REVIEW ARTICLES

BACTERIOPHAGE THERAPY IN AQUACULTURE

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Introduction

Aquaculture has emerged as a world's fast-growing enterprise with an average compound growth rate of 10–20 % annually since 1984 and as a viable alternative to the concurrent decline in wild capture fisheries. The global aquaculture production was estimated about 52,546,205 metric tonnes by weight, excluding aquatic plants in 2008 (FAO, 2010). As a result of intensification of aquaculture to meet the growing demand, farming practices have shifted from extensive culture to intensive culture, as a result of which problems in terms of disease have become a matter of concern. Bacterial diseases have remained a serious threat to the aquaculture industry in the recent years. *Lactococcus garvieae* infection of yellowtail, *Aeromonas salmonicida* infection in salmon culture (Hiney *et al.*, 1994), *Flavobacterium psychrophilum* infections of trout (Ostland *et al.*, 2000), Vibriosis by *Vibrio harveyi*, *V.angullarium*, *V. salmonicida* on formed finfish and shell fish culture (Kraxenberger – Beatty *et al.*, 1990, Saeed, 1995, Baticados *et al.*, 1990) are few examples of bacterial infectious diseases which have caused severe economic damage to the aquaculture industry. At present, antibiotics such as oxytetracycline, potentiated sulfonamides, fluroquinolones and florfenicol are being used to control bacterial diseases in aquaculture (Baticados and Paclibare, 1992; Morrison and Rainnie, 2004). The continuous use of antibiotics in hatcheries and farms has lead to the emergences of antibiotic resistant strains of bacteria in the aquatic environment (Karunasagar *et al.*, 1994). Moreover, the accumulation of antibiotics both in the environment and in formed shrimp tissues can be potentially risky to consumers and the environment (Alderman and Hastings, 1998). Such adverse effects have prompted scientists to search for alternative to replace antibiotics in controlling diseases in aquaculture. One of the potential alternatives is bacteriophage based approach – phage therapy.

Bacteriophages – an overview

Bacteriophages, kingdom of viruses which infect the bacterial cells, are very distinct from the plant and animal viruses. Phages were discovered independently by Frederick Twort in England in 1915 and by Felix d'Herelle at the Pasteur Institute in Paris in 1917. They are widely distributed in nature and found in water, in soil, in humans or in the cells of microbes. Bacteriophages are known for their simplified structure; composed of nucleic acid surrounded by a protein coat. They undergo two types of reproduction: *lytic* and *lysogenic*. Lytic phages infect the bacterial cells, reproduce to produce numerous progeny and get released by lysing the cell wall. In the process, each phage particle can produce approximately 200 daughter lytic phages per lytic cycle which can infect other bacteria (Carlton, 1999). Lysogenic bacteriophages incorporate their genome into the host genome and divide along with the host bacterial cells. They need an induction event like physiological stress to switch into the lytic cycle. In this addition there are some filamentous phages which simply leak out of cells without killing them. Obviously, lytic phages are the best candidates for bacteriophage therapy as they undergo rapid growth, disrupt bacterial metabolism and reproduction and importantly lyse the bacterial cells.

Phage therapy

The application of phages to treat bacterial diseases has extended from medical applications into the fields of agriculture, aquaculture, food industry and waste water treatment. The history

of bacteriophage therapy dates back to the pre-antibiotic era. After the discovery of bacteriophages, d'Herelle (1917) used phages to treat dysentery. The four patients treated with single dose of bacteriophage recovered fully within few days (Sulakvelidze *et al.,* 2001). The first report on the bacteriophage therapy came in 1921 by Richard Bruynoghe and Joseph Maisin who used bacteriophages to treat Staphylococcus skin disease. Several similar interesting studies followed and encouraged by these results, d'Herelle continued working on avian typhosis, septicemic disease (barbone), bubonic plague and cholera in India. However, due to the wide spread success and development of broad spectrum antibiotics and some inconsistent therapeutic results with the bacteriophages, research on bacteriophage therapy lost its importance. In 1980's, increasing concerns over the antibiotic resistant bacteria prompted researchers to reconsider bacteriophage therapy as an alternative to antibacterial agents. Smith and colleagues (1982, 1983 and 1987) reported that *E. coli* phage can be used to effectively treat experimental *E. coli* infection in mice and calves and could be used for prophylaxis as well. Barrow *et al.*, (1998) supported the results of Smith and colleagues by treating the experimental *E. coli* infection in chicken. Soothil (1992) reported the utility of phages to treat experimental disease in mouse models with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumanii* for which antibiotic resistance is a frequent problem. Slopek *et al.,* (1987) conducted series of experiments on the application of bacteriophages for the treatment of human bacterial infections.

The use of bacteriophage therapy in aquaculture seems very promising and practical. It gained importance due to limited number of licensed antibiotics and resistance of pathogenic bacteria to antibiotics and drugs. Attempts have been made to use bacteriophages to treat diseases in aquaculture. Wu and Chao, (1982) examined the effect of a phage, ÖET-1, isolated from a pond water in Taiwan, on *Edwardsiella tarda*. In *in vitro* experiments, phage killed 25 of 27 *E. tarda* strains and reduced the bacterial count to less than 0.1% when a bacterial suspension of 1.2×10^{12} cells/ml was infected with ÖET-1 at multiplicity of infection (MOI) of 0.08 after 8 hour. The studies of Park *et al.* (1997) and Nakai *et al.*, (1999) have shown that bacteriophage could be used to control *Lactococcus garvieae* infections of yellowtail and other marine fishes. The research group of Park and Nakai continued their interest to bacteriophages of *Pseudomonas plecoglossida*, the causative agent of bacterial hemorrhagic ascites disease in cultured Ayu fish. They reported that oral administration of phage impregnated feed to Ayu resulted in protection against experimental infection of *Pseudomonas plecoglossida* (Park *et al.,* 2000). The potential of bacteriophages to control luminescent bacterial disease caused by *V. harveyi* in shrimp hatcheries has been explored in reports of Vinod *et al.,* (2006). Study of Karunasagar *et al.*, (2007) demonstrated the lytic ability, effect on *V. harveyi* biofilm and hatchery trials of bacteriophage for control of luminous bacterial disease.

The use of bacteriophage therapy particularly in aquaculture has a few great advantages. Apart from reducing the bacteria in the infected fishes, bacteriophages can also reduce the environmental load of the pathogen as they are as effective in the environment as they are in the fish. Therefore, they can also be used prophylactically. There is also an opinion that bacteriophages could reduce the carrier states of bacterial pathogens of finfish and shellfish. Therefore, there is a great scope for application of bacteriophage therapy in aquaculture systems. Advantageous properties of phage as therapeutic agents include self-replication, which results in increased concentrations as infection persists, and the narrow host range of phage, which

prevents harm to beneficial, naturally occurring microflora (Mathur *et al.*, 2003). Antibiotics usually target both pathogenic and normal microflora. Thus, phage therapy is safer and there is no need of repeated administration as phages can replicate as long as the host cells are available. On the contrary, antibiotics undergo metabolic destruction and if at stable, they need in numerous molecules to act on bacteria. Phage resistance may be a problem, but if the changes occur in the receptor or capsule, it may lead to virulence attenuation. Because antibiotics are broad spectrum in action, they tend to provoke resistance in several species of bacteria. Development of phage is inexpensive and can be accomplished in a few days compared to the development of antibiotics which take years and is very expensive.

However, there are also some disadvantages with the phage therapy. In cases of disease outbreaks with unknown disease causing bacterium, high specificity may be a problem because the causative agent has to be established to identify the phage which can effectively infect this bacterium. Bacteriophages are rapidly cleared by the spleen, liver and other filtering organs of reticuloendothelial system. This therapy cannot be used for intracellular bacteria. There are also concerns that phages might mediate genetic exchange among bacteria.

Conclusion

There is a great scope for application of bacteriophage therapy in aquaculture systems. A serious and systematic research effort is required to understand their efficacy in aquaculture systems.

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CHICKEN ANTIBODIES (IgY) AS AN ALTERNATIVE TO MAMMALIAN ANTIBODIES

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Introduction

Antibodies presently available for research, diagnostic and therapies are mostly mammalian monoclonal or polyclonal antibodies. Traditionally, bigger animals such as horses, sheep, pigs, rabbits and guinea pigs were used for the production of polyclonal antibodies, while mice and rats were used for the production of monoclonal antibodies. Both technologies have their advantages and disadvantages. Both technologies also involve some steps each of which causes distress to the animals involved