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Antimicrobial peptides used in Aquaculture

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Abstract

Antimicrobial peptides (AMPs) are a promising alternative to traditional antibiotics in aquaculture, addressing the issue of antimicrobial resistance (AMR). These peptides, essential in the innate immune system, exhibit broad-spectrum activity against bacteria, fungi, viruses and parasites. Their membrane-disrupting mechanism reduces resistance risk, making them a sustainable option for disease control. AMPs, classified by structure, amino acid composition, source and action mode, come from diverse sources including mammals, amphibians, insects, microorganisms, plants and aquatic organisms. Their actions include membrane targeting, non-membrane targeting and immunostimulatory effects. AMP-based therapies offer an eco-friendly solution for managing infections and improving the health of farmed aquatic species.

keywords: Antimicrobial peptides (AMPs), Antimicrobial resistance (AMR), Fish disease management, Broad-spectrum activity, Peptide classification, Sustainable aquaculture, Aquatic-derived AMPs

Introduction

Aquaculture production reached 130.9 million tonnes, becoming vital to the global food economy and livelihood security (FAO, 2024). To meet rising demand, the industry adopted intensive methods, heavily using antibiotics, which led to increased antimicrobial resistance (AMR) in bacterial pathogens (Debbarma et al., 2024). This raised concerns about shrimp disease control, prompting a search for alternatives. Antimicrobial peptides (AMPs) emerged as a solution, enhancing immune responses and rapidly killing pathogens (Ohta et al., 2006; Dlamini et al., 2019). This was crucial in fish farming, where species like teleosts relied mainly on innate immunity due to underdeveloped adaptive immunity (Masso-Silva & Diamond, 2014).

What are AMPs?

Antimicrobial peptides (AMPs) were small proteins essential to innate immunity, offering broad-spectrum activity against microbes and cancer cells (Zasloff, 2002; Hancock & Sahl, 2006). Ranging from 10 to 100 amino acids, they were positively charged and amphipathic, enabling microbial membrane disruption (Ganz, 2003; Semreen et al., 2018). AMPs showed potential as antibiotic alternatives due to lower resistance and environmental impact (Schittek et al., 2001). Marine organisms, like crustaceans, provided potent AMPs for disease control in aquaculture, offering a sustainable approach to improving aquatic species' health (Hancock & Sahl, 2006; Ingermann et al., 2010; Kosa et al., 2011)

Importance of AMPs

Antimicrobial peptides (AMPs) have emerged as a promising alternative to antibiotics in aquaculture due to their multiple advantages. One of the key benefits of AMPs is their low risk of resistance development, as they disrupt microbial membranes, making it difficult for pathogens to adapt and ensuring long-term efficacy (Schittek et al., 2001). Additionally, AMPs exhibit potent antimicrobial activity even at minimal concentrations, reducing environmental impact while maintaining efficiency (Hancock & Sahl, 2006; Semreen et al., 2018). These peptides are particularly valuable in targeting multi-drug-resistant pathogens such as Vibrio and Aeromonas, which pose significant threats to aquatic species (Dlamini et al., 2019). Their broad-spectrum activity enables them to combat a wide range of pathogens, including bacteria, fungi, viruses and parasites, providing comprehensive protection in aquaculture (Ganz, 2003; Hancock & Sahl, 2006). Furthermore, AMPs play a crucial role in controlling viral diseases and parasitic infections, enhancing the overall health and resilience of farmed aquatic species (Malafaya et al., 2007). These properties make AMPs a sustainable and effective alternative to traditional antibiotics in aquaculture.

Classification of AMPs

The diversity of natural AMPs made their classification difficult. AMPs were classified based on their peptide structure, amino acid-rich species, source of origin and modes of action.

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4.1 According to their peptide structure

AMPs could be categorized into four main categories based on their peptide structures: 1) α-helical AMPs, 2) β-sheet AMPs, 3) linear extended structure AMPs and 4) mixed AMPs (Huan et al., 2020).

1. α-helical AMPs: α-helical AMPs included molecules like cecropins, magainins, pleurocidins and others, primarily derived from insects, amphibians and teleost fish (Park et al., 1997; Douglas et al., 2001; Lauth et al., 2002). These peptides were rarely found in invertebrates, though certain tunicate hemocytes were exceptions (Lee et al., 1997).
2. β-sheet AMPs: β-sheet AMPs formed disulfide bonds and included defensins, mytilins and others (Saito et al., 1995; Charlet et al., 1996).
3. Extended Linear Structure AMPs: These AMPs had a linear, extended structure and were rich in amino acids like histidine, proline, tryptophan and arginine. Their antimicrobial properties stemmed from interacting with intracellular proteins, like heat shock proteins (Nayab et al., 2022).
4. Mixed AMPs: This category combined both α-helical and β-sheet structures, with a high content of proline and arginine (Nayab et al., 2022).

4.2 According to the amino acid-rich species

- AMPs were also classified based on their amino acid-rich species into five categories: proline-rich, tryptophan-rich, arginine-rich, histidine-rich and glycine-rich AMPs.
- Proline-rich AMPs (PrAMPs) entered bacterial cells through the inner membrane transporter SbmA and targeted ribosomes, interfering with protein synthesis (Mattiuzzo et al., 2007; Seefeldt et al., 2015). Examples included Tur1A and Bac5 (Imjongjirak et al., 2017).
- Tryptophan- and arginine-rich AMPs used the properties of these amino acids to enhance antimicrobial effects. Examples included indolicidin and triptrpticin (Walrant et al., 2020; Chan et al., 2006).
- Histidine-rich AMPs like HV2 and L4H4 had antibacterial and anti-inflammatory effects (Dong et al., 2019; Lointier et al., 2020).
- Glycine-rich AMPs, including attacins, played a role in immune activation and stability (Wang et al., 2015).

4.3 According to their source origins

AMPs could be classified according to their source origins into six categories:

Mammalian AMPs were found in humans, cattle and other vertebrates, with cathelicidins and defensins as examples (Reddy et al., 2004). Amphibian-derived AMPs came from amphibians, such as frogs, protecting them from pathogens (Rollins-Smith, 2009). Insect-derived AMPs enhanced insect

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survival against infections (Abdel-Tawwab et al., 2020). Microorganism-derived AMPs came from fungi and bacteria like *Lactococcus lactis* and *Bacillus subtilis* (Huan et al., 2020). Plant-derived AMPs were part of plant defenses, such as cyclotide from *Bauhinia rufescens* (Nganso et al., 2020). Aquatic-derived AMPs were isolated from aquatic organisms like fish, crabs and shrimp, with immunomodulatory and antimicrobial properties (Valero et al., 2020; Brockton & Smith, 2008).

4.4 According to their modes of action

AMPs could act through three proposed mechanisms:

1. Membrane-active mechanisms: AMPs disrupted microbial membranes, leading to leakage and cell death (Huan et al., 2020; Sengupta et al., 2008).
2. Non-membrane-active mechanisms: These AMPs targeted internal cell functions, including inhibiting protein synthesis and disrupting nucleic acids (Mardirossian et al., 2014; Subbalakshmi & Sitaram, 1998).
3. Immunostimulatory effects: AMPs stimulated cytokine production, enhanced cell-mediated immunity and improved humoral immunity.

5. Mechanism of Antimicrobial Peptides (AMPs)

AMPs kill microbes through various mechanisms, targeting the cytoplasmic membrane and interfering with essential cellular molecules. This multi-hit approach reduces the chances of resistance.

Barrel-Stave Model:

In this model, AMPs inserted themselves vertically into the membrane and assembled to create a channel or pore. This channel allowed ions and other molecules to pass through, disrupting the membrane's integrity. Pardaxin and alamethicin were examples of AMPs that used this mechanism (Harder & Schroder, 2016).

Toroidal Pore (Wormhole) Model:

Unlike the barrel-stave model, pore formation here didn't primarily rely on AMPs interacting directly with each other. Instead, the AMPs induced curvature and bending in the lipid bilayer of the membrane. The resulting pore was formed by both the AMPs and the phospholipid head groups of the membrane (Gaspar & Castanho, 2016). This model disrupted the bilayer structure but didn't necessarily create a distinct, protein-lined channel like the barrel-stave model. Protegrin-1, melittin, and magainin-2 were examples of AMPs that acted through this mechanism. A key difference from the barrel-stave model was that the bilayer was significantly disturbed in the toroidal model but remained relatively intact in the barrel-stave model (Di Somma, 2020).

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Carpet Model:

In this model, AMPs accumulated on the surface of the bacterial membrane, acting like a carpet. Once a certain concentration of AMPs was reached, they exerted a detergent-like effect, disrupting the membrane by forming micelles (clusters of lipids and AMPs). This led to membrane breakdown and cell death. LL-37 and cecropin were examples of AMPs that utilized this mechanism (Geitani, 2020).

6. Benefits and Applications of AMPs in Aquaculture

AMPs offer a promising alternative to antibiotics in aquaculture, helping to manage diseases and reduce antimicrobial resistance. They are used for treating bacterial, fungal, parasitic and viral infections in fish, shrimp and mollusks through water, feed, or topical applications. AMPs produced by probiotics also promote gut health and maintain balanced microbiomes (Sung et al., 2008; Yang et al., 2011).

Antifungal Activity

Fish-derived AMPs, like piscidin-2 and hepcidins, showed effectiveness against fungi such as *Candida albicans* and *Aspergillus niger* (Sung et al., 2008; Yang et al., 2011; Zhuang et al., 2017). Histone-like peptides also targeted *Saprolegnia parasitica* (Robinette et al., 1998).

Antiparasitic Activity

AMPs like piscidin-2 and "ecPis-3" were effective against ectoparasites and protozoan parasites such as Trichodina and *C. irritans* (Colorni et al., 2008; Ruangsri et al., 2012). "HbβP-1" from *Ictalurus punctatus* also demonstrated strong antiparasitic effects (Noga et al., 2002).

Antiviral Activity

AMPs such as piscidins and β-defensins showed antiviral effects, including activity against the channel catfish virus and spring viremia of carp virus (Chinchar et al., 2004; Garcia-Valtanen et al., 2014). Synthetic SA-hepcidin-2 exhibited antiviral effects against Rhabdoviruses and Reoviruses (Gui et al., 2016).

Antibacterial Activity

AMPs demonstrated significant antibacterial properties. For example, fortified diets with AMPs reduced mortality in shrimp infected with *Vibrio harveyi* (Gyan et al., 2020). LEAP-2 inhibited *Edwardsiella tarda* and *Streptococcus agalactiae* (Lei et al., 2020) and NKL-24 from zebrafish showed antibacterial activity against *Vibrio parahaemolyticus* (Shan et al., 2020).

Limitations of AMPs

While AMPs show potential in aquaculture, several challenges limit their use. These include instability in varying pH conditions, potential toxicity to host organisms and high production costs. The lack of comprehensive data on their toxicology and pharmacokinetics, as well as the inhibition of

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AMP activity by ions and serum components, are additional concerns. Environmental factors like ocean acidification and protease degradation also affect AMP effectiveness. Moreover, the potential for pathogen resistance, delivery challenges and environmental impact must be further addressed for widespread use.

Future Perspectives

Future research on AMPs in aquaculture focused on enhancing their stability, broadening their pathogen activity range and reducing toxicity to host organisms. Efforts were directed at developing advanced delivery systems, such as nanoparticles and microencapsulation, to protect AMPs from degradation and target specific tissues in aquatic animals. Additionally, AMPs were explored for use in diagnostic tools to rapidly detect pathogens. Further research on AMP-pathogen-host interactions aimed to improve AMP design and treatment strategies. Finally, addressing environmental concerns related to AMP use required studies to assess impacts on non-target organisms and develop mitigation strategies.

Conclusion

Antimicrobial peptides (AMPs) showed great potential in improving aquaculture sustainability by preventing diseases and enhancing fish health. As eco-friendly alternatives to antibiotics, AMPs help address antimicrobial resistance. They also support immune function, reduce stress and promote growth in aquatic species. Despite challenges like stability, affordability and effective delivery, ongoing research in genetic engineering, peptide synthesis and probiotics is expanding their applications. Further development of AMPs can help create a more sustainable aquaculture industry, reduce antibiotic use and ensure a healthier seafood supply for the future.

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