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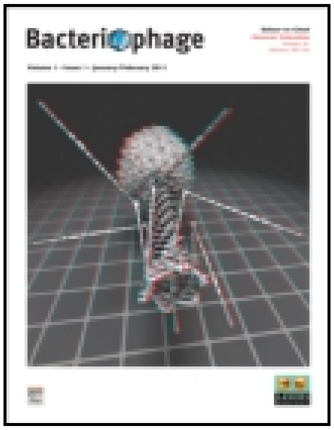

Bacteriophage
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kbac20

Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology

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Accepted author version posted online: 31 Oct 2014.Published online: 20 Dec 2014.

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To cite this article: Gary P Richards (2014) Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology, Bacteriophage, 4:4, e975540, DOI: 10.4161/21597081.2014.975540

To link to this article: http://dx.doi.org/10.4161/21597081.2014.975540

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Bacteriophage remediation of bacterial pathogens in aquaculture: a review of the technology

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Keywords: aquaculture, bacterial infections, fish disease, mariculture, phage therapy, shellfish

Abbreviations: CFU, colony forming units; i.m., intramuscular; i.p., intraperitoneal; MOI, multiplicity of infection; PFU, plaque forming units.

Bacteriophages have been proposed as an alternative to antibiotic usage and several studies on their application in aquaculture have been reported. This review highlights progress to date on phage therapies for the following fish and shellfish diseases and associated pathogens: hemorrhagic septicemia (Aeromonas hydrophila) in loaches, furunculosis (Aeromonas salmonicida) in trout and salmon, edwardsiellosis (Edwardsiella tarda) in eel, columnaris disease (Flavobacterium columnare) in catfish, rainbow trout fry syndrome or cold water disease (Flavobacterium psychrophilum) in trout and salmon, lactococcosis (Lactococcus spp.) in yellowtail, ulcerative skin lesions (Pseudomonas aeruginosa) in freshwater catfish, bacterial hemorrhagic ascites disease (Pseudomonas plecoglossicida) in ayu fish, streptococcosis (Streptococcus iniae) in flounder, and luminescent vibriosis (Vibrio harveyi) in shrimp. Information is reviewed on phage specificity, host resistance, routes of administration, and dosing of fish and shellfish. Limitations in phage research are described and recommended guidelines are provided for conducting future phage studies involving fish and shellfish.

Introduction

Increasing global demand for fish and shellfish can only be met through intensive aquaculture production. Worldwide, aquaculture produced 59.9 million metric tons (59.9 billion Kg) of food fish and shellfish in 2010 at a farmgate value estimated at $119.4 billion.[1] Intensive culturing of marine and freshwater organisms has its challenges due in large part to the presence of a host of bacterial pathogens which can kill or damage aquaculture products, leading to an economic burden on the industry and product shortages in the marketplace. These losses can occur in hatcheries and larval rearing facilities or during any part of the grow-out process. The introduction of pathogens to fish and shellfish may be through the feed, the water, contaminated surfaces, aerosols, or by spread from one animal to another. Many pathogens in aquaculture are opportunistic and may remain undetected until some stress makes the animals susceptible to infection. Stresses commonly include improper temperature, pH, or salinity or rapid shifts in these parameters; poor oxygenation; buildup of toxic chemicals, like ammonia; overcrowding; over or under feeding; excessive handling; and overall poor water quality.

A long list of bacteria can lead to opportunistic infections of fish and shellfish.[2] Vaccination methods have been applied in some fish species (reviewed by Almeida et al.[3]) with varying levels of success. Reductions in losses have most often been achieved with antibiotic treatment; however, long-term antibiotic usage has led to antibiotic resistant bacterial strains and increasing ineffectiveness of such treatments.[4-7] Although antibiotics are commonly used (overused) in many countries, there is a need to move away from antibiotics to more natural, probiotic treatments.[5,8,9] One such treatment involves the use of bacteriophages (phages) to reduce morbidity and mortalities in various aquaculture settings.

Phages are naturally-occurring bacterial viruses which infect specific species or strains of bacteria. There are 2 general types of phages, lytic and lysogenic. Lytic phages infect host bacteria through a process involving attachment of the phage to the bacterium; insertion of the phage genome into the host cell; cessation in the synthesis of host components; host mediated replication of phage components including capsid proteins and nucleic acids; assembly of new phage particles; lysis of the host; and release of progeny phages. Since lytic phages replicate quickly and rapidly cause death and lysis of the host, they are ideal for the development of phage therapies for use in treating animal infections and in reducing pathogens in various foods and the environment.

In contrast, lysogenic phages may replicate in a manner similar to that of the lytic phages, but can also integrate their DNA into the host's chromosomes, a process referred to as lysogenization. The lysogenized host cells may replicate normally for generations, however, at some point they may spontaneously or through induction by chemicals, radiation, carcinogens, etc. excise the phage DNA, and synthesize new phage particles, which

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Submitted: 08/28/2014; Revised: 10/03/2014; Accepted: 10/07/2014
http://dx.doi.org/10.4161/21597081.2014.975540

in turn lyse the host, releasing more lysogenic viruses into the surrounding medium. This process of phage DNA integration into the host genome can enhance the virulence of the host, as in the case of a Myoviridae integrating into Vibrio parahaemolyticus,[10] or a myovirus-like phage integrating into Vibrio harveyi,[11-13] or the filamentous phage CTXɸ integrating into Vibrio cholerae and Vibrio mimicus.[14] In addition, during the excision of the phage DNA from the host chromosome, host DNA may become incorporated into the phage DNA. Thus, lysogenic phages can facilitate the horizontal transfer of bacterial genes from one bacterium to another to enhance bacterial virulence. For these reasons, lysogenic phages should never be used in phage therapy. Advances in whole genome sequencing of phages are facilitating the identification of genetic components involved in lysogeny to ward off the use of lysogenic phages in commercial applications.[15] Lytic phages, on the other hand, do not integrate into the host's DNA and do not enhance the host's virulence, making them ideal candidates for therapeutic use.

Phages have been used for decades to effectively treat human wound and gastrointestinal infections in Eastern Europe and countries of the former Soviet Union.[16,17] Phages are now commercially available for: treating bacterial diseases in humans, animals, and agricultural crops; reducing pathogens to enhance food safety; and for aquaculture (reviewed in Housby and Mann,[18] Hodgson,[19] and Ly-Chatain[20]). Only one company, Phage Biotech Ltd. in Israel, was listed as developing a phage treatment in aquaculture and that was for Vibrio harveyi in shrimp.[19] Intralytix Inc. in Baltimore, MD, is also developing a phage treatment against V. tubiashii and related pathogens in larval oyster and clam hatcheries (personal communication).

There are numerous reviews on the use of phage therapy for various animals; however, there are relatively few reviews on the use of phages for treating fish and shellfish in aquaculture settings including those by Almeida et al.,[3] Oliveira et al.,[21] Sanmukh et al.,[22] Nakai and Park,[23] and Nakai[24]. This paper reviews available literature on the use of phages for treating specific pathogens in aquaculture products.

Phage Research on Bacterial Disease Remediation in Aquaculture Products

About 150 different bacterial pathogens of farmed and wild-caught fish have been identified.[2] Some of these are common causes of morbidity and mortality in aquaculture operations. Studies on phage control of pathogens in aquaculture have shown mixed results, where some bacteria appear to be more easily controlled than others. A listing of the most significant pathogens for which phage therapy has been evaluated on fish or shellfish is shown in **Table 1**. Considering the total number of fish pathogens and the wide range of fish species subject to aquaculture, it is clear that research in this area is in its infancy. The following review of the available literature demonstrates successes and failures experienced thus far in phage applications for the prevention or remediation of disease in aquaculture products. Descriptions of these pathogens, the diseases they cause, symptoms of the diseases in various fish and shellfish species, and results of phage studies to prevent or treat these illnesses are as described below.

Aeromonas hydrophila

Aeromonas hydrophila is a Gram-negative, facultatively anaerobic, motile rod and the causative agent of tail and fin rot and hemorrhagic septicemia, also known as motile Aeromonas

Table 1. Studies involving phage therapy in fish and shellfish

Pathogen	Name of Illness	Fish/shellfish evaluated	Treatment effective	Reference
Aeromonas hydrophila	Hemorrhagic septicemia; tail and fin rot; red-fin disease	Loach (Misgurnus anguillicaudatus)	Yes	Wu et al. (1981)[26]
			Yes	Jun et al. (2013)[29]
Aeromonas salmonicida	Furnunculosis	Brook trout (Salvelinus fontinalis)	Yes	Imbeault et al. (2006)[30]
		Rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar)	No	Verner-Jeffreys et al. (2007)[31]
Edwardsiella tarda	Edwardsiellosis	Loach (Misgurnus anguillicaudatus)	Yes	Wu et al. (1981)[26]
			Yes	Wu and Chao (1982)[43]
Flavobacterium columnare	Columnaris disease	Catfish (Clarias batrachus)	Yes	Prasad et al. (2011)[47]
Flavobacterium psychrophilum	Rainbow trout fry syndrome and in salmonids bacterial coldwater disease	Rainbow trout (O. mykiss)	Yes	Madsen et al. (2013)[51]
		Rainbow trout and Atlantic salmon (Salmo salar)	Yes	Castillo et al. (2012)[52]
Lactococcus spp.	Lactococcosis	Yellowtail (Seriola quinqueradiata)	Yes	Nakai et al. (1999)[54]
Pseudomonas aeruginosa	Ulcerative lesions on skin	Freshwater catfish (Clarias gariepinus)	Yes	Khairnar et al. (2013)[56]
Pseudomonas plecoglossicida	Bacterial hemorrhagic ascites disease	Ayu (Plecoglossus altivelis)	Yes	Park et al. (2000)[57]
			Yes	Park and Nakai (2002)[58]
Streptococcus iniae	Streptococcosis	Japanese flounder (Paralichthys olivaceus)	Yes	Matsuoka et al. (2007)[60]
Vibrio harveyi	Luminous vibriosis	Shrimp (Penaeus monodon)	Yes	Vinod et al. (2006)[69]
			Yes	Karunasagar et al. (2007)[70]

septicemia, in freshwater and, to a lesser extent, in marine fish.[2] Symptoms of hemorrhagic septicemia include surface lesions often leading to erosion of the fins and the loss of scales; hemorrhaging of the gills and vents; abscesses and ulcers; abdominal distension; an accumulation of ascites fluid; anemia; and damage to internal organs and musculature with generalized liquefaction.[2] A study by Hsu et al. used phages to treat A. hydrophila in unfiltered fish pond water.[25] Treatment was successful in reducing 99% of the A. hydrophila in the water within 8 h when the multiplicity of infection (MOI) was 0.23. Some phage-resistant strains developed over time. This study did not evaluate the use of phages to prevent or treat A. hydrophila infection in fish.

In perhaps the earliest phage therapy treatment of an aquaculture species, Wu et al. isolated and partially characterized 8 bacteriophages against A. hydrophila.[26] One of the phages with the strongest lytic activity, referred to as AH1, was used to treat A. hydrophila-infected loaches (Misgurnus anguillicaudatus). Known by some as the oriental weather loach, the pond loach, the Japanese loach, and the cyprinid loach, M. anguillicaudatus is a major aquaculture species in Japan, Korea and other countries, where it typically inhabits rice paddies, ditches and streams.[27-29] Wu et al. grew A. hydrophila to log phase and then infected them with phage AH1 at an MOI of 2.[26] The infected bacteria were then centrifuged and the pellet resuspended in Ringer's solution before being injected into the loach at 3 h intervals. The site and manner of injection were not disclosed. When this host-phage preparation was allowed 3, 6, 9, or 12 h to incubate before injection into the loaches, there were no infections or mortalities, even after 7 d. In contrast, control loaches injected with just A. hydrophila showed 100% infection, where infection was defined by the development of inflammation and necrosis around the site of injection within 24 h. The mortality rate for these same fish was 65% after 7 d. This study did not report the amount of bacteria or phages that were injected into the fish, but only listed the MOI as 2 (2 phage particles to each A. hydrophila). Another part of this study reported the effects of MOI on the necrosis and mortality of loaches with a 3 h preincubation of the bacteria and phages before injection into the loaches. At MOIs of 0.01, 0.1 and 1.0, there was no infection or mortality; whereas, with an MOI of only 0.001 there was 40% infection and 25% mortality. Bacteria-only controls for this experiment gave 95% infection and 60% mortality. Data from both sets of experiments indicate that MOIs of 0.01 to 2.0 effectively eliminated disease in loaches, when the bacteria and phages were combined and injected 3 h later.[26] Unfortunately, pretreatment of the bacteria and phages, as performed in this study, is not a practical solution to treating loaches in aquaculture. Nevertheless, the addition of phage AH1 to aquaculture waters may serve as a preventive measure to eliminate or control A. hydrophila in loach farming.

Recently, Jun et al. isolated 2 Myoviridae, designated pAh1-C and pAh6-C, against A. hydrophila and used them in experiments on M. anguillicaudatus.[29] Healthy loaches with a mean weight of 15 g were injected intraperitoneally (i.p.) with either 2.6×10^6 (trial 1) or 2.6×10^7 (trial 2) CFU of A. hydrophila/fish followed immediately by injection with either 3.0×10^7 PFU of phage pAh1-C/fish or 1.7×10^7 PFU of pAh6-C/fish. Control fish received only injections of A. hydrophila at the above concentrations. After 7 d, control fish gave 39% and 100% mortality in trials 1 and 2, respectively. In fish treated with phages pAh1-C or pAh6-C, there were no mortalities in trial 1. In trial 2, with the higher initial dosage of A. hydrophila injected, mortalities were approximately 43% and 17% for pAh1-C and pAh6-C, respectively.

A second set of experiments was also performed by Jun et al. using the same conditions as for the first experiment, except the phages were introduced into food pellets and fed to the fish.[29] The A. hydrophila was still injected at the same levels. Mortalities in controls without phages were 38% (trial 1) and 96% (trial 2) after 7 d. With phage treatment, mortalities in trial 1 were 17% and 12% with pAh1-C and pAh6-C treatments, respectively. In trial 2 of the feeding study with fish injected with the higher level of A. hydrophila, mortalities were 47% and 27% for treatments with pAh1-C and pAh6-C, respectively. Thus, phage therapy provided a protective effect against A. hydrophila-induced mortalities in the loach with roughly equivalent results when phages were administered by i.p. injection or through the food. Additionally, an experiment was conducted to determine if healthy loaches treated with the phages at a concentration of 10^{10} PFU/fish would have any effects on loach survival or physical condition over a one-month period. Results showed that the phage treatments did not have any negative effects on the fish.[29] Unfortunately, they did not report on any studies using a combination of the 2 phages, but they did acknowledge the possibility that combining the phages might enhance the efficiency of phage therapy.

Aeromonas salmonicida

Aeromonas salmonicida subsp. salmonicida is a Gram-negative, facultatively anaerobic, non-motile, rod-shaped bacterium and is the causative agent of furnunculosis, also called typical furunculosis, which causes acute or chronic hemorrhagic septicemia. This bacterium has broad host specificity, infecting not only salmonids as the name implies, but a wide range of fresh and saltwater fish. There are multiple forms of furunculosis in fish. Acute furunculosis is the most common form affecting aquaculture, where mortalities occur within just a few days. Symptoms of this systemic infection include a darkening of the fish (melanosis), lethargy, lack of appetite, and hemorrhages at the base of the fins. Internal hemorrhaging is common in the heart, viscera, and over the abdominal walls. Sub-acute or chronic furunculosis is more common in older fish and would be of less concern in aquaculture. Internally, it causes hemorrhaging of the musculature and organs. Externally, the sub-acute form can cause reddened fins, bloody discharge from the nares and vents, lethargy and protrusion of the eyes.[2] Adult fish tend to recover from the sub-acute form. Furunculosis also causes white nodules on the kidneys and characteristic boil-like skin lesions (furuncles). Other forms of furunculosis have also been described, but will not be covered here.

Imbeault et al. used a myovirus known as HER110 in an effort to combat A. salmonicida infection in brook trout (Salvelinus fontinalis).[30] Year-old trout challenged with A. salmonicida alone developed furnuculosis and died or had to be euthanized by the end of the study (day 45). These trout were challenged with A. salmonicida simply by adding known amounts to the water. All (100%) of untreated fish were severely sick or dead by day 45. Phages were added to some tanks 5 and 6 d post-infection at an MOI of 1. These trout showed mortalities or serious illness in only 10% of the fish at day 45. Negative and phage-only controls showed no signs of infection and all survived for the duration of the study. Experiments were conducted in 70-liter aquaria containing gravel on the bottom. Aeromonads were found to persist in the interstitial water of the gravel bed and in the circulating water where phage levels between 6 and 20 d were about 10^{12} PFU/ml of interstitial water within the gravel and 10^{9} PFU/ml in the circulating water. It was suggested that this persistence of aeromonads may have been due to the formation of a biofilm on the gravel and other surfaces – a biofilm which slowly leached some bacteria into the water. Alternatively, the possible development of phage-resistant Aeromonas mutants was suggested as a potential mechanism for A. salmonicida persistence. To prevent the formation of mutants, Imbeault et al. recommended that phage treatments should contain multiple phages, so that if the target pathogens become resistant to one or another phage type, the remaining phages will be sufficient to give long-term bacterial inactivation.[30] Another factor that might improve the effectiveness would be to increase the MOI of the phage inoculum to a level higher than 1.

Verner-Jeffreys et al. conducted a study on rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar) in which 2 sets of experiments were presented to determine the efficacy of using phages to combat furunculosis in juvenile fish.[31] Fish were initially challenged with A. salmonicida subsp salmonicida by i.p. injection followed by injection with a cocktail of 3 lytic phages (designated B, O and Q by Rodgers et al.[32]) against A. salmonicida subsp salmonicida. The family from which phages B, O and Q belonged was not specified in Rodgers et al.[32] or Verner-Jeffreys et al.[31]; however, judging from electron micrographs provided by Rodgers et al., they appear to be either Myoviridae or Syphoviridae as they had icosahedral heads and long tails. Fish injected with the phage treatment lived longer but still died of furunculosis by the end of the study (within 96 h). The time until death was dependent on how long the phage was administered after injection of the bacterium. The sooner the phage was given, the longer the fish lived. In one set of experiments, phages were reportedly administered at an MOI of 1.9×10^{5}, far higher than the MOI of 1 that was used by Imbeault et al.[30] For rainbow trout, low phage titers were observed in the kidney and spleen within 4 d of phage injection, but no phages could be detected 7 d after injection. Levels of phage persisted in the stomach, upper gut and lower gut of rainbow trout for 96 h, the duration of this study. In contrast, phage could not be detected in these tissues in Atlantic salmon 96 h post inoculation.

In the second efficacy study, juvenile Atlantic salmon were infected by subjecting them to water taken from a tank of fish dying of furunculosis.[31] The amount of A. salmonicida in the water was not disclosed and the MOI of treatments could not be determined. Experiments were performed evaluating the effectiveness of phage therapy by 3 delivery routes (oral, immersion, or intraperitoneal). Oral administration was with phages that had been incorporated into the food. Treatment of healthy control fish with phages showed that phage administration by all 3 methods was safe for the fish. The continuous oral administration of phages to Atlantic salmon via their food did not prevent clinical signs of furunculosis or death for salmon grown in A. salmonicida-contaminated water, even when the treatment was given prophylactically (i.e., before introduction of A. salmonicida).[31] Likewise, i.p. treatment and daily treatment for 1 h by immersion in a phage bath were not effective in preventing death. One reason provided for the ineffectiveness of the treatments in Atlantic salmon was that A. salmonicida subsp salmonicida is highly infectious even in very low doses. One limitation of the study was the lack of data on the amount of A. salmonicida in the fish and water at the time of phage addition. Nevertheless, under the conditions of this study, phage treatment was ineffective in preventing or treating furunculosis in Atlantic salmon and rainbow trout (**Table 1**).

More recently, Kim et al. isolated and characterized bacteriophage PAS-1, a myovirus which has broad infectivity toward 15 A. salmonicida subsp. salmonicida as well as A. salmonicida subsp. achromogenes and A. salmonicida subsp. masoucida, but not toward any of 10 isolates of A. hydrophila.[33] This isolate was recommended for consideration for use in aquaculture in Korea, but may have broader applications worldwide.

Edwardsiella ictaluri

Edwardsiella ictaluri is a member of the Enterobacteriae family of bacteria. It is a small, Gram-negative, weakly motile, pleomorphic rod, which is associated with freshwater species of fish, especially farm-raised channel catfish (Ictalurus punctatus). It has been reported to cause nearly half of the reported illnesses in channel catfish along the US Gulf Coast. Infected catfish often develop small, red and white ulcers on their skin, petechial hemorrhaging on their ventral side, and long, raised, red pimples between their eyes.[34] Infected catfish stop eating and swim in tight circles due to infection of the brain with E. ictaluri. They can hang in the water column almost vertically or spin rapidly in circles. The abdomen of the fish may become swollen and their eyes may protrude to form a popeyed appearance. Efforts have been made to identify and characterize lytic phages against E. ictaluri[35,36] and to sequence them[35,37]; however, there is little to no information on the effectiveness of these phages to reduce illnesses or mortalities in cultured catfish or other species.

Edwardsiella tarda

Edwardsiella tarda is another major pathogen producing the disease known as edwardsiellosis, also known as enteric septicemia of catfish (ESC) or emphysematosis putrefactive disease of catfish (EPDC) in a variety of freshwater and marine fish, including channel catfish. Infected fish develop excessive mucus secretion, lesions on their skin, muscle abscesses which become filled with gas and an appreciable amount of necrotic tissue.[38] Edwardsiella tarda is also an opportunistic pathogen in humans causing gastroenteritis, nausea, vomiting and low-grade fever and in severe cases may lead to enterocolitis or bacillary dysentery.[39] As in the case of E. ictaluri, most of the research on phages against E. tarda has been on their isolation, characterization, and sequencing.[40-42]

A study by Hsu et al. evaluated bacteriophages that were isolated from streams and fish ponds and tested them against E. tarda and Aeromonas hydrophila that had been obtained from eels (Anguilla japonica), eel pond water, and from commercial sources.[25] Experiments performed in unfiltered pond water spiked with 1.6×10^4 CFU of E. tarda/ml and an equal number of phages (MOI = 1.0) gave a significant (about 1-log) reduction over 4 h and 1.5-log reduction over 8 h. There was no reduction in E. tarda titer over phage-negative controls when the MOI was reduced to 0.1. Other work by Wu and Chao identified a phage (ϕET-1) against E. tarda and demonstrated its ability to reduce E. tarda in water by 99.9% in 8 h at an MOI of 0.08.[43] They also evaluated loach (Misgurnus angullicaudatus) survival with phage therapy. In this experiment, E. tarda was infected with ϕET-1 at an MOI of 0.1 and allowed an 8 h incubation period for the phage to replicate and kill the host cells. Loaches were then immersed for 1 h in the mixture of host and phage. Fish survival was 90% after 4 d. In contrast, shorter incubation of the host and phage before immersion of the fish gave poor results (0% survival if E tarda and phage were mixed together and immediately used to treat the fish, and only 5% survival if the incubation period was only 2 h[43]).

Flavobacterium columnare

Flavobacterium columnare is the causative agent of columnaris disease in a variety of freshwater fish, including carp, channel catfish, eel, goldfish, perch, salmonids, and tilapia. It is a major cause of illness and death in the channel catfish industry in the US, second only to E. ictaluri.[44] Flavobacterium columnare is a long, Gram-negative, filamentous, aerobic bacterium which exhibits flexing movement.[45] It expresses itself as an acute to chronic infection of the gills, skin and fins and the progression of the disease depends on the virulence of the pathogen and the age of the fish, with juvenile fish mostly affected by rapid damage to the gills, but with little skin or fin involvement. In adult fish, necrotic tissues are more commonly observed followed by gill damage. Mouth rot also occurs with oral lesions rendering the fish unable to eat.[44] One study isolated and characterized phages against F. columnare from fish farms, but did not apply them to treat fish.[46]

In a study in India by Prasad et al., they isolated F. columnare from naturally-infected fish from Sub Himalayan waters and 9 lytic phages from waters and sediments from rivers, reservoirs and fish farms.[47] A phage with broad host specificity, referred to as FCP1, was identified (misidentified) as a podovirus with a "hexagonal head and non-contractile long tail," which by definition is more likely to be a member of the Syphoviridae family, since Podoviridae family members have short tails. Catfish (Clarias batrachus) that were approximately 20–25 cm long and weighed 25–30 g were injected intramuscularly (i.m.) with a highly infectious strain of F. columnare. Twenty-four hours later, the fish were treated with phages administered by 3 routes: intramuscular injection, by immersion in a bath, and orally using phage-impregnated food. Injection and bath treatments were administered only once whereas the food was given twice daily, presumably for the duration of the study (4 days). All treatments resulted in a reduction in F. columnare in the gills, liver, kidney, and serum of the catfish with concomitant and dramatic increases in phages in the same tissues. It was reported that 100% of the fish survived and that clinical symptoms of columnaris disease were absent after treatment. Unfortunately, no information was provided on the mortality rate of control fish that were injected with F. columnare but which did not receive phage treatment. Nevertheless, this paper makes promising claims that phage treatment cured or prevented columnaris disease in fish injected with a virulent strain of F. columnare.[47]

Flavobacterium psychrophilum

Flavobacterium psychrophilum is a bacterial disease of cold-water fish, like rainbow trout and ayu. In trout, it causes rainbow trout fry syndrome (RTFS) and in salmonids it causes cold water disease (CWD).[48] It produces a variety of symptoms including saddle-like skin lesions near the dorsal fin, darkening of the fish, and in some fish species, necrosis of the mouth, swollen and darkened spleens, bloody ascites fluid, and abdominal hemorrhaging.[49] In rainbow trout fry, F. psychrophilum caused 90% mortality with symptoms including anorexia, darkened pigmentation of the caudal peduncle, and a distended abdomen.[49]

A variety of phages have been isolated and characterized against F. psychrophilum.[48,50] Madsen et al. reported on a preliminary study where 10^4 CFU of F. psychrophilum were injected i.p. into rainbow trout fry followed 24 h later by i.p. injection of 10^4 PFU of bacteriophage per fish.[51] Results indicated that phage treatment did not significantly affect fish survival. Contrary to these findings, Castillo et al. in Chile evaluated the effects of phage therapy on the mortalities of rainbow trout and salmon (S. salar) and showed enhanced survival after phage treatment.[52] In their study, juvenile fish (15–30 g) were simultaneously injected i.p. with 10^8 CFU of F. psychrophilum and 10^9 phages followed by monitoring for 15 d. In 20 g salmon, controls inoculated with F. psychrophilum alone experienced 45% mortalities, but with simultaneous injection of fish with both bacteria and phage, mortalities were only 18% for an overall reduction in mortalities of 60%. In another experiment with smaller salmon (10 g), 13% mortality was observed without phage intervention and no mortality was observed when phages were simultaneously administered. In the case of trout that weighed 15 g, 47% mortality was obtained with bacteria alone, but was reduced to 20% mortality when phages were administered. Other tests involving i.p. injection of F. psychrophilum into trout showed 80% mortality without phage intervention, but with 2 different phages (1H and 6H) injected i.p., mortalities were reduced to 47 and 67%, respectively.[52]

Lactococcus garvieae

Lactococcus garvieae, formerly known as Enterococcus seriolicida and Streptococcus sp., causes lactococcosis in yellowtail (Seriola quinqueradiata) and other fish. It is a non-motile, facultatively anaerobic, Gram-positive bacterium that is coccoid shaped and forms short chains. It causes disease in both freshwater and marine fish. Internal signs of the disease are not always apparent and depend in part on the fish species. In yellowtail, symptoms include damage to the kidneys, liver, intestine and spleen and production of ascites fluid. In rainbow trout, eye hemorrhaging was observed, while in some marine fish, blood was observed in the peritoneal cavity, livers were pale and the fish exhibited enteritis, but kidneys were unaffected.[2]

A lytic phage against L. garvieae was isolated, identified, and characterized by Park et al.[53] Designated PLgY, it was obtained from diseased fish and was identified as a member of the Siphoviridae family. Nakai et al. evaluated the effectiveness of PLgY in reducing mortalities caused by L. garvieae in juvenile or young yellowtail by 3 routes of phage administration (oral via the feed, i.p., and by anal intubation).[54] Control fish consisted of yellowtail that were infected with Lactococcus, but no phage. After i.p. injection of yellowtail (50 g fish) with $10^{7.2}$ PFU of phage followed by i.p. injection with $10^{8.7}$ CFU of L. garvieae, a 90% survival rate was observed compared to a 45% survival rate for control fish which did not receive phage. Effectiveness of the treatment was better when phages were administered at the time of bacterial challenge (100% survival) compared to 80% and 50% survival when phages were administered 1 h and 24 h after the L. garvieae, respectively.[54] The successful treatment of lactococcosis in yellowtail by oral administration of phages demonstrates the potential utility of phages in therapeutic and prophylactic treatment of lactococcosis in yellowtail.

The persistence of PLgY-16 phage and 2 other phages against L. garvieae (PLgY-30 and PLgY-1) in water was also determined.[54] In unsterilized seawater, autoclaved seawater, autoclaved artificial seawater, and autoclaved distilled water, phages persisted at high levels for 8 weeks except in the unsterilized seawater, where phages persisted at high levels for only 3 days, followed by a precipitous drop to negligible levels within a week.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative, aerobic, motile, rod-shaped bacterium that is ubiquitous in the marine environment. It is a common human pathogen, often acquired in hospital settings,[55] but has been reported to infect fish as well. A study from India evaluated the ability of phages to cure ulcerative lesions reportedly caused by P. aeruginosa on the surface of freshwater catfish (Clarias gariepinus).[56] Twenty P. aeruginosa isolates were obtained from catfish lesions. They all showed multiple drug resistance, and one produced metallo-β-lactamase, making this isolate carbapenem-resistant too. A lytic phage against this P. aeruginosa isolate was obtained from sewage and was characterized. The phage was sequenced and submitted to GenBank as accession number KC969441. This phage showed 99% identity with Pseudomonas phage PT2 (GenBank accession number EU236438.10) and 98% identity with Pseudomonas phage phiKMV (accession number AJ505558.1) and was used to treat skin lesions on catfish by swabbing the lesions.[56] In 8–10 days, lesion sizes were significantly reduced ($P < 0.001$) in phage-treated fish compared to control fish that did not receive phage treatment. This corresponded to a 7-fold reduction in the size of the lesion in treated fish. The study demonstrated for the first time an effective treatment against a highly antibiotic resistant P. aeruginosa. Since antibiotic resistance is an ever increasing problem in the aquaculture industry, this study demonstrates limited effectiveness of phage therapy in situations where antibiotics have become ineffective.

Pseudomonas plecoglossicida

Pseudomonas plecoglossicida is a Gram-negative, aerobic, motile, rod-shaped bacterium and is the causative agent of bacterial hemorrhagic ascites disease in cultured, freshwater ayu fish (Plecoglossus altivelis). The detection of bloody ascites fluid is a characteristic clinical sign of infection by P. plecoglossicida. Lesions may occur in the kidney, liver, spleen, heart, intestines, and gills accompanied by necrosis in the spleen, liver and kidneys.[2] Extensive studies were performed by Park et al.[57] and Park and Nakai[58] on the potential for remediation of ayu mortalities using phage therapy. In the study by Park et al., 2 phages, one Myoviridae (designated PPpW-3) and one Podoviridae (designated PPpW-4), were isolated from pond water.[57] Host specificity studies showed that they both infected 27 different strains of P. plecoglossicida, but did not infect related pseudomonads (P. anguilliseptica, P. fluorescens, or P. putida), or A. hydrophila, A. salmonicida, E. tarda, Vibrio anguillarum or Vibrio ordalii.[57] Their broad specificity toward such a wide assortment of P. plecoglossicida strains suggested that they might make good candidates for phage therapy of ayu. Phage resistant P. plecoglossicida variants that developed over time were shown to be non-pathogenic toward ayu.

Pseudomonas plecoglossicida was administered orally to ayu (mean fish weight was 10 g) by the incorporation of live bacteria into commercial dry pelleted food.[57] Fifteen minutes later, fish were fed pellets containing a mixture of phages PPpW-3 and PPpW-4. With phage treatment, an average of 22.5% mortality was noted compared with 65.0% mortality for controls without phage treatment. In a second experiment, Park et al. fed bacteria-containing pellets to the fish (mean fish weight 2.4 g) followed 1 h or 24 h later with phage-impregnated pellets.[57] No mortalities were observed in 50 fish when phage was administered 1 h after the bacterium and only 13% mortality was observe when phage was administered 24 h after the bacterium. Mortality rates for the 2 experiments where fish were treated with phages showed 22.5% mortality with phage addition at time 0 and 0% mortality with phage addition 1 h after the administration of the bacteria. These differences were attributed to the treatment of different sized fish (10 g vs. 2.4 g, respectively). In contrast, the mortality rate for control fish that did not receive phage intervention averaged 65% in the first experiment and 79% in the second experiment. The protective effect of phage treatment was significant ($P = 0.05$).

A follow-up to the above work was performed by Park and Nakai where 2.7 g ayu were treated with an oral dose of P. plecoglossicida through their feed followed by the introduction of phage PPpW-3, or PPpW-4, or both, or neither (control).[58] After 2 weeks, mortalities were as follows: 93.3% in the controls without phage treatment, 53.3% in fish treated with PPpW-3 only, 40.0% in fish treated with PPpW-4 only, and 20.0% for fish receiving a mixture of both phages. A large-scale test was also performed with 120,000 ayu (mean weight of 20 g each) in a commercial fish pond. These fish were naturally infected with P. plecoglossicida and were treated with both phages together in food pellets on days 0, 1, and 8. Over the course of 2 weeks, mortality rates dropped about one-third; however, it was determined that the ayu were co-infected with Flavobacterium psychrophilum which may have contributed to about 30% of the remaining mortalities. The antibiotic sulfisozole was used to treat the F. psychrophilum-infected fish, which was a common commercial practice; however, such treatment typically makes ayu more susceptible to P. plecoglossicida infection.[57,58] Thus, the effectiveness of the phage treatment in the absence of F. psychrophilum would have likely been greater. Phages were detected in the kidneys of fish within 3 h of administration while P. plecoglossicida could not be detected in live fish after 1 week. Park and Nakai also showed that there was no neutralizing antibody against the phages in fish serum, whether the fish were inoculated orally or intramuscularly.[58] It was unfortunate that the fish in the pond study were co-infected by 2 significant fish pathogens, confounding the results and leading to uncertainty as to the actual reduction in mortalities caused by the phage therapy. However, this serves to highlight the need to develop and employ phages for the treatment of multiple bacterial illnesses simultaneously.

Streptococcus iniae

Streptococcus iniae is a Gram-positive, β-hemolytic, zoonotic bacterium that causes streptococcosis in fish as well as cellulitis, endocarditis, meningitis, and arthritis in humans.[59] Transmission to humans is usually from handling infected fish. Streptoccosis has been associated with 30–50% of the deaths in some fish ponds and is particularly invasive toward tilapia (Oreochromis spp.), rainbow trout, coho salmon (Oncorhynchus kisutch), and yellowtail (reviewed in Weinstein et al.[59]). Matsuoka et al. performed studies on the Japanese flounder (Paralichthys olivaceus) by i.p. injection of S. iniae followed 1 h later by an injection of either 2 or 4 phage isolates.[60] Phage-treated fish had significantly fewer (mean of around 50%) mortalities after 15 d compared to untreated fish which had 100% mortalities. Injection of flounder with S. iniae followed 12 h or 24 h later with an injection of a mixture of 4 phages showed approx. 40% and 30% survival, respectively, after 15 d compared with near total losses for the S. iniae-inoculated controls. Phage-treated fish that died during the trials often contained phage-resistant S. iniae, indicating that further research is needed to establish the effectiveness of phage therapy.

Vibrio harveyi

Vibrio harveyi (also known as Vibrio carchariae) is a motile, Gram-negative, curved rod. It is probably best known as the epidemiological agent responsible for luminosis vibriosis in shrimp, a disease characterized by slow growth, loss of appetite, and high shrimp mortality. It has also been associated with diseases in abalone (Haliotis tuberculata),[61] cobia (Rachycentron canadum),[62] rock lobster (Jasus verreauxi),[63] grouper (Epinephelus coioides),[64] red drum (Scaienops ocellatus),[65] salmonids,[66] summer flounder (Paralichthys dentatus),[67] pearl oysters (Pinctada maxima),[68] and other species of fish and shellfish. Depending on the fish and shellfish species, V. harveyi can also cause a host of other symptoms ranging from hemorrhaging, necrotizing enteritis, and gastroenteritis, to high mortalities without overt symptoms. Vibrio harveyi is naturally bioluminescent, giving infected shrimp and other species a characteristic luminescence. A number of phages against V. harveyi have been isolated and partially characterized.[69-76]

Two studies were conducted on the biocontrol of V. harveyi in shrimp hatcheries.[69,70] Vinod et al. isolated a Siphoviridae with lytic activity toward a broad range of V. harveyi strains and used this phage to treat shrimp (Penaeus monodon) larvae that were infected with V. harveyi.[69] In a laboratory study, 20 post-larval (18 day) shrimp were added to plastic containers (tubs) containing 1 L of filter-sterilized seawater and then infected with 10^5 vibrios/ml. Phages were added at approx. 10^5 PFU/ml to: a) 3 tubs at 0 h and 24 h, b) a second set of tubs only at 0 h, and c) no phages were added to a third set of tubs to serve as a phage-negative control. Shrimp receiving 2 doses of the phages showed 80% survival and a 3-log reduction in V. harveyi counts. Shrimp receiving only one phage treatment had 70% survival with a 2-log reduction in V. harveyi, while shrimp with no phage treatment had only about a 25% survival and an increase in V. harveyi counts of 1 log. These trials were concluded in only 48 h, which may have been too short a period to measure overall mortality rates. Nevertheless, longer term (17 day) commercial hatchery trials were performed on 35,000 nauplii in 500 L tanks. These larvae were naturally infected with V. harveyi. After 17 d of daily phage treatment, the mean survival rate was 86%, but was only 17% in control tanks without phage treatment.[69] Overall, this study showed that treatment of the water in which shrimp were raised reduced mortalities appreciably. In side by side comparisons with antibiotic usage (oxytetracycline at 5 ppm/day and kanamycin at 10 ppm/day), survival rates were only 40%, showing an advantage to using phage therapy.[69]

In a study by Karunasagar et al., 4 phages against V. harveyi were isolated from oyster tissues and shrimp hatchery water.[70] Two of these were characterized as Siphoviridae and were used in large-scale trials in a commercial shrimp hatchery. Ten ton tanks of seawater containing 5×10^5 post-larval stage 5 (PL 5) shrimp larvae (P. monodon) with signs of luminous vibriosis (high mortality and luminescence) were treated with one of 2 phages (designated Viha10) on days 1 and 3, while the other phage (Viha8) was used to treat the shrimp on days 2 and 4. Each treatment was with 2×10^6 PFU/ml. Strain Viha10 was previously shown to lyse 70% of 100 V. harveyi isolates obtained from oysters, while Viha8 lysed 68% of 100 isolates obtained from hatchery water. Using duplicate tanks, larval shrimp survival was 88% in one tank and 86% in the other, compared to survivals of 68% and 65% in tanks treated with oxytetracycline or kanamycin, respectively. Unfortunately, no uninoculated controls were included in this experiment, presumably due to the high losses in production yield that would have likely resulted. This study also evaluated the effectiveness of phage Viha10 treatment against V. harveyi in biofilms and showed a 1-log reduction in 6 h and a 3-log reduction in 18 h.

Phage Specificity, Host Resistance, Routes of Administration and Dosing

Host specificity varies from one phage to another. Myoviridae are considered by some to have broader specificity than Podoviridae and Siphoviridae,[77,78] although from personal experience, some Myoviridae are highly specific toward Vibrio strains (personal observations). Mixtures or cocktails of phages with different host specificities may be useful to prevent the development of phage resistant pathogens. Imbeault et al. suggested that to reduce the likelihood of the development of phage resistance, that different combinations of phages should be used each year on farms.[30] Cocktails of phages are widely viewed as a practical approach to combating phage resistance while providing an effective treatment against a range of pathogens or strains.[20,24,48,79] Polyvalent phages, capable of infecting multiple strains within a species, are also a desirable treatment option.[20] The application of phages by the aquaculture industry may be easier than for other animal types, since live fish may be treated via their feed, by injection, or by immersion in water containing the phages. Unlike terrestrial animals, aquaculture species and their surrounding aqueous environment may be subjected to phages to simultaneously reduce pathogens both within the animal and in its immediate environment. Treatment regimens, including the dosage and frequency of phages applied and their route of administration (oral, immersion, injection, swabbing, etc.), will likely affect therapeutic outcomes. Surface swabbing with phages was effective for treating ulcerative skin lesions caused by P. aeruginosa in catfish[56]; however, for deep, systemic infections, injection of phages was commonly employed. Multiple treatments or continuous phage treatment via the feed may enhance therapeutic efficacy over single treatment scenarios. Ly-Chatain suggested microencapsulation of phages to extend their viability at the infection site or as they travel to the site of infection.[20] Microencapsulation could be designed for the timed release of phages at a controlled rate to optimize their persistence and effectiveness.

Clearly, some routes of administration are impractical if not impossible. For instance, injection of minute larvae or tiny fish would not be feasible. Likewise, the immersion of fish in high titers of phages would be impractical if the fish were contained in very large volumes of water or in flow-through systems. Nakai et al. made the point that natural routes of infection may be necessary for optimal therapeutic benefit, as in the case of L. garvieae, which naturally infects yellowtail via the oral route.[54]

Phage dosage is likely to be a major factor in the effectiveness of treatment. Literature shows a wide variety of doses administered in laboratory and field testing. The ability to prepare enough phages for treatment may not be feasible if very high MOI's are required. An MOI of 1 was reportedly sufficient to reduce A. salmonicida-induced mortality of brook trout by 90%[30] and an MOI of 0.01 (10^6 PFU of phage injected i.m. to 10^8 CFU of F. columnare) totally eliminated symptoms of columnaris disease in catfish.[47] These MOI's may be feasible for implementation in commercial operations; whereas, higher MOI's may be too costly for practical application, depending, in part, on the design of the facility. Research should be directed toward the isolation and identification of phages with high replication rates and burst sizes in order to facilitate efficient infection of host cells.[48] Overall, the effectiveness of phage intervention in aquaculture will depend in large part on a variety of factors including the age of the fish, the stressors allowing the opportunistic pathogen to become established in the system, specificity of the phages to the infecting bacteria, early diagnosis and treatment of disease, the concentration of the pathogen, the site of infection, the dose of phages applied, the route of phage administration, and environmental conditions. These are all areas in need of additional investigation.

Limitations in Phage Research and Applications

Phages used for therapeutic applications must be carefully scrutinized to ensure that they are lytic phages. Lysogenic phages should never be used. Lysogenic phages have been shown to enhance the virulence of pathogens, as in the case of 2 studies of phages against V. harveyi in shrimp.[11,13] Some studies have demonstrated that the development of phage resistance by some pathogens is achieved at the expense of host virulence.[57,80-82]

Many fish and shellfish pathogens are likely to be opportunistic, invasive only when animals have been stressed. The effectiveness of phage therapy may vary depending on the degree to which the animals are stressed, with better therapeutic results in minimally stressed animals (those able to fight off some of the pathogens) and poorer results for animals more stressed or simultaneously infected with multiple pathogens. Clearly, early treatment appears to be a key to a successful outcome.[54,58] Prophylactic application of phages in aquaculture may also be highly beneficial in some fish species,[54,58] but not necessarily in others.[31]

Once aquaculture products are infected, it is critically important to be able to diagnose the infectious agent so the appropriate treatment tools may be employed. In the event of a bacterial etiology, it is imperative that phage treatment be initiated quickly, that phage treatment includes a mix of 2 or more phages against the particular pathogen to reduce the likelihood of resistance development, that monitoring for secondary infections by other opportunistic pathogens be implemented, and that secondary treatment with phages against other secondary pathogens also be applied as needed. As more and more phages are isolated, identified, and characterized against bacterial pathogens of fish and shellfish, it seems likely that treatments will employ mixtures of phages for the simultaneous treatment of many different bacterial

Table 2. Recommended guidelines for research and reporting on phage studies in fish and shellfish. The following information should be considered when designing research studies and should be noted and reported in any publications

- Bacterial pathogen under study
  - Species, genus, source and accession numbers, when available
  - General characteristics
  - Known virulence factors (if infecting fish with lab strains)
- Fish or shellfish
  - Species and common name
  - Size, age, and life history stage
  - Health status at beginning of experiment (healthy, diseased, immune compromised, etc.)
  - Stocking density (for experiment)
- Phage characteristics
  - Source and characteristics of phage(s) to be used
  - Lytic (or lysogenic)
  - Phage family (if known): Myoviridae, Siphoviridae, Podoviridae, etc.
  - Mixture of phages or single phage to be used in treatment
  - Phage titers
- Configuration of aquaculture tanks or lab-scale system
  - Tank or pond volume and dimensions
  - Average depth of pond
  - Number of tanks or ponds used for the experiment
- Source water
  - Source and general quality of water
  - Water treatment before use (if applicable), like filtration, UV disinfection, etc.
  - Month or season collected and used
- Water parameters during experiment
  - Range and mean of water temperature, salinity, pH, and dissolved oxygen
  - Use of aeration
  - Flow rates
  - Use of antibiotics (if applicable)
- Fish and shellfish challenge
  - Route of administering bacterial pathogen(s) to fish or shellfish (natural contamination or through feed, bath, swab, or injection). If injection, indicate site location and how (i.m. or i.p., etc).
  - Route of administering phages (via feed, bath, swab, injection (i.m. or i.p., etc.).
  - Means of incorporating bacteria or phages into feed, if applicable
  - Titer of bacteria added and frequency of addition (if added more than once)
  - Titer of phage added and frequency of addition (if added more than once)
  - Duration between initial exposure of fish to bacteria and the addition of phages
  - Whether treatment is prophylactic, or administered early after infection (before symptoms), or during early or late infection (after symptoms appear)
  - Feeding regime: type of feed, amount and frequency administered
  - Photoperiod, especially for indoor aquaculture operations or laboratory experiments
  - Negative controls used (uninoculated fish and/or fish inoculated with phages only)
  - Positive controls (fish inoculated with bacterial pathogen only)
- Data collection
  - Report the frequency of collection of physical and chemical water quality parameters
  - Indicate assay methods used (standard methods, if available) for bacterial and phage testing as well as how frequently tests were conducted
  - Report health condition and mortalities of fish at regular intervals, if possible
  - Report beginning and final counts of illnesses or mortalities for each experiment.
  - Describe the symptoms of ill fish or shellfish
  - Report beginning and ending titers of bacteria and phages in fish and water.
- Waste product treatment
  - Method of treating waste water
  - Method of carcass disposal
- Quality control
  - Know and report the health status of the fish or shellfish before the experiment begins.
  - Monitor and report any background levels of target pathogen and any other possible (likely) contaminating pathogens before initiation of experiment and during experiment, as needed
  - Report complete methods used for analyses
- Data reporting and statistics
  - Collect and report data for periods sufficient to show long-term success or failure of phage treatments
  - Perform sufficient testing (number of experiments and enough replicates) to make valid statistical claims and report the results
  - Provide information on statistical tests performed to evaluate the data
  - Disclose all of the above information in papers submitted for publication

pathogens. The broad-scale use of phages in aquaculture and other applications may lead to risks if these phages are released into the environment (reviewed in Meaden and Koskella[83]). The wholesale release of phages from aquaculture operations into environmental waters may lead to an imbalance in natural bacterial flora with potential negative consequences, particularly if the phages have broad host specificity. In the event that lysogenic phages are inadvertently employed in phage studies, there could be horizontal transmission of genes from the pathogen to the phage. Through the release of such phages, these genes could further transfer to bacteria in the environment, thus increasing their virulence, drug resistance, and threat to the community. Additionally, some bacterial pathogens of fish are also human pathogens, like S. iniae, P. aeruginosa and E. tarda[39,55,59]; therefore, safe handling of fish and knowledge of their role in potential disease transmission are essential. Disinfection is the key to phage and pathogen containment. For safety, discharge water should be disinfected by appropriate methods: UV-treatment, etc. for pond water, and chlorine bleach or sterilization for lab-scale systems. All aquaria, tanks, piping, tubing, etc. used during the experiments should be disinfected to prevent the spread of disease. Likewise, all dead or diseased fish and shellfish should be disinfected or discarded in a sanitary manner to prevent disease spread. Finally, all unwanted bacteriological and phage cultures should be sterilized by autoclaving before they are discarded. These precautions will foster enhanced safety in both the research laboratory and in various aquaculture settings.

Future Research Needs

It is difficult to compare results of the various studies on the use of phages in aquaculture because there are no standardized methods for the conduct of the work or for analysis of samples. Some published papers fail to provide key information, like the dosage of phages and bacteria, or the MOI's, or the overall health status of the fish at the start of the study. Age and size of the fish or shellfish and information on the setup of the aquaculture experiment should be provided in all published works. **Table 2** recommends some of the important data that should be collected and reported in future phage trials on fish and shellfish in order to better compare and contrast results among studies.

A number of phage treatments have been reported in this review. Before any of these can be applied commercially, they must undergo efficacy testing to demonstrate their effectiveness and safety. Testing should strive to identify: effective phage dosages for the particular fish and pathogens to be treated, administration procedures, ages of fish to be treated, single vs. multiple treatments, phage specificity, overall reduction in fish illness or mortality, and cost. The most effective treatment is useless if it is cost prohibitive. Since each aquaculture facility is different, testing will need to be performed in each facility to ensure treatment efficacy. The efficacy of treatment will also be highly dependent on the general health of the fish. In cases where fish are infected with organisms that compromise their immune systems or are subjected to unfavorable environmental conditions, phage treatment may be rendered ineffective, thus 2 identical experiments performed on healthy fish versus stressed fish may give 2 entirely different outcomes. Fish that are overcrowded, under- or overfed, mishandled, or subject to poor water quality, may not respond to phage therapy. A balance must be struck between production demands and the general health and well-being of the fish. Quality control practices should be put in place in all aquaculture facilities to ensure stable and supportive growth conditions for cultured product. Controls should be supplemented with routine bacterial testing to identify baseline levels of bacteria and the occasional elevated levels of pathogens in the system. Routine monitoring for specific fish pathogens will allow corrective actions to be taken on a timely basis. Under most conditions, early treatment may be the key to significantly reducing morbidity and mortality. In addition, research is needed to identify environmental risks and to develop safeguards to mitigate such risks.

Currently, antibiotics are losing their effectiveness as antibiotic-resistant strains of a variety of bacteria have been identified. This leads to the realization that alternative treatments, like phage therapy, must be explored. Most of the studies reviewed in this paper showed an overall protective effect of phage therapy on fish and shellfish (**Table 1**), thus providing an optimistic outlook on future benefits of phage-based technologies for treating diseases in aquaculture. It is hoped that the recommended research and reporting guidelines provided in **Table 2** will facilitate more rapid progress in this field. Once the efficacy of phages in treating specific bacterial diseases has been established, commercial scale-up of therapeutic phage production by biotech companies and receipt of regulatory approvals to license and distribute products will be needed to place emerging phage technologies into the hands of users.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was made possible through intramural funding from the USDA, ARS under CRIS project 1935-42000-065-00D.

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