form transmembrane channels. Antimicrobial peptides with α-helical structures are ubiquitous and found in many organisms. They were common compound of innate defense mechanisms in the animal kingdom and help to control microbial flora and combat pathogens (Tossi *et al*., 2000) Smaller natural antimicrobial peptides exist acidic cyclic dodecapeptide (Romeo *et al*., 1988) and recently, it has been demonstrated that even smaller artificial peptides (6 or 8 amino acids) can generate pores in membranes by assembling into nanotubes (Fernandez *et al.,* 2001).

Antimicrobial peptides may enter the target cell by disrupting the membrane and bind to the intracellular molecules and interfere with their metabolic function. Finally, some peptides may generate pores that admit water but do not allow osmotically active substances to pass. The entry of water generates osmotic pressure that eventually stretches and breaks the microbial membrane (Lehner *et al*., 1997). It has been suggested that mode of action of these compounds on the membranes of bacteria, fungi, protozoa and artificial lipid bilayers may be similar and that they may involve the formation of ion channel pores that span membranes without requiring a specific target receptor (Wade *et al*., 1990).

Recent reports demonstrated that the site of the antibacterial action of the peptides is the cytoplasmic membrane (Moore *et al.,* 1996; Vaara *et al*., 1996). The innate immunity system, which first evolved in lower animals unlike adaptive immunity, occurs throughout invertebrate and vertebrate species. Current knowledge of this system remains limited, especially with regard to the host defense mechanisms used upon initial pathogen presentation.

Innate immunity must act quickly to mount a first line of defense to hold the pathogen in check before the adaptive response matures (Medzhitov *et al*., 2000). Although defense strategies are diverse for different pathogens, many of them are evolutionarily conserved, including production of an array of antimicrobial peptides (AMPs), activation of phagocytic cells, and production of toxic metabolites. AMPs, the best-studied defense effectors, are rapidly elicited after microbe presentation. These ancient weapons play crucial roles in combating microbial infections in invertebrates, vertebrates, and plants (Tzou *et al.,* 2000).

Disease causing microbes that have become resistance to conventional antibiotics are an increasing public health problem. There is evidence that about 70% of bacteria causing infections in hospitals are resistant to atleast one of the commonly used antibiotics. There are also multi-resistant microorganisms, some of which are resistant to nearly all approved antibiotics (Finch *et al*., 2006). Antimicrobial chemotherapy is severely troubles by the emergence and rapid spread of multiresistant bacteria (Cohen, 1992; Neu, 1992).

Staphylococcus aureus remains one of the most intensively investigated bacterial species. As a human and animal pathogen, it can cause a variety of nosocomial and community-acquired infections ranging from minor skin abscesses to serious,

potentially life-threatening diseases, such as bone and soft tissue intra-surgical infections, and invasive endocarditis (Chambers, 2001; Lowy, 1998). In terms of resistance, *Staphylococcus aureus* infection posses an ever increasing problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) has spread worldwide, and it has the capacity to adapt to different environmental condition (Waldvogel, 2000). Over the past few years, studies have shown an increase in antibiotic-resistant bacteria is gentamycin-resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) (Witte, 1999). Recent reports of S*taphylococcus* isolates with intermediate or complete resistance to vancomycin proved a chemotherapeutic era in which effective bactericidal antibiotics against this organism may no longer be readily available (Hiramatsu *et al*., 1997).

As current antibiotic therapy options are becoming limited for staphylococcal infections, there is an urgent need for new antimicrobial agents to combact these resistant pathogens. Antimicrobial peptides are now promising class of antimicrobial agents derived from naturally occurring peptides (Hanoc, 1997). The marine surface environment is a site of intense competition for living space by a wide variety of organisms. Bacteria are generally recognized as primary colonizers of this habitat (Bryers, 1982). The microbial diversity in the sea is yet to be revealed. In the last few years marine microorganisms emerged as a new field for the discovery of novel biologically active compounds (Fenical, 1997). Marine natural products have an exceedingly bright future in the discovery of life saving drugs. The first antibiotic from marine bacterium was identified and characterized in 1966 (Burkholder *et al*, 1996).

Microbial natural products are an important source of both existing and new drugs. Among the producers of commercially important metabolites, bacteria have proven to be a prolific source with a surprisingly small group of taxa accounting for the vast majority of compounds discovered. Since in 1950s, many structurally diverse natural products with outstanding bioactivities have been discovered from marine organisms (Blunt *et al.,* 2007). Marine microorganisms, namely, marine microbial symbionts, have become one of the research hotspots of marine microbiology and marine natural products (Schmidt 2005; Salomon *et al*., 2004).

A novel antimicrobial protein, designated Enterolysin A, was purified from an *Enterococcus faecalis* LMG 2333 culture. Enterolysin A inhibits growth of selected *Enterococci, Pediococci, Lactococci* and *Lactobacilli*.

Killing of sensitive bacteria by Enterolysin A showed dose-response behavior, and the bacteriocin has a bacteriolytic mode of action. Enterolysin A was purified, and the primary structure was determined by combined amino acid and DNA sequencing. The nucleotide sequence of Enterolysin A determined has been deposited in the Gen Bank nucleotide data bank under accession number AF249740. Mature Enterolysin A consists of 316 amino acids and has a calculated molecular weight of 34,501 Da and the theoretical pI is 9.24. (Trine Nilsen *et al.*, 2003).

Ribosomally synthesized peptides with antimicrobial activity are produced by prokaryotes, plants, and a wide variety of animals, both vertebrates and invertebrates. These peptides represent an important defense against micro-organisms. Although the peptides differ greatly in primary structures, they are nearly all cationic and very often amphiphilic, which reflects the fact that many of these peptides kill their target cells by permeabilizing the cell membrane. Moreover, many of these peptides may roughly be placed into one of three groups: (1) those that have a high content of one (or two) amino acid(s), often proline, (2) those that contain intramolecular disulfide bonds, often stabilizing a predominantly β-sheet structure, and (3) those with amphiphilic regions if they assume a α-helical structure. (Jon *et al.,* 1997).

In Ross *et al.,*(2009) investigation it has been said that the biologically pure culture of *Lactobacillus acidophilus* strain DPC6026, a sample which has been deposited at the National collection of Industrial and Marine Bacteria are capable of producing peptides having antimicrobial activity from milk or milk products.

Zhang *et al*., (2009) reported recently that Non ribosomal peptide synthetase (NRPS) Adenylation (A) domain genes were investigated by polymerase chain reaction for 109 bacteria isolated from four South China Sea sponges, *Stelletta tenuis*, *Halichondria rugosa, Dysidea avara*, and *Craniella australiensis*. Fifteen bacteria were found to contain NRPS genes and grouped into two phyla Firmicutes (13 of 15) and Proteobacteria (two of 15) according to 16S rDNA sequences. Based on the phylogenetic analysis of the conserved A domain amino acid sequences, most of the NRPS fragments (11 of 15) showed below 70% similarity to their closest relatives suggesting the novelty of these NRPS genes. All of the 15 bacteria with NRPS genes have antimicrobial activities, with most of them exhibiting activity against multiple indicators including fungi, gram positive and gram negative bacteria. Phylogenetic analysis based on the representative NRPS genes shows high diversity of marine NRPS genes.

A biofilm-forming marine bacterium, D2, isolated from the surface of the *Tunicate ciona* intestinalis, was found to produce a novel, 190-kDa protein with antibacterial activity. The protein contained at least two subunits of 60 and 80 kDa, joined together by non covalent bonds, and was shown to be released by D2 cells into the surrounding medium during stationary phase. N-terminal sequence analysis revealed no close similarity of this protein to any other proteins within the Swiss Prot database. Bactericidal activity against a wide variety of marine and medical bacterial isolates was observed, 77% of the strains tested being sensitive to the protein. Bacterial strains varied in their resistance to the D2 protein. The ability of the D2 bacterium to produce an antibacterial factor in addition to its inhibitory effects on marine invertebrates and algae indicates that D2 has the potential to greatly affect the survival of a range of colonizers of the marine surface environment (Sally *et al., 1996*)

Ozgur Ceylan (2008) reported that fifteen *Streptomycete* isolates which exhibited antimicrobial activity against at least two of the test organisms were characterized by conventional methods and five isolates were highly active against *S. aureus* strains including Methicillin Resistant *Staphylococcus aureus* (MRSA). Twelve *Streptomycete* isolates showed anticandidal activity against *Candida albicans* and ten isolates were highly active with an inhibition zone more than 30 mm in diameter.

A novel antimicrobial polypeptide was isolated and characterized from loach, *Misgurnus anguillicaudatus*. The polypeptide, named MAPP is a single-chain polypeptide with molecular weight of about 9,800 Da and pI about various bacteria including *Bacillus subtilis, Escherichia coli and Staphylococcus aureus*. (Xian e*t a.l,* 2003).

Juan *et al.,* (1994) identified a core undecapeptide of sapecin B having antimicrobial activity. Based on the structure the peptide has been synthesized systematically that consists of terminal basic motifs and internal oligo-leucine sequences and examined their antimicrobial activities. Of these peptides, RLKLLLLLRLK-NH2 and KLKLLLLLKLK-NH2 were found to have potent microbicidal activity against *Staphylococcus aureus*, *Escherichia coli*, Methicillin-Resistant *S. aureus* and *Candida albicans* in liquid medium. He also systematically synthesized the Denantiomer of KLKLLLLLKLK-NH2 which was resistant to tryptic digestion and persisted longer in the culture medium, showing greater antimicrobial activity than the original peptide.

A *Bacillus* sp. strain producing a bacteriocin like substance was characterized by biochemical prowling and 16S rDNA sequencing. The phylogenetic analysis indicated that this strain has low sequence similarity with most *Bacillus* spp., suggesting a new species was isolated. The antimicrobial activity was detected starting at the exponential growth phase, and maximum activity was observed at stationary phase. The substance was inhibitory to a broad range of indicator strains, incluing pathogenic and food spoilage bacteria such as *Listeria monocytogenes*, *B. cereus*, *Aeromonas hydrophila*, *Erwinia carotovora*, *Pasteurella haemolytica*, *Salmonella Gallinarum*, among other. The antibacterial substance was stable over a wide pH range, but it was sensitive to pronase E and lipase (Amanda *et al.,* 2007). The bacterium P34

strains producing an antimicrobial substance that inhibits the pathogen *Listeria monocytogenes*. This antimicrobial substance has a molecular mass of 1,456 Da, was relatively heat stable and sensitive to proteolytic enzymes, suggesting a lipopetide molecule (Motta *et al.,* 2007).

Lavermicocca *et al.,* (1999) stated that *Pseudomonas syringae* strain NCPPB2355 was found to produce a bacteriocin inhibitory against strains of *P. syringae* sub *sp. savastanoi*, the causal agent of olive knot disease. The purification of the bacteriocin obtained by ammonium sulphate precipitation of culture supernatant fluid, membrane ultrafiltration, gel filtration and preparative PAGE, led to the isolation of a high molecular weight proteinaceous substance. The bacteriocin analysed by SDS-PAGE revealed three protein bands with molecular weights of 76, 63 and 45 kDa, respectively.

Yumiko Sano and Makoto Kageyama (1981) reported that Pyocin AP41, a protease-sensitive bacteriocin produced by *Pseudomonas aeruginosa* PAF41, was purified to a homogeneous state and characterized. The molecular weight of this pyocin was about 95,000 which was a complex of two kinds of polypeptides which showed two protein bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and their apparent molecular weights were 90,000 and 6,000 to 7,000, respectively. Amino acid compositions of these components were determined. Sensitive cells were killed by this pyocin only under growing conditions and the pyocin-treated cells lysed in about 30 min with concomitant production of their resident pyocins or phages.

Phospholipase C (heat-labile hemolysin) was purified from *Pseudomonas aeruginosa* culture supernatants to near homogeneity by ammonium sulfate precipitation followed by a novel application of DEAE-Sephacel chromatography. The enzyme was highly active toward phospholipids possessing substituted ammonium groups (e.g., phosphatidycholine, lysophosphatidylcholine, and sphingomyelin). The phospholipase C from *P. aeruginosa* exhibits high affinity for substituted ammonium groups, but requires an additional hydrophobic moiety for optimum binding. The specific activity of the purified enzyme preparation increased 1,900-fold compared with that of culture supernatants. The molecular weight of the phospholipase C was estimated to be 78,000 by both sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Sephacryl S-200 column chromatography and was 76,000 by high-performance size exclusion chromatography. The isoelectric point was 5.5. Amino acid analysis showed that phospholipase C was rich in glycine, serine, threonine, aspartyl, glutamyl, and aromatic amino acids, but was cystine free (Randy *et al.,* 1982).

Microcin D93 is an antibiotic substance produced by *Escherichia coli* strains which harbor the 5.5-kilobase plasmid pMccD93 is purified based on gel permeation chromatography and reverse-phase high-pressure liquid chromatography. The antibiotic appears to be a small, hydrophilic, basic peptide, active on *E. coli* and *Proteus, Citrobacter*, and *Pseudomonas* species and much more active on recA strains than on their isogenic wild type. Diminution of the rate of DNA biosynthesis without any apparent effect on other macromolecules appears to be a primary effect in the action of microcin D93. (Jose *et al*., 1986)

Paula (2004) stated that *Escherichia coli* Microcin J25 (MCC J25) is a 2107-Da peptide antibiotic whose uptake into E. coli is mediated by the outer membrane receptor FhuA and the inner membrane proteins TonB, ExbB, ExbD, and SbmA. *E. coli* MccJ25 targets RNA polymerase, in *S. Typhimurium* which inhibits not only RNA synthesis but also cell respiration. Fluorescence viability staining showed that *S. Typhimurium* cells exposed to MccJ25 remain viable but are unable to form colonies.

Phoebe *et al.,* (2001) said that one of the characteristics of the host defense of insects is the rapid synthesis of a variety of potent antibacterial and antifungal peptides. Seven types of inducible antimicrobial peptides (AMPs) have been characterized in Drosophila. The importance of these peptides in host defense is supported by the

observation that flies deficient for the Toll or Immune deficiency (Imd) pathway, which affects AMP gene expression, are extremely susceptible to microbial infection. The constitutive expression of a

single peptide in some cases is sufficient to rescue Imd; spittle susceptibility to microbial infection, highlighting the important role of AMPs in Drosophila adult host defense.

Drosocin is a 19 amino acid peptide secreted by Drosophila in response to septic injury. The sequence (GKPRPYSPRPTSHPRPIRV) contains six Pro and four Arg residues which are incorporated into three repeated triplet sequences Pro-Arg-Pro. The peptide is glycosylated at Thr 11 and has potent antimicrobial activity. The solution conformation of drosocin and its non-glycosylated derivative were determined by NMR spectroscopy and structure calculations. (Mc Manus *et al.,* 1999)

Ramesh *et al.,* (2009) recently reported that, the antimicrobial peptide was isolated from the haemolymph of the crab *Thalamita crenata*. In antibacterial activity the highest zone of inhibition was observed in the haemolymph of *T.crenata* against *Proteus mirabilis* (17 mm) and lowest zone of inhibition was observed in against *Klebsiella oxytoca* and *Lactobacillus vulgaris* (14mm). But there was no activity against the tested fungal pathogens. On the basis of TLC observations on further confirmation with 1H NMR peptide, fractions were subjected to tandem mass spectrometry (ESI /MS/MS), which resulted in the identification of peptides. The molecular mass of the purified haemolymph showed presence of several molecular mass range of m/z 88 to 507. To retrieve a sequence, the mass spectrometric data of the peptides were applied Mass difference between two adjacent peaks showed precisely fit the mass of an amino acid residue. Based on the arrangement of amino acid in the haemolymph of *T.crenata* species sample categorized peptide.

Meylears *et al.,* (2002) reported that antimicrobial compounds have been isolated from insects, with molecular masses less than 1 kDa. One of such low molecular mass compounds isolated from the flesh fly *Neobellieria bullata* has antimicrobial properties of β-alanyl-tyrosine.

An extracellular inhibitory substance produced by the marine *Alteromonas* strain P-31 (NCMB 2144) was isolated and purified. The inhibitor was a macromolecule with a molecular weight of 90,000 estimated by Sephadex G-100 chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The inhibitory activity was antagonized by proteinase K and I-amylase and inactivated by heating at 80°C for 30 min and the antimicrobial compound was characterized as a thermolabile glycoprotein. The substance exhibited a broad inhibitory spectrum, being active against clinical and environmental isolates from related and non related taxonomical bacterial groups as well as against the producer strain and other similar marine bacterial strains. The inhibitory glycoprotein did not display cytotoxicity toward mammalian and fish cell lines. (Juan *et al.,* 1989).

Nataliya *et al.,* (2004) examined the ability of marine Proteobacteria from the *Pseudoalteromonas* genus and *Alteromonas macleodii* which produced low-molecular-weight, biologically active compounds with antimicrobial and surface-active properties. A new marine bacterium, *Pseudoalteromonas issachenkonii*, exhibited a high level of biological activity and produced antifungal and hemolytic compounds. Four of the 15 strains studied (*P. luteoviolacea, P. rubra, P. undina, and P. issachenkonii*) produced cell-bound, two (*P. elaykovii and P. carrageenovora*) produced extracellular, and one strain (*P. citrea*) produced cell-bound and extracellular fatty acids and phospholipids with surface activity.

The thermophilic bacteria which exhibit antimicrobial activity were isolated from hot spring Jordan valley. Those gram- negative rods shaped were found to be *Yersinia sp*.1 and *Aeromonas hydrophilia*. Extracts from aqueous phases of the two strains were tested for antimicrobial activity by agar well diffusion method against 6 microorganisms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter sp.* and *Candida albicans*). The highest antimicrobial activity was recorded by *Yesinia sp*.1 giving (16 mm) inhibition zone against *Pseudomonas aeruginosa,* where *Aeromonas hydrophilia* produce no activity against the test organisms. Extracts from the two thermophilic strains showed relatively high antibacterial activity against *Staphylococcus aureus*.

Motta and Brandelli (2002) observed the antimicrobial activity in *Brevibacterium linens* ATCC

9175 is at the exponential growth phase. A crude bacteriocin obtained from the culture supernatant inhibited the growth of *Listeria monocytogenes* ATCC 7644*, B. linens* ATCC 9172 and *Corynebacterium* NCTC 7547, but was inactive against the Gram-negative bacteria

and yeast tested. The bacteriocin was stable at 30°C but the activity was lost when the temperature reached
50°C. It was sensitive to the proteolytic action of trypsin, papain and pronase E and was active between pH 6.0 and 9.0. This bacteriocin was used as a bio preservative in food systems, food protection against pathogens and spoilage micro-organisms.

Leandro *et al.,* (2008) isolated thirty out of 8,000 different colony morphotypes from soil samples and selected based on their ability to produce antimicrobials. Most of the psychrotolerant isolates were phylogenetically related to *Serratia proteamaculans* (96.4–97.9%) while the psychrophilic isolated 8H1 was closely related to *Pseudomonas sp*. (90–94% similarity). Produced antimicrobials showed a promising wide spectrum of activity both against gram-positive and gram-negative pathogenic bacteria. They were suspected to be microcin-like compounds (Mwt 2,000 Da) and showed a marked tolerance to heat (1 h in boiling water bath) and pH-treatments (1–12).

Kazuhisa Ouhara *et al*.,(2005) reported the antimicrobial peptide activity of human β-defensin-1 (hBD1), hBD2, hBD3 and LL37(CAP18) against oral bacteria which included *Actinobacillus actinomycetemcomitans* (20 strains), *Prophyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarius, Streptococcus mitis* and *Lactobacillus casei.*

In vitro antimicrobial activities of the seven peptides were further tested against bacteria (*E.coli* and *Staphylococcus aureus*) and fungi (*Candida albicans and Cryptococcus neoformans*). Each peptide exerted activity against at least two organisms. Generally, the peptides were more potent against bacteria than fungi. (Quan *et al*., 2002).

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