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Review

Bacteriophages for aquaculture: Are they beneficial or inimical

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ABSTRACT

World fisheries production is projected at 164 million tonnes in 2020, and a major increase in the quantity of fish produced is expected to originate from aquaculture. Diseases represent a severe threat to aquaculture productivity and natural/organic strategies to combat infectious diseases are gaining new ground to address the issues of antibiotic resistance of bacteria and issues related to antibiotic residues in aquatic food animals. Phage therapy is currently considered as a viable alternative to antibiotics for treatment of bacterial infections in aquaculture systems. A cocktail of lytic phages and a synergistic combination of phages and other antimicrobials are viable options to control bacterial infections and at the same time evade phage resistance. However, lysogenic phages have the ability to transform non-virulent bacterial strains in to virulent strains which may cripple aquatic food production and also threaten food safety. The review focuses on the roles of bacteriophages and their implications for aquatic food production and food safety.

Contents

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1. Introduction

World fisheries production is projected at 164 million tonnes in 2020, and major increases in the quantity of fish produced will originate from aquaculture at an estimated annual growth rate of 2.8% (OECD/FAO, 2011). Indian fishery exports witnessed impressive growth from 37,175 tonnes in 1970 to 983,756 tonnes in 2013–14. In terms of value the increase was from US $ 47.38 million in 1970 to US $ 5007.70 million in 2013–14. Frozen shrimp continued to be the major export value item accounting for a share of 64.12% of the total US $ earnings of which the contribution of cultured shrimp was 73.31% (www.mpeda.com). Disease outbreaks are increasingly recognized as a significant constraint to aquaculture production and trade and are affecting economic development of the sector in many countries of the world. In the aquatic food production system, bacteriophages are becoming increasingly relevant today to control bacterial infections due to the emergence of antibiotic resistant superbugs and the presence of veterinary residues in the aquatic food products.

Bacteriophages (commonly referred as phages, Φ) are natural enemies of bacteria and hence mans friend to fight bacterial infections. Albeit, it has been nearly hundred years since Twort (1915) and d'Herelle (1917) discovered bacteriophages independently (Duckworth, 1976), interest on bacteriophages has been rekindled recently mainly as natural alternative antimicrobial tool to control antibiotic resistant superbugs (Breitbart, 2012; Casas et al., 2010; Housby and Mann, 2009; Jones et al., 2012; Kutateladze and Adamia, 2010; Kutter et al., 2010; Mahony et al., 2011; Martinez-Diaz and Hipolito-Morales, 2013; Meaden and Koskella, 2013; Payet and Suttle, 2014; Sanmukh et al., 2012; Svircev et al., 2011). The regulatory approval for application of bacteriophages in food (Listshield™, Listex P100) and agriculture (Agriphage™) has been another reason for renaissance of bacteriophage research (Bren, 2007; Carlton et al., 2005; Coffey et al., 2010; Fortuna et al., 2008; Lang, 2006; O'Flaherty et al., 2009). List-Shield ™, from Intralytix was approved by USFDA for treatment of food products prior to market and in the specific context the phages were classified as Generally Recognized As Safe (GRAS) (FDA, 2013). Since their advent, antibiotics have become the most relied treatment regimen to control bacterial infections in human and veterinary medicine. Unrestricted use of antibiotics led to the emergence of multidrug resistant superbugs. Rapid Alert System for Food and Feed (RASFF) of the European Commission has issued alert notifications for the presence of veterinary drugs (chloramphenicol, nitrofuran metabolites) in fish and fishery products imported in to the European Union (https://webgate.ec.europa.eu/rasff-window/portal/).

Bacteria resistant to antibiotics have been found in the aquatic environment (Kummerer, 2009) and higher level of antibiotic resistance was reported in bacteria associated with aquaculture farms than nearby coastal regions. (Labella et al., 2013). The use of bacteriophages to control bacterial infections in aquatic food production system has the promising potential to address the twin problem of controlling bacterial infections and at the same time avoiding residue contamination. The present review focuses on the possible roles of bacteriophages and their implications for aquatic food production and food safety.

1.1. Phage diversity in the aquatic environment

Spencer (1955) isolated the first phage from the marine environment and majority of the viruses in the oceans are believed to be phages (Breitbart, 2012). Phages are more resistant to the environmental stress than bacteria (Aertsen et al., 2005; Sano et al., 2004). It is predicted that marine phages are among the most diverse with a Shannon index between 7 to 8 for water and > 9 for marine sediments (Breitbart et al., 2002, 2004). In the aquatic environment suspended particles are hot spots of phage production as high viral abundance of 10^5 to 10^{11} per ml was demonstrated on suspended particles (Weinbauer et al., 2009) probably due to higher densities of bacteria on particles (Grossart et al., 2007). Bacteriophages infect phylogenitically distant heterotrophic and autotrophic bacteria (Clokie et al., 2010). Although, phages are thought to be host specific predators, the true host range of most phages is completely uncharacterized. De Corte et al. (2010) observed a decrease in viral density when moving from shallow to deep waters indicating relatively wider host range of deep sea viruses. Metagenomics (Streit and Schmitz, 2004) may be the means of accessing the diversity of phage community.

1.2. Phage structure

Bacteriophages exist in three basic structural forms namely icosahedral (20 sided) head with a tail; icosahedral head without a tail and a filamentous form. The most prevalent bacteriophages in the aquatic environment belong to the *Myoviridae*, *Siphoviridae* and *Podoviridae* (Paul and Sullivan, 2005). *Myoviridae* phages have dsDNA, icosahedral symmetrical head and a helical contractile tail separated by neck. *Siphoviridae* phages have dsDNA, icosahedral capsid and filamentous non-contractile tail. *Podoviridae* phages have dsDNA, icosahedral symmetrical head, very short non-contractile tail. *Inoviridae* phages have circular + sense ssDNA as genome and have rod or filamentous shape.

2. Bacteriophage lifestyles

Bacteriophages adopt any of the two lifestyle options when it infects a bacterium. The most apparent lifestyle is the lytic cycle, a violent form of infection resulting in the destruction of the bacterial host. The lytic cycle comprises a series of events that occur between attachment of phage particle to a bacterial cell and its subsequent release of daughter phage particles. It consists of four phases: adsorption of phage to host cell, penetration of phage nucleic acid, intracellular development and final release of daughter phage particles. The lysogenic cycle, on the other hand comprises replication of phage nucleic acid together with the host genes for several generations without major metabolic consequences for the cell. This is a latent mode of infection and it occurs at a very low frequency. The phage genes in this state may occasionally revert to lytic cycle, leading to release of phages particles, this property is known as lysogeny and phage that can develop both lytically and lysogenically are said to be temperate phages. The bacteriophage maintained in the lysogenic lifestyle can be induced into the lytic lifestyle, triggered by physical and chemical inducers such as UV irradiation, pesticides, PCBs, Mitomycin C etc. (Cochran et al., 1998).

2.1. Lytic phages: the protectors from bacterial infections

Lytic phages are obligate predators of bacteria resulting in the destruction of the bacterial host. This feature makes lytic phages suitable as invisible weapons against microscopic enemies. Lytic life cycle may be as short as 20 to 60 min. Lytic phages continue to amplify at the site of infection as long as there are sensitive bacterial hosts to infect. In nature, lytic bacteriophages influence bacterial diversity by 'Killing the Winner', a scenario in which the abundance of most active bacterial host is controlled by an increase in abundance of its phages (Breitbart, 2012).

2.1.1. Phage therapy

Bacteriophages were employed as therapeutic agents way back in 1929 (d'Herelle, 1929). *Vibrio cholerae* was the first bacteria against which phage therapy was tried but the activity of phage was found to be much higher in vitro than in vivo (Adams, 1959). These studies were later abandoned due to the introduction of broad spectrum antibiotics which were cheap and an easy alternative. However, phage preparations were used extensively for diagnosis, prophylaxis and therapy of many bacterial infections in Eastern Europe (Kutter et al., 2010). Lytic phages have the potential for very specific control of bacterial pathogens without negative environmental impact compared to antibiotics and hence recognized as biotherapeutic agents (Mahony et al., 2011). Phage