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Phage-Derived Antimicrobials: A Sustainable Approach to Combat Bacterial Infections in Aquaculture

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ABSTRACT

Phage-derived antimicrobials are emerging as a sustainable alternative to traditional antibiotics in aquaculture, offering targeted solutions to combat bacterial infections while mitigating the environmental and public health issues associated with antibiotic use. This review examines the role of bacteriophages and their derivatives, such as lysins and tail-like bacteriocins, in addressing the rising threat of antimicrobial resistance (AMR) and bacterial pathogens in aquaculture systems. We explore recent advancements in phage engineering, particularly CRISPR-based phage modification, and their potential integration with sustainable practices like probiotics and vaccines. Additionally, the review highlights the practical challenges of phage therapy, including production scalability, regulatory barriers, and environmental stability. Current research and case studies demonstrate successful phage applications in fish, shrimp, and shellfish farming, underscoring the potential of phage-derived antimicrobials to improve aquaculture health without leaving harmful residues. Finally, we advocate for increased research, policy support, and investment in phage-based solutions as a means to enhance aquaculture productivity sustainably and reduce AMR spread.

Introduction

Overview of Aquaculture and Its Importance in Global Food Security

Aquaculture, the farming of fish, crustaceans, mollusks, and aquatic plants, plays a critical role in global food security by meeting the growing demand for protein and providing a sustainable alternative to capture fisheries. With a production of over 80 million tons annually, aquaculture contributes significantly to the global economy, especially in developing countries where it serves as both a source of livelihood and nutrition (FAO, 2021). The industry's rapid expansion highlights the need for sustainable practices to ensure long-term viability (Bostock et al., 2010).

Current Challenges in Aquaculture: Focus on Bacterial Infections and Antimicrobial Resistance (AMR)

Despite its benefits, aquaculture faces considerable challenges, particularly bacterial infections, which are among the primary causes of disease outbreaks, leading to economic losses and threats to animal welfare (Bondad-Reantaso et al., 2005). To manage these infections, antibiotics have been widely used; however, this reliance has contributed to the emergence of antimicrobial resistance (AMR) in aquatic pathogens, posing risks to both aquaculture sustainability and public health due to the potential transfer of resistant bacteria to humans (Cabello, 2006). This growing issue has led to calls for alternative, sustainable solutions in disease management.

Brief Introduction to Bacteriophages and Phage-Derived Antimicrobials

Bacteriophages, viruses that specifically infect and lyse bacterial cells, have garnered attention as an effective and eco-friendly alternative to antibiotics. Phage-derived antimicrobials, including phage enzymes like lysins and tailocins, offer a targeted approach to combat bacterial pathogens without harming beneficial microbes in the environment (Chan et al., 2013). Their specificity and adaptability make them particularly suitable for managing bacterial infections in aquaculture.

Objectives of the Review

This review explores the potential of phage-derived antimicrobials as a sustainable alternative to traditional antibiotics in aquaculture. The focus will be on evaluating their effectiveness against bacterial infections, assessing their environmental impact, and discussing future perspectives on their large-scale application in the industry.

2. Background on Bacterial Infections in Aquaculture

Common Bacterial Pathogens Affecting Aquaculture

Bacterial pathogens are a major threat to aquaculture, frequently causing disease outbreaks that impact productivity and fish health. Among the most common pathogens are *Vibrio*, *Aeromonas*, and *Pseudomonas* species, which are associated with high morbidity and mortality in fish and shellfish populations (Austin & Austin, 2007). *Vibrio* species, for instance, are often implicated in vibriosis, a serious disease in shrimp and fish, while *Aeromonas* species can cause hemorrhagic septicemia and skin ulcers, especially in freshwater fish. *Pseudomonas* species are also known for causing fin rot and other opportunistic infections, particularly under stressful or crowded aquaculture conditions (Roberts, 2012).

Current Treatment Methods: Antibiotics, Chemical Treatments, and Their Limitations

Traditional management of bacterial infections in aquaculture has relied heavily on antibiotics and chemical treatments. Antibiotics, such as oxytetracycline and sulfonamides, have been widely administered to treat infections and sometimes as prophylactics to prevent outbreaks. Additionally, chemical disinfectants like formalin and hydrogen peroxide are used to manage bacterial loads in water (Smith et al., 2008). However, these treatments have notable drawbacks. The overuse and misuse of antibiotics in aquaculture have led to increased antibiotic resistance among aquatic pathogens, diminishing the efficacy of these treatments (Cabello, 2006). Chemical disinfectants, while effective, can accumulate in the environment, potentially harming non-target organisms and contributing to ecological imbalances (Martinez, 2009).

Rising Concerns of AMR in Aquaculture and Environmental Impact

The prevalence of antimicrobial resistance (AMR) in aquaculture is a growing global concern, as resistant bacterial strains can be transferred from aquaculture environments to other ecosystems, including human populations. AMR in aquaculture pathogens, such as *Vibrio* and *Aeromonas*, not only complicates disease management but also poses risks for public health through foodborne transmission (Schar et al., 2021). Additionally, antibiotic residues can disrupt microbial communities in the environment, leading to further ecological consequences and reduction in biodiversity (Kümmerer, 2009). These issues underscore the need for sustainable and effective alternatives to conventional antimicrobial practices in aquaculture.

3. Phage Therapy as an Alternative

Explanation of Bacteriophages and Their Specificity to Bacteria

Bacteriophages, or phages, are viruses that specifically infect and replicate within bacteria. Unlike broad-spectrum antibiotics, phages target specific bacterial strains or species, injecting their genetic material into bacterial cells to hijack their cellular machinery, ultimately leading to bacterial cell lysis and death (Abedon, 2015). This specificity is a unique and valuable feature, as phages can eliminate harmful pathogens without affecting beneficial bacteria in the surrounding environment, making them particularly suited for applications in aquaculture where ecological balance is crucial (O'Flaherty et al., 2009).

Advantages of Phage Therapy

Phage therapy offers several advantages over traditional antimicrobial approaches:

- **Specificity**: Phages can selectively target pathogenic bacteria, reducing collateral damage to beneficial microbes. This is especially beneficial in aquaculture settings, as it minimizes disruptions to the microbiome of aquatic animals and ecosystems (Clokie et al., 2011).
- **Low Environmental Impact**: Phages are naturally occurring entities that degrade in the environment after their target bacteria are eliminated, minimizing residual impact compared to antibiotics, which can persist and accumulate (Sulakvelidze, 2011).
- **Adaptability**: Phages are naturally adaptable, evolving alongside bacterial populations. This evolution can be harnessed to overcome bacterial resistance, allowing phages to remain effective against evolving bacterial strains (Chan et al., 2013).

Limitations and Challenges Associated with Phage Therapy

Despite its potential, phage therapy faces several limitations and challenges:

- **Phage-Host Specificity**: While specificity is an advantage, it also requires precise identification of bacterial pathogens before effective treatment can be administered. This specificity can limit the range of bacteria a particular phage can target, necessitating phage cocktails or tailored solutions for different bacterial infections (Rohde et al., 2018).
- **Regulatory Hurdles**: The regulatory frameworks for approving phage therapy for use in aquaculture are not yet well-established in many regions. This creates delays and obstacles in bringing phage-based products to market (Pirnay et al., 2015).
- **Potential for Phage Resistance**: Just as bacteria develop resistance to antibiotics, they can evolve resistance to phages. However, this challenge can be managed by using phage cocktails or through genetic engineering to enhance phage efficacy and prevent resistance (Kutter & Sulakvelidze, 2004).

4. Types of Phage-Derived Antimicrobials

Phage Lysins: Mechanism of Action and Specificity

Phage lysins, also known as enzybiotics, are enzymes produced by bacteriophages that break down the peptidoglycan layer of bacterial cell walls, causing cell lysis and death. These enzymes exhibit high specificity, targeting particular bonds in the bacterial cell wall that vary among bacterial species, allowing precise elimination of pathogenic bacteria without affecting beneficial microbiota (Fischetti, 2010). The ability of phage lysins to target specific bacteria makes them a promising tool in treating bacterial infections in aquaculture without impacting the surrounding microbial ecosystem (Nelson et al., 2001).

Phage Endolysins and Peptidoglycan Hydrolases: Their Role and Effectiveness

Endolysins are a specific type of phage-derived lysin that acts internally during the final stages of the phage lytic cycle, facilitating phage release by degrading the host bacterial cell wall. These enzymes target peptidoglycan hydrolases, which break down essential cell wall components, leading to rapid and effective bacterial cell lysis (Schmelcher et al., 2012). Their effectiveness and specificity against Gram-positive bacteria, combined with their ability to function independently of intact phages, make endolysins valuable as standalone antimicrobials in aquaculture (Borysowski et al., 2006).

Phage Tail-Like Bacteriocins and Tailocins

Phage tail-like bacteriocins, also known as tailocins, are protein complexes that resemble the tail structures of bacteriophages and can puncture bacterial cell walls, causing membrane depolarization and cell death. Tailocins are produced by certain bacteria and possess specificity similar to phage particles, targeting and lysing closely related bacterial strains (Ghequire & De Mot, 2014). Tailocins are particularly useful in aquaculture for targeting bacterial pathogens without the risks associated with broader-spectrum antimicrobials (Lood et al., 2015).

Genetic Engineering in Enhancing Phage-Derived Antimicrobials

Advances in genetic engineering have enabled the enhancement of phage-derived antimicrobials by modifying phage enzymes or constructing synthetic phages to improve efficacy, host range, and stability. For instance, engineering lysins to enhance binding affinity or modifying phage genomes to target antibiotic-resistant bacteria are promising innovations (Lu & Collins, 2007). Additionally, combining phage elements with antimicrobial peptides through genetic manipulation can yield more effective and customizable phage-derived products suited to the unique needs of aquaculture (Pirnay et al., 2015).

5. Mechanism of Action in Aquatic Environments

Interaction of Phages with Bacterial Biofilms and Planktonic Cells

In aquatic environments, phages can target both planktonic (free-floating) bacterial cells and biofilm-associated bacteria, though biofilms present additional challenges due to their complex structure and protective extracellular matrix. Biofilms protect bacteria from environmental stressors and antimicrobial agents, making infections more persistent and harder to treat. Phages have shown effectiveness in penetrating and disrupting biofilms by producing enzymes that degrade biofilm components, leading to the lysis of bacteria embedded within the matrix (Harper et al., 2014). This ability makes phages particularly valuable for combating biofilm-associated infections in aquaculture, where biofilms often harbor pathogenic bacteria that infect fish and other aquatic organisms (Curtin & Donlan, 2006).

Factors Affecting Phage Activity in Aquaculture: Temperature, Salinity, and pH

Phage activity in aquaculture is influenced by several environmental factors.

- **Temperature** is critical, as each phage strain has an optimal temperature range for infection; deviations can reduce phage efficacy or lead to inactivity (Jończyk et al., 2011).
- **Salinity** also impacts phage viability, as some phages are more stable in saline environments, while others are optimized for freshwater systems (Weinbauer & Rassoulzadegan, 2004).
- **pH levels** affect phage adsorption and stability, with most phages being stable within a pH range of 6–8, typical for many aquaculture environments (Kokjohn et al., 1991). These factors highlight the importance of selecting phages that are well-suited to the specific environmental conditions of an aquaculture operation.

Use of Phage Cocktails and Engineered Phages to Overcome Bacterial Resistance

To combat bacterial resistance, which can develop when bacteria evolve mechanisms to evade specific phages, researchers have developed phage cocktails and genetically engineered phages. Phage cocktails, composed of multiple phage strains targeting various bacterial receptors, reduce the likelihood of resistance by ensuring that any bacterial population contains targets for multiple phages (Chan et al., 2013). Engineered phages offer another approach, as genetic modifications can expand their host range, improve lytic activity, and enhance biofilm-degrading capabilities (Pires et al., 2016). These methods enhance the adaptability and effectiveness of phage therapy, making it a more robust solution for managing bacterial infections in diverse aquaculture settings.

6. Current Research and Case Studies

Case Studies of Phage Application in Aquaculture Settings

Several case studies have demonstrated the practical application of phage therapy in aquaculture, where phages have been used to control bacterial infections effectively. For instance, a study in Thailand showed the successful use of a phage cocktail targeting *Aeromonas hydrophila*, a common pathogen in tilapia farming. The phage treatment led to a significant reduction in bacterial load and mortality rates among infected fish, highlighting phage therapy as a viable alternative to antibiotics in aquaculture (Khaw et al., 2016). Another case study in Chile focused on phage use against *Vibrio anguillarum* in salmon farming, where phage treatment significantly improved survival rates, demonstrating the effectiveness of phages in controlling vibriosis outbreaks (Kim et al., 2020).

Recent Research Findings on Phage-Derived Antimicrobials in Aquaculture

Recent research has advanced our understanding of phage-derived antimicrobials, exploring their mechanisms, environmental impacts, and application strategies. A study by Silva et al. (2021) reviewed the use of phage lysins as an alternative to live phages, showing that lysins could degrade bacterial cell walls with high specificity, effectively controlling pathogens like *Vibrio* and *Pseudomonas* without the risk of releasing live phages into the environment. Another study investigated the combination of phage therapy with probiotics, finding that this combined approach can enhance fish immune responses and reduce infection rates more effectively than either treatment alone (He et al., 2021).

Examples of Successful Phage Therapy Applications for Specific Bacterial Pathogens

Phage therapy has been successfully applied to control various bacterial pathogens across different aquaculture species:

- **Fish Farming**: Phage application has shown success against *Aeromonas salmonicida* in trout farms, leading to reduced mortality and infection rates (Culot et al., 2019). Phages have also been used against *Yersinia ruckeri*, which causes enteric redmouth disease in salmonids, with positive outcomes in both infection control and fish recovery (Nakai & Park, 2002).
- **Shrimp Farming**: Phages have been employed to target *Vibrio harveyi*, a pathogenic bacterium in shrimp farms, significantly reducing mortality rates and improving water quality without harming the shrimp or other beneficial microbial communities (Moriarty, 2014).
- **Shellfish Farming**: In oyster farms, phage therapy has been applied to combat *Vibrio splendidus*, a pathogen responsible for oyster mortalities. Treatment with specific phages led to decreased bacterial load and improved oyster health, underscoring the potential of phages for sustainable shellfish farming (King et al., 2020).

7. Advantages and Sustainability Aspects

Environmental Benefits of Phage-Derived Antimicrobials Over Traditional Antibiotics

One of the primary advantages of phage-derived antimicrobials is their minimal environmental impact compared to traditional antibiotics. Phages are naturally occurring entities that break down after they lyse their target bacteria, leaving no residual environmental contaminants. This biodegradability contrasts with antibiotics, which often persist in aquatic systems, accumulating in sediments and impacting non-target organisms. Phages, therefore, offer a more eco-friendly alternative, helping maintain biodiversity and ecosystem balance in aquaculture environments (Nobrega et al., 2015).

Reduction in AMR Spread in Aquaculture and Natural Water Bodies

Phage therapy can play a crucial role in reducing the spread of antimicrobial resistance (AMR) in aquaculture. Unlike antibiotics, which exert selective pressure that drives the evolution of resistant bacterial strains, phages specifically target pathogenic bacteria with minimal impact on surrounding microbial communities. This specificity reduces the risk of cross-resistance, as phages only affect the intended bacterial hosts, rather than contributing to a broader resistance pool in aquaculture and nearby ecosystems (Goodridge & Abedon, 2003). Studies have demonstrated that the use of phages in aquaculture results in fewer resistance issues and prevents AMR from spreading to natural water bodies through effluents, thus supporting global efforts to combat AMR (Loc-Carrillo & Abedon, 2011).

Potential for Maintaining Aquaculture Productivity Without Harmful Residues

Phage-derived antimicrobials support sustainable aquaculture by maintaining productivity without introducing harmful chemical residues. Antibiotic use can leave residues that enter the food chain, leading to health concerns for consumers. In contrast, phages do not leave chemical traces in fish or shellfish, enabling the production of safer, high-quality seafood (Sulakvelidze et al., 2001). Additionally, because phages specifically target pathogens rather than exerting a broad biocidal effect, they allow aquaculture operations to maintain beneficial microbial communities that contribute to water quality and animal health, further supporting sustainable aquaculture productivity (Mateus et al., 2014).

8. Challenges and Limitations

Challenges in Large-Scale Production and Purification of Phage-Derived Antimicrobials

Producing phage-derived antimicrobials on a large scale poses significant technical challenges. Unlike traditional pharmaceuticals, phage production involves cultivating large quantities of bacterial hosts to propagate phages, which can be labor-intensive and costly. Additionally, the purification of phages to remove endotoxins and other bacterial by-products is a complex process, requiring advanced filtration and chromatography techniques to ensure product safety and efficacy (Clark & March, 2006). This complexity in production and purification has implications for the cost-effectiveness and scalability of phage-based therapies for aquaculture (Gill & Hyman, 2010).

Regulatory and Legal Considerations

The regulatory landscape for phage therapy is still evolving, creating challenges for its widespread adoption. Currently, phage products face diverse regulatory classifications worldwide, with some countries treating them as therapeutic agents requiring clinical approval and others as biological products with less rigorous standards. These inconsistencies can create barriers to market entry and limit international trade, as regulations may differ based on local perceptions of phages as “natural” versus engineered entities (Pirnay et al., 2015). Establishing clear, harmonized guidelines for phage therapy products is essential for their adoption in aquaculture and to ensure compliance with safety and efficacy standards (Verbeken et al., 2012).

Issues with Phage Stability and Storage in Aquatic Settings

Phage stability in aquatic environments is influenced by various factors, including temperature, pH, and salinity, which can affect their viability and effectiveness over time. Maintaining stable phage preparations in the variable conditions of aquaculture settings is challenging, as phages may lose activity when exposed to fluctuating environmental parameters. Additionally, phages are sensitive to UV light and can degrade in direct sunlight, requiring careful storage and application strategies to preserve their efficacy (Jończyk et al., 2011). The need for stable formulations or encapsulation methods to protect phages in diverse aquatic conditions adds further complexity to their use in large-scale aquaculture applications (Vinner et al., 2019).

9. Future Perspectives and Potential Solutions

Advancements in Phage Therapy: CRISPR-Based Phage Engineering and Synthetic Biology

Recent advancements in CRISPR technology and synthetic biology are expanding the possibilities for phage therapy by enabling the precise engineering of phages with enhanced effectiveness and adaptability. CRISPR-based phage engineering allows scientists to edit phage genomes, improving their ability to target antibiotic-resistant bacteria and broadening their host range (Citorik et al., 2014).

Synthetic biology techniques are also being employed to design phages that deliver targeted antibacterial agents or disrupt biofilm formation more effectively, addressing some limitations associated with natural phages (Lu & Collins, 2007). These innovations could significantly enhance the potential of phage therapy in aquaculture, making it a more reliable and versatile alternative to antibiotics.

Potential Integration with Other Sustainable Aquaculture Practices

Phage-derived antimicrobials show promise when combined with other sustainable aquaculture practices, such as probiotics and vaccines. For instance, combining phage therapy with probiotics can help maintain healthy gut microbiota in fish, boosting immune responses and preventing pathogen colonization. This complementary approach has shown potential in reducing the occurrence and severity of infections while promoting overall fish health (Nayak, 2010). Similarly, the use of vaccines alongside phages could provide a layered defense, as vaccines offer long-term immunity while phages address acute infections, together creating a more resilient aquaculture system (Hansen & Olafsen, 1999).

Opportunities for Phage-Derived Antimicrobials in Integrated Pest Management (IPM) Systems for Aquaculture

Integrated pest management (IPM) in aquaculture aims to control disease outbreaks with minimal environmental impact. Phage-derived antimicrobials fit well within IPM frameworks, as they can be used in targeted applications that do not disrupt beneficial microbial communities in the environment (Abedon, 2015). Incorporating phages into IPM strategies allows for the selective control of specific bacterial pathogens, reducing the reliance on chemical disinfectants and antibiotics. Furthermore, as phages can be used in conjunction with natural biological controls, their integration into IPM systems can provide a more sustainable approach to managing disease outbreaks in aquaculture (Defoirdt et al., 2011).

10. Conclusion

Phage-derived antimicrobials hold significant promise as a sustainable solution for combating bacterial infections in aquaculture. By specifically targeting pathogenic bacteria while leaving beneficial microbes intact, phages offer an environmentally friendly alternative to traditional antibiotics, which are associated with harmful residues and antimicrobial resistance (Goodridge & Abedon, 2003). The development of engineered phages and phage cocktails has further enhanced their adaptability and effectiveness, making them a viable component in integrated pest management strategies aimed at maintaining aquatic health without disrupting ecosystem balance (Defoirdt et al., 2011).

Looking ahead, the future of phage therapy in aquaculture appears promising. Advancements in CRISPR-based phage engineering and synthetic biology are expanding the potential of phages to address an even broader range of pathogens, while also improving stability and delivery in aquatic environments (Citorik et al., 2014). However, realizing the full potential of phage therapy requires a concerted effort in research, regulatory development, and financial investment. Establishing clear policies and guidelines will facilitate safe and effective phage applications across diverse aquaculture settings, supporting sustainable production and reducing dependency on antibiotics. Further research and dedicated funding will be essential to optimize phage formulations, improve large-scale production techniques, and demonstrate long-term efficacy in varied aquaculture environments, ultimately paving the way for phage-derived antimicrobials to become a mainstream tool in sustainable aquaculture practices.

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