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Bacteriófagos: aliados para combatir enfermedades bacterianas en acuicultura. Un primer punto de partida en la acuicultura ecológica

Bacteriophages: allies to combat bacterial diseases in aquaculture. A first starting point in organic aquaculture

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Resumen

La acuicultura ha tenido un gran crecimiento debido a la mayor demanda de productos acuícolas, sin embargo, se ve amenazada por la presencia de bacterias resistentes a los antibióticos que generan gran mortalidad y pérdidas económicas. Una alternativa para combatir estos problemas es el uso de bacteriófagos. Los cuales son virus que infectan en el interior de una bacteria y la lisan. En este artículo se revisa el uso de bacteriófagos como alternativa al uso de antibióticos para combatir infecciones bacterianas en la acuicultura. Los bacteriófagos son aislados de mar, ríos, lagos, aguas residuales y muestras de tejido, asimismo estos virus presentan mejor desempeño al suministrarse en el agua respecto al alimento. La posibilidad de eliminar las infecciones provocadas por bacterias patógenas en sistemas acuícolas con cocteles de fagos está siendo un fenómeno notable, debido a que es rentable, ecológico, y seguro tanto para la acuicultura, el ser humano y animales. Sin embargo, existe poca regulación en cuanto a su uso y hay controversia en la fago resistencia. En ese sentido, antes de la aplicación de los fagos a nivel industrial, se necesitan más estudios que determinen ciertos estándares para lograr una mayor productividad, y beneficio económico, ofreciendo productos inocuos y ecológicos.

Abstract

Aquaculture has had a great growth due to the greater demand for aquaculture products, however, it is threatened by the presence of bacteria resistant to antibiotics that generate high mortality and economic losses. An alternative to combat these problems is the use of bacteriophages. Which are viruses that infect inside a bacterium and lyse it. This article reviews the use of bacteriophages as an alternative to the use of antibiotics to combat bacterial infections in aquaculture. Bacteriophages are isolated from the sea, rivers, lakes, sewage, and tissue samples, and these viruses also perform better when supplied in water than in food. The possibility of eliminating the infections caused by pathogenic bacteria in aquaculture systems with phage cocktails is being a remarkable phenomenon because it is profitable, ecological, and safe for both aquaculture, humans, and animals. However, there is little regulation regarding its use and there is controversy in phage resistance. In this sense, before the

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phage-resistance,
environmental safety,
food safety.

application of phages at an industrial level, more studies are needed to determine certain standards to achieve greater productivity and economic benefit, offering safe and ecological products.

Introduction

In 1917 phages or bacteriophages were discovered[1-3] and 91 years ago they were used as therapeutic agents[4], after that, it was discovered that their activity had more effect *in vitro* compared to *in vivo*, for *Vibrio cholerae*[5]. However, the detail study of bacteriophages was abandoned, with the appearance of cheaper broad-spectrum antibiotics, but discovering that its prolonged use generates the appearance of multi-resistant bacteria to antibiotics and leads to large economic losses, again, the use of natural origin bacteriophages has returned[6,7].

Aquaculture is one of the industries that is known worldwide as an economy for improve the sector of disadvantaged countries[8]. It has been seen average production growth of 9.2% per year since 1970 worldwide[9]. In 2015 projected world fish production at 164 million tons for this 2020[10], but it is expected to exceed that projection because in 2018 the FAO reported that 156 million tons were intended for human consumption[11].

Aquaculture is the sector that currently has the highest growth in the food industry, however, intensive rearing looks threat by the appearance of bacterial diseases caused by *Aeromonas*, *Pseudomonas*, *Vibrios* and *Flavobacterium*, which are the cause of mortality and economic losses[12-15]. In 1997 estimated a loss of 3 billion per year globally and recently, global economic losses are estimated from 1.05 to 9.58 billion dollars a year in aquaculture[16,17]. For this reason, antibiotics are used as a treatment, however, studies reveal that the excessive and inappropriate use of these compounds have caused bacterial resistance to antibiotics, presence of antibiotic residues costs in aquaculture products and by-products, sediment, wild fish, in addition, wastewater or discharges from aquaculture production centers play an important role in the transfer of resistance genes[18-21].

Faced with this situation, different alternatives such probiotics[22], prebiotics[23-26], phytobiotics[27,28] and bacteriophages have been proposed to fight those diseases[29-31], additionally, these show synergism when are used together with probiotics and show greater effectiveness than them, by reducing pathogenic bacteria[32-34]. The use of bacteriophages to prevent bacterial infections in aquaculture could help in aquaculture healthiness and to provide a product safe for the consumer, without fear of consuming food with antibiotic residues.

For this reason, this review postulates employment of mixed phage cocktails as an alternative to the use of antibiotics in aquaculture.

Development

Bacteriophages or phages are highly specific viruses that infect, be replicate in bacterial cells without invading other cells and they can have different infection cycles[32,35,36].

These viruses are found in large numbers in the environment and are the natural predators of bacterias[37]. Also, phages have different infective cycles within the bacteria: infection lytic, lysogenic, pseudo-lysogenic and chronic[38,39].

Lytic infection is the only one that does not allow bacterial multiplication, while the others cycles of infection allow it, when these are in low population density. According to the cycle of infection, phages can be classified as virulent or lytic and lysogenic or temperate. Furthermore, current approaches to phage therapy in aquaculture are oriented to the use of lytic phages, which belong to the *Caudovirals* that include the families *Myoviridae*, *Podoviridae*, and *Siphoviridae*[40].

Phage infection begins with recognition of specific receptors on the bacterial membrane and the consequent adhesion of the virus, after that, the phage introduces its genome into the bacterium and later it is replicated within her. Finally, release holins and endolysins (diverse group of small proteins produced by bacteriophages dsDNA) whose function is to form pores in the membrane, trigger and control degradation of the host cell wall at the end of the cycle lytic, causing cell lysis and release of new phages[40,41].

Phages in aquaculture. It has been justified that the phages belonging to the families *Myoviridae*, *Podoviridae* and *Siphoviridae* are part of the intestine microbiome of the fish, being in greater more temperate phages than lytic ones[42]. The phages used in various studies were isolated from the sea, rivers, lakes, sewage and tissue samples (table 1 and 2), so it is reasonable to that phage isolated from liquid media perform better by supplying them in the water than through food, the advantage of using them in water is that it controls bacteria in the environment (water), of the animals in production[43,44].

Table 1 In vitro studies of bacterial phages that affect aquaculture

Host bacterium	Phage	Phage family	Reference
Aeromonas salmonicida AS01	PAS-1	*Myoviridae*	45
Flavobacterium psychrophilum 950106-1/1	FpV-1, FpV-2, FpV-3, FpV-4, FpV-9, FpV-13, FpV-15, FpV-17, FpV-19, FpV-21, FpV-22.	*Podoviridae*	46
Aeromonas hydrophila L372	2L372X, 2L372D, 4L372D, 4L372XY.	*Myoviridae*	
	4L372X.	*Siphoviridae*	47
Aeromonas rivipollensis D05	2D05, 4D05.	*Myoviridae*	
Aeromonas hydrophila	13AhydR10PP, 14AhydR10PP, 85AhydR10PP, 50AhydR11PP, 60AhydR13PP.	*Myoviridae*	
	25AhydR2PP.	*Podoviridae*	48
Pseudomonas fluorescens	22PfluR64PP, 67PfluR64PP, 71PfluR64PP, 98PfluR60PP	*Podoviridae*	

Likewise, the time lapse between infection and treatment, the dose used and the route of administration influence the results (table 2). Although they have been used in food[43], intraperitoneally and immersed, the literature indicates that the best protective effects are observed when applied intraperitoneally, however, the most practical way in commercial production is by immersion or in feed, but the first form of application is the one that allows to obtain the best results (table 2). However, phages are not part of the formulation of diet so they do not go through the extrusion process, but rather the food is immersed in phages[33].

Table 2 Effects of the use of phages in fish

Host bacterium	Bacterial dose	Phage family	Phage	Phage dose	IT[1]	Via	Animal	Effect	Reference
Aeromonas hydrophila	2.6x10^{6} UFC/animal	*Myoviridae*	pAh6-C	1.7x10^{7} UFP/ animal	24 hours	I.P.[2]	Dojo fish	Mortality drop from 39 % to 0%	43
						Feed pellets[3]		Mortality drop from 38% to 11%	
Aeromonas hydrophila	3.7x10^{9} UFC/ml	*Podoviridae*	ΦZH1, ΦZH2	8.1x10^{9} UFP/ml	24 hours	I.P.[2]	Nile tilapia	Mortality drop from 68% to 18%	53
Streptococcus parauberis	No desafiado	*Siphoviridae*	Str-PAP-1	2x10^{5} UFP/g	Not challenged	Feed pellets[3]	Olive flounder	Mortality drop from	54
V. harveyi MO10	1x10^{6} UFC/ml	*Siphoviridae*	vB_VhaS-tm	1x10^{2} UFP/ml	4 hours	Immersion	Greenlip abalone	Mortality drop from 100% to 30%	55
Aeromonas hydrophila N17	3.2x10^{6} UFC/animal	*Myoviridae*	Φ2, Φ5	3.2x10^{8} UFP/ animal	Immediate	I.P.[2]	Striped Catfish	Mortality drop from 81% to 0%	50
		Podoviridae	Vpms1						
V. parahaemolyticus ATCC 17802		*Siphoviridae*	A3S					Mortality drop from 40% to 0%	
	1x10^{6} UFC/ml	*Leviviridae*	Aie	1x10^{7} UFP/ml	Immediate	Immersion	Brine shrimp		56
		Podoviridae	F12						
V. harveyi EC11								Mortality drop from 50% to 0%	
		Leviviridae	F8						
		Myoviridae	50AhydR13PP, 60AhydR15PP.						
Aeromonas hydrophila									
	0.9x10^{8} UFC/animal	*Podoviridae*	25AhydR2PP.	1x10^{5} UFP/ml	24 hours	Immersion	European eel	Mortality drop from 60% to 20%	57
Pseudomonas fluorescens		*Podoviridae*	22PfluR64PP, 67PfluR64PP, 71PfluR64PP, 98PfluR60PP.						

[1]IT: intervalo de tiempo entre infección y tratamiento, [2]I.P.: intraperitoneal, [3]pellet impregnado con fagos

On the other hand, individual phages have been studied, phage cocktails and mixed phage cocktails. The difference between individual phages and phages cocktails is in the virus variety, it is considered as a cocktail from two phages, independently of belonging or not to the same family. Likewise, the difference between cocktails and mixed cocktails lies in the variety of host bacteria, where considered as a mixed cocktail from two genres bacterial.

There is extensive information on phage work individual than in cocktails, although there are enough hundreds of phage studies of *Aeromonas, Pseudomonas* and *Vibrios,* so we already have mixed cocktails (table 1 and 2). The best way to start using phages in commercial breeding centers is by mixed lithic phage cocktails, due to the high specificity of phages and the difficulty of know all the bacterial strains present in a production center, either to use them as growth promoters or as a treatment for diseases[33].

Finally, entrepreneurial oriented jobs are needed. use of mixed lithic phage cocktails as growth promoters and the effects on productive parameters, nutrient digestibility and development of intestinal villi. In addition, phages have confirmed synergism when be used with probiotics and be more effective than these by reducing pathogenic bacteria in other animal species[32,33]. For that reason, you should make a comparison and combination of the phages with other alternatives, such as probiotics, prebiotics, essential oils and organic acids as performed in other animal species[32,34].

Phage-resistance. Bacteriophages and bacteria have a predator and prey relationship since both exist, which led to a coevolution, where bacteria have found strategies to elude their predators and phages forms of neutralize these strategies. There is a controversy on phage resistance[43,45,49-51] and its various mechanisms have been studied such as: production of polysaccharides, modification of phage receptors, loss of phage receptors, CRISPR-Cas system and apoptosis, which have a genetic nature[52].

Despite these bacterial strategies, the modification and loss of phage receptors serve to prevent phage adhesion, but it has a high opportunity cost as it reduces their multiplication and phages can change their tail fibers to find the newly altered receptors[58]. Regarding the production of polysaccharides, bacteria use them to prevent adhesion of phages, however, these can produce depolymerases that degrades give[59,60].

The CRISPR-Cas system is one of the most studied since it is part of the adaptive immune system of bacteria and use it to degrade the Phage DNA, however, some phages can protect your genetic material with a protective cover "core type" theory[61]. Finally, to fight phage-resistance you have mixed phage cocktails and quorum quenching[56,62,63].

Current status of phages in aquaculture. The economic losses associated with the treatment of infections bacterial, has prompted scientists to seek new treatment alternatives with strategies sustainable. One of them is phage cocktail therapy, known to be an ecological alternative that helps in the prevention and control of pathogenic bacteria diseases[64]. Phage cocktails provide the means to evade resistance to the presence of a single phage and allow the treatment of diverse pathogens at the same time[65,66]. For example, a study showed that using two and three phage cocktails is more efficient than using a single one, in *Vibrio* control in aquaculture[64]. By adding 75 µg / mL of Vplys60 (encoded endolysin enzyme by phages) inhibits the formation of biofilms and reduces the bacterial population, which increases the survival rate of *Artemia Franciscana* and reduces the burden of *Vibrio*[67]. In a pilot study, were able to determine that the use of phage cocktail is a safe and viable way to fight Vibrio infections (Vibrio *alginolyticus, V. cyclitrophicus and V. splendidus*) in sea cucumber (*Apostichopus japonicus*)[68]. Scientific reports indicate that the use of phages in aquaculture could reduce pathogen levels and not cause harmful damage to the structure of the community microbial quality of the gastrointestinal tract of the individual indirectly and indirectly improve productivity.

Regulations on the use of phages in aquaculture. Phage therapy is being limited by the lack of a regulatory framework that is specific and designed taking into account the nature of the bacteriophages[69]. Despite the attributes that it is considered bacteriophages as antimicrobials, present self-replication capabilities and features like self-restriction and are non-toxic[70,71]. This gives an overview that cannot be classified or regulated as antibiotics. In that sense, the limited knowledge and poor regulation led to classify them as substances that interfere with clinical trials[72]. Faced with this situation, in Europe, researchers are motivated to demand the adequate regulation that allow the generation of efficient treatments with the use of phages or bacteriophages[73]. A report indicates that no product based on phage is approved for use in humans, except in the countries that make up the Union of Soviet Socialist Republics. This is partly due to the lack of a regulatory framework and the limited availability of data on large-scale use magnitude[72]. However, its use is being approved for its application in agriculture by the Food and Drug Administration from the United States (FDA) and the United States Department of Agriculture (USDA for its acronym in English).

Commercial products. While the use of phage is not approved for use in humans, there are products aimed at improving the food safety or its application for pest reduction in agriculture. For example, the product whose trade name is Listex TM, composed mainly of Antilisteria monocytegenes phage P100. Another product is Biotector® created by Cheil Jedang Corporation, AgriphageTM for plant biocontrol, EcoShieldTM focused on *Escherichia coli*, are being commercialized[74-77].

With regard to aquaculture, Aquaphage and Enviphage are projects funded by the European Union, in order to create a network of researchers for the development of phage therapy in aquaculture and determine the environmental effects caused by industrial use[78,79]. They were able to verify the effectivity of the bacteriophage Listex P100 in reducing of *Listeria monocytogenes* from the fillet surface of salmon and fresh catfish[80,81]. In European eel (*Anguilla anguilla*) organism tolerance to BAFADOR® was achieved, which stimulated the parameters of cellular and humoral immunity, and reducing post-experiment mortality[57].

Advantages and disadvantages

Advantages. Bacteriophages are effective and specific because they act directly on the pathogenic agent and without negative impact on the fish's health (intestinal flora) or human beings regarding antibiotics that destroy all flora[65]. They are considered of natural origin and this is translated as an organic product. After self reply are easy to isolate and spread[82]. The use is to combat gram positive pathogenic bacteria and gram negative[83]. Direct application with water or spray makes it easy to use[84]. The preparation of multiple phage components leads to being synergistic in cocktail. Its compatibility with food makes it easy to use. Cocktails can be used for therapy and bio-healing. No effect has been reported in use, which makes it viable and omnipotent products[85]. The cocktails are currently relatively cheap[10,86].

Disadvantages or inconvenient of use. The use of phages in the treatment of bacterial infections requires exact identification of the bacterial species to be controlled, its phage application needs regulatory approvals. In this strategy, as with antibiotics in general, is the bacteria's potential that can develop antibacterial drug resistance[87]. Resistance, even if it's being used without prior consultation of the consumer regarding their acceptance. The genetic manipulation for the incorporation of genes into the cell leads to gene transfer which could generate pathogenicity and virulence factors[10,88]. Regarding the latter, scientists suggest choosing phages without the ability to genetic transmission or be modified to eliminate the natural process[89,90].

Discussion

The application of phage therapy has been one of the better alternatives for the treatment of pathogenic bacterial infections. It is a viable alternative that can replace, in the not too distant future, to the antibiotics currently used in the aquaculture. Its use has been around for a century, which was first discovered[1] and currently its application is helping to overlap the big health problems in aquaculture. Currently, scientists are working with repetitions Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based on a series of proteins (Cas) in order to identify adaptive immunity of bacteriophages. Example, in a study discovered a giant phage from *Serratia* that achieves to evade Type I CRISPR-Cas systems, but it was sensitive to type III immunity[52]. These tools could assist in identifying and creating phage cocktails for applicability purposes in the treatment and control of bacterial infections. In that sense, phage therapy could be an alternative method to reduce overuse of antibiotics in aquaculture. By reducing the infections could be improved production by better feed conversions and higher weight gain and obtain a better profit at the end the process.

To obtain a successful and effective phage therapy, certain factors must be standardized and take into account such as post-application profitability, evaluate the impact on the environment in the short, medium and long-term, method of use or administration, age of animals, proper selection of pathogen to be treated and the level of affectation in the flock. Standardization of use should be evaluated by each altitudinal floor presented by the regions of the world and existing species. That is why, a monitoring allows early identification of diseases that can help counteract problems faster and leads to an acute more sustainable aquiculture over time. In that sense, it is important to have a balance of production and the maintenance of the integral health of the systems aquaculture.

For several decades, humans have used antibiotics to protect aquaculture systems from many diseases, but overuse of these has allowed bacteria to build resistance to such drugs. Due to the excessive use of antibiotics in aquaculture, may induce tolerance in animals to these drugs, affecting the health of living beings, and for them are considered emerging pollutants which are threats to ecosystems[91,92]. Therefore, it is essential to reduce the use of antibiotics and to apply other methods that are more viable in the social, environmental and economic aspect. Phage-therapy plays an important role as one of the best alternatives, since there are still no regulatory problems in their use in aquaculture. The possibility to fight pathogenic bacteria in aquaculture systems with phage cocktails is being a remarkable phenomenon, due to the fact that it is profitable, safe for aquaculture and for humans and animals that benefit from it. Although a certain degree of phage-resistance has been reported[93,94], and that lysogenic phages can carry antibiotic resistance genes capable of bring resistance to a bacterial strain[95], these negative effects may be negligible with respect to resistance developed by applying antibiotics always before and when the mechanisms behind are identified of the spread of these resistance genes to antibiotics and identify new genes sooner from becoming public health problems. Therefore, studies on the possible impacts on the environment such as material transfer genetics through transduction and disruption of the microbiome should be considered.

Although long-term experience in environmental therapies with phages is short, the majority of published investigations fail to highlight any risk that is associated with the interruption of the microbial community measured by phages. It is possible due to its host specificity. Despite its apparent safety, it is necessary to evaluate the effect of each commercial bacteriophage on the microbial community treated before use at the industrial level. This will identify the effectiveness and productive, social and environmental security.

Conclusions

Bacterial diseases cause mortality and economic losses in aquaculture, but the lithic phages are an alternative to fight the antimicrobial resistance. They are an alternative to antibiotics as growth promoters because they don't affect beneficial microorganisms or the animal, and also don't generate toxic residues.

Treatment with phage cocktails is currently considered a viable alternative to antibiotics for the treatment of bacterial infections in aquaculture. The use of bacteriophages in aquaculture does not affect either the fish or consumer's intestinal health. However, there is a possibility of phage resistance in the future, for this before the application of bacteriophages on an industrial scale efficacy and safety should be analyzed under a regulatory framework.

The current scientific, social and economic context is directed to the use of bacteriophages in the aquaculture activity, but you must constantly update the phage's libraries used because pathogens are constantly evolving and these may vary between countries and ecological and latitudinal zones. In that sense, more studies are needed to strictly indicate a healthy environment and food safety of products treated with phages for humans and thus be able to identify the ideal phage for specific cases in the aquaculture.

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Conflicts of interest

The present research has no conflicts of interests.

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Ethical aspects

The authors declare that the writing of the research is developed using carefully the integrated input course of previous studies in the literature and recognized them through the respective cited authors and the sources.

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