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REVIEW

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Antimicrobial Peptides from fish: Main Forces for Reducing and Substituting Antibiotics

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Abstract

Fish antimicrobial peptides (AMPs) are small molecular peptides with biological activity that play an important role in the fish immune system. They not only have broad-spectrum activity against a variety of viruses, bacteria, fungi, parasites, and cancer cells but also show immunomodulatory activity in different fish. However, the defects of natural fish AMPs, such as their high hemolytic activity and low stability, hinder their application. Therefore, many scientists have tried to optimize the sequence and structure of fish AMPs to meet actual clinical needs. In addition, as the most promising alternative to antibiotics, fish AMPs have good application prospects in animal husbandry, food, biomedicine, and other fields. Many fish AMPs are being researched and developed by scholars around the world. This paper summarizes literature reports regarding the classification of antibacterial peptides and their biological functions and applications, emphasizing their transformation and optimization and the latest research progress to provide a theoretical basis for the research and development of antibacterial peptides as well as technical support for the production of related products.

Introduction

It is well-known that antibiotics have been used as antibacterial agents since their discovery, and in recent decades they have also been widely used in animal husbandry and aquaculture (Lu et al., 2022). However, intensive aquaculture practices have led to an increasing number of diseases in fish, and the regulatory conditions for the use of antibiotics in aquaculture are not very standardized (Xiong et al., 2019, Bhat et al., 2022). The inappropriate use of antibiotics will lead to the development of antimicrobial-resistant bacteria, and the prolonged use of antibiotics that are lower than the minimum inhibitory concentration will lead to horizontal gene transfer between human and fish pathogens, thus raising concerns about environmental pollution and human safety (Bhat et al., 2022). Therefore, numerous researchers have explored several types of antibiotic alternatives, among which antimicrobial peptides have won the favor of researchers due to their broad antimicrobial activity and non-induction of resistance (Chen et al., 2020b). Natural antimicrobial peptides (AMPs) are evolutionarily conserved genetically encoded molecules with functional and structural diversity, usually with a positive net charge (generally ranging from +2 to +11) and a significant proportion of hydrophobic residues (generally 50%) (Moretta et al., 2021). They usually exhibit an amphiphilic structure due to the inclusion of hydrophobic and hydrophilic regions (Moretta et al.,

2021). AMPs not only stimulate the innate immune system against challenging pathogens, but also have effective resistance against multiple types of infectious and non-infectious pathogenic agents (bacteria, fungi, viruses, parasites) (Xia et al., 2018; Xia et al., 2019; Naiel et al., 2023). The study reported that the top three antimicrobial peptides of animal origin were amphibians (45.85%), arthropods (23.63%), and mammals (13.43%), followed by a smaller percentage of human and fish origin, which were divided into 5.77% and 5.57%. Finally, mollusks (1.92%), reptiles (1.84%), birds (1.76%), and bacteria (0.25%) (Wang et al., 2022b). Fish is an important branch of animals, rich in antimicrobial peptides. Fish and higher vertebrates have two immune systems, non-specific immunity and adaptive immunity, and fish antimicrobial peptides are non-specific immune compounds (Valero et al., 2013). A large number of studies have shown that there are four main methods to obtain antibacterial peptides: natural extraction, chemical synthesis, enzymatic hydrolysis, and genetic engineering (Wang, 2019). Genetic engineering has become a popular method among many researchers because of its low production cost and easy large-scale industrialization (Wang, 2019). However, fish antimicrobial peptides, whether gene synthesis or chemical synthesis, are difficult to carry in industrial production (Li et al., 2016). Fish antimicrobial peptides are unstable under certain conditions (Li et al., 2016). Therefore, the optimization of fish antimicrobial peptides is particularly important.

An overview of antimicrobial peptides from fish

To adapt to the living requirements of the water environment, fish must have a strong biological defense function to protect themselves from the complex microorganisms in the water environment (Shabir et al., 2018). As part of the innate immune response, antimicrobial peptides are considered to be the most powerful tool against fish pathogens, especially in the early stages of egg and larval development of the immune system (Valero et al., 2020a). Previously, Pardaxin was isolated from the Red Sea flatfish *Pardachirus marmoratus* (Primor et al., 1980). The number of discovered antimicrobial peptides in fish is increasing rapidly. In particular, high-throughput screening based on bioinformatics such as homology of transcriptome and genome datasets and *de novo* identification of tandem mass spectrometry can predict more antimicrobial peptides (Chen et al., 2020a). Studies have found that fish antimicrobial peptides are mainly derived from the mucus layer, which is a protective film against pathogen invasion (Chaturvedi et al., 2020). Fish antimicrobial peptides can also be found in fish saliva, circulatory system, and intestinal flora (Rajanbabu and Chen, 2011b; Dong et al., 2017). Fish antimicrobial peptides have antiviral, antibacterial, antifungal, antiparasitic, anti-tumor, and immunomodulatory effects (Chaturvedi et al., 2020; Zermeño-Cervantes et al., 2020; Valero et al., 2020b). Among them, antibacterial activity has been widely reported (Fernandes et al., 2004; Chen et al., 2019; Neelima et al., 2021). The antibacterial activity of antimicrobial peptides is achieved by complex mechanisms, which can be regulated by the cell wall, cell membrane, anti-biofilm formation, different intracellular targets, and host immune system (Li et al., 2022). The interaction between cationic antimicrobial peptides and cell membrane, or membrane permeability, is considered to be one of the potential and recognized mechanisms (Lei et al., 2019). As Lei described, cell damage usually includes membrane perforation and non-membrane perforation (Lei et al., 2019). Membrane perforation contains the barrel-stave model, carpet-like model, aggregate channel model, and toroidal-pore model (Figure 1). The fish collagen antimicrobial peptide collagen in forms a β-sheet structure under hydrophobic conditions interacts with anionic (phosphatidylglycerol) and amphoteric (phosphoethanolamine and phosphatidylcholine) lipids, and remains at the membrane-water interface, following the carpet-like model (Ennaas et al., 2016). In addition, studies have reported that the mechanism and site of action, the antimicrobial activities of AMPs depend on the interaction between their cations and the negative charges on the microbial surface (Lina et al., 2020).

Classification of fish antimicrobial peptides

Fish can produce different types of AMPs, which are divided into 5 families according to their structure: β-defensins, cathelicidins, histone-derived peptides, hepcidins, and fish-specific piscidins (Table 1) (Chaturvedi et al., 2020).

β-Defensins

β-defensins are characterized by six conserved cysteine-mediated polarized hydrophobic regions and hydrophilic regions, net cationic charge, β-sheet structure, and three intramolecular disulfide bonds (Das et al., 2022). The disulfide bond plays an important role in the structural integrity of β-defensins (Das et al., 2022). As reported by Ma et al., disulfide bonds play a key role in maintaining the conserved motifs and secondary structure of β-defensins in Olive flounder (Ma et al., 2020). According to literature reports, the earliest fish defensins were found in zebrafish *Danio rerio*, *Takifugu rubripes,* and *Tetraodon nigroviridis* (Zou et al., 2007). Later, β-defensins were also found in *Oncorhynchus mykiss* (Casadei et al., 2009), *Paralichthys olivaceus* (Nam et al., 2010), *Sparus aurata* (Cuesta et al., 2011), *Siniperca chuatsi* (Wang et al., 2012), *Megalobrama amblycephala* (Liang et al., 2013), *Paramisgurnus dabryanus* (Chen et al., 2013), *Oreochromis niloticus* (Dong et al., 2015), *Trachinotus ovatus* (Zhou et al., 2019), *Salmo salar* (Harte et al.,

2019), *Odonus niger* (Neelima et al., 2021), *Dicentrarchus labrax* (Barroso et al., 2021a), *Ctenopharyngodon Idella* (Feng et al., 2023). β-Defensins are mainly expressed in immune and mucosal tissues, such as the gill, kidney, spleen, skin, and liver. It is also expressed in some fish's pituitary, testis, swim bladder, and peritoneum (Katzenback, 2015). In fish with multiple β-defensin gene copies, some gene copies are connected and exist on the same chromosome, while other β-defensin genes have separate chromosomes, which provides a theoretical basis for fish to have multiple β-defensin genes (Katzenback, 2015). As Harte et al reported, four β-defensin genes were found in *Oncorhynchus mykiss,* and five β-defensin genes were found in Atlantic salmon *Salmo salar* (Harte et al., 2019). In addition, A large amount of data has shown that fish β-defensins have antibacterial (Shabir et al., 2018; Neelima et al., 2021), antiviral (Guo et al., 2012), anti-cancer (Neelima et al., 2021), immunoregulatory (Chaturvedi et al., 2020), chemotaxis (Cuesta et al., 2011), regulation of reproductive function and endocrine function (Jin et al., 2010).

Cathelicidins

Cathelicidin was first isolated from the intestinal tissue of *Myxine glutinosa* (Atlantic hagfish) (Uzzell et al., 2003). Cathelicidins are composed of a C-terminal mature peptide and an N-terminal signal peptide with a highly conserved cathelin domain in the middle. Most cathelicidins contain 4 cysteine residues and have a molecular mass of approximately 12 kDa (Katzenback 2015; Wu and Wang, 2021). Antibacterial peptides from bony fish can be divided into cathelicidin 1 and cathelicidin 2 based on the presence or absence of cysteines and disulfide bonds (Katzenback, 2015). Bridle et al., found that cathelicidin 1 and cathelicidin 2 from Atlantic salmon can stimulate peripheral blood leukocytes and increase the transcription and expression of the chemokine interleukin-8 (Bridle et al., 2011). Acosta et al reported that Salmo salar cathelicidin 1 derived peptide induced IFN-γ expression in head kidney leukocytes, indicating that it can be used to enhance the immune response of fish (Acosta et al., 2019). Chen et al., isolated two cathelicidins, CATH_BRALE, and codCath1, from zebrafish (*Danio rerio*), which have strong antibacterial activity against common aquatic Gram-positive and Gram-negative bacteria, and the antibacterial effect is very rapid, killing bacteria in 60 minutes (Chen et al., 2019). They also demonstrated their strong immunomodulatory function by inhibiting the expression of bacterial-induced zebrafish TNF-alpha, IL-1β, and IL-6 pro-inflammatory cytokines and stimulating the expression of IL-8

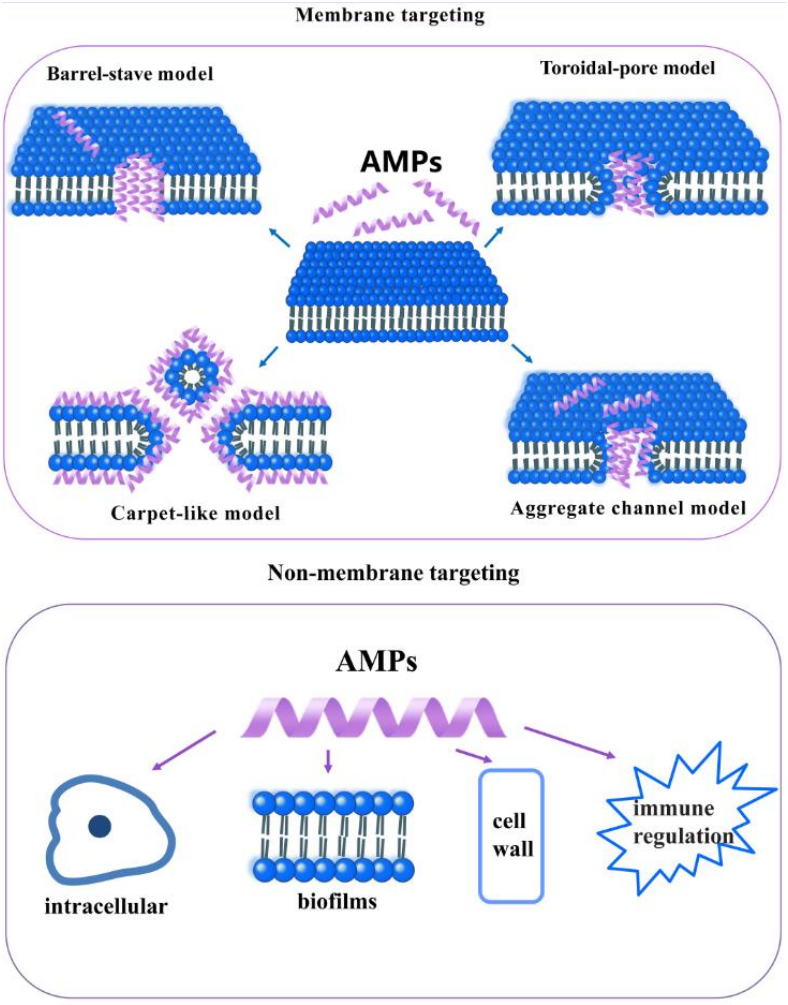

Figure 1. The interaction between antimicrobial peptides and microbial membrane.

Table 1. Classification of fish antimicrobial peptides

Family	Genome structure	Molecular structure	Amino acid composition	Biological activity	References
β-Defensins	Three exons or two introns	Consists of 6 cysteines and has three disulfide bonds between C1-C5, C2-C4 and C3-C6	The precursor is composed of 18-24 amino acid signal peptide and 39-45 amino acid mature peptide.	Antifungal, antiviral, chemotaxis, immune regulation and reproductive regulation	Katzenback (2015); Neelima et al. (2021)
Cathelicidins	Four exons and three introns	Cathelin domain, highly conserved N-terminal propeptide, contains a single disulfide bond, most have 4 cysteine residues	Mature peptide contains 47~69 amino acids	Selective targeting action on Gram-positive bacteria and Gram-negative bacteria with certain antifungal activity	Katzenback (2015); Preeti et al. (2020); Wu and Wang (2021)
Hepcidins	Conserved 3 exons and 2 introns	C-Terminal mature peptide, rich in cysteines, N-terminal signal peptide, intermediate propeptide composition. Contains a β-hairpin structure and an intramolecular disulfide bond	generally contains 20~26 amino acids with Cys in the majority	Antiviral, with some antibacterial, anticancer, and immune regulation; also involved in the regulation of fish iron homeostasis	Katzenback (2015); Li et al. (2019); Qi et al. (2020); Wu and Wang (2021)
Histone-derived peptides		Derived from C-terminal peptide fragments of the N-terminal H1 and H2A histones	> 10 kDa peptide fragment	Broad-spectrum antibacterial activity, anticancer, antiviral and participates in immune regulation and innate immunity	Katzenback (2015); Wu and Wang (2021)
Piscidins	Four exons and three introns	Amphiphilic α-helical conformation where the hydrophilic side is cationic and rich in Phe, His, Ile	18 to 46 amino acid residues	Antifungal, antibacterial (G^+ and G^-), antiparasitic and antiviral	Katzenback (2015); Stefi et al. (2020); Wu and Wang (2021)

chemokines (Chen et al., 2019). *In vivo,* challenge experiments showed that antimicrobial peptides could significantly reduce the number of bacteria and enhance the survival rate of zebrafish (Chen et al., 2019). In addition, Zhang et al. found that the biological activity of antimicrobial peptides Cathelicidins from rainbow trout is mediated by the N-terminal, and the unique repetitive motifs of the salmon family improve the structural flexibility of antimicrobial peptides, further enhancing the antibacterial and IL-18 stimulation activity (Zhang et al., 2015).

Hepcidins

They were first isolated from the gills of hybrid striped bass (Li et al., 2019; Qi et al., 2020). Hepcidin antibacterial peptides generally contain 20~26 amino acids, are composed of four disulfide bond β-folding structures, and are mainly expressed in the liver with the minority in the gills, skin, blood, and heart (Wu and Wang, 2021). Chen et al. reported that hepcidin 2 has a stronger bactericidal effect than hepcidin 1 in *Boleophthalmus pectinirostris* and has immunomodulatory activity on monocytes/macrophages (MO/MΦ) (Chen et al., 2018). Rajanbabu et al., identified the tilapia antibacterial peptide hepcidin (TH) 1-5 and found that it can regulate the expression of virus-selective response genes such as IL and annexin and control salmon embryonic cells (cese-214) infected by infectious pancreatic necrosis virus (IPNV) through direct antibacterial and immune regulation, indicating that hepcidin (TH) 1-5 has antiviral activity (Rajanbabu and Chen, 2011a). Research reports indicate, according to the phylogenetic analysis of sequence clustering, fish Hepcidin antimicrobial peptide (HAMP) can be divided into two types: HAMP1 and HAMP2 (Xu et al., 2012). HAMP1 is a mammalian HAMP homologous gene and exists in each fish species, while the HAMP2 subtype only appears in acanthopterygians, representing a diverse and complex population (Xu et al., 2012). Furthermore, the evolutionary direction of HAMP1 and HAMP2 in fish may be affected by the specific living environment and surrounding pathogens (Xu et al., 2012). Studies have found that fish hepcidin has a dual role in iron metabolism regulation and bacterial infection (Rodrigues et al., 2006). Liu et al. obtained a new HAMP1 hepcidin subtype Tf-Hep in roughskin sculpin (*Trachidermus fasciatus*), which has antibacterial activity against four Gram-negative bacteria and four Gram-positive bacteria (Liu et al., 2017). Barroso et al. tested the administration of sea bass (*Dicentrarchus labrax*)-derived hepcidins (hamp1 or hamp2) in a model of infection and iron overload (Barroso et al., 2021b). The results showed that hamp2 can significantly reduce fish mortality and bacterial load and is a feasible antibiotic substitute, while hamp1 affects iron metabolism regulation and seems to reduce the effect of iron overload (Barroso et al., 2021b).

Histone-derived Peptides

Fish histone-derived peptides originate from the N-terminal and C-terminal of H1 and H2A histones and are present in skin and skin mucus (Katzenback, 2015). Histone derivatives were first identified as AMPs isolated from the skin mucus of *Ictalurus punctatus* (Robinette et al., 1998). Fernandes et al. extracted a new antimicrobial peptide oncorhyncin II from the skin secretion of rainbow trout, with a molecular weight of 7.2 kDa. It is active against both Gram-positive and Gram-negative bacteria but has no hemolysis to red blood cells (Fernandes et al., 2004). Histone H1 and its fragment peptides isolated from coho salmon mucus and serum can enhance the immune activity of pleurocidin and other natural antibiotics in fish against pathogens (Patrzykat et al., 2001). Histone-derived peptides are gradually discovered, as Chaithanya et al. found H2A-derived AMPs sequences in *Tachysurus jella* and *Cynoglossus semifasciatus* (Chaithanya et al., 2013). Dhanya Lenin et al. also obtained histone H2A-derived AMPs sequences in *Lates calcarifer*, *Glossogobius giuris*, *Mugil cephalus,* and *Carangoides malabaricus* (Dhanya Lenin et al., 2023). Data have shown that fish histone derivatives are produced in the presence of lipopolysaccharide, epidermal damage, or some Gram-negative bacteria and have extensive antibacterial activities against both Gram-positive and Gram-negative bacteria. Histones are involved in transcriptional regulation, apoptosis signal transduction, and other biological functions, indicating that histone derivatives play a key role in fish immunity. However, compared with other antibacterial peptides, there are few studies on their antibacterial mechanism, immune regulation, and signal sites in fish (Katzenback, 2015; Preeti et al., 2020).

Piscidins

Piscidins are a class of antimicrobial peptides unique to fish. They have cationic, linear, amphiphilic, α-helix polypeptides and are composed of highly present histidine, isoleucine, and phenylalanine (Chaturvedi et al., 2020; Valero et al., 2020). The members of the Piscidins family include piscidins, gaduscidin, dicentracin, pleurocidin, misgurin, epinecidin and moronecidin. Although they have similar structures, the sequence homology between them is low (Katzenback, 2015). The first member of this family, pleurocidin, was isolated from the skin mucus secretion of the winter flounder (Cole et al., 1997). According to existing research, piscidins 1~7 have been isolated, and they exist in various fish as different isomers. Among them, piscidin 1 and piscidin 2 continue to have high activity against *Staphylococcus aureus* in the presence of high concentrations of ions (Raju et al., 2021). Su et al. reported that zebrafish overexpression of tilapia piscidin 3 (TP3) can effectively inhibit the pro-inflammatory response and increase the production of C3b and further

speculated that TP3 may play an anti-inflammatory role by enhancing TLR4-mediated negative regulation of NF-κB (Su et al., 2018). This study found that the expression of piscidin 4 in healthy fish was significantly higher than that in stressed fish or diseased fish, indicating that the expression of piscidin can be used as one of the conditions for judging the health status of fish (Corrales et al., 2009). Bae et al., cloned a gene (Rbpisc) of piscidin in rock bream and found that it was expressed in many tissues of healthy fish. However, its expression in gill tissue displayed an upward trend after infection by pathogenic bacteria, showing specific antibacterial activity against Gram-positive and Gram-negative bacteria, which indicated that Rbpisc played an important role in the innate immune response of fish (Bae et al., 2016). Piscidins have also been reported to regulate the expression of proinflammatory factors and immune-related genes such as IL-1β, IL-10, NF-κB, and TLR3 in fish (Liu et al., 2019). In addition, piscidins have not only antibacterial, antiviral, and antiparasitic activities but also low cytotoxicity, high salt tolerance, and thermal stability (Li et al., 2015b).

Biological Functions of Fish Antimicrobial Peptides

A large number of studies have shown that fish AMPs have not only relatively familiar biological activities, such as antibacterial, antifungal, antiviral, antitumor, and antiparasitic properties but also some little-known characteristics and functions, such as host defense peptides, immunogenic pharmaceutical active compounds, antioxidants and adjuvants (Table 2) (Yulema et al., 2020).

Antibacterial Activity

As early as 1996, Oren et al. reported that Pardaxin isolated from *Pardachirus marmoratus* has high antibacterial activity (Oren et al., 1996). Subsequently, more and more reports have shown the antibacterial activity of fish antimicrobial peptides. A novel linear antimicrobial peptide pelteobagrin was purified from the skin mucus of yellow catfish (*Pelteobagrus fulvidraco*) and showed antimicrobial activity against both Gram-positive and Gram-negative bacteria (Su, 2011). Two antibacterial peptides, CsRG12 and CsLC11, were obtained from a fish piscidin protein and could destroy the formation of biofilms of *Bacillus cereus* and *S. aureus* and inhibit their growth (Stefi Raju et al., 2020). *Clarias gariepinus*, an antimicrobial peptide isolated from the skin mucus of African catfish, has antibacterial activity against *S. aureus* and *Escherichia coli* (Okella et al., 2021). The epinecidin-1 plasmid inserted into zebrafish can effectively inhibit the growth of *Streptococcus agalactiae* and *Vibrio vulnificus* (Peng et al., 2010). Hepcidin synthesized *in vitro* has certain antibacterial activity against *Vibrio anguillarum* (Álvarez et al., 2016). The antibacterial peptide of catfish mucus synthesized *in vitro* can destroy the structures of *Shewanella putrefaciens* and *E. coli* and cause the leakage of intracellular substances, thus showing its significant antibacterial activity (Liu et al., 2020). The antimicrobial peptide hepcidin gene was obtained from caspian trout (*Salmo caspius*), and Caspian trout (CtHep) was synthesized *in vitro* to inhibit pathogenic bacteria *S iniae* and *Aeromonas hydrophila* (Shirdel et al., 2019). In addition, it has been reported that piscidin extracted from tilapia is used for wound healing caused by a bacterial infection (Raju et al., 2021). In summary, fish AMPs exhibit certain lethality to common pathogenic microorganisms (Gram-positive bacteria, Gram-negative bacteria), which greatly increases the survival rate of fish and improves their living environment.

Antiviral Activity

The number of research reports on the antiviral activity of fish AMPs is lower than that of their antibacterial activity, but there are still many reports confirming the antiviral activity of fish AMPs. Guo et al., cloned defensin from the liver of *Epinephelus coioides*, which directly interacted with virus particles, notably inhibited the infection and replication of Singapore grouper iridovirus (SGIV) and viral nervous necrosis virus (VNNV) and induced the related reaction of type I interferon *in vitro* (Guo et al., 2012). According to the literature, the piscidin isolated from hybrid striped bass has antiviral activity against channel catfish virus (CCV) and frog virus 3. Epinecidin-1, an antibacterial peptide isolated from *Epinephelus coioides*, can inhibit Japanese encephalitis virus (JEV), foot and mouth disease virus (FMDV), SGIV, and other viruses (Chinchar et al., 2004; Chee et al., 2019). Huang et al. treated BHK-12 cells with epinecidin-1 and found that the 50% cytotoxic concentration (CC_{50}) was 19.5 μg/mL, the effective concentration of 50% EC_{50} was 0.6 μg/mL, and the selection index (CC_{50}/EC_{50}) was 31.4. At higher concentrations, epinecidin-1 exhibited antiviral activity against FMDV, and the effect was more pronounced during virus attachment (Huang et al., 2018). Antiviral detection of two hepcidins, SA-hepcidin 1 and SA-hepcidin 2 cloned from *Scatophagus argus*, showed that SA-hepcidin 2 was effective against *Micropterus salmoides* reovirus (MsReV) and *Siniperca chuatsi* rhabdovirus (SCRV), but SA-hepcidin 1 has no such activity (Gui et al., 2016). In addition, TO17 was recently reported as a novel antimicrobial peptide, as the infectious spleen and kidney necrosis virus was incubated with TO17 and injected into the red drum. After 3-7 days of infection, the viral load in the spleen was significantly lower than that in the control group, which proved that TO17 had antiviral activity (He et al., 2018).

Antiparasitic Activity

Pis1, pis2, and a new splicing variant pis2-b from the piscidin of Atlantic cod were identified as having

Table 2. Functions of fish antimicrobial peptides

Family	Antimicrobial peptide	Species	Expression and distribution	Biological activity	Reference
β-Defensins	fBDI-1-5	*Paralichthys olivaceus*	Head kidney	Antibacterial	Nam et al. (2010)
	β-Defensin	*Paramisgurmus dabryanus*	Eyes, gills, skin	Antibacterial	Chen et al. (2013)
	On-Def	*Odonus niger*	Gill	Antibacterial, anticancer, immune regulation	Neelima et al. (2021)
	EcDefensin	*Epinephelus coioides*	Liver	Antiviral, immune regulation	Guo et al. (2012)
	zfBD2	*Danio rerio*		Antiviral, immune regulation	Garcia-Valtanen et al. (2014)
	β-defensin (ScBD)	*Siniperca chuatsi*	Head kidney, gill, spleen	Antiviral	Wang et al. (2012)
	β-defensin (TroBD)	*Trachinotus ovatus*	head kidney, spleen	Antiviral, antiviral immunity	Zhou et al. (2018)
	β-defensin (Onβ-defensin)	*Oreochromis niloticus*	Skin, kidney, spleen	Antiviral	Dong et al. (2015)
	β-defensins (BDs)	*Ctenopharyngodon idella*	skin	Antiviral	Feng et al. (2022)
Cathelicidins	Cathelicidins	Codfish		Resistant to fungi and Gram-negative bacteria	Wu and Wang (2021)
	CATH_BRALE codCath1	*Danio rerio*		Antibacterial, immune regulation	Chen et al. (2019)
	HFIAPs 1, 2, 3	Atlantic hagfish	Intestinal tissue	immune regulation	Uzzell et al. (2003)
	Cathelicidin	rainbow trout	mucosal layer of gut, head kidney	Antiviral	Zhang et al. (2015)
Hepcidins	Hepcidin	Miiuy croaker *Dicentrarchus labrax*	Spleen, intestines, kidneys	Antibacterial, iron metabolism regulation	Rodrigues et al. (2006); Nam et al. (2010); Valero et al. (2020a)
	BpHep-1 BpHep-2	*Boleophthalmus pectinirostris*		Antibacterial, immune regulation	Chen et al. (2018)
	TH1-5	Tilapia		Antibacterial, immune regulation	Rajanbabu and Chen (2011)
	Hep1, Hep2, Hep3, Hep4	*In vitro* synthesis	Liver, kidney, spleen	Antibacterial	Alvarez et al. (2016)
Histone-derived peptides	Oncorhyncin II	Rainbow trout	Skin mucus	G^+ and G^- resistance	Fernandes et al. (2004)
	Oncorhyncin III	*Oncorhynchus mykiss*	Skin secretion	G^+ and G^- resistance	Fernandes et al. (2003)
	Hipposin	*Hippoglossus hippoglossus L.*	Skin mucus	G^+ and G^- resistance	Birkemo et al. (2003)
Piscidins	Piscidin 1	*Morone saxatilis* × *M. chrysops*		Bacteriostasis and anticancer	Cheng et al. (2020)
	Piscidin 2	Hybrid striped bass	Columnar cells	Antiparasitic, antibacterial	Colorni et al. (2008); Wu and Wang (2021)
	Piscidin 3	Tilapia		Anti G+ (*Streptococcus agalactiae*), antitumor	Bor-Chyuan et al. (2018); Wu and Wang (2021)
	Piscidin 4	Hybrid striped bass *Tilapia mossambica*	Gill	Antibacterial (Gram-positive and Gram-negative bacteria) including bacteria that cause babellosis and Lactococcus diseases, anticancer	Corrales et al. (2009); Ting et al. (2019)
	Piscidin 5	Striped bass and white bass		Antibacterial	Salger et al. (2016); Stefi et al. (2020)
	Piscidin 6	Striped bass and white bass		Antibacterial, antifungal, antiparasitic	Salger et al. (2016); Stefi et al. (2020)
	Piscidin 7	Striped bass and white bass		Antibacterial	Salger et al. (2016); Stefi et al. (2020)
	Pleurocidin	Winter flounder	Skin mucus	G^+ and G^- resistance	Cole et al. (1997)
	Moronecidin	Hybrid striped bass	Gills, skin, intestines, spleen, blood cells	Antibacterial	Lauth et al. (2002)
Piscidins	Epinecidin-1	*Epinephelus coioides*		Antibacterial, antifungal, immune regulation, anticancer, wound healing, antiparasitic	Neshani et al. (2019); Chee et al. (2019); Gui et al. (2016)
	Misgurin	*Misgurnus anguillicaudatus*	Skin	G^+ and G^- resistance, antifungal	Park et al. (1997)
	Chrysophin	*Chrysophrys major*	Gill, skin	Antibacterial	Stefi et al. (2020)
	Dicentracin	*Dicentrarchus labrax*	Macrophages, granulocytes, monocytes, macrophages	Antibacterial	Salerno et al. (2007)
	Gaduscidin	*Gadus morhua*	Brain, spleen, kidney, gill	Antibacterial	Browne et al. (2011)
	Myxinidin	Hagfish	Skin mucous	Antibacterial, yeast	Subramanian et al. (2009)

antiparasitic activity against *Tetrahymena pyriformis* (Ruangsri et al., 2012). Piscidin 2 isolated from the columnar cells of hybrid striped bass is lethal to three protozoan ectoparasites, namely, the ciliates *Cryptocaryon irritans* and *Trichodina* sp., and the dinoflagellate *Amyloodinium ocellatum*. Parasite death usually occurs within 60 minutes after contact, which indicates its strong antiparasitic activity (Colorni et al., 2008). Huang et al. reported that Epinecidin-1 antimicrobial peptide can act on *Trichomonas vaginalis in vitro* and *in vivo* (Huang et al., 2019a). EcPis-3, a homolog isolated from *Epinephelus coioides* piscidin, has strong antibacterial activity during the infection period of *Cryptocaryon irritans*, and ecPis-2L and ecPis-4L have inhibitory effects on parasites and fungi (Zhuang et al., 2017). Of-Pis1, a piscidin homolog isolated from rock bream (*Oplegnathus fasciatus)* by Umasuthan et al., has high activity against *Miamiensis avidus* (Umasuthan et al., 2015).

Antifungal Activity

Piscidin 2 produced by the mast cells of hybrid striped bass has effective antifungal activity against human pathogenic fungi (Sung et al., 2008). Myxinidin, a new antibacterial peptide isolated from the acidic mucus extract of the hagfish epidermis, has a fungicidal effect on *Candida albicans*, and its antifungal activity is 2~16 times greater than that of the fish antifungal peptide pleurocidin (Subramanian et al., 2009). Zahran et al. found that piscidin 2 had strong activity against *Saprolegnia,* it has a partial synergistic effect in the presence of copper (Zahran et al., 2010). Wang et al. reported that antimicrobial peptides (parasin I, pelteobagrin, and SaRpAMP) in catfish inhibited *Candida albicans* with MICs of (1μg/mL, 64μg/mL, 64μg/mL) (Wang et al., 2022b). Nikapitiya et al. designed and synthesized ctominin with AMPs structural characteristics according to the cDNA sequence of Octopus minor defense protein 3. It has effective against *C. albicans*, and the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of 50 and 200 μg/mL (Nikapitiya et al., 2020). Veedu et al. characterized the mature peptide *St*-hep of *Sorsogona tuberculata* AMPs hepcidin by bioinformatics database and found that *St*-hep has many biological activities such as antifungal activity (Veedu et al., 2021).

Antitumor Effects

In recent years, an increasing number of studies have indicated that fish AMPs can induce apoptosis or inhibit the growth of tumor cells. Morash et al., reported that some members of the pleurocidin family had selective antitumor activity against human leukemia cells *in vitro*, and they were also applied for tumor treatment in a zebrafish model (Morash et al., 2011). Ting et al., found that piscidin 4 in tilapia can induce the expression of FBJ murine osteosarcoma viral oncogene homolog B (FOSB) caused by Ca^{2+} homeostasis disorder through mitochondrial damage, and overexpression of the FOSB protein leads to the death of non-small and fine lung cancer cells, which indicates that piscidin 4 has the potential to prevent cancer (Ting et al., 2019). Huang et al., studied the antibacterial peptide pardaxin secreted from flatfish by proteomics and found that the regulatory protein pardaxin mainly played roles in the folding protein reaction, oxidative stress, and cytoskeleton distribution to finally induce apoptosis in human cervical cancer cells (HeLa) (Huang and Chen, 2013b). Piscidin 1, isolated from hybrid striped bass (*Morone saxatilis × M. chrysops*) by Cheng et al., did not affect noncancerous cells but was more cytotoxic to osteosarcoma cells (OSA) than lung and ovarian cancer cells (Cheng et al., 2020). Piscidin 1 can regulate mitochondrial active oxygen species and reduce mitochondrial transmembrane potential and the mitochondrial antioxidant manganese superoxide dismutase, which leads to mitochondrial dysfunction and apoptosis in OSA cells (Cheng et al., 2020). Epinecidin-1 isolated from *Epinephelus coioides* can not only inhibit the proliferation and induce the apoptosis of human leukemia U937 cells but also stimulate the expression of interleukin, which may be the result of multiple actions of the mitochondrial pathway and death receptor pathway (Chen et al., 2009).

Immune Regulation

Fish AMPs have not only activity against pathogenic microorganisms but also powerful immunomodulatory functions. Pan et al., studied epinecidin-1, a recombinant antibacterial peptide of Epinecidin-1, on *Epinephelus coioides* and *Danio rerio* infected with *Vibrio vulnificus* and found that the recombinant antibacterial peptide epinecidin-1 could significantly increase the expression of tumor necrosis factor (TNF-α) in *Epinephelus coioides* and that of NOS-2, IL-1β, and Toll-like receptor 4 (TLR-4) in *Danio rerio* (Pan et al., 2012). Moreover, García-Valtanen et al., reported that the zebrafish antibacterial peptide β-defensin 2 regulates immune cell function by inducing NF-κB translocation from the cytoplasm to the nucleus and activating the NF-κB pathway (Garcia-Valtanen et al., 2014). Zhang et al., reported that the antibacterial peptide cathelicidin from rainbow trout may stimulate chemotactic IL-8 and recruit immune cells to the infected site, thus causing an immune response (Zhang et al., 2015). Lei et al. cloned TroLEAP-2 from golden pompano (*Trachinotus ovatus*), which can induce the expression of non-specific immune-related genes, and overexpression of TroLEAP-2 can also significantly improve the activation of macrophages *in vivo* (Lei et al., 2020). Liu et al. obtained the Hepcidin gene sequence in Coregonus ussuriensis berg and analyzed it (named CuHepc) (Liu et al., 2022). The expression of immune-related genes IL-10, TLR3, and NF-κB in the liver and intestine was up-regulated after the administration of

CuHepc peptide *in vivo*, indicating that Hepcidin plays an important role in the immune response of *Coregonus ussur*iensis berg (Liu et al., 2022). In addition, researchers used recombinant Artemia (an aquaculture feed) to express epinecidin-1 and found that adding recombinant Artemia to adult or larval zebrafish can significantly improve their enhanced immunity against acute infection and increase their survival (Ting et al., 2018; Jheng et al., 2015).

Reconstruction and Optimization of Fish Antimicrobial Peptides

Based on the antibacterial, antiviral, antiparasitic, and other biological activities of antimicrobial peptides, coupled with the extensive use of antibiotics and the emergence of drug-resistant microorganisms, antimicrobial peptides have broad application markets in the fields of medicine, food, agronomy, animal husbandry, and aquaculture (Huan et al., 2020). However, there are still many problems to be solved regarding their development and applications, which has led to scientists trying to optimize and transform natural AMPs. In recent years, the development of optimization strategies and molecular design, as well as the rapid rise of nanotechnology, have improved the biological and chemical properties of AMPs (Tan et al., 2021). Such as genetic engineering, amino acid deletion, addition, replacement, peptide cutting, conjugation with fatty acid or other biomolecules, and formulation with antimicrobial synergizing agents. nanotechnology and encapsulation and other methodological strategies. It helps to improve the quality and production of AMPs, enhance antibacterial activity, improve stability, and improve proteolytic enzyme resistance. All of the above optimization design methods provide good suggestions for the modification of fish AMPs. It is therefore expected that the modified AMPs can provide more properties to meet actual needs.

Genetic Engineering

Compared with traditional separation and chemical synthesis methods, genetic engineering is considered to be one of the important strategies to improve the quality and yield of antimicrobial peptides. This method has the potential advantages of large-scale production and low cost. At present, the use of bacteria, yeast, transgenic animals, and plants to express AMP has begun to explore (Sinha et al., 2018; Huang et al., 2019b). For example, Patiño-Rodríguez et al. used *Agrobacterium tumefaciens*-mediated transient expression system to express broad-spectrum AMPs protegrin-1 (PG-1) in *Nicotiana tabacum* (tobacco) using magnICON. The results showed that the expression of PG-1 helped to inhibit 72% of *Mycobacterium bovis* BCG, 70.2% of *Staphylococcus aureus*, 70% of *Candida albicans*, 56.6% of *Escherichia coli*, 53.2 % of *Klebsiella pneumoniae* (Patiño-Rodríguez et al., 2013). Genetic engineering is paving the way for the synthesis and production of various AMPs, but this field has great potential for development. Such as gene editing technology CRISPR-Cas combined with existing genetic engineering methods, for more easily and faster production of AMP (Sinha et al., 2018).

Amino Acid Deletion, Addition, Replacement

As reported in the literature, Lee et al., designed an analog of PIs-1, PG, based on piscidin 1 isolated from mast cells of hybrid striped bass and replaced the original glycine (Gly) residue with proline (Pro) at the 8th position, which endowed it with stronger antibacterial activity and cell selectivity (Lee et al., 2007). Lu et al. synthesized a cationic AMP derivative Pep05 (KRLFKKLLKYLRKF) by replacing L-amino acids with D-amino acids and unnatural amino acids. The results showed that both D-amino acids and unnatural amino acids enhanced the stability of peptides to proteases (Lu et al., 2020). Zhang et al., found adopted the method of replacing isoleucine (Ile) with lysine (Lys) on melittin-related peptide (AR-23), which is a positively charged residue, on the nonpolar side of the peptide. The results showed that the amphipathic properties, hydrophobicity, helicity, hemolysis, and ability to penetrate the human cell membrane of this antibacterial peptide could be notably changed (Zhang et al., 2016). As predicted by CAMPr3, iAMPprep, and molecular docking, replacing amino acids at specific positions may increase the antibacterial activity of antimicrobial peptides and can be used to solve microbial infections in aquaculture (Bhat et al., 2022). Hu et al. used sequence template and amino acid mutation to optimize and mutate the fish antimicrobial peptide hepcidin. The results showed that the optimized antimicrobial peptide could enhance the antibacterial activity (Hu, 2021). Liu et al., found that the antibacterial peptide from catfish mucus synthesized *in vitro* can kill a variety of bacteria, but its bactericidal effect still has much room for improvement (Liu et al., 2020). Therefore, it was proposed that its sequence can be used as a template for the amino acid deletion or effective, high-quality amino acid addition at specific positions, so its bactericidal effect is expected to be greatly improved (Liu et al., 2020). Research finds modification of antimicrobial peptides by replacing, deleting, or adding amino acids can form a perfect amphiphilic structure (Tan et al., 2021). Nevertheless, the perfect amphiphilic structure has the best activity but enhances antibacterial cytotoxicity. while the rational distribution of imperfect amphiphile residues is more conducive to the optimization of cell selectivity (Tan et al., 2021). Wang et al., reported that an antibacterial peptide with an incomplete amphiphilic palindrome structure (RRIRIIIRIRR-NH2) had high antifungal and antibacterial activities, and most of the incomplete amphiphilic antibacterial peptides had enhanced antibacterial activities against microorganisms (Wang et al., 2018).

Peptide Cutting

More and more reports have pointed out that truncated peptides can improve antibacterial activity. Such as Namvar Erbani et al. reported the antifungal and antibacterial effects of hybrid AMPs CecropinA-Magenin2 (CE-MA) and truncated derivatives (CMt1-CMt3). CMt1 has good antifungal and antibacterial effects, and the toxicity is much smaller than that of the parent CE-MA, CMt3 had the weakest antibacterial activity (Namvar Erbani et al., 2021). It also shows that if the truncation is carried out correctly, it will become a good strategy to reduce sensitization, toxicity, inflammation, and synthesis cost and improve the stability of AMPs (Namvar Erbani et al., 2021). Lv et al. synthesized a 24-residue short peptide GI24 by truncating the AMPs PMAP-36. GI24 has the similar high antibacterial activity to the parent PMAP-36 (Lv et al., 2014). However, it has also been reported that the truncated peptide has a weak antibacterial activity, but the potency of the original peptide can be retained after other modification methods (Hansen et al., 2020). Similar to this, Wang et al. obtained a peptide analogue GK-1 by truncating 11 amino acids at the C-terminus of AMP Pleurocidin, but GK-1 directly truncating Pleurocidin had no antibacterial activity (Wang et al., 2022a). GK-2, GK-3, and GK-4 were obtained by amino acid substitution. Among them, GK-4 has the same antibacterial activity and safety as the parent Pleurocidin and the resistance to pepsin hydrolysis is higher (Wang et al., 2022a). In addition, the removal of N-terminal human beta-defensin 3 (hBD-3) under high NaCl exposure was more resistant to *Escherichia coli* and *Enterococcus faecium* than the parent (Li et al., 2015). Self-assembled antimicrobial peptides have also been widely studied.

Conjugation with Fatty Acid or Other Biomolecules

Zhong et al., connected fatty acid chains of various lengths (4-16 carbon chains) to the side chain of a D-amino acid and found that the new peptides with 8-12 conjugated carbon chains had better antibacterial activity and antibiofilm activity and could better resist bacterial resistance, moreover, the designed peptide has higher stability to serum, salt, trypsin and different concentrations of pH environment (Zhong et al., 2020). Storck et al., replaced the hydrophobic motifs of a synthetic antimicrobial peptide (PEP19-4LF) with fatty acids and linked saturated fatty acids with different chain lengths before verifying its antibacterial activity. The results stated that the antibacterial activity of this peptide was directly related to the length of the fatty acid chains, and the antibacterial activity of 11 carbon chain lengths is the best (Storck et al., 2020). The results were consistent with those of Zhong (Zhong et al., 2020), which proved that coupling fatty acid chains to the side chains of amino acids was a potential strategy for designing antibacterial peptides. Tan et al., treated the WKKIWKDPGIKKWIK peptide segment of a specific targeted antimicrobial peptide (STAMP) with a single factor and carried out a series of tests on the antibacterial activity, hemolysis, cytotoxicity, and sensitivity (Tan et al., 2020b). The results showed that it is difficult to achieve the expected transformation effect by changing only a certain factor (Tan et al., 2020). To this end, a new template modification method was proposed; that is, the phage display peptide recognizes and binds to *E. coli* cells and connects to the end of the sequence. Using this method to optimize STAMP can improve its antibacterial activity and stability (Tan et al., 2020b). According to other reports, the stability of peptides can be improved by introducing D-amino acids, N-terminal amidation, unnatural amino acids, and cyclization or by adding unusual amino acids to the binding sites of proteases and modifying peptide bonds through nitrogen bond alkylation to prevent degradation (Preeti et al., 2020). After N-terminal acetylation and C-terminal amidation of β-hairpin AMP tachyplesin I, compared with the parent tachyplesin I, the modified AMP was resistant to human serum protein degradation, but showed cytotoxicity to tumor cells and normal human cells (Kuzmin et al., 2017).

Formulation with Antimicrobial Synergizing Agents

Zharkova et al. used AMPs (porcine protegrin 1, human cathelicidin LL-37, beta-defensins, etc.) with different structures and modes of action in combination with various antibiotics, confirming that the combination of antimicrobial peptides with antibiotics or other antimicrobial agents is a promising strategy (Zharkova et al., 2019). Pleurocidin (Ple) and C-terminal amidation derivative Pleurocidin-amide (Ple-a), isolated from winter flounder Pleuronectes americanus by Hsu et al., have antibacterial activity against Gram-positive and Gram-negative bacteria (Hsu et al., 2022). When combined with conventional antibiotics with different mechanisms of action, Ple-a shows synergistic effects on multidrug-resistant (MDR) *E.coli* (Hsu et al., 2022). Doolin et al. reported that histone H2A and antimicrobial peptide LL-37 had different antibacterial effects, but formed a synergistic antibiotic mechanism (Doolin et al., 2020). LL-37 forms pores, allowing H2A to enter bacteria. H2A can stabilize AMP-induced pores, recombine bacterial chromosome DNA after entering bacteria, and inhibit transcription to kill bacteria (Doolin et al., 2020). The effect of the LL-37/H2A combination is much greater than their respective antibacterial effects (Doolin et al., 2020). Masadeh et al. reported a new modified hybrid peptide MAA-41, which was formed by the combination of BMAP-28 and LL-37 αhelixes and then modified with amino acids. The combination of MAA-41 with conventional antibiotics could improve the antibacterial activity of the combination drugs, and the synergistic or additive effects showed that the MIC was significantly reduced to 0.25 μM (Masadeh et al., 2022).

Nanotechnology and Encapsulation

Nanotechnology can protect AMP from degradation and improve its efficacy. Currently, various metal nanoparticles (gold, silver), lipid nanoparticles (liposome), polymer nanoparticles [chitosan, hyaluronic acid, and poly (glycolide-co-lactide) or PLGA] have been used to deliver AMP, and the use of AMP nanoparticle delivery carriers can enhance biological stability (Sowers et al., 2023). As reported by Wadhwani et al., when the peptide is connected to metal nanoparticles, the peptide chain can maintain the degree of freedom of its skeleton conformation, and the stability of trypsin digestion is significantly improved compared with the free state (Wadhwani et al., 2017). Jayathilaka et al. used an ionotropic gelation technique to encapsulate the antimicrobial peptide Octominin with chitosan (CS) and carboxymethyl chitosan (CMC) as a nano-drug delivery system. Compared with Octominin, Octominin-encapsulated CS nanoparticles (Octominin-CNPs) have lower toxicity and stronger antibacterial activity against *C. albicans* and *A. baumannii* (Jayathilaka et al., 2022). Cubic liquid crystalline gel (cubosomes) is also used as a drug delivery carrier for LL-37. Cubosomes can protect LL-37 from proteolysis to the greatest extent, but the bactericidal ability after encapsulation is only effective for Gram-negative bacteria (Boge et al., 2017). Yu et al. reported a strategy to induce the self-assembly of AMP T9W into nanostructured antimicrobial micelles that can enhance pharmacological properties. Compared with the parent molecule, T9W ($CT9W_{1000}$) formed by PEGylatio at the C-terminus was more effective against *Pseudomonas aeruginosa*, and the range of antibacterial spectrum was expanded. The CT9W1000 micelles have high stability in serum, saltions, and acid-base environment. Most importantly, the CT9W self-assembled structure is resistant to trypsin degradation (Yu et al., 2023). Ko et al. used fish-derived AMP pleurocidin (PLE) C-terminal amidation and poly (lactic-co-glycolic acid) (PLG) polymer to further encapsulate to form PLG-PLE (am) microparticles, which maintained the release of AMP PLE and effectively protected fish from pathogenic bacteria (Ko et al., 2019).

Applications of fish Antimicrobial Peptides

As an essential component of fish innate immunity, AMPs form the natural defense barrier of the skin and can continuously prevent most infectious microorganisms from invading. Additionally, AMPs are involved in a variety of practical applications as small molecules with unique functions.

Feed Additives

Research has reported that aquatic feed additives (those for immune stimulation and novel feed additives) added to conventional feed can be involved in regulating the growth, quality, immunity, antioxidative stress, and disease-resistant abilities of aquatic animals, not only providing better economic benefits for the majority of aquaculture farmers but also promoting good environmental benefits (Zhao et al., 2020). Therefore, fish AMPs can be used as novel feed additives. Huang et al., generated yeast cells with recombinant expression of piscidin 4 into granules and added them to ordinary feed as a novel feed additive. Fish supplemented with 3% and 5% piscidin 4 had significantly improved survival rates, weight gain rates, and liver indices. It was also found that the antimicrobial peptide could increase the expression level of IgM and increase the activities of antioxidant enzymes (SOD, GPx, hydrogen peroxide) (Huang et al., 2020). In addition, it has been reported that the antibacterial peptide Hepcidin gene engineering expression product of *Pseudosciaena croce*, was added to the feed of *Acanthopagrus schlegelii* as a feed additive. After one year of safe feeding, it was found that PC-HEPC can not only inhibit harmful microorganisms in the intestine *in vivo*, and maintain normal intestinal metabolism and immune function but also promote growth (Tan et al., 2020a).

Food Safety

As early as 2004, pleurocidin was isolated from *American plaice*, and its potential application in food was evaluated. The results showed that pleurocidin had good antibacterial activity against food-borne microorganisms such as *E. coli*, *Listeria monocytogenes*, *Saccharomyces cerevisiae,* and *Penicillium expansum* without clear cytotoxicity to human intestinal cells. These results indicated the versatility of AMPs, which could be used as a potential food preservative (Burrowes et al., 2004). Gu Chentao observed that spraying crude AMPs on the surface of red plums could significantly delay senescence and decay. The freeze-dried powder of crude isolated fish scale AMPs was placed in freshly squeezed cucumber juice, which effectively inhibited microbial growth and maintained the colors of fruit and vegetable juices. The results showed that adding fish scale AMPs had a good effect on the preservation of fruits and vegetables (Gu, 2019). Wang et al., combined pleurocidin, an antibacterial peptide isolated from the skin mucus of winter flounder, with microfiber mats by electrostatic spinning and successfully applied it to cider, which had a good inhibitory effect on *E. coli* (Wang et al., 2015).

Biomedicine

Fish AMPs have been recognized as a safe and potential source of antibacterial drugs, and for this application, it is most important to ensure that they are non-toxic. Wu et al., examined the antitumor activity of pardaxin from *Pardachirus marmoratus* by using mN-11 tumor cells from mice as the research model. The soft AGAR assay, qPCR, ELISA and other experimental data showed that pardaxin could delay the development of

tumors by inhibiting the proliferation and differentiation of tumor blood vessels, which indicated that the antimicrobial peptide induced cancer cell apoptosis through the original apoptotic pathway, thus highlighting its great potential as an antitumor drug (Wu et al., 2012). Huang et al., synthesized epinecidin (Epi)-1 and evaluated the inactivated EPI-1 vaccine, in which Epi and JEV were simultaneously injected into mice. JEV was added for attack 14 days later, and the survival rate of the mice was 100%. As an inactivated vaccine combination therapy, EPI-1 can inhibit JEV activity, regulate the expression of the immune response genes IL-6, IL-10, IL-12, TNF-α, and INF-γ, and increase the level of serum anti-JEV neutralizing antibodies, suggesting that an EPI-1-modified vaccine is a promising strategy (Huang et al., 2011). Epinecidin-1 from *Epinephelus coioides* has antitumor activity against HT1080 cells *in vitro* and has the function of peptide cleavage, making it a likely effective drug against human fibrosarcoma cells in the future (Lin et al., 2009). Huang et al, tested marine pardaxin combined with inactivated mouse bladder cancer cell lysate (MBT-2) in a mouse model and found that cocoa enhanced the secretion of endogenous nitric oxide (NO) in mouse macrophages and regulated the levels of MCP-1, IL-6, and IL-12. It was also proven that the addition of pardaxin to the cell culture medium could inhibit the proliferation of MBT-2 *in vitro*, which further indicated its potential as a cancer vaccine (Huang et al., 2013a). β-Defensin 2 is an effective molecular adjuvant for viral DNA vaccines in *Danio rerio* (Garcia-Valtanen et al., 2014). However, when fish AMPs are used as pharmaceutical products, difficulties such as the high cost of peptide synthesis, protease instability, and serum instability need to be overcome.

Conclusions and Future Challenges

AMPs cannot only help the body defend against the attack of foreign pathogens but also play an indispensable role in neutralizing endotoxin, regulating cell death, promoting wound healing, and regulating immune function (Xia et al., 2018; Xia et al., 2019; Xia et al., 2021a,b). Considering that fish is an important source of AMPs that have a variety of biological functions, many scholars have conducted in-depth research on AMPs in the fields of animal husbandry, food, medicine, and other related areas and have achieved certain results. The broad-spectrum antibacterial activity and membrane-breaking mechanism of AMPs highlight their prospects as antibiotic substitutes. However, antibacterial peptides also face some challenges in the research and development process. (1) The first problem is the source of fish AMPs. The content of natural AMPs in fish is low, the separation process is complicated, and the expression by genetic engineering is also low. Additionally, the structures of synthetic AMPs may be different from those of natural AMPs, and their cost is high. (2) The second issue is AMP stability in the body. Antibacterial peptides are easily degraded by trypsin and Proteolytic enzymes in animal intestines and cannot reach the hindgut to exert their biological activity. (3) Fish AMPs have many functions, but their specific functional mechanism is not clear, and the interaction between different types of AMPs needs further study. (4) The correlation between AMP structure and antibacterial activity, hemolytic activity and cytotoxicity is unknown. (5) Compared with traditional antibiotics, the antibacterial activity of antibacterial peptides is slightly inadequate, especially protein antibacterial peptides, which are easily denatured and inactivated during production, transportation, administration, etc. How to maintain their antibacterial activity also needs further research. The theoretical system of structure-activity relationships of antibacterial peptides established by combining big data with antibacterial peptide activity modification technology is an effective way to improve the antibacterial activity of antibacterial peptides. Antibacterial peptides that resist enzymatic hydrolysis obtained by nanotechnology induce amphiphilic antibacterial peptides to self-assemble into nano assemblies, and coating technology and special amino acids to avoid restriction sites are stable to proteases and can resist hydrolysis by various enzymes *in vivo*. The key endogenous antibacterial peptides can be generated through *in vitro* synthesis, which is also a good way to stimulate the secretion of endogenous antibacterial peptides by adding certain functional substances (such as short-chain fatty acids, amino acids, and probiotics). In the future, it will remain necessary to further explore the molecular modifications, scientific syntheses and efficient and low-cost production technology of antibacterial peptides and establish a eukaryotic expression system to adequately express the target antibacterial peptides to obtain fish antibacterial peptides with improved safety, higher activity, lower cost, and more stable expression to efficiently utilize fish antibacterial peptides and ensure animal food safety and human health.

Ethical Statement

This study does not require an ethics committee report.

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Author Contribution

Lei Wang and Ting Qin are contribute equally to this work. Lei Wang, Ting Qin and Xiaojing Xia designed the review and contributed to manuscript preparation. Yuxin Zhang, Huihui Zhang, Jianhe Hu and Likun Cheng wrote the manuscript. Ting Qin, Likun Cheng and Xiaojing Xia revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that we have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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