Mechanism of Action, Applications, and Improvement Strategies of Antimicrobial Peptides[1] Postprint

Authors: Wang Wujing, Gao Jinyan, Tong Ping, Chen Hongbing

Date: 2017-11-08T00:00:00+00:00

Abstract

Antimicrobial peptides are a class of polypeptide substances exhibiting antibacterial activity, characterized by numerous advantages including safety and non-toxicity, broad antimicrobial spectrum, excellent stability, low bactericidal concentration, small molecular mass, and weak allergenicity, thereby emerging as a prominent research focus in the biological sciences. This paper reviews the current research status and applications of antimicrobial peptides, analyzes existing challenges and improvement strategies, and discusses future application prospects.

Full Text

Antimicrobial Peptides: Mechanism of Action, Applications, and Improvement Strategies

WANG Wujing[1],[2], GAO Jinyan[1]*, TONG Ping[2], CHEN Hongbing[2],[3]

[1]School of Food Science and Technology, Nanchang University, Nanchang 330047, China
[2]State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China
[3]Sino-German Joint Research Institute, Nanchang University, Nanchang 330047, China

Abstract

Antimicrobial peptides are a class of polypeptide substances with antibacterial activity that have become a research hotspot in the biological field due to numerous advantages, including safety, non-toxicity, broad antimicrobial spectrum, good stability, low bactericidal concentration, small molecular weight, and

weak allergenicity. This paper reviews the current research status and applications of antimicrobial peptides, analyzes existing problems and improvement strategies, and discusses future prospects.

Keywords: antimicrobial peptides; bacteriostatic activity; application

---

Introduction

In recent years, the abuse of antibiotics has led to increasingly serious problems such as bacterial resistance, drug residues, and environmental pollution. Concurrently, public concern regarding food, agricultural, and environmental safety has grown annually, making the search for novel antimicrobial agents urgent. Antimicrobial peptides are diverse and, because their antibacterial mechanism differs from traditional antibiotics, they are less likely to induce resistance. Consequently, the antimicrobial mechanisms of these peptides have become a major research focus. Antimicrobial peptides offer multiple advantages, including low allergenicity, no residues, and low bactericidal concentrations, meeting the requirements for food safety and safe production of animal products. They show potential as food and feed additives, and their low toxicity combined with various pharmaceutical functions enables extensive medical applications.

Based on domestic and international research reports in recent years, this review synthesizes current knowledge on antimicrobial peptide mechanisms of action, application status, existing problems, and improvement strategies.

1.1 Overview of Antimicrobial Peptides

Antimicrobial peptides are generally defined as positively charged, helical, short-sequence substances with antibacterial effects, typically isolated from various organisms including unicellular organisms, plants, insects, fish, birds, and mammals. They consist of 12–60 amino acid residues with molecular weights generally below 10 kDa and are considered important components of innate immunity for resisting invasion by foreign microorganisms. Due to their abundance of hydrophobic groups, antimicrobial peptides typically contain both hydrophobic and hydrophilic regions, exhibiting amphipathicity toward cell membranes.

Numerous scholars have isolated antimicrobial peptides from food-derived proteins, with the earliest study dating to 1966 when antibacterial peptides with activity against various bacteria were isolated from bovine casein. In 1972 and 1980, Swedish scientists Boman and Hultmark discovered similar antibacterial substances through induction in pupae of the cecropia moth (*Hyalophora cecropia*). The first true antimicrobial peptide, named cecropin by Boman, inhibited both Gram-positive and Gram-negative bacteria. Since then, various antimicrobial substances have been discovered, with insects and certain invertebrates being extensively studied. To date, over 2,000 naturally occurring antimicrobial peptides have been identified.

1.2 Mechanism of Action of Antimicrobial Peptides

The emergence of antibiotic-resistant bacteria due to antibiotic abuse has highlighted that antimicrobial peptides can inhibit certain drug-resistant strains, suggesting their mechanism differs from conventional antibiotics. While no definitive mechanism has been established, researchers have proposed various hypotheses broadly categorized into three types: cell membrane damage, intracellular bactericidal activity, and immunomodulatory effects.

1.2.1 Cell Membrane Damage Mechanism The cell membrane damage mechanism involves two primary steps: first, selective adsorption of antimicrobial peptides to negatively charged bacterial cell membranes, followed by killing through either pore-forming or non-pore-forming modes. Pore-forming modes can be further divided into four models.

The first is the barrel-stave model, where peptides embed their hydrophobic groups into the cell membrane after adsorption, forming channel structures that cause bacterial cell contents to leak out, leading to death (e.g., Ctx-Ha). The second is the carpet model, where peptides alter membrane surface tension, causing deformation and eventual membrane disintegration; studies show that aurein 1.2 operates through this mechanism. The third is the toroidal-pore model, where peptides aggregate and insert into the membrane, inducing bending of the bacterial phospholipid monolayer to form ring-shaped pores approximately 1–2 nm in diameter, ultimately causing bacterial death (e.g., Lacticin Q). The fourth is the aggregate channel model, where peptides bind with phospholipid molecules on the membrane surface to form peptide-lipid polymers that enter the cell, causing bacterial damage (e.g., Maculatin 1.1).

Non-pore-forming mechanisms propose that peptides bind to the bacterial cell membrane surface and disrupt normal physiological functions, such as affecting DNA replication, RNA transcription, or protein synthesis, thereby causing cell death.

1.2.2 Intracellular Bactericidal Mechanism Research demonstrates that some antimicrobial peptides can penetrate bacterial cell membranes and enter the cytoplasm, affecting cellular biochemical processes to exert bactericidal effects intracellularly. For example, the synthetic antimicrobial peptide NK-18, derived from NK-lysin, inhibits both Gram-negative and Gram-positive bacteria. Mechanistic studies reveal that NK-18 can kill bacteria not only by disrupting cell membranes but also by entering the cytoplasm and binding to DNA to disrupt physiological activity. This dual-action mechanism endows NK-18 with high activity and stability.

1.2.3 Immunomodulatory Mechanism Not all antimicrobial peptides function through direct bactericidal action; some exert activity through immunomodulatory effects. These include: reducing endotoxin-induced inflammatory responses, inducing cytokine secretion to recruit macrophages and other

immune cells, promoting pro-inflammatory factor synthesis, and regulating adaptive immunity. Although these peptides cannot directly kill bacteria, they enhance the body' s ability to resist infection.

The ability of antimicrobial peptides to exert antibacterial effects without harming normal cells primarily stems from their positive surface charge interacting with negatively charged bacterial cell membranes, whereas eukaryotic cell membranes consist of neutral phospholipids, sphingomyelin, and cholesterol. Therefore, the interaction between antimicrobial peptides and anionic bacterial membrane surfaces is the key factor in bacterial killing. Additionally, researchers believe that the amino acid composition of antimicrobial peptides determines their charge, amphipathicity, and hydrophobicity—properties that significantly influence their mode of action and selective effects on microbial cells.

In summary, regardless of the specific mechanism, antimicrobial peptide activity is undoubtedly closely related to the structure and properties of bacterial surface cell membranes.

2. Applications of Antimicrobial Peptides

Beyond broad antibacterial spectra, antimicrobial peptides exhibit selective inhibitory effects against fungi, parasites, and even viruses, while also demonstrating antitumor, antiprotozoal, wound-healing-promoting, and immune-modulating activities. Consequently, they have wide-ranging applications across various industries.

2.1 Medical Applications Antimicrobial peptides have extensive medical applications. In tumor and cancer therapy, many drugs affect normal cells while inhibiting or killing cancer cells. However, some antimicrobial peptides can selectively inhibit tumor and cancer cells without affecting normal cellular activities, making them suitable drug candidates. For instance, lactoferrin-derived peptides inhibit tumor cells, breast cancer cells, and gastric cancer cells.

Peptide drugs offer advantages including small molecular weight, simple structure-activity relationships, easy modification, and protein-like activity with significant therapeutic efficacy, making them ideal candidates for drug development, production, and application. Several antimicrobial peptide drugs have already entered the market; for example, daptomycin, an anionic antimicrobial peptide approved in 2003, is used for skin infections caused by *Staphylococcus aureus* and other Gram-positive bacteria.

Antimicrobial peptides can also serve as drug carriers. Laszlo demonstrated that homologs of the insect antimicrobial peptide pyrrochoricin have vaccine delivery potential, offering a new application strategy. In medical device maintenance, Rai et al. coated gold nanoparticles with cecropin-melittin hybrids for use in medical equipment, proving they prevent device-related infections while exhibiting low toxicity to humans. Immobilized antimicrobial peptides could significantly reduce the substantial costs associated with equipment maintenance.

In conclusion, the multiple advantages of antimicrobial peptides promise good application prospects in pharmaceuticals and medical devices.

2.2 Agricultural and Livestock Applications Due to their unique advantages, antimicrobial peptides have promising applications in agriculture and animal husbandry. In agriculture, they can cultivate disease-resistant varieties while extending the shelf life of agricultural products. In animal husbandry, they can eliminate pathogenic bacteria, improve immunity, enhance animal production performance, and do so safely without residues, causing no harm to animals or environmental pollution, thereby generating considerable economic benefits for the breeding industry.

During growth, plants suffer from varying degrees of microbial damage. Through genetic engineering, introducing antimicrobial peptide genes into crops can inhibit phytopathogen growth and cultivate new disease-resistant varieties. For example, Osusky et al. introduced insect antimicrobial peptide genes into potatoes, obtaining plants resistant to bacterial and fungal infections for approximately one year. Researchers transferring the antimicrobial peptide Shiva-1 into tobacco found it could resist *Pseudomonas solanacearum* infection, reduce wilting symptoms, and decrease tobacco mortality.

Antimicrobial peptides also provide post-harvest protection; potatoes expressing the MsrA3 gene not only showed reduced disease during growth but could be stored for over two years without pathogen damage, significantly improving crop preservation.

In animal husbandry, antimicrobial peptides as feed additives inhibit pathogenic bacteria such as *E. coli* and *Salmonella*, promote livestock growth, and improve product quality. For example, Cao Liting et al. administered nisin into the mammary glands of cows with mastitis, finding it promoted mammary tissue repair and improved milk synthesis capacity, suggesting nisin' s potential as a mastitis therapeutic agent.

Antimicrobial peptides can eliminate bacteria and viruses in animals, improving production performance. Liu Hongjian et al. found that adding small amounts of antimicrobial peptides to sow feed significantly improved piglet survival rates and average weight gain. Hou Zhenping et al. fed weaned piglets with *E. coli* followed by lactoferricin B and cecropin P1, finding these peptides improved growth performance, reduced diarrhea rates, and alleviated weaning stress. Huang Mujia et al. replaced plasma protein powder with 2% antimicrobial peptide preparation, observing increased daily weight gain, improved feed conversion, effective relief of weaning stress syndrome, reduced diarrhea rates, and significantly improved piglet health.

Antimicrobial peptides also enhance animal immunity. Jia et al. injected *Vibrio* into coho salmon and fed them with two antimicrobial peptides, demonstrating that the peptides improved fish resistance to infection and reduced mortality. Jiang Shan et al. found that adding appropriate amounts of antimicrobial pep-

tides to tilapia feed promoted growth, improved immunity, and increased survival rates, though excessive doses caused side effects. Lü Zunzhou et al. showed that adding antimicrobial peptides to feed improved serum immune levels in chickens and promoted interleukin-2 gene expression in spleens, thereby enhancing immunity. Yang Yurong et al. added ostrich skin antimicrobial peptides to chick drinking water, finding they promoted immune organ development, increased T lymphocyte numbers, and enhanced cellular immunity.

2.3 Food Industry Applications In the food industry, antimicrobial peptides serve as natural preservatives, effectively reducing diseases caused by *E. coli* and *Salmonella* while better preserving food flavor compared to heat treatment. Due to their thermal stability, some peptides can be used in foods requiring heating. Nisin, a peptide secreted by *Streptococcus lactis* that inhibits certain Gram-positive bacteria, is a safe, natural food preservative with good antibacterial effects in various foods. Many countries, including the UK and France, use nisin as a food preservative without limiting its addition.

Beyond serving as natural preservatives, antimicrobial peptides can be used as additives to improve food quality. For example, adding modified lacticin 3147 to cheese improved flavor by maintaining the balance of non-fermentative lactic acid bacteria flora.

Additionally, antimicrobial peptides can be incorporated into food packaging or sprayed directly onto food surfaces to achieve bioactive packaging. This approach is safe and hygienic, effectively inhibits pathogen growth, and extends shelf life. For vegetables and meat products, spraying edible antimicrobial peptides on surfaces can reduce moisture loss and maintain good appearance. Quintieri et al. coated cheese packaging materials with lactoferrin-derived peptides, inhibiting *Pseudomonas* growth and effectively extending shelf life.

With scientific and technological development, antimicrobial peptides will have higher utilization value across various industries.

3. Problems and Solutions in Antimicrobial Peptide Applications

Despite significant advantages including safety, broad antimicrobial spectrum, low bactericidal concentration, good stability, small molecular weight, and weak allergenicity, along with efficacy against drug-resistant bacteria, current research on antimicrobial peptides remains primarily at the laboratory and in vitro levels. Market and medical applications require solving a series of problems. This section analyzes existing problems and improvement strategies based on recent research.

3.1 Source Issues Antimicrobial peptides are obtained through four main approaches: (1) Direct isolation and purification from organisms. Although widely sourced, their natural abundance is low with limited resources, and purification requires multiple chromatographic techniques, resulting in high production costs

and difficulty in large-scale manufacturing. (2) Enzymatic hydrolysis of antimicrobial proteins. While operationally simple and inexpensive with highly active products, extraction steps are cumbersome and inefficient with high purification costs. Key challenges include achieving directed enzymatic cleavage and rapid, high-throughput preparation. (3) Chemical synthesis based on known amino acid sequences. This method enables rapid acquisition of target peptides but suffers from high costs and difficulty maintaining natural structures. (4) Genetic engineering by introducing antimicrobial peptide genes into host organisms for expression. This approach yields diverse products in large quantities, but the resulting peptides are susceptible to degradation by host proteases, making pure product acquisition difficult, and potential host toxicity requires investigation.

All four methods share disadvantages of high cost and low efficiency. Rapid isolation and preparation are key to widespread application. Therefore, developing new, efficient preparation methods based on antimicrobial peptide characteristics warrants further investigation. For example, rational design of amino acid sequences can produce peptides with stronger antibacterial activity than parent peptides. For genetically engineered peptides, selecting appropriate host strains can improve expression levels and activity, or using fusion expression can reduce damage to host cells.

3.2 Safety Issues Current antimicrobial peptide research is primarily based on in vitro models and limited clinical trials, with insufficient toxicological studies. Medical applications require further investigation of toxicity, teratogenicity, and allergenicity, as well as clinical trials to determine dosage and administration routes. For example, polymyxins and gramicidin S, known peptide antibiotics for clinical topical application derived from bacteria with structural modifications, are effective against infections but too toxic for systemic use.

Structure significantly influences antimicrobial peptide properties. To ensure safety, structural modifications can maintain activity while reducing toxic side effects. Avitabile et al. substituted 1–2 amino acid residues in Temporin-B from the European common frog with alanine and added lysine, finding the modified peptide showed significantly enhanced inhibition of Gram-positive bacteria without hemolytic activity. Truncating active peptides to improve antibacterial activity while reducing toxic side effects is also effective. Dong et al. truncated avian β-defensin 4, improving antibacterial activity while reducing hemolytic toxicity.

Researchers found that antimicrobial peptide Polybia-MPI had antitumor activity but was susceptible to enzymatic degradation and toxic. Substituting amide bonds with thioamide bonds yielded MPI-1 with high activity, stability, and low toxicity. PEGylation is another method to reduce hemolysis. Morris et al. created hybrid peptides from human and cecropin antimicrobial peptides, and after PEGylation, found significantly reduced toxicity to lung epithelial cells.

3.3 Activity and In Vivo Stability Issues Although antimicrobial peptides show high activity, many are not yet suitable for market applications compared to antibiotics. Researchers have extensively studied activity enhancement, with conjugation being a common and effective method. Li Ting et al. added glucose and sucrose to yellow croaker protein antimicrobial peptides, improving inhibitory activity against indicator bacteria through Maillard reactions. Since charge quantity affects binding to bacterial membranes, substituting negatively charged amino acids with positively charged ones within a certain range may enhance antibacterial activity.

Co-administration with other substances may also yield higher activity through synergistic effects. For example, the antimicrobial peptide indolicidin and antibiotic levofloxacin can be amide-linked, with the conjugate showing significantly enhanced inhibition of Gram-positive bacteria.

Many studies have demonstrated antimicrobial peptide stability under high temperature, acid-base conditions, and freeze-thaw cycles. Wang Chen et al. designed novel cecropin-like antimicrobial peptides that maintained good antibacterial activity after heating at 100 °C for 2 hours. However, antimicrobial peptides are susceptible to degradation by human proteases, resulting in poor stability. Grönberg et al. found that antimicrobial peptide LL-37 lost its antibacterial activity after 6 hours of incubation with trypsin.

Understanding protease characteristics can help improve in vivo stability. Human proteases fall into two categories: exopeptidases acting on peptide chain termini and endopeptidases acting on internal peptide bonds. Modifying antimicrobial peptides to reduce protease binding can enhance stability. For peptides susceptible to exopeptidases, terminal blocking methods can prevent degradation, including C-terminal amidation, N-terminal acetylation, and deamination. To prevent endopeptidase hydrolysis, D-amino acids can replace L-amino acids near specific cleavage sites, or linear peptides can be cyclized to maintain structural stability.

Carmona et al. used D-amino acids to replace L-amino acids in antimicrobial peptide Pin2, maintaining antibacterial activity while preserving high activity in serum and trypsin environments. Chan et al. cyclized antimicrobial peptide Gomesin, significantly improving peptide stability and enhancing antitumor and antimalarial activities. Additionally, immobilization can increase stability. Dutta et al. immobilized antimicrobial peptides onto polyhydroxyethyl methacrylate, significantly reducing cytotoxicity.

In summary, the charge properties and hydrophobicity of antimicrobial peptides affect their interaction with bacterial membranes, thereby influencing antibacterial activity and stability. Structural modification to improve activity is a worthwhile research direction. Additionally, selecting appropriate animal models and administration routes is crucial when studying in vivo activity, stability, and safety. Ensuring safety, high activity, and optimal preparation methods will facilitate better applications in medicine, food, and animal husbandry, enabling

more efficient discovery of beneficial peptides.

4. Summary and Future Prospects

Current research on discovered antimicrobial peptides is extensive, but the relationship between their mechanisms and structure remains unclear. However, with scientific and technological advances and improving biotechnology, we will gain more accurate understanding of antimicrobial peptide mechanisms and discover more highly active and safe peptides from various organisms.

Researchers are actively seeking antimicrobial peptides to replace commercially available antibiotics that have developed resistance, or to combine antimicrobial peptides with traditional antibiotics for synergistic effects. Future optimization will focus on reducing toxicity and production costs while expanding antimicrobial spectra and improving performance. Some antimicrobial peptides do not act directly on pathogens but instead clear pathogens by modulating host immune systems, offering a new therapeutic approach for infections.

In food applications, spraying safe antimicrobial peptides on vegetables and other foods to extend shelf life and sensory quality, or directly applying them to packaging materials, will ensure food safety and preservation. In animal husbandry, numerous trials demonstrate antimicrobial peptides can serve as feed additives to improve livestock product performance, though safety, pharmacokinetics, and efficacy require further investigation, as do peptide modification strategies.

In conclusion, research on antimicrobial peptide mechanisms, safety, antibacterial activity, and stability remains a formidable task. However, we believe that with deepening research, the value of antimicrobial peptides will be better utilized across industries, existing problems will be solved, and they will ultimately benefit all humanity.

References

[1] HUANG Haifeng, GOU Sanhu, MA Yinyun, et al. Research progress on evaluation methods for antibacterial activity and toxicity of antimicrobial peptides in bacterial infection animal models[J]. Progress in Veterinary Medicine, 2016, 37(3): 103-108.
[2] SPLITH K, NEUNDORF I. Antimicrobial peptides with cell-penetrating peptide properties and vice versa[J]. European Biophysics Journal, 2011, 40(4): 387-397.
[3] KATZIRKATCHALSKY A, LAHAV E, SODE MORGENSEN M. Polypeptidic anti-biotic substances derived from casein: US Patent 3764670[P]. 1970-10-28.
[4] BOMAN H G, NILSSON I, RASMUSON B. Inducible antibacterial defence system in Drosophila[J]. Nature, 1972, 237(5352): 232-235.
[5] HULTMARK D, STEINER H, RSMUSON T, et al. Insect immunity: purification and properties of three inducible bactericidal proteins from

hemolymph of immunized pupae of Hyalophora cecropia[J]. European Journal of Biochemistry, 1980, 106(1): 7-16.
[6] YI H Y, CHOWDHURY M, HUANG Y D. Insect antimicrobial peptides and their applications[J]. Applied Microbiology and Biotechnology, 2014, 98(13): 5807-5822.
[7] MIKUT R, RUDEN S, REISCHL M, et al. Improving short antimicrobial peptides despite elusive rules activity[J]. Biochimica Biophysica (BBA): Biomembranes, 2016, 1858(5): 1024-1033.
[8] ZHENG H, FEI D, LIU B H, et al. R-Thanatin inhibits growth and biofilm formation of methicillin-resistant staphylococcus epidermidis in vivo and in vitro[J]. Antimicrobial Agents and Chemotherapy, 2013, 57(10): 5045-5052.
[9] WANG Weidong. Design, screening and activity study of novel antibacterial proteins[D]. Master' s thesis. Hangzhou: Zhejiang Sci-Tech University, 2016.
[10] CESPEDES G F, LORENZON E N, VICENTE E F, et al. Mechanism of action and relationship between structure and biological activity of Ctx-Ha: a new ceratotoxin-like peptide from Hypsiboas albopunctatus[J]. Protein and Peptide Letters, 2012, 19(6): 596-603.
[11] FERNANDEZ D I, LE BRUN A P, WHITWELL T C, et al. The antimicrobial peptide aurein 1.2 disrupts model membranes via the carpet mechanism[J]. Physical Chemistry Chemical Physics, 2012, 14(45): 15739-15751.
[12] SHENKAREV Z O, BALANDIN S V, TRUNOV K I, et al. Molecular mechanism of action of β-hairpin antimicrobial peptide arenicin: oligomeric structure in dodecylphosphocholine micelles and pore formation in planar lipid bilayers[J]. Biochemistry, 2011, 50(28): 6255-6265.
[13] PARTON D L, AKHMATSKAYA E V, SANSOM M S P. Multiscale simulations of the antimicrobial peptide maculatin 1.1: water permeation through disordered aggregates[J]. The Journal of Physical Chemistry B, 2012, 116(29): 8485-8493.
[14] ZHANG L J, GALLO R L. Antimicrobial peptides[J]. Current Biology, 2016, 26(1): R14-R19.
[15] YAN J X, WANG K R, DANG W, et al. Two hits are better than one: membrane-active and DNA binding related double-action mechanism of NK-18, a novel antimicrobial peptide derived mammalian NK-Lysin[J]. Antimicrobical Agents Chemotherapy, 2013, 57(1): 220-228.
[16] ZHANG Dongdong, SHANG Dejing. Potential and application prospects of antimicrobial peptides as novel anti-infective drugs[J]. Chinese Journal of Biochemical Pharmaceutics, 2016(1): 178-182.
[17] SONG D G, ZONG X, ZHANG H W, et al. Antimicrobial peptide Cathelicidin-BF prevents intestinal barrier dysfunction in a mouse model of endotoxemia[J]. International Immunopharmacology, 2015, 25(1): 141-147.
[18] NARAYANA J L, HUANG H N, WU C J, et al. Epinecidin-1 antimicrobial activity: in vitro membrane lysis and In vivo efficacy against Helicobacter pylori infection in a mouse model[J]. Biomaterials, 2015, 61: 41-51.
[19] NIYONSABA F, USHIO H, HARA M, et al. Antimicrobial peptides human β-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic

cytokine IL-31 by human mast cells[J]. The Journal of Immunology, 2010, 184(7): 3526-3534.
[20] VELDHUIZEN E J A, SCHNEIDER V A F, AGUSTIANDARI H, et al. Antimicrobial and immunomodulatory activities of PR-39 derived peptides[J]. PLoS One, 2014, 9(4): e95939.
[21] MALANOVIC N, LOHNER K. Gram-positive bacterial cell envelopes: the impact on the activity antimicrobial peptides[J]. Biochimica Biophysica (BBA): Biomembranes, 2016, 1858(5): 936-946.
[22] FINDLAY B, ZHANEL G G, SCHWEIZER F. Cationic amphiphiles, a new generation of antimicrobials inspired by the natural antimicrobial peptide scaffold[J]. Antimicrobial Agents and Chemotherapy, 2010, 54(10): 4049-4058.
[23] HOANG V L T, KIM S K. Antimicrobial peptides from marine sources[J]. Current Protein and Peptide Science, 2013, 14(3): 205-211.
[24] LU Y, ZHANG T F, SHI Y, et al. PFR peptide, one of the antimicrobial peptides identified from derivatives lactoferrin, induces necrosis leukemia cells[J]. Scientific Reports, 2016, 6: 20823.
[25] SONG D W, KIM S H, KIM H H, et al. Multi-biofunction of antimicrobial peptide-immobilized fibroin nanofiber membrane: implications wound healing[J]. Acta Biomaterialia, 2016, 39: 146-155.
[26] MANGONI M L, BHUNIA A. Editorial: Antimicrobial peptides in medicinal chemistry: advances applications[J]. Current Topics Medicinal Chemistry, 2016, 16(1): 2-3.
[27] SHAGAGHI N, PALOMBO E A, CLAYTON A H A, et al. Archetypal tryptophan-rich antimicrobial peptides: properties and applications[J]. World Journal of Microbiology and Biotechnology, 2016, 32: 31.
[28] ZHANG Wei, SONG Jingjing, ZHANG Bangzhi, et al. Research progress on strategies for new peptide drug development[J]. Scientia Sinica Chimica, 2013, 43(8): 941-952.
[29] JUNG D, POWERS J P, STRAUS S K, et al. Lipid-specific binding of the calcium-dependent antibiotic daptomycin leads changes lipid polymorphism of model membranes[J]. Chemistry and Physics of Lipids, 2008, 154(2): 120-128.
[30] OTVOS Jr, L, CUDIC M, CHUA B Y, et al. An insect antibacterial peptide-based drug delivery system[J]. Molecular Pharmaceutics, 2004, 1(3): 220−232.
[31] RAI A, PINTO S, EVANGELISTA M B, et al. High-density antimicrobial peptide coating with broad activity and low cytotoxicity against human cells[J]. Acta Biomaterialia, 2016, 33: 64-77.
[32] OSUSKY M, ZHOU G Q, OSUSKA L, et al. Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance phytopathogens[J]. Nature Biotechnology, 2000, 18(11): 1162-1166.
[33] JAYNES J M, NAGPALA P, DESTÉFANO-BELTRáN L, et al. Expression of a cecropin B lytic peptide analog in transgenic tobacco confers enhanced resistance to bacterial wilt caused by Pseudomonas solanacearum[J]. Plant Sciences, 2002, 89(1): 43-53.
[34] OSUSKY M, OSUSKA L, HANCOCK R E, et al. Transgenic potatoes expressing a novel cationic peptide resistant blight rot[J]. Transgenic Research,

2004, 13(2): 181-190.
[35] WANG Qing, ZHANG Weitao, XU Yanzhao, et al. Research progress on antimicrobial peptide activity mechanisms and applications in aquaculture[J]. Henan Agricultural Sciences, 2016, 45(10): 6-9, 23.
[36] CAO Liting, HU Songhua. Changes in daily milk yield and main milk components after nisin antimicrobial peptide treatment of clinical mastitis in dairy cows[J]. Chinese Journal of Veterinary Science, 2010, 30(12): 1686-1688, 1693.
[37] LIU Hongjian, XU Yaping, NI Yongcheng, et al. Effects of antimicrobial peptides on sow production performance and piglet growth performance[J]. Journal of Agriculture, 2014, 4(8): 77-80.
[38] HOU Zhenping, YIN Yulong, WANG Wenjie, et al. Effects of lactoferricin B and cecropin P1 on growth and intestinal microflora of weaned piglets challenged with Escherichia coli[J]. Chinese Journal of Animal Nutrition, 2011, 23(9): 1536-1544.
[39] HUANG Mujia, LIU Wenjuan, LI Yongxin. Effects of antimicrobial peptide preparation replacing plasma protein powder on growth performance and health status of weaned piglets[J]. China Feed, 2011(3): 43-44.
[40] JIA X, PATRZYKAT A, DEVLIN R H, et al. Antimicrobial peptides protect coho salmon from Vibrio anguillarum infections[J]. Applied Environmental Microbiology, 2000, 66(5): 1928-1932.
[41] JIANG Shan, WANG Baojie, LIU Mei, et al. Effects of dietary recombinant antimicrobial peptides on growth performance and immunity of genetically improved farmed tilapia (GIFT)[J]. Journal of Fishery Sciences of China, 2011, 18(6): 1308-1314.
[42] LÜ Zunzhou, YUAN Xiaoxiao, CAI Zhaowei, et al. Effects of antimicrobial peptides on serum immune indices and spleen interleukin-2 mRNA expression in laying hens[J]. Chinese Journal of Animal Nutrition, 2011, 23(12): 2183-2189.
[43] YANG Yurong, LIANG Hongde, WEI Hongli. Preliminary study on effects of ostrich skin antimicrobial peptides on immune organ index and T lymphocyte count in chicks[J]. Chinese Agricultural Science Bulletin, 2009, 25(20): 46-48.
[44] LI Youqi. Research progress on nisin application in food preservation[J]. Food Research and Development, 2012, 33(4): 233-235.
[45] SUDA S, D COTTER P D, HILL C, et al. Lacticin 3147-biosynthesis, molecular analysis, immunity, bioengineering applications[J]. Current Protein and Peptide Science, 2012, 13(3): 193-204.
[46] LIU Huairan, WEN Sai, XU Dandan. Natural antimicrobial peptides and their applications in food industry[J]. Feed Research, 2016(14): 5-12.
[47] TORNUK F, HANCER M, SAGDIC O, et al. LLDPE based food packaging incorporated with nanoclays grafted with bioactive compounds to extend shelf life of some meat products[J]. LWT-Food Science and Technology, 2015, 64(2): 540-546.
[48] QUINTIERI L, PISTILLO B R, CAPUTO L, et al. Bovine lactoferrin and lactoferricin on plasma-deposited coating against spoilage Pseudomonas spp.[J].

Innovative Food Science & Emerging Technologies, 2013, 20: 215-222.
[49] WANG Junjie, ZHAO Yan, TU Yonggang, et al. Research progress on egg-derived antimicrobial peptides[J]. Food Science, 2013, 34(9): 399-403.
[50] LI Y F. Recombinant production of antimicrobial peptides in Escherichia coli: a review[J]. Protein Expression and Purification, 2011, 80(2): 260-267.
[51] LI Guannan, XIA Xuejuan, LONG Yaohang, et al. Research progress and application of antimicrobial peptides[J]. Chinese Journal of Animal Nutrition, 2014, 26(1): 17-25.
[52] AVITABILE C, NETTI F, OREFICE G, et al. Design, structural and functional characterization of a Temporin-1b analog active against Gram negative bacteria[J]. Biochimica et Biophysica Acta (BBA): General Subjects, 2013, 1830(6): 3767-3775.
[53] DONG N, MA Q Q, SHAN A S, et al. Novel design of short antimicrobial peptides derived bactericidal domain avian β-defensin-4[J]. Protein and Peptide Letters, 2012, 19(11): 1212-1219.
[54] ZHANG W, LI J, LIU L W, et al. A novel analog of antimicrobial peptide Polybia-MPI, with thioamide bond substitution, exhibits increased therapeutic efficacy against cancer and diminished toxicity in mice[J]. Peptides, 2010, 31(10): 1832-1838.
[55] MORRIS C J, BECK K, FOX M A, et al. Pegylation of antimicrobial peptides maintains the active peptide conformation, model membrane interactions, and antimicrobial activity while improving lung tissue biocompatibility following airway delivery[J]. Antimicrobial Agents and Chemotherapy, 2012, 56(6): 3298-3308.
[56] REINHARDT A, NEUNDORF I. Design and application of antimicrobial peptide conjugates[J]. International Journal of Molecular Sciences, 2016, 17(5): 701.
[57] LI Ting, JIANG Xiaowan, YE Qing, et al. Improving antibacterial activity of yellow croaker protein antimicrobial peptides by Maillard reaction[J]. Meat Research, 2012(1): 14-17.
[58] GHAFFAR K A, HUSSEIN W M, KHALIL Z G, et al. Levofloxacin and indolicidin for combination antimicrobial therapy[J]. Current Drug Delivery, 2015, 12(1): 108-114.
[59] WANG Chen, ZHANG Wufan, NIU Mingfu, et al. Design and activity determination of cecropin-like antimicrobial peptide CLP[J]. Chinese Journal of Preventive Veterinary Medicine, 2016, 38(8): 600-604.
[60] GRÖNBERG A, ZETTERGREN L, ÅGREN M S. Stability of the cathelicidin peptide LL-37 in a Non-healing wound environment[J]. Acta Dermato-Venereologica, 2011, 91(5): 511-515.
[61] QI Li, JIANG Ning, ZHANG Aizhong, et al. Current status and modification strategies of antimicrobial peptide research and development[J]. China Animal Husbandry and Veterinary Medicine, 2016, 42(2): 450-456.
[62] CARMONA G, RODRIGUEZ A, JUAREZ D, et al. Improved protease stability of the antimicrobial peptide substituted with d-amino acids[J]. The Protein Journal, 2013, 32(6): 456-466.
[63] CHAN L Y, ZHANG V M, HUANG Y H, et al. Cyclization of the

antimicrobial peptide gomesin native chemical ligation: influences stability bioactivity[J]. ChemBioChem, 2013, 14(5): 617-624.
[64] DUTTA D, KUMAR N, WILLCOX M D P. Antimicrobial activity of four cationic peptides immobilised to poly-hydroxyethylmethacrylate[J]. Biofouling, 2016, 32(4): 429-438.

Note: Figure translations are in progress. See original paper for figures.