THE SCIENCE WORLD
A Monthly e Magazine

ISSN:2583-2212
August, 2022; 2(8), 1453-1458

Popular Article

Phage therapy: An alternate biomedicine against bacterial pathogens in aquaculture

Alka Nokhwal[a]*, Ravikant[a], Taruna Anand[b] and R. K. Vaid[b]
[a]Department of Zoology and Aquaculture, COBS&H, CCS Haryana Agricultural University, Hisar 125004, India
[b]National Centre for Veterinary Type Cultures, ICAR-National Research Centre on Equines, Sirsa Road, Hisar 125001, India
https://doi.org/10.5281/zenodo.7014724

Abstract

Aquaculture endows the rising tide of antimicrobial resistance among bacterial pathogens along with increasing incidences of bacterial disease outbreaks which calls for the urgent requirement of alternative anti-bacterial agents than antibiotics. One such promising treatment is the use of phage therapy in aquatic farming, which is re-gaining huge scientific attention since the past few decades and great bactericidal potential is reported by extensive *in vitro* and *in vivo* studies. Phage therapy holds potential as a relatively inexpensive and low environmental impact biocontrol approach. Therefore, we aim to document the important aspects of phage therapy including the advantages as well as possible drawbacks associated with their use. A logical selection of phages based on the outlined characteristics is necessary to meet requirements of a successful treatment and global acceptance.

Keywords: Bacteriophage, phage therapy, antimicrobial resistance, fish diseases, aquaculture

Introduction

The cultivation of different species of fish, shrimps, and crustaceans has made the aquaculture industry a major contributor to the economy and an important food source worldwide. Of the global production of aquatic animals i.e., 178 million tonnes (MT) in 2020, 49% was harvested in aquaculture, which further retrieved 61.8% from inland waters and 37.6% from marine waters (FAO, 2020). Total aquaculture production of 88 MT in 2020 was up from 85.2 MT in 2019 and 82.5 MT in 2018. The total first sale value of the global production was estimated at USD 265 billion for aquaculture (FAO, 2022). In India, the fish production has increased from 8.67 MMT in FY 2011-12 to 14.73 MMT in 2021-22 (GOI, 2021-22). However, along with great development in this industry, from disease management practices to quality production methods, there has been a surge in the infectious diseases accompanied by the emergence and re-emergence of multidrug-resistant MDR bacterial pathogens, which limits the further intensification and causes severe economic loss. According to estimates, 34% of infections are caused by bacteria (Lafferty et al., 2019).

Table: Nota

Bacterial pathogen	Disease
Aeromonas spp.	Furunculosis and motile aeromonas septicaemia
Edwardsiella spp.,	*Edwardsiella* septicaemia
Flavobacterium spp.,	Columnaris, rainbow trout fry syndrome, and bacterial cold-water disease
Lactococcus spp.,	Lactococcosis, hyperacute, and haemorrhagic septicaemia
Pseudomonas spp.,	Red skin disease
Streptococcus spp.,	Haemorrhagic septicaemia
Vibrio spp.	Luminous vibriosis

These bacterial causative agents may also be responsible for human diseases transmitted from fish used as food or through the handling of culture systems. The major disease protection mechanism of aquaculture farming i.e., administration of antibiotics as prophylactic (as well as growth promoters) and therapeutic agents, due to their extensive use, has resulted in the selection, prevalence, and spread of antibiotic-resistant bacteria. The selection pressure is further intensified by a disturbed pathogen-host-environment, large-scale production facilities, and high-density cultivation conditions. Considering growing global concerns about the marked antibiotic resistance genes, antibiotic-resistant bacteria, and demand for consumable products free of chemical residues, also, regarding the “one health concept” of Govt. of India, there is an urgent need to look for more natural and environment-friendly approaches for prevention and control of fish bacterial diseases in aquaculture.

1. Bacteriophages: the nano-medicines

Among several biocontrol strategies, one noteworthy biomedicine is the use of bacteriophages (phages), which are the natural viral predators of bacteria and obligate intracellular parasites (Abedon, 2009). Bacteriophages are the most abundant candidates in the ecosystem, widespread in every possible habitat ranging from the depth of oceans to the soil, the water we drink, and the food we eat, with total numbers estimated to be more than 10^{30}. The concept of phage therapy was introduced in the early 1900s, but the studies were largely discontinued due to a surge in active interest in antibiotics but now the research concerns in phage therapy have regrown owing to several advantages over antibiotics and other antibacterial strategies.

Bacteriophages are highly host specific through ligand-receptor interactions which limits their spectrum of activity and prevents unwanted damage to the normal healthy microflora of the host. They multiply only if a specific host is available, otherwise are degraded in the environment itself. Phages deploy two mechanisms for their replication- lytic cycle (virulent phage) or lysogenic cycle (temperate phage). While virulent phages infect, multiply and cause "lysis" of the host bacterial cells, temperate phages do not immediately kill the cell and establish a permanent infection in the host cell by incorporating part of their genome into that of the host cell and multiplying with the process of host cell division. The lytic action releases them free to invade other susceptible bacteria in the surroundings i.e., single dose potential, which requires no booster dosage and is an auto replicative mode of action, unlike vaccines. To date, bacteriophages have been employed as novel therapeutic and biosensing tools and find various applications in biotechnology and medical science such as quick bacterial detection and disease diagnosis (phage typing), disease prophylaxis (phage vaccine), treatment (phage therapy), and biocontrol agents. Whole phage virions, as well as phage derived products such as endolysins can be explored as potent bactericidal agents. In aquaculture, phages are well known for long to selectively inhibit fish pathogens and reduce fish mortality.

Advantages of phage therapy

- No/minimal disruption of the normal host microflora
- Targets AMR strains and able to eradicate biofilms
- Stimulation of host immune response
- Auto-dosing, able to multiply in *in vivo* conditions
- Abundantly present in the environment, easy detection protocols and inexpensive isolation
- No known inherent toxicity to humans, plants, animals and environment
- Lacks cross-reactivity with antimicrobial agents

Challenges for phage therapy

- Narrow host range; poorly applicable to treat systemic diseases
- Phage resistance development; horizontal gene transfer; difficulty of lifecycle determination
- Production of exotoxins by some phages
- Clearance of phages from the organism by the immune system
- Requirement of an effective phage formulation for targeted phage delivery

- Possible sensitivity to various physico-chemical parameters resulting in loss of phage viability
- Lack of standardized regulatory guidelines for phage licensing and approval for use

2. Phage-preparation formulations and mode of phage administration

Essentially, one should take into account variables like the titre of bacteriophages, timing of phage administration, availability of a bacterial host, their stability, uniqueness of biological systems, and phage kinetics among others, when planning phage therapy or phage-based prophylaxis in aquaculture.

Phage delivery methods in aquaculture include injection, bath immersion, feed formulation, and topically applied phages with varying levels of success (Gon Choudhury *et al*., 2017). The type of disease and the stage of infection progression have a significant impact on the process. Additionally, an appropriate selection of phages includes aspects such as their strictly lytic nature, toxin free metabolism, capacity to survive in the stomach or cross the epithelial barrier. Low pH levels in the fish stomach environment, where the pH ranges from 2 to 7, may greatly reduce the action of phages during therapy.

3. Phage sensitivity to environmental variables

Although it is not difficult to discover new bacteriophages in the environment, their stability poses a considerable obstacle to their use in commercial products. The solution's composition (the presence or absence of specific ions), production process variables (temperature, pressure), or the environment's pH can all have an impact on the activity or native structure or adequate stability of bacteriophages. This could, for instance, change how the phage tail is assembled and affect how well it can connect to host receptors, or it could even completely destroy the bacteriophage structure. The salt concentration is another element crucial for phage stability in aquaculture water. Some bacteriophages are said to require low salt concentrations for the infection process since high amounts may cause osmotic shock, which would render the bacteriophages inactive. Using best practices makes it easy to extract and multiply phages, and sequencing in combination with cutting-edge bioinformatics techniques makes it quick to choose viruses that may be able to combat bacterial diseases.

4. Phage resistance mechanisms and other safety elements

The appearance of bacterial mutants that are insensitive to bacteriophages, on the other hand, is one of the challenges faced by phage therapy. Nevertheless, phage-resistant bacteria are not resistant to other phages with a comparable target range, and the likelihood for bacteria to develop resistance is approximately ten times slower than in the case of antibiotics. Because establishing resistance to more than one phage would result in a higher fitness cost for the bacteria, the use of bacteriophage cocktails or mixtures of bacteriophages with other antimicrobials appears to be a successful method to prevent bacterial resistance. The possibility of horizontal gene transfer during the processes of conjugation, transformation, or transduction (general/specialized) should be taken into account as another crucial safety factor while designing phage therapy.

5. Host pharmacokinetics, immune response and removal from the body

It is well known that phages cause the body's immune system to respond by triggering both specific and general immunological reactions resulting in fast clearance from the body organs. The effectors include most importantly the phagocytes, lymphocytes and antibodies among others which checks the virus from targeting the bacteria. However, it is also important to note that because lower vertebrates are thought to be resistant to endotoxic shock, no negative side effects have been reported to date when the phage therapy has been used in aquaculture. Studying each case and selecting the method of delivery, dose, buffers, and period of exposure to phages with care are some potential answers to the immune response problem. To keep the phages unaltered in the system they could be microencapsulated, protective agents could be used, or the right buffers might be used, among other options. Another concept involves searching for phage mutants (using genetic or chemical techniques), with reduced immunogenicity of surface proteins to make phages evade the host immune system and use of phage cocktails to enable phage survival even in presence to antibody-mediated neutralization.

6. Regulatory approvals and future perspectives

Overall, bacteriophages appear to be a good option in a future pool of antimicrobials due to unique properties. The current regulatory system is the impediment, which necessitates a new analysis for each case and largely restricts the widespread use of phage therapy. Phage cocktail registration is highly challenging because, in the majority of nations, phages could only be registered individually. Few recognized phage preparations exist currently for aquaculture such as

BAFADOR® (against *Aeromonas hydrophila* and *Pseudomonas fluorescens*). The biggest barrier to the widespread acceptance of phage cocktails is still their safety, thus additional research is required in this area, particularly the issues of gene transfer and the environmental impact of phages which may lead to unintended consequences. A better understanding of the fish immune system, phage-host interactions, collaboration with high-throughput technologies, and comprehensive food safety regulations are required altogether to design an adequate phage therapy strategy for application in aquatic food production systems.

References

1. Abedon, S. T. (2009). Phage evolution and ecology. *Advances in applied microbiology*, *67*, 1-45. https://doiorg/101016/S0065-2164(08)01001-0
2. FAO (2020) The State of World Fisheries and Aquaculture Sustainability in action. FAO Fisheries and Aquaculture Department, Rome. https://doiorg/104060/ca9229en
3. Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, *Annual Report*. Government of India, 2021-22.
4. Gon Choudhury, T., Tharabenahalli Nagaraju, V., Gita, S. et al. (2017). Advances in bacteriophage research for bacterial disease control in aquaculture. *Reviews in fisheries science and aquaculture*. *25*(2), 113-125. https://doiorg/101080/2330824920161241977
5. Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., et al. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual review of marine science*, *7*(1), 471-496. https://doi.org/10.1016/S0065-2164(08)01001-0