Bacteriophage Therapy in Aquaculture: An Overview

20

Md. Idrish Raja Khan and Tanmoy Gon Choudhury

Abstract

In the present scenario, the development of drug-resistant bacteria poses a global threat to all living kinds including aquatic animals. The phenomenon calls for prompt action, through development and timely adoption of alternative strategies in order to sustain the quality as well as to ensure safety of the aquatic produce. In view of antimicrobial resistance especially antibiotic abuse, efforts made towards the advancement of the biological control approaches such as probiotic, symbiotic, and bacteriophage have been accelerated. In recent times, the employment of the biocontrol approach through the applications of lytic bacteriophages for therapy of bacterial infection have leaped over other bioagents. Bacteriophages are bacteria-specific viruses that precisely infect host bacteria and ultimately kill them. Ever since their discovery in the early nineteenth century, the phage therapy enjoyed fleeting popularity in western countries owing to exploratory researches and scientific explanation with regard to their successful clinical trials. In the post antibiotic discovery era, the significance of the phage was ignored. However, after the emergence of antimicrobial resistance, a new craze for therapy was appeared either as prophylactic or therapeutic approach including the aquaculture industry. Most of the therapy in aquaculture is still in the laboratory stage, and is limited to in vitro characterisation and lab-based efficacy which have emerged as the major obstacle in its adoption at the farm level. In this chapter, an effort has been made to draw a connecting line between the current state of information about bacteriophages and what could be the possible strategies for the development of field-based therapy towards the sustenance of aquaculture.

M. I. R. Khan · T. G. Choudhury (✉)
Department of Aquatic Health and Environment, College of Fisheries, Central Agricultural University (Imphal), Lembucherra, Tripura, India

P. K. Pandey, J. Parhi (eds.), *Advances in Fisheries Biotechnology*,
https://doi.org/10.1007/978-981-16-3215-0_20

Keywords

Antimicrobial resistance · Phage therapy · Biocontrol · Prophylactic or therapeutic

20.1 Introduction

Over the past few decades, the aquaculture sector has served the nutritional needs of the people throughout the globe. The contribution from Asian subcontinent was maximum, i.e. 89% of total volume and 79% of the total value of fish production globally (Bostock et al. 2010). However, there are several factors which continue to play a crucial role in limiting the aquaculture production such as infectious diseases, especially those of bacterial origin. As per an assessment of Lafferty et al. (2014), the bacterial infection alone accounts for about 34% of total outbreaks encountered in the aquaculture system. Additionally, the indiscriminate use of chemotherapeutics to mitigate the disease problem has caused the rise in antimicrobial resistance (AMR) strain and the situation can exaggerate by the emergence of superbugs. According to Van Boeckel et al. (2019), the application of chemical therapeutics, especially antibiotics, for rearing of the farm animals including aquatic animals, accounts for about 73% of all antibiotic usage throughout the globe. In the recent past, various chemical agents have been used either as a prophylactic treatment or as growth enhancers. This would have paved the way that, due to the emergence of drug-resistant aetiological agents, the pathological condition that was resolved easily earlier is becoming a major setback to aquaculture production (Gelband et al. 2015). Consequently, researchers all over the world have been engaged with the development of alternative treatment approaches. In light of the investigation for substitute, the biocontrol strategy via bacteriophages could be considered as a sustainable option. The phage therapy, however, is an aged approach but the latest developments in the identification of potential isolates and their multidimensional application strategies have also fuelled the investigations towards the use of bacteriophages as a biological tool for health management in aquaculture.

20.2 Brief About Bacteriophages

Bacteriophages are the viruses which are obligate intracellular parasites of bacteria; they ultimately kill or lyse the host cell and release new progenies (Al-Sum and Al-Dhabi 2014). Bacteriophages are informally called phages, which is derived from a Greek word "phagein" meaning "to devour". They utilize the bio-machinery of the bacterial host for all kinds of metabolic support in order to survive (Al-Sum and Al-Dhabi 2014). As the natural environment is replete with loads of bacterial host, the occurrence of phages is natural and can flourish in soil up to $10^{7–8}$ virions g^{-1} and in water approximately 10^7 virions mL^{-1} either in fresh or saline environment (Ninawe et al. 2020; Park et al. 2020). According to Abedon et al. (2011), the total count of bacteriophages on the earth is about 10 times the total bacterial host thriving

in different environments, which accounts for about $10^{30–31}$. The International Committee on the Taxonomy of Viruses (ICTV) is responsible for the typing of phages and they have classified bacteriophages into 19 families, among which a few are well characterized including *Microviridae*, *Myoviridae, Inoviridae, Podoviridae* and *Siphoviridae* (Simmonds et al. 2017; Adriaenssens et al. 2018; Walker et al. 2019). The vast abundance and diversity of phages in the biosphere provides an already equipped resource to mine for the potential phages for a variety of purposes (Nikolich and Filippov 2020). Employment of precise killing capability of phages to control lethal bacterial pathogens is called as phage therapy or phagotherapy. The putative phages are composed of proteinous outer shell/capsid measuring about 24 to 200 nm in size, which contains proteins and nucleic acids (either DNA or RNA) ranging 17 and 700 kb in length (Ackermann 2003; Sharma et al. 2017). The majority of phages possess a tail (variable in size) in their structure with tail fibres on it which helps in the precise identification and adherence to the bacterial host (Kowalska et al. 2020).

The life cycle of bacteriophages can be categorized into two stages, first is lytic (virulent) and second, temperate. In the first lytic cycle, the phages adhere themselves to bacterial host followed by taking control of the host's bio-molecular machinery to proliferate and ultimately kill the host bacteria, concurrently releasing its progeny phages. The lytic phages are responsible for the production of two specific proteins to kill the host, "holins and endo-lysins". The protein, holins work in synergy with the endo-lysins and are responsible for the perforation on the bacterial cell followed by the destruction of cell wall after phage multiplication (Cisek et al. 2017). In the second temperate lysogenic stage, after the infection of bacterial host the phage genome shifts to dormant stage "prophage" which can exist within the host in the form of a plasmid and can last for many generations and can make its genes (including virulent genes) functional for the host bacterium. However, any sudden exposure or any triggering factor such as DNA damage, UV exposure and antibiotic treatment might lead the conversion of lysogenic phage to lytic stage (Letchumanan et al. 2016; Kowalska et al. 2020). Temperate phages are favourable to bacteria because they might encode for antibiotic resistance gene or some other potent genes; additionally, these lethal genes can be horizontally transferred to another bacterium in the residing environment (Lin et al. 2017). On the contrary, virulent lytic phages kill the bacterial cells directly where the possibility of any genes transfer is limited, which make lytic phages a desirable candidate for therapeutic bacteriophage therapy (Jassim and Limoges 2014; Letchumanan et al. 2016). However, according to the report of Freifelder (1987), the prevalence of lysogenic phage compared to lytic phages is as more as 90% in nature, which makes phage isolation a crucial state in development of phage therapy. There are few literature who vote for another third phage variant, a carrier state of the lysogenic stage termed as pseudolysogenic cycle, where the phage genetic material does not replicate but instead remains inactivated within the host till the occurrence of favourable condition (such as nutrient availability which hinders the bacteriophage gene expression). Once the favourable situation prevails, carrier state might be

initiated with either the lytic cycle or the commencement of true lysogeny (Sieiro et al. 2020).

20.3 History of Bacteriophage Researches

Ernst Hankin in 1896 was the first one to demonstrate the presence of certain unidentified antimicrobial compounds against *Vibrio cholera* which are heat labile, filterable and transmissible, from the waters sample of the Ganges river system of India (Hankin 1896); however, he was not able to come to a conclusion regarding the reason behind anti-bacterial activity (Twort 1915; D'Hérelle 1917; Summers 2005). Later, in 1915, Frederick Twort, a British pathologist, was the first to demonstrate the presence of an "ultra-microscopic virus" that could affect bacteria; however, he also failed to explain the phenomenon, including the existence of virus (Summers 2005). Two years later in the year 1917, a French-Canadian microbiologist Felix d'Herelle observed a similar clear zone phenomenon in stool samples of bacillary dysentery patients. Unlike Twort, this time, d'Herelle was able to explain the presence of "invisible microbe", a virus which he termed as "Bacteriophage" (Brunoghe and Maisin 1921). Later, during the 1920s, various clinical trials on phagotherapy were carried out in Eastern Europe and the Soviet Union, where therapy was used for the treatment of variety of diseases including bubonic plague and cholera in India (Nikolich and Filippov 2020). Despite encouraging initial success of the page therapy, their application as antimicrobial approach was declined because of the discovery of antibiotics in the mid-nineteenth century.

20.4 Bacterial Diseases in Aquaculture and Its Control Measures

Despite the fact that aquaculture is one of the fastest rising food-production sectors in the world, it is currently plagued by frequent and severe outbreaks of diseases. The sector is under threat from several groups of pathogen such as bacteria, fungi, viruses, and parasites. Among all these concerns, the bacterial pathogens can endure well in both fresh water and marine water aquatic ecosystem without their host; and the attribute favours them as major impediments to the aquaculture industry. The situation is further exaggerated by the adopted intensive culture practices and human anthropogenic activities which has led the foundation for the adulteration in the optimal physico-chemical quality of the aquatic environment (Pridgeon and Klesius 2012). Till now, about 13 bacterial genera have been identified as pathogenic to aquatic organisms including fish, which comprises both gram-negative pathogens (*Edwardsiella*, *Aeromonas*, *Vibrio*, *Flavobacterium*, *Pseudomonas*, *Yersinia*, *Francisella*, *Piscirickettsia*, *Photobacterium* and *Tenacibaculum*) and gram-positive (*Renibacterium*, *Lactococcus* and *Streptococcus*) (Pridgeon and Klesius 2012; Gui and Zhang 2018).

To control bacterial disease outbreak in an aquatic system, feeding fishes with drug-medicated feed, especially antibiotics, is a general practice. At present, the

addition of various kinds of nutraceuticals or functional food is very well accepted to remediate the situation either as a prophylactic or therapeutic agent (Pridgeon and Klesius 2012). However, the approach is usually expensive and maybe ineffective for therapeutic purposes as infection-weaken fish do not accept any kind of feed especially medicated feed. Additionally, frequent and sub-therapeutic level of chemical additives or drugs over an extended period led the base for the development of AMR among pathogens (Cunha 2009). Substitutes for antimicrobial agents with similar or enhanced protection are therefore urgently needed to provide robust protection against variety of bacterial aetiological agents in target organisms. At present, the application of various kinds of vaccines, immunostimulant of natural or chemical origin is very well accepted in commercial aquaculture farms, along with several biocontrol strategies such as application of probiotic, bacteriophages and symbiotic. Among these alternative strategies, phagotherapy emerges as a sustainable substitute to chemical therapeutics, since phage application has the potential to not only eliminate the virulent pathogens precisely but can also to help in the creation of homeostasis in aquatic environment by minimizing the application of chemicals and other remedial drugs to achieve the goals of "One Health" approach of WHO.

20.5 Research on Bacteriophage Therapy in Aquaculture

Although bacteriophages were discovered way back at the beginning of the nineteenth century, however, the focus of research on its therapeutic potential against bacterial diseases was limited to a certain part of the world because of the poor understanding of phage life cycle and bacteria-phage interactions (Almeida et al. 2009). Furthermore, with the discovery of antibiotics, the application of phages remains underexplored. However, in some places such as Eastern Europe and in the Soviet Union, they successfully demonstrated several clinical trials on human patients which laid the foundation to the future work (Park et al. 2020). Moreover, the emergence of multi-drug resistant bacteria has substantially encouraged researchers to explore the potential of phagetherapy; because, phages can be employed as bioagents against wide range of bacterial pathogens. Owing to the specificity of phages to their host, the probability of disrupting natural microflora of aquatic environment or host inhabiting beneficial bacteria will be null which is very unlikely with the administration of common broad-spectrum antibiotics (Fortuna et al. 2008). The very first attempt to employ phage therapy in aquaculture was made in the year 1981 in Taiwan against *Aeromonas hydrophila* in loach (*Misgurnus anguillicaudatus*) (Wu et al. 1981). Nowadays, work associated with the phagotherapy against bacterial pathogens in aquaculture has been accepted worldwide and encouraging researchers to explore the application and efficacy of phage therapy in different circumstances under various culture conditions (Table 20.1).

Table 20.1 Isolation and application of bacteriophage in aquaculture

Pathogen	Disease (lesion)	Organism	Bacteriophage	Phage administration	Treatment	Reference
Bacteriophage application in finfish						
Aeromonas salmonicida	Furunculosis	Brook trout (*Salvelinus fontinalis*)	HER 110	Immersion	The treatment at MOI 100 not only delayed the onset of infection by 7 days; additionally, bacteriophage reduced the total mortality from 100% to 10%	Imbeault et al. (2006)
		Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*)	O, R and B	Intraperitoneal injection, oral feeding and immersion	No adverse effect was observed. However, using a combination of all three phages by injection only delayed the death, but didn't affect the result as none of the treatments was able to provide protection against infection	Verner-Jeffreys et al. (2007)
		Rainbow trout (*Oncorhynchus mykiss*)	PAS-1	Intramuscular injection	Fish treated with MOI of 10,000 showed a significant survival rate of 26.7%. The surviving fish did not show ulcerative lesions and remained healthy until 14 days post administration	Kim et al. (2015)

		Senegalese sole (*Solea senegalensis*)	AS-A	Immersion	After 72 h of infection, fish juveniles treated with phages at MOI of 100 showed no mortality contrary to 36% mortality in the untreated control group	Silva et al. (2016)
Edwardsiella ictaluri	Edwardsiellosis or enteric septicaemia	Japanese eel (*Anguilla Japonica*)	Phages ET-1	–	Phages were very effective with lysing capacity of 92.6% against 27 bacterial hosts. Additionally, at MOI 0.08 phages were able to reduce down the bacterial count by 99.9% in water	Wu (1982)
		Channel catfish (*Ictalurus punctatus*)	ΦeiDWF, ΦeiAU and ΦeiMSLS (*Siphoviridae*)	–	The in vitro analysis reveals the lysing capacity of phages, which can be used for therapeutic application	Carrias et al. (2011)
		Ayu (*Plecoglossus altivelis*)	–	Intraperitoneal injection	Higher protection was observed in fish that were first injected with phages and then 1 h later injected with the pathogen, whereas the fish that was first injected with the pathogen and then the phages only showed	Mahmoud and Nakai (2012)

(continued)

Table 20.1 (continued)

Pathogen	Disease (lesion)	Organism	Bacteriophage	Phage administration	Treatment	Reference
					delayed mortality compared with the control	
E. tarda	Edwardsiellosis or Edwardsiella septicaemia	Zebrafish (*Danio rerio*)	ETP-1 (*Podoviridae*)	Immersion	The fish were bath exposed to phages for 12 days and concurrently infected with *E. tarda*, the result revealed the elevated survival in treatment in comparison to control until 4 days post challenge	Nikapitiya et al. (2020)
E. tarda and *A. hydrophila*	Hemorrhagic septicaemia and Edwardsiellosis	Japanese eel (*A. japonica*)	Different bacteriophages combination	Immersion	At MOI of 11.5 the bacterial count was reduced 3 times within 2 h of exposure. Whereas in pond water, 250-folds reduction at MOI of 0.23 in 8 h. Additionally, the count of *E. tarda* was dropped by 85% even in the absence of phage in the pond water after 48 h of exposure	Hsu et al. (2000)
A. hydrophila	Haemorrhagic septicaemia or		pAh1-C and pAh6-C		Both of the intraperitoneal and oral	Jun et al. (2013)

	Motile Aeromonas Septicaemia (MAS)	Cyprinid loach (*Misgurnus anguillicaudatus*)		Intraperitoneal injection and oral feeding	administration improved the survival	
		Striped catfish (*Pangasianodon hypophthalmus*)	*A. hydrophila* Φ2 and *A. hydrophila* Φ-5	Intraperitoneal injection	The survival rate of catfish at MOI 100 was 100%, compared to the 18.3% survival in the control devoid of phage treatment	Le et al. (2018)
		Loach (*Misgurnus anguillicaudatus*)	Akh-2 (*Siphoviridae*)	Immersion	Mortality rates were 16%, 53%, 57% and 56.67% after 24, 48, 72 and 96 h, respectively when compared to the control group with 100% mortality; most of the surviving fish showed no disease symptoms	Akmal et al. (2020)
A. hydrophila and *Pseudomonas fluorescens*		Rainbow trout (*Oncorhynchus mykiss*)	–	Bacteriophage cocktail BAFADOR®, containing 3 bacteriophages against *A. hydrophila* and 4 against *P. fluorescens* was used for immersion or feeding of fish	Stimulation of non-specific immune system and reduction of mortality	Schulz et al. (2019a)

(continued)

Table 20.1 (continued)

Pathogen	Disease (lesion)	Organism	Bacteriophage	Phage administration	Treatment	Reference
		European eels (*Anguilla anguilla*)	–	Fish were fed with bacteriophage cocktail BAFADOR® containing 3 bacteriophages against *A. hydrophila* and 4 against *P. fluorescens*	Stimulation of cellular and humoral immunity and reduction in mortality	Schulz et al. (2019b)
Flavobacterium columnare	Columnaris disease	Catfish (*Clarias batrachus*)	FCP1–FCP9 FCP1 (Podoviridae)	Intramuscular injection, bath and oral feeding	Phage treatment led to the disappearance of gross clinical signs, negative bacteriological test, detectable phage and 100% survival	Prasad et al. (2011)
		Rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*)	FCL-2	–	Reduced mortality	Laanto et al. (2015)
F. psychrophilum	Systemic bacterial coldwater disease (CWD)	Rainbow trout (*Oncorhynchus mykiss*) and other species of trouts	FpV-1 to FpV-22	–	Significant lytic capacity against with broad host range	Stenholm et al. (2008)
		Ayu fish (*Plecoglossus altivelis*)	PFpW-3, PFpC-Y (*Myoviridae*) PFpW-6, PFpW-7 (*Podoviridae*) PFpW-8 (*Siphoviridae*)	–	PFpW-3 displayed significant lytic capacity	Kim et al. (2010)

		Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*)	–	Intraperitoneal injection	Mortality decreased in the range of 16% to 100%	Castillo et al. (2012)
Lactococcus garvieae	Lactococcosis	Yellowtail (*Seriola quinqueradiata*)	PlgY, PLgY-16, PLgY-30, PLgW-1 (*Siphoviridae*)	Intraperitoneal injection and oral feeding	Both administered phage prevented fish from experimental *L. garvieae* infection. Mortality drops from 90% to 45% (for injection), whereas for oral mortality drop from 65% to 10%.	Nakai et al. (1999)
P. aeruginosa	Ulcerative lesions	Catfish (*Clarias gariepinus*)	–	On-spot treatment	The therapy efficiently cured the infected fish within 8 to 10 days with a sevenfold reduction of the lesion with untreated infection control	Khairnar et al. (2013)
P. plecoglossicida	Bacterial haemorrhagic ascites disease	Ayu (*Plecoglossus altivelis*)	PPpW-3 (*Podoviridae*) PPpW-4 (*Myoviridae*) and a combination of both PPpW-3/ PPpW-4	Oral feeding	At MOI 1, mortality drop from 65% to 22%	Park et al. (2000)
		Ayu (*Plecoglossus altivelis*)	PPpW-3, PPpW-4	Oral	Phage-receiving fish showed high protection against infection and	Park and Nakai (2003)

(continued)

Table 20.1 (continued)

Pathogen	Disease (lesion)	Organism	Bacteriophage	Phage administration	Treatment	Reference
					mortality drop from 90 to 26%	
Streptococcus iniae	Streptococcosis	Japanese flounder (*Paralichthys olivaceus*)	PSiJ31, PSiJ32, PSiJ4, and PSiJ42	Intraperitoneal injection	Mortalities of fish receiving phages were significantly lower than the control, ranging from 80% to 0%	Matsuoka et al. (2007)
Streptococcus agalactiae	–	Nile tilapia (*Oreochromis niloticus*)	–	Immersion	Treated fish had survival rates of 60% with a delayed mean death time of about 3 days in comparison to control	Jun et al. (2017)
Vibrio anguillarum	Vibriosis	Atlantic salmon (*S. salar*)	ALMED, CHOED, ALME, CHOD, CHOB	Immersion	At MOI of 1 and 20, the treatment increased the survival of fish up to 100%. Mortality drop from 95 to 30% at MOI 1 and at MOI 20 from 95% to 0%	Higuera et al. (2013)
		Atlantic cod (*G. morhua*) and turbot (*Scophthalmus maximus*) larvae	KVP40	Immersion	The maximum reduction in mortality varied from 29% to 92% for turbot and from 49% to 86%; notably, reduction in mortality	Rørbo et al. (2018)

					was not significant in the majority of cases	
Bacteriophage application in shellfish						
V. alginolyticus	Skin ulceration and viscera ejection	Sea cucumber (*Apostichopus japonicus*)	–	Immersion	Increased survival in a range of 73, 50 and 47% at MOI of 10, 1 and 0.1, respectively, whereas the no phage treatment group only had 3% of survival rate	Zhang et al. (2015)
		Live prey (*Artemia salina*)	jSt2 and jGrn1	Immersion	At MOI 100, 93% reduction of presumptive *Vibrio* concentration after 4 h of treatment	Kalatzis et al. (2016)
V. harveyi	Luminous vibriosis	Larvae of *Penaeus monodon*	VHLM (*Myoviridae*)	Immersion	The laboratory trial showed that survival was enhanced up to 80% with two doses of bacteriophage, whereas survival rate in control was only 25%	Vinod et al. (2006)
		Larvae of *P. monodon*	Viha8, Viha10 (*Siphoviridae*) Viha9, Viha11	Immersion	Mortality drops from 88% to 32% compared to antibiotic treatment	Karunasagar et al. (2005, 2007)
		Penaeid shrimp	Viha1 to Viha7 (six from *Siphoviridae* and one Viha4 from *Myoviridae*)	–	All the phages were found to be highly lytic with different lytic spectrum. Three of the phages (Viha1, Viha3 and Viha7) caused 65%	Shivu et al. (2007)

(continued)

Table 20.1 (continued)

Pathogen	Disease (lesion)	Organism	Bacteriophage	Phage administration	Treatment	Reference
					of the strains to lyse while Viha2, Viha4 and Viha6 caused 40% of the host strains to lyse. Viha5 had a narrow spectrum (14%)	
		Tropical rock lobster (*Panulirus ornatus*)	VhCCS-06 (*Siphoviridae*)	–	Phages were able to eliminate the host bacterial count up to 1.2×10^7 CFU mL^{-1} compared to control 9.3×10^7 CFU mL^{-1}	Stomps et al. (2010)
		Shrimp larvae (*P. monodon*)	Bacteriophages VHM1, VHM2 and VHS1	Immersion	The phages were applied alone and in different cocktail combinations. Larval survival was in a range of 60%–88.3% after 96 h in the phage treatment group, compared to 26.6% to 35% survival in the control treatments without phage	Stalin and Srinivasan (2017)
		Abalone (*Haliotis laevigata*)	vB_VhaS-a, vB_VhaS (Siphoviridae)	Immersion	The treatment was revealed survival of about 70%	Wang et al. (2017)
			VHP6b	Immersion	After 10 days, mortality in the treated group was	Patil et al. (2014)

		Black tiger shrimp (*P. monodon*)			20% when compared to >70% of control treatment	
		Black tiger shrimp (*P. monodon*)	Phage V	Immersion	Optimum activity of *V. harveyi* phage was observed at salinity of 25 ppt, pH of 7, TDS of 11.25 mg mL^{-1} and temperature of 30 °C. Combination of recombinant shrimp lysozyme and *V. harveyi* phage significantly improved the phage activity	Choudhury et al. (2012, 2019)
V. parahaemolyticus	Vibriosis	Brine shrimp (*Artemia franciscana*)	–	–	Single dose was efficient enough to eliminate the pathogens. However, when the phage treatment was delayed, it was ineffective to control the mortality	Martinez-Diaz and Hipólito-Morales (2013)
		Whiteleg shrimp (*Litopenaeus vannamei*) larvae	A3S and Vpms1	Immersion	At MOI of 0.1, the infection was counteracted and an early application (at 6 h post-infection) was	Lomelí-Ortega and Martínez-Díaz (2014).

(continued)

Table 20.1 (continued)

Pathogen	Disease (lesion)	Organism	Bacteriophage	Phage administration	Treatment	Reference
					effective to avoid mortality	
		Shrimp (*Penaeus vannamei*)	–	Oral diet and immersion	Mortality in groups treated 1 h after bacterial infection was 100%, whereas prophylactic use of phages resulted in mortality varied from 25% to 50%	Luo et al. (2018)
		Blue mussels (*Mytilus edulis*)	–	Immersion	Phage cocktail was effective in significantly reducing *V. parahaemolyticus* to undetectable numbers in mussels	Onarinde and Dixon (2018)
		Oysters	*Siphoviridae* pVp-1	Immersion and surface application	After 72 h of phage application with bath immersion, bacterial growth was reduced up to 1.4×10 CFU mL^{-1} in the treatment group as compared to control (8.9×10^6 CFU mL^{-1}). Whereas, after 12 h of phage surface application, the bacterial growth was	Jun et al. (2014)

					inhibited by 1.94 CFU mL^{-1} of the treatment group to 1.44×10^6 CFU mL^{-1} in the control group	
Vibrio sp. VA-F3		Shrimp (*L. vannamei*)	ValLY-3, VspDsh-1, VspSw-1, VpaJT-1 and ValSw4–1 (*Siphoviridae*)	–	Survival rate assessed after 7 days of cultivation reached 91.4% when compared to 20% rate in the untreated control group	Chen et al. (2019)
V. splendidus	Severe epizootics Skin Ulceration Syndrome (SUS)	Sea cucumber (*Apostichopus japonicus*)	vB_VspS_VS-ABTNL-1 (PVS-1), vB_VspS_VS-ABTNL-2 (PVS-2) and vB_VspS_VS-ABTNL-3 (PVS-3)	Oral feeding	Survival rate during the next 10 days was 18% for the control group, whereas 82% for the phage cocktail, and 65%, 58% and 50% for the three phages applied alone	Li et al. (2016a, b)
V. cyclitrophicus	–	Sea cucumbers (*A. japonicus*)	vB_VcyS_Vc1	Oral feeding	Reduced mortality	Li et al. (2016a, b)
V. coralliilyticus	Massive mortality of Pacific oyster larvae	Pacific oyster larvae (*Crassostrea gigas*)	pVco-14 (*Siphoviridae*)	–	Significantly higher survival rate in treatments compared to the untreated control	Kim et al. (2019)

20.6 Phage-Based Products for Therapy in Aquaculture

The potential and efficacy of phages have encouraged some private companies/institutes to develop phage-based product for commercial application to treat bacterial diseases in aquaculture which is tabulated below (Table 20.2).

20.7 Strategic Guideline for the Development of Phage Therapy in Aquaculture

For the development of bacteriophages therapy in aquaculture, a set of standard protocols need to be followed (Nakai and Park 2002; Choudhury et al. 2017) (Fig. 20.1). This includes isolation and characterization of phage (Fig. 20.2), in vivo and in vitro therapeutic potentiality testing, safety testing and regulatory approval, etc.

20.8 Dose and Mode of Application for Phage Therapy

There are several modes of application of phage therapy reported by many researchers since its discovery. However, the application of phage in the aquaculture system includes direct release of phages in the culture system, injection through intramuscular or intraperitoneal mode, immersion, oral administration through feed, anal intubation, etc. Among all these reported modes, release of phages directly into the culture system is the most preferred method (Shivu et al. 2007; Choudhury et al.

Table 20.2 Phage-based products for therapy in aquaculture

Name of the Company/ Institute	Product description	References
Intralytix	Phage therapy (as cocktail of phage) to control *Vibrio tubiashii* and *V. coralliitycis* infections in oyster	Intralytix I (2018)
Phage Biotech Ltd	Phage therapy to treat *V. harveyi* infections in shrimp	Phage Biotech (2017)
Mangalore Biotech Laboratory	Phage formulation (LUMI-NIL MBL) to control luminous vibriosis in shrimp	Mangalore Biotech Laboratory (2019)
Fixed Phage Ltd	Binds the phages in feed pallets for phage therapy aquaculture.	Mattey (2020)
ACD Pharma	Phage-based solutions against Yersiniosis in Atlantic salmon	ACD Pharma (2017)
Proteon pharmaceutical	Phage-based product BAFADOR® to targets aquaculture pathogens *Pseudomonas* spp. and *Aeromonas* spp. via immersion	Grzelak (2017)
ICAR-CIBA	LUMI^{PHAGE} for biocontrol of luminous bacteria in shrimp larvae	ICAR-CIBA (2017)

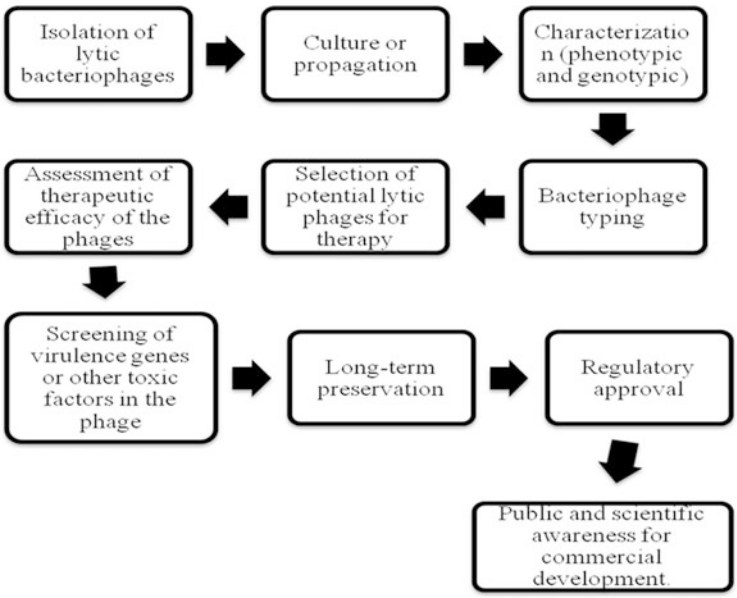

Fig. 20.1 Strategies for bacteriophage therapy in aquaculture

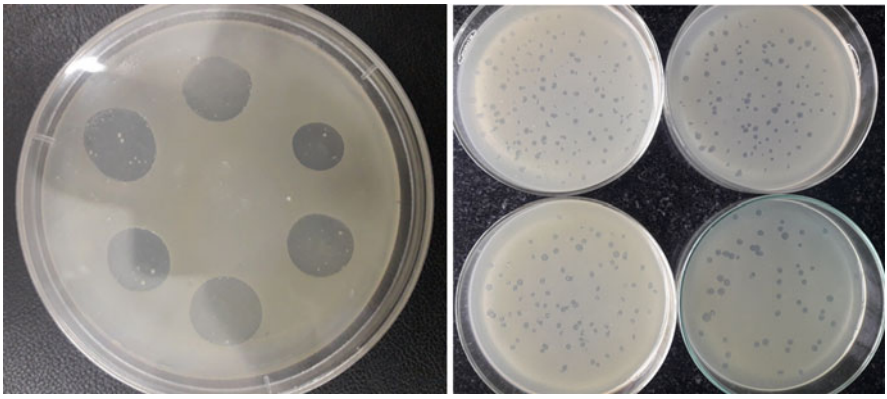

Fig. 20.2 Zone of lysis by bacteriophages and plaques formed by bacteriophage

2017; Silva et al. 2016). In recent times, various combination of phage as "cocktail" has gained a lot of interest among researchers as futuristic bacteriophage approach. Cocktail of diverse combinations such as phage-phage, phage-probiotic, phage-immunostimulant and phage-antibiotic are demonstrated in the literature (Fischetti et al. 2006; Chan et al. 2013; Choudhury et al. 2019). There are advantages and disadvantages to each mode of application; which often depends on the nature of the bacterial pathogen (Martinez-Diaz and Hipólito-Morales 2013; Richards 2014).

For effective phage therapy, it is important to know the exact dose of application. Various doses have been reported by researchers for both laboratory and field condition. However, in most cases, the dose of application depends on the type of pathogen, state of phage, multiplicity of infection (MOI) of phage or lytic capability, etc. For effective phage therapy, researchers may attempt to isolate phage with a high replication rate, broad host range with high lytic capacity at lower doses (Choudhury et al. 2017).

20.9 Positives and Negatives of Phage Therapy

Several well-established advantages of phage treatment include (Barrow et al. 1998; Nakai 2010):

1. Because of the natural abundance, phage isolation is comparatively easy and cheap.
2. Bacteriophages have narrow host range indicating that phages are very specific to host and do not harm the endemic intestinal or environmental microflora.
3. No inherent toxicity and environment friendly.
4. Self-replicating capability eliminates the necessity of multiple administrations.
5. Effective against biofilm-forming bacteria.
6. Bacteriolytic capability of phages allows them to eliminate MDR (multi-drug resistant) bacteria.
7. Because of the high specificity, phages do not contribute to the development of resistance among pathogens.
8. Administration of phages can be very feasible because of the multimodal application such as oral, aerosols, immersion, injection, and topical.

Bacteriophage application has an immense potential but even then, the feasibility, accessibility and field efficacy still remains a concern, which roots to several drawbacks in phage therapy:

1. Because of the high specificity of phages, the pathogenic bacteria must be identified before therapy, which may prove to be a realistic and practical challenge in the field condition.
2. Difficult to extrapolate in vivo efficacy in comparison to in vitro results.
3. Temperate phages can transfer lethal or toxic genes to harmless bacteria.
4. Because of the robust nature of the host bacteria phage resistance can be developed by bacteria.
5. Contradictory opinion on interaction with the immune responses of fish/shellfish.
6. There might be practical difficulties, e.g. injecting large numbers of animals, acceptance of phage mediated feed to diseased fish.
7. Conversion of lytic phage to lysogenic state is still a mystery among phage experts and may be a concern prior to application.

20.10 Conclusion

Bacteriophage therapy has been reintroduced in the system after the rise of drug-resistant bacteria and to cater the necessity of finding an alternative to chemotherapeutic application. Owing to the host specificity of phage and lytic capability, it can prove to be an attractive approach in that it provides a ray of hope against AMR. At present, the potential phagotherapy has established its efficacy in preventing or controlling the bacterial infections in both freshwater and marine water in various target species of fish and shellfish origin. Bacteriophage therapy has been intensively researched and developed against various clinical conditions in the area of biomedical application. However, in aquaculture, the therapy is not yet fully investigated. The lack of in vitro and in vivo research on optimization and efficacy in different culture condition existing in diverse aquatic environments has led to the challenge we are facing today, with the development of effective field-based formulation. It is high time that attempts are made to address the concerns that have arisen over time, and research efforts should therefore be conceptualized and aimed at establishing sustainable phage therapy.

References

Abedon ST, Kuhl JS, Blasdel BG, Kutter EM (2011) Phage treatment of human infections. Bacteriophage 1:66–85

ACD Pharma (2017) Bacteriophage therapy—ACD Pharma. https://acdpharma.com/2021/nyhet/acd-pharmas-work-with-bacteriophages/. Accessed 9 Jul 2021

Ackermann HW (2003) Bacteriophage observations and evolution. Res Microbiol 154(4):245–251

Adriaenssens EM, Wittmann J, Kuhn JH, Dann Turner D, Sullivan MB, Dutilh BE, Jang HB, Zyl LJV, Klumpp J, Lobocka M, Switt AIM (2018) Taxonomy of prokaryotic viruses: 2017 update from the ICTV bacterial and archaeal viruses subcommittee. Arch Virol 166:1125–1129

Akmal M, Rahimi-Midani A, Hafeez-Ur-Rehman M, Hussain A, Choi TJ (2020) Isolation, characterization, and application of a bacteriophage infecting the fish pathogen *Aeromonas hydrophila*. Pathogens 9(3):215

Almeida A, Cunha A, Gomes NCM, Alves E, Costa L, Faustino MAF (2009) Phage therapy and photodynamic therapy: low environmental impact approaches to inactivate microorganisms in fish farming plants. Mar Drugs 7:268–313

Al-Sum AB, Al-Dhabi NA (2014) Isolation of bacteriophage from Mentha species in Riyadh, Saudi Arabia. J Pure Appl Microbiol 8(2):945–949

Barrow P, Lovell M, Berchieri A (1998) Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin Diagn Lab Immunol 5:294–298

Biotech P (2017) Developments. Phage Biotech, Tel Aviv

Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K, Little D, Ross L, Handisyde N, Gatward I, Corner R (2010) Aquaculture: global status and trends. Philos Trans R Soc Lond B Biol Sci 365(1554):2897–2912

Brunoghe R, Maisin J (1921) Essais de therapeutique au moyen du bacteriophage du staphylocoque. C R Soc Biol 85:1020–1021

Carrias A, Welch TJ, Waldbieser GC, Mead DA, Terhune JS, Liles MR (2011) Comparative genomic analysis of bacteriophages specific to the channel catfish pathogen, *Edwardsiella ictaluri*. Virol J 8:6

Castillo D, Higuera G, Villa M, Middelboe M, Dalsgaard I, Madsen L, Espejo RT (2012) Diversity of *Flavobacterium psychrophilum* and the potential use of its phages for protection against bacterial cold water disease in salmonids. J Fish Dis 35(3):193–201

Chan BK, Abedon ST, Loc-Carrillo C (2013) Phage cocktails and the future of phage therapy. Future Microbiol 8:769–783

Chen L, Fan J, Yan T, Liu Q, Yuan S, Zhang H, Yang J, Deng D, Huang S, Ma Y (2019) Isolation and characterization of specific phages to prepare a cocktail preventing *Vibrio* sp. Va-F3 infections in shrimp (*Litopenaeus vannamei*). Front Microbiol 10:2337

Choudhury TG, Maiti B, Venugopal MN, Karunasagar I (2012) Effect of Total Dissolved Solids and Temperature on Bacteriophage Therapy against Luminous vibriosis in Shrimp. Bamidgeh 64:761

Choudhury TG, Tharabenahalli Nagaraju V, Gita S, Paria A, Parhi J (2017) Advances in bacteriophage research for bacterial disease control in aquaculture. Rev Fish Sci Aquac 25(2):113–125

Choudhury TG, Maiti B, Venugopal MN, Karunasagar I (2019) Influence of some environmental variables and addition of r-lysozyme on efficacy of *Vibrio harveyi* phage for therapy. J Biosci 44 (1):8

Cisek AA, Dąbrowska I, Gregorczyk KP, Wyżewski Z (2017) Phage therapy in bacterial infections treatment: one hundred years after the discovery of bacteriophages. Curr Microbiol 74 (2):277–283

Cunha BA (2009) Antibiotic resistance. Med Clin N Am 84:1407–1429

D'Hérelle F (1917) Sur un microbe invisible antagoniste des bacilles dysentériques. C R Acad Sci 165:373–375

Fischetti VA, Nelson D, Schuch R (2006) Reinventing phage therapy: are the parts greater than the sum? Nat Biotechnol 24(12):1508–1511

Fortuna W, Miedzybrodzki R, Weber-Dabrowska B, Gorski A (2008) Bacteriophage therapy in children: facts and prospects. Med Sci Monit 14(8):RA126–RA132

Freifelder DM (1987) Microbial genetics. Jones and Bartlett, Portolla Valley, CA

Gelband H, Miller-Petrie M, Pant S, Gandra S, Levinson J, Barter D, White A, Laxminarayan R (2015) The state of the World's antibiotics 2015. Wound Heal S Afr 8:30–34

Grzelak J (2017) BAFADOR® presented at the international bacteriophage conference in Tbilisi. https://www.proteonpharma.com/bafador-at-the-international-bacteriophageconference-in-tibilisi/. Accessed 9 Jul 2021

Gui L, Zhang QY (2018) Disease prevention and control. In: Aquaculture in China: success stories and modern trends, pp 577–598

Hankin EH (1896) An outbreak of cholera in an officers' mess. Br Med J 2:1817–1819

Higuera G, Bastías R, Tsertsvadze G, Romero J, Espejo RT (2013) Recently discovered *Vibrio anguillarum* phages can protect against experimentally induced vibriosis in Atlantic salmon, *Salmo salar*. Aquaculture 392:128–133

Hsu CH, Lo CY, Liu JK, Lin C (2000) Control of the eel (Anguilla japonica) pathogens, *Aeromonas hydrophila* and *Edwardsiella tarda*, by bacteriophages. J Fish Soc Taiwan 27(1):21–31

ICAR-CIBA (2017). http://www.ciba.res.in/images/aquaticdiv/adv/EHP,WSSV,%20Lumiphage%202.pdf

Imbeault S, Parent S, Lagacé M, Uhland CF, Blais JF (2006) Using bacteriophages to prevent furunculosis caused by *Aeromonas salmonicida* in farmed brook trout. J Aquat Anim Health 18 (3):203–214

Intralytix I (2018) Intralytix, Inc. http://www.intralytix.com/

Jassim SAA, Limoges RG (2014) Natural solution to antibiotic resistance: bacteriophages "the living drugs". World J Microbiol Biotechnol 30:2153–2170

Jun JW, Kim JH, Shin SP, Han JE, Chai JY, Park SC (2013) Protective effects of the *Aeromonas* phages pAh1-C and pAh6-C against mass mortality of the cyprinid loach (*Misgurnus anguillicaudatus*) caused by *Aeromonas hydrophila*. Aquaculture 416–417:289–295

Jun JW, Kim HJ, Kil Yun S, Chai JY, Park SC (2014) Eating oysters without risk of vibriosis: application of a bacteriophage against *Vibrio parahaemolyticus* in oysters. Int J Food Microbiol 188:31–35

Jun JW, Han JE, Giri SS, Tang KF, Zhou X, Aranguren LF, Kim HJ, Yun S, Chi C, Park SC (2017) Phage application for the protection from acute Hepatopancreatic necrosis disease (AHPND) in *Penaeus vannamei*. Indian J Microbiol 58:114–117

Kalatzis PG, Bastias R, Kokkari C, Katharios P (2016) Isolation and characterization of two lytic bacteriophages, φSt2 and φGrn1; phage therapy application for biological control of *Vibrio alginolyticus* in aquaculture live feeds. PLoS One 11(3):e0151101

Karunasagar I, Vinod MG, Kennedy B, Vijay A, Deepanjali A, Umesh K, Karunasagar I (2005) Biocontrol of bacterial pathogens in aquaculture with emphasis on phage therapy. In: Diseases in Asian Aquaculture V, Fish Health Section, Asian Fisheries Society, Proceedings of the Fifth Symposium on Diseases in Asian Aquaculture

Karunasagar I, Shivu MM, Girisha SK, Krohne G, Karunasagar I (2007) Biocontrol of pathogens in shrimp hatcheries using bacteriophages. Aquaculture 268(1–4):288–292

Khairnar K, Raut MP, Chandekar RH, Sanmukh SG, Paunikar WN (2013) Novel bacteriophage therapy for controlling metallo-beta-lactamase producing *Pseudomonas aeruginosa* infection in catfish. BMC Vet Res 9(1):264

Kim JH, Gomez DK, Nakai T, Park SC (2010) Isolation and identification of bacteriophages infecting ayu *Plecoglossus altivelis* altivelis specific *Flavobacterium psychrophilum*. Vet Microbiol 140:109–115

Kim JH, Choresca CH, Shin SP, Han JE, Jun JW, Park SC (2015) Biological control of *Aeromonas salmonicida* subsp. Salmonicida infection in rainbow trout (*Oncorhynchus mykiss*) using Aeromonas phage PAS-1. Transbound Emerg Dis 62:81–86

Kim HJ, Jun JW, Giri SS, Chi C, Yun S, Kim SG, Kim SW, Kang JW, Han SJ, Kwon J, Oh WT (2019) Application of the bacteriophage pVco-14 to prevent *Vibrio coralliilyticus* infection in Pacific oyster (*Crassostrea gigas*) larvae. J Invertebr Pathol 167:107244

Kowalska JD, Kazimierczak J, Sowińska PM, Wójcik EA, Siwicki AK, Dastych J (2020) Growing trend of fighting infections in aquaculture environment—opportunities and challenges of phage therapy. Antibiotics 9(6):301

Laanto E, Bamford JKH, Ravantti JJ, Sundberg LR (2015) The use of phage FCL-2 as an alternative to chemotherapy against columnaris disease in aquaculture. Front Microbiol 6:1–9

Lafferty KD, Harvell CD, Conrad JM, Friedman CS, Kent ML, Kuris AM, Powell EN, Rondeau D, Saksida SM (2014) Infectious diseases affect marine fisheries and aquaculture economics. Ann Rev Mar Sci 7:471–496

Le TS, Nguyen TH, Vo HP, Doan VC, Nguyen HL, Tran MT, Tran TT, Southgate PC, Kurtböke Dİ (2018) Protective effects of bacteriophages against *Aeromonas hydrophila* causing motile Aeromonas septicemia (MAS) in striped catfish. Antibiotics 7(1):16

Letchumanan V, Chan KG, Pusparajah P, Saokaew S, Duangjai A, Goh BH, Ab Mutalib NS, Lee LH (2016) Insights into bacteriophage application in controlling vibrio species. Front Microbiol 7:01114

Li Z, Li X, Zhang J, Wang X, Wang L, Cao Z, Xu Y (2016a) Use of phages to control *Vibrio splendidus* infection in the juvenile sea cucumber *Apostichopus japonicus*. Fish Shellfish Immunol 54:302–311

Li Z, Zhang J, Li X, Wang X, Cao Z, Wang L, Xu Y (2016b) Efficiency of a bacteriophage in controlling Vibrio infection in the juvenile sea cucumber *Apostichopus japonicus*. Aquaculture 451:345–352

Lin DM, Koskella B, Lin HC (2017) Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther 8(3):162–173

Lomelí-Ortega CO, Martínez-Díaz SF (2014) Phage therapy against *Vibrio parahaemolyticus* infection in the whiteleg shrimp (*Litopenaeus vannamei*) larvae. Aquaculture 434:208–211

Luo X, Liao G, Liu C, Jiang X, Lin M, Zhao C, Tao J, Huang Z (2018) Characterization of bacteriophage HN 48 and its protective effects in Nile tilapia *Oreochromis niloticus* against *Streptococcus agalactiae* infections. J Fish Dis 41(10):1477–1484

Mahmoud M, Nakai T (2012) Bacteriophage therapy of *Edwardsiella ictaluri* infection in ayu *Plecoglossus altivelis*. In: Proceedings of the 5th global fisheries and aquaculture research conference, Faculty of Agriculture, Cairo University, Giza, Egypt, 1-3 October 2012

Mangalore Biotech Laboratory (2019) MANGALORE BIOTECH LAB: Products. http://mangalorebiotech.com/products.html

Martinez-Diaz SF, Hipólito-Morales A (2013) Efficacy of phage therapy to prevent mortality during the vibriosis of brine shrimp. Aquaculture 400:120–124

Matsuoka S, Hashizume T, Kanzaki H, Iwamoto E, Park SC, Yoshida T, Nakai T (2007) Phage therapy against β-hemolytic streptococcicosis of Japanese flounder *Paralichthys olivaceus*. Fish Pathol 42:181–189

Mattey M (2020) Fixed Phage Ltd. Treatment of bacterial infections in aquaculture. U.S. Patent 10,849,942

Nakai T (2010) Application of bacteriophages for control of infectious diseases in aquaculture. In: Bacteriophages in the control of food-and waterborne pathogens. American Society of Microbiology

Nakai T, Park SC (2002) Bacteriophage therapy of infectious diseases in aquaculture. Res Microbiol 153:13–18

Nakai T, Sugimoto R, Park KH, Matsuoka S, Mori K, Nishioka T, Maruyama K (1999) Protective effects of bacteriophage on experimental *Lactococcus garvieae* infection in yellowtail. Dis Aquat Organ 37:33–41

Nikapitiya C, Chandrarathna HPSU, Dananjaya SHS, De Zoysa M, Lee J (2020) Isolation and characterization of phage (ETP-1) specific to multidrug resistant pathogenic *Edwardsiella tarda* and its in vivo biocontrol efficacy in zebrafish (*Danio rerio*). Biologicals 63:14–23

Nikolich MP, Filippov AA (2020) Bacteriophage therapy: developments and directions. Antibiotics 9(3):135

Ninawe AS, Sivasankari S, Ramasamy P, Kiran GS, Selvin J (2020) Bacteriophages for aquaculture disease control. Aquac Int 28(5):1925–1938

Onarinde BA, Dixon RA (2018) Prospects for biocontrol of *Vibrio parahaemolyticus* contamination in blue mussels (*Mytilus edulus*)—a year-long study. Front Microbiol 9:1043

Park SC, Nakai T (2003) Bacteriophage control of *Pseudomonas plecoglossicida* infection in ayu *Plecoglossus altivelis*. Dis Aquat Organ 53(1):33–39

Park SC, Shimamura I, Fukunaga M, Mori KI, Nakai T (2000) Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. Appl Environ Microbiol 66(4):1416–1422

Park SY, Han JE, Kwon H, Park SC, Kim JH (2020) Recent insights into *Aeromonas salmonicida* and its bacteriophages in aquaculture: a comprehensive review. J Microbiol Biotechnol 30 (10):1443–1457

Patil JR, Desai SN, Roy P, Durgaiah M, Saravanan RS, Vipra A (2014) Simulated hatchery system to assess bacteriophage efficacy against *Vibrio harveyi*. Dis Aquat Organ 112:113–119

Prasad Y, Kumar AD, Sharma AK (2011) Lytic bacteriophages specific to *Flavobacterium columnare* rescue catfish, *Clarias batrachus* (Linn.) from columnaris disease. J Environ Biol 32(2):161–168

Pridgeon JW, Klesius PH (2012) Major bacterial diseases in aquaculture and their vaccine development. Anim Sci Rev 7:1–16

Richards GP (2014) Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology. Bacteriophage 4(4):e975540

Rørbo N, Rønneseth A, Kalatzis PG, Rasmussen BB, Engell-Sørensen K, Kleppen HP, Wergeland HI, Gram L, Middelboe M (2018) Exploring the effect of phage therapy in preventing *Vibrio anguillarum* infections in cod and turbot larvae. Antibiotics 7(2):42

Schulz P, Pajdak-Czaus J, Robak S, Dastych J, Siwicki AK (2019a) Bacteriophage-based cocktail modulates selected immunological parameters and post-challenge survival of rainbow trout (*Oncorhynchus mykiss*). J Fish Dis 42:1151–1160

Schulz P, Robak S, Dastych J, Siwicki AK (2019b) Influence of bacteriophages cocktail on European eel (*Anguilla anguilla*) immunity and survival after experimental challenge. Fish Shellfish Immunol 84:28–37

Sharma S, Chatterjee S, Datta S, Prasad R, Dubey D, Prasad RK, Vairale MG (2017) Bacteriophages and its applications: an overview. Folia Microbiol 62(1):17–55

Shivu MM, Rajeeva BC, Girisha SK, Karunasagar I, Krohne G, Karunasagar I (2007) Molecular characterization of *Vibrio harveyi* bacteriophages isolated from aquaculture environments along the coast of India. Environ Microbiol 9:322–331

Sieiro C, Areal-Hermida L, Pichardo-Gallardo Á, Almuiña-González R, de Miguel T, Sánchez S, Sánchez-Pérez Á, Villa TG (2020) A hundred years of bacteriophages: can phages replace antibiotics in agriculture and aquaculture? Antibiotics 9(8):493

Silva YJ, Moreirinha C, Pereira C, Costa L, Rocha RJM, Cunha Â, Gomes N, Calado R, Almeida MA (2016) Biological control of *Aeromonas salmonicida* infection in juvenile Senegalese sole (*Solea senegalensis*) with phage AS-A. Aquaculture 450:225–233

Simmonds P, Adams MJ, Benko M, Breitbart M, Brister JR, Carstens EB, Davison AJ, Delwart E, Gorbalenya AE, Harrach B, Hull R (2017) Consensus statement: virus taxonomy in the age of metagenomics. Nat Rev Microbiol 15:161–168

Stalin N, Srinivasan P (2017) Efficacy of potential phage cocktails against *Vibrio harveyi* and closely related Vibrio species isolated from shrimp aquaculture environment in the south east coast of India. Vet Microbiol 207:83–96

Stenholm AR, Dalsgaard I, Middelboe M (2008) Isolation and characterization of bacteriophages infecting the fish pathogen *Flavobacterium psychrophilum*. Appl Environ Microbiol 74:4070–4078

Stomps CC, Hoj L, Bourne DG, Hall MR, Owens L (2010) Isolation of lytic bacteriophage against *Vibrio harveyi*. J Appl Microbiol 108:1744–1750

Summers WC (2005) Bacteriophage research: early history. In: Kutter E, Sulakvelidze A (eds) Bacteriophages: biology and applications. CRC, Boca Raton, FL

Twort F (1915) An investigation on the nature of ultra-microscopic viruses. Lancet 186:1241–1243

Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, Gilbert M, Bonhoeffer S, Laxminarayan R (2019) Global trends in antimicrobial resistance in animals in low-and middle-income countries. Science 365(6459):eaaw1944

Verner-Jeffreys DW, Algoet M, Pond MJ, Virdee HK, Bagwell NJ, Roberts EG (2007) Furunculosis in Atlantic salmon (*Salmo salar* L.) is not readily controllable by bacteriophage therapy. Aquaculture 270(1–4):475–484

Vinod MG, Shivu MM, Umesha KR, Rajeeva BC, Krohne G, Karunasagar I, Karunasagar I (2006) Isolation of *Vibrio harveyi* bacteriophage with a potential for biocontrol of luminous vibriosis in hatchery environments. Aquaculture 255(1–4):117–124

Walker PJ, Siddell SG, Lekowitz EJ, Mushegian AR, Dempsey DM, Dutilh BE, Harrach B, Harrision RL, Hendrickson RC, Junglen S, Knowles NJ (2019) Changes to virus taxonomy and international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses. Arch Virol 164:2417–2429

Wang Y, Barton M, Elliott L, Li X, Abraham S, O'Dea M, Munro J (2017) Bacteriophage therapy for the control of *Vibrio harveyi* in greenlip abalone (*Haliotis laevigata*). Aquaculture 473:251–258

Wu JL (1982) Isolation and application of a new bacteriophage, ET-1, which infect *Edwardsiella tarda*, the pathogen of edwardsiellosis. Rep Fish Dis Res (Taiwan) 4:8–17

Wu JL, Lin HM, Jan L, Hsu YL, Chang LH (1981) Biological control of fish bacterial pathogen, *Aeromonas hydrophila*, by bacteriophage AH 1. Fish Pathol 15(3–4):271–276

Zhang J, Cao Z, Li Z, Wang L, Li H, Wu F, Jin L, Li X, Li S, Xu Y (2015) Effect of bacteriophages on *Vibrio alginolyticus* infection in the sea cucumber, *Apostichopus japonicus* (Selenka). J World Aquac Soc 46(2):149–158