

Vibriosis in Shrimp Aquaculture A Review

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ABSTRACT

Aquaculture is the growing food sector globally and is established itself as high protein resource fulfill the food demand since the natural resources exhibits over exploitation but presently the biggest problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non-biological agents. among the groups of microorganisms that cause serious losses in shrimp culture, bacterial diseases mainly due to vibrio have been reported in penaeid shrimp culture systems implicating at least 14 species viz *Vibrio Harveyi*, *V. Splendidus*, *V. Parahaemolyticus*, *V. Alginolyticus*, *V. Anguillarum*, *V. Vulnificus*, *V. Campbellsi*, *V. Fischeri*, *V. Damsella*, *V. Pelagicus*, *V. Orientalis*, *V. Mediterrani*, *V. Logei* etc.

Keywords: Aquaculture, Shrimp, *Vibrio Harveyi*, Luminous Bacteria

I. INTRODUCTION

Shrimp aquaculture has become one of the fastest developing industries in recent times, more than 80,000 hac have been brought under shrimp culture in the coastal areas of India. While the traditional type of shrimp farms are being improved, new extensive and semi-intensive farms are being established at a rapid pace. Most of these new farms stock shrimps at high densities and use compounded pelleted feed in order to achieve higher production rates. These modern methods impose stress on the shrimps making them susceptible to diseases. The most important predisposing factors leading to disease outbreaks in shrimp culture include

- ✓ Adverse environment (poor soil and water quality)
- ✓ High stocking density with limited water exchange facilities.
- ✓ Nutritional deficiency / poor nourishment.
- ✓ Accumulation of unutilized feed followed by its putrefaction by the native heterotrophic microorganisms.
- ✓ Inadequate aeration.
- ✓ Suboptimal or heavy algal blooms in the pond.
- ✓ Physical injury and
- ✓ Presence of virulent pathogen in high counts.

These and many other less known stress factors lead to outbreaks of disease, sometimes of epizootic proportions in shrimp farms and hatcheries causing heavy mortalities.

Vibriosis is one of the major disease problems in shellfish and finfish aquaculture. *Vibriosis* is a bacterial disease responsible for mortality of cultured shrimp worldwide (Lightner & Lewis 1975; Admas, 1991; Lightner *et al.*, 1992; Lavilla-Pitago *et al.*, 1996; Lavilla-pitago *et al.*, 1998; chen *et al.*, 2000). *Vibrio* species are widely distributed in cultured facilities throughout the world. *Vibrio* related infections frequently occur in hatcheries, but epizootics also commonly occur in pond reared shrimp species. *Vibriosis* is caused by gram negative bacteria in the family *Vibrionaceae*. Outbreaks may occur when environmental factors trigger rapid multiplication of bacteria already tolerated at low levels with shrimp blood (sizemore & Davis 1985) or by bacterial penetration of host barriers. The exoskeleton provides an effective physical barrier to pathogens trying to penetrate the external surface of crustaceans, as well as the foregut and hindgut.

Vibrio harveyi a gram negative luminous bacterium is one of the etiologic agents of mass mortalities of *Penaeus monodon* larval rearing systems. A large number shrimp hatcheries along the coastline of our

country involved in shrimp seed production often suffer setbacks due to luminescent bacterial disease and suffer enormous economic losses. Among the *V.harveyi* isolates some are virulent and some are not, suggesting a great deal of molecular and genetic variation in this group of bacteria. The pathogenic mechanism has also been recently attributed to bacteriophage. *Vibriosis* is caused by a number of vibrio species of bacteria including; *Vibrio harveyi*, *V. splendidus*, *V. parahaemolyticus*, *V. alginolyticus* (Brock and Lightner ,1990;Ishimaru *et al.*,1995). There have been occasional reports of *vibriosis* caused by *V. damsella*, *V. fluvalis* and other undefined vibrio species.(Lightner,1996).

II. METHODS AND MATERIAL

Vibrio Diseases

Bacterial Septicemia

This is one of the severe systemic diseases caused by bacteria. The affected shrimps are lethargic and show abnormal swimming behavior. The pereopods and pleopods may appear reddish due to expansion of chromatophores and the shrimps may show slight flexure of the abdominal musculature. In severely affected shrimp, the gill covers appear flared up and eroded. In more severe cases extensively melanised black blisters can be seen on the carapace and abdomen. The disease was caused by *Vibrio alginolyticus*, *V. anguillarum*, or *V. parahaemolyticus*. The disease was diagnosed based on gross signs and symptoms and confirmed by isolation of pathogen from haemolymph by standard microbiological methods and histopathology. The outbreak can be prevented by maintaining good water quality and reduce the organic load by increased water exchange. The disease was controlled by increase water exchange with good quality sea water feed shrimps with antibiotic fortified feeds (only after ascertaining in vitro sensitivity of the pathogen).

Necrosis of Appendages

The disease was characterized by necrosis on the tips of walking legs, swimmerets, and uropods and become brownish black. The setae, antennae and appendages may be broken and melanized. Necrosis of appendages caused by *Vibrio spp*, *Pseudomonas spp*, *Aeromonas spp*,

and *Flavobacterium spp* and were diagnosed by based on gross signs and symptoms and prevented by maintaining good water quality. Stock at optimum density. Avoid unnecessary handling of the shrimps, which may lead to injuries and necrosis controlled by induce molting by applying 5-10 ppm teased cake.

Vibriosis

Vibriosis infected shrimp larvae show necrosis of appendages, expanded chromatophores, empty gut, absence of faecal strands and poor feeling. Cumulative mortalities may be very high reaching up to 80% within few days caused by *V.alginolyticus*, *V.parahaemolyticus* diagnosis was made by microscopic demonstration of motile bacteria in the body cavity of moribund shrimp larvae, and confirmed isolation and identification of pathogenic bacteria by standard microbiological methods. The disease can be prevented by maintaining good water quality and reduce organic load in the water by increased water exchange and outbreaks can be controlled by 10-15 ppm ethylene diamine tetra acetic acid (EDTA) to the rearing water.

Brown Spot Diseases :(Shell Disease or rust Disease)

The disease was characterized by the affected animals show the presence of brown to black encrusted areas on the body surface and appendages. It is caused by the bacterium *Aeromonas spp*, and *flavobacterium spp.*, with chitinolytic activity and were diagnosed by isolation and identification of bacteria from the site of infection by standard microbiological methods and prevented by reduce organic load in water increased water exchange avoid unnecessary handling and overcrowding to minimize chances of injury and infection controlled by induce molting by applying 5-10ppm teased cake. Improve water quality by increasing water exchange. If there is no improvements shrimp may be fed with antibiotic fortified feeds at 2-10% of the biomass for 10-14 days.

Filamentous Bacterial Disease

The disease was characterized by affected shrimp larvae fouling of gills, setae, appendages and body surface. Moulting is impaired and the larval shrimp may die due to hypoxia. It is caused by filamentous bacteria such as *Leucothrix mucor*. It is diagnosed by microscopically demonstrating filamentous bacterial fouling of body

surface and appendages of shrimp larvae. It is prevented by maintaining good water quality with optimal physical – chemical conditions controlled by 0.25 – 1 ppm copper sulphate bath treatment for 4 -6 hours.

Disease Control

The disease control programmers in aquaculture must include examination of diseases and mortalities in a holistic manner and consider various factors such as stocking densities, environment (turbidity, temperature, P^H, salinity, dissolved oxygen, H₂S, NH₃, NO₂, etc of water and redox potential of soil) rate of water exchange, presence of bottom dwelling algae, the type of feed and its rate of consumption by the shrimps, phytoplankton bloom, physiological status of shrimps, etc. most of the disease control methods are based on preventive measures. They are;

- i. Good husbandry practices,
- ii. Use of adequate balanced diets,
- iii. Quarantine measures,
- iv. Use of genetically resistant stock for culture,
- v. Use of prophylactic vaccines and
- vi. Use of drugs.

Disease prevention by adapting good husbandry practices and by providing balanced diets has been adequately mentioned in the earlier section.

Treatment

Vibriosis is controlled by rigorous water management and sanitation to prevent the entry of vibrios in the culture water(Baticodos *et al.*, 1990) and to reduce stress on the shrimps (Lightner ,1993). Good site selection ,pond design and pond preparation are also important (Nash *et al.*1992).An increase in daily water exchanges and a reduction in pond biomass by partial harvesting are recommended to reduce mortalities caused by *Vibriosis*. Draining drying and administering lime/ dolomite to ponds following harvest is also recommended (Anderson *et al.*, 1988).

Probiotics as Disease Controlling Agents

Probiotics are administrated directly into the water or via feeds. Immunostimulants have had success in reducing shrimp mortalities associated with *Vibriosis* (Itami, 1996). Jiravani Ichpasical and chuayuhuwong

(1997) reported the use of an effective treatment of *Lactobacillus sp* against *vibriosis* and white spot diseases in *P.monodon* . the investigated the growth of some probiotic bacteria, and their survival in the 20ppt sea water for at least 7 days. Inhibiting activity of two *Lactobacillus sp* against *Vibrio sp*, *E.coli*, *Staphylococcus sp* was determined.

Probiotic Bacteria

The use of beneficial bacteria (Probiotics) to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture. The term Probiotic has been defined as a Probiotic is a mono or mixed culture of live microorganisms that, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora of bacteria modify the bacterial composition of the water and sediment. The health of animals is thus improved by the removal or decrease in population density of pathogens and by improving water quality through the more rapid degradation of waste organic matter.

Microbial ecology and biotechnologies have advanced in the last decade, to the point that commercial products and technologies are available for treating large areas of water and land to enhance population densities of particular microbial species or biochemical activities. The practice of bioremediation is applied in many areas, but success varies greatly, depending on the nature of products used and the technical information available to the end user. The bacteria that are added must be selected for specific functions that are amenable to bioremediation and be added at a high enough population density and under the right environmental conditions, to achieve the desired outcomes. Bioaugmentation and the use of probiotics are significant management tools for aquaculture but their efficacy depends on understanding the nature of competition between particular species or strains of bacteria. They are same concepts that are used successfully for soil bioremediation and probiotic usage in the animal industry.

Probiotic Application in Aquaculture

Bacterial species composition in shrimp ponds, which are large water bodies up to a hectare or more in size,

hatchery tanks and shrimp guts can easily be changed and thus result in an improvement in shrimp production. In particular, luminous vibrio can be controlled in this manner. To my knowledge there has not been any rigorous study made of vibrio populations in shrimp on farms, in relation to antibiotic probiotic usage.

Use of Probiotic as Controlling Agents

The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B.subtilis*, *B.lechniformis*, *Nitrobacter sp*, *Aerobacter* and *Sacharomyces carvevisiae*, application of probiotic through water of tanks and ponds may also have an effect on fish health by improving several qualities of water, since they modify the bacteria composition of the water and sediments (Ashraf 2000; Venkateswara,2007). When probiotics are applied in culture water they multiply and over grow the pathogenic organism present in the water.

Application through injection

Application of probiotics by injection is a possibility Austin et al., (1995) suggested the possibility of freeze drying the probiont like vaccine and applied either through bathing or injection. Except the experimental purposes application of probiotic by injection has not been widely reported. According to Yassir *et al.*, (Yassir 2002 ; Nikoskelainen 2003). The use of probiotics stimulate rainbow trout immunity by stimulating phagocytes activity complement mediated bacterial killing and immunoglobulin production (Noh 1994).

Developing Probiotics for Aquaculture in Disease Control

Table 1 : Prospective probiotics evaluated for shrimp aquaculture applications

Strain	Source	Evaluated for	Effective dose/ mode of application	References
Bacillus S11	Black tiger shrimp	Growth and survival of black tiger shrimp <i>Penaeus monodon</i>	1kg wet wt (-100 g dry wt)of BS11(-1010CFU g-1)in 3kg	Rengpipat <i>et al.</i> 2003
Bacillus subtilis BT 23	Shrimp culture ponds	Against the growth of <i>Vibrio harveyi</i> isolated by agar antagonism assay from <i>Penaeus monodon</i>	106-108 CFU ML-1 for 6d	Vaseeharan and Ramasamy 2003
Pseudomonas sp PM 11 Vibrio fluvialis PM 17	Gut of farm reared sub-adult shrimp	Immunity indicators of <i>Penaeus monodon</i>	Pseudomonas sp.PM11 @103 bacterial cells	Alvandi <i>et al.</i> 2004

It has been widely published that a probiotic must possess certain properties (Verschuere) *et al.*, 2000 a) .These properties were proposed in order to aid correct establishment of new, effective and safe products. The properties include;

The probiotic should not be harmful to the host it is desired for.

- i. It should be accepted by the host, e.g. through ingestion and potential colonization and replication within the host.
- ii. It should reach the location where the effect is required to take place.
- iii. It should actually work in vivo as opposed to in vitro findings.
- iv. It should preferably not contain virulence resistance genes or AB resistance genes.

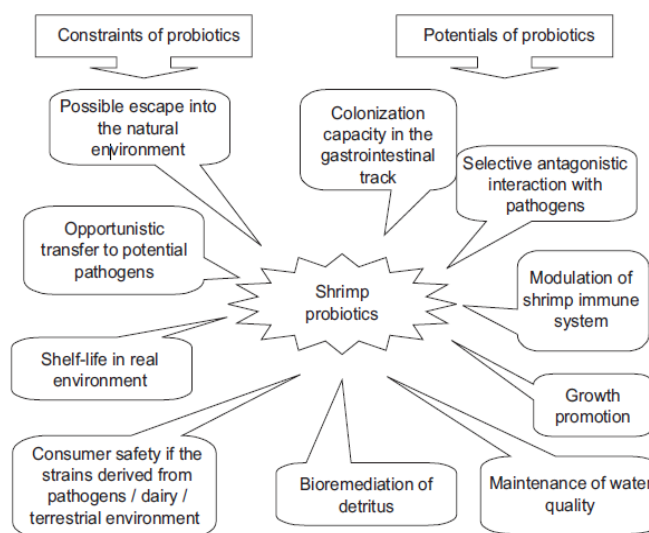


Figure 1. Potentials and constraints of probiotics in shrimp aquaculture

			ml-1 fo 3 days and <i>V.fluvalis</i> PM 17@10 ³ bacterial cells ml ⁻¹ for seven days	
Arthrobacter XE-7	Isolated from <i>Penaeus chinensis</i>	Protection of <i>Penaeus chinensis</i> post-larvae from pathogenic vibrios such as <i>V.parahaemolyticus</i> , <i>V.anguillarum</i> and <i>V.nereis</i>	106 CFU/ml	Li <i>et al.</i> 2006
Bacillus subtilis and B.megaterium	Marine environment	Production of digestive enzymes proteases, carbohydrases, and lipases	Potential application in shrimp feeds	Solano and Soto 2006
Paenibacillus spp,B.cereus and Pa.polymyxa synechocystis MCCB 114 and 115	Sea water,sediment and marine fish gut samples seawater	Against pathogenic Vibrios. Antagonism against <i>V.harveyi</i>	10 ⁴ and 10 ⁵ CFU ml ⁻¹	Ravi <i>et al</i> 2007
Bacillus licheniformis	Shrimp pond	Intestinal microbiota and immunity of the white shrimp <i>Litopenaeus vannamei</i>	Post larvae fed on the cyanobacterial cultures <i>B.licheniformis</i> suspension of 10 ⁵ CFU ML ⁻¹ for 40 days	Preetha <i>et al.</i> 2007
Lactic acid bacteria	Shrimp gut	Survival of marine shrimp, <i>Litopenaeus vannamei</i> challenged with <i>V.harveyi</i> .	Liquid diet supplemented with B6 strain at 10(8)CFU/ml	Vieria <i>et al.</i> 2007 Li <i>et al.</i> 2007
Lactobacillus plantarum	Shrimp isolate	Immune response and microbiota of shrimp, <i>Litopenaeus vannamei</i> challenged with <i>V.alginolyticus</i> ,and <i>V.harveyi</i>	10 ¹⁰ CFU /kg diet/10 ⁸ CFU/kg feed	Chiu <i>et al.</i> 2007
Vibrio alginolyticus UTM 102 ,Bacillus subtilis UTM 126,Roseobacter gallaeciensis SLV22	Gastrointestinal tract of adult shrimp <i>Litopenaeus vannamei</i>	Antagonism against the shrimp-pathogenic bacterium , <i>V. parahaemolyticus</i> PS -017	Feed supplement	Balcazar <i>et al.</i> 2007
Bacillus subtilis UTM 126	Shrimp culture pond	Protection against vibriosis in juvenile <i>Litopenaeus vannamei</i> PS-017	105 CFU/g	Balcazar and Rojas Luna 2007
Pediococcus acidilactici	Strain MA 18/5 M,CNCM	Survival of <i>Litopenaeus stylirostris</i> against <i>Vibriosis</i> caused by <i>V.nigripuchritudo</i>	Probiotic coated pellet feed	Mathieu <i>et al.</i> 2008
B.subtilis	Not available	Growth and digestive enzyme activity of <i>Litopenaeus vannamei</i>	1.5 to 7.5% supplemented to the feed	Gomez and Shen 2008.

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