



Litopenaeus Vannamei Their Bacterial Diseases Outbreak At Bhal Bhavnagar Aquaculture, Gujarat

¹Heenaba Jadeja, ²Apexa Patadia, ³Bharatsinh Gohil

¹Research scholar, ²Assistant Professor, ³Head of the department

¹Microbiology Department,

¹Atmiya University, Rajkot, India

Abstract: This research focused on India's shrimp culturing system, which is no less essential than shrimp culturing in other nations. India ranks second in aquaculture farming, however there has been a significant fall in shrimp production from 2019-21. Andhra Pradesh is India's top shrimp-producing state. The shrimp causes various illnesses, which prevents production from reaching its height in India. Climate, location, land, water parameters, correct feed, probiotics, and environmental characteristics are just a few of the aspects that shrimp growers must consider. Furthermore, illness outbreaks are a serious impediment. *Litopenaeus vannamei*, a white shrimp farmed in the Bhal area near Bhavnagar, was heavily affected with blackish spot infections despite the use of probiotics and strict adherence to environmental criteria that promote shrimp growth. In this investigation, samples were collected, and white shrimp from infected and healthy shrimps were isolated and compared. After homological, phylogenetic, and morphological analysis, the organism identified from diseased shrimp was *Acinetobacter indicus* CIP 110367 strain A648 and *Bacillus subtilis* strain NCDO 1769 from healthy shrimp gut flora.

Index Terms - “*Litopenaeus vannamei*, Gut flora, Diseased Shrimp, Phylogenetically, *Acinetobacter indicus*, *Bacillus subtilis*”

I. INTRODUCTION

Shrimp farming is one of the most exciting and popular farming enterprises today. Shrimps' quick development can lead to the most economically viable farming enterprise for aquaculture growers. Unfortunately, the advent of many illnesses has turned into an economic danger and has slowed industrial progress. This caused aquaculture growers to reconsider continuing in the same manner. Aquaculture farming is critical to world economics in a variety of ways. As a result, there should be limited options for overcoming this setback. There are numerous different species of shrimps, and aquaculture farmers all throughout the world raise various shrimps based on their atmospheric survival. White shrimp (*Litopenaeus vannamei*) are frequently utilized in aquaculture farming in Gujarat. Bacterial infections in shrimp have been reported for many years; typically, bacterial infection occurs. Bacterial disease is most serious threat and often caused mass mortality in shrimp larvae which greatly influenced the sustainable supply of healthy fry [1]

II. SHRIMP FARMING

Shrimps are often grown in ponds or impoundments on land. To do this, the ponds are first dried and tilled (to eliminate bugs and predators and metabolized organic debris) before being limed (to correct the pH and to keep the bottom free from microorganisms). The natural food species known as plankton (floating) and benthos are then developed using inorganic fertilisers such as urea and super phosphate (bottom living). Contamination is caused by plankton in aquaculture ponds, and the water is generally green or greenish-brown in color. Throughout the cultural era, this color is retained. Shrimp post larvae are stocked at different densities (numbers per sq meter) depending on the amount of development after these preparations. Supplement meal consists of pelleted meals containing roughly 40% protein. The meal comes in three sizes: starter, growth, and completion, depending on the size of the shrimp. Feed trays are used to quantify the level of feed to be supplied, and it is altered based on the rate of growth. Water quality is thoroughly monitored, and the ideal levels of critical factors like dissolved oxygen, pH, and salinity are maintained by frequent water exchange. The real exchange rate is determined by the amount of shrimp stocked and the pond's water quality. Some farmers utilize mechanical or electrical aerators to aerate their ponds. The significance of aquaculture in the global food production sector, aquatic resource planning, and the socio-economic growth of coastal remote regions is now well acknowledged. Globally, substantial progress has been achieved in making shrimp aquaculture development efficient and sustainable.

III. PROBIOTICS AND ITS SIGNIFICANCE IN SHRIMP FARMING

Parker (1974) coined the term "probiotic," which he defined as organisms that contribute to the gut microbial balance. Fuller (1989) extended the concept of probiotics to include a live microbial feed additive that improves the intestinal microbial balance of the host animal. The necessity of living cells as a crucial component of a prospective probiotic has been stressed in this updated definition, which also clarifies the uncertainty caused by the usage of the term substances. Probiotics is generally used to denote bacteria that promote health benefits of other organisms [2]. Probiotics were defined by Lilley and Stillwell (1965) as probiotic as substances secreted by one microorganism, which stimulated the growth of another. Probiotics are living bacteria that give a health advantage to the host when taken in sufficient amounts, according to an expert from the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (FAO, 2001).

Abbreviations and Acronyms

FAO- Food and Agriculture organization, **WHO-** World health organization, **TCBS-** Thiosulfate citrate bile salts sucrose agar, **SS-Salmonella Shigella agar**, **KOH-Potassium hydroxide**, **BLAST-** Basic Local Alignment Search Tool, **PCR-Polymerase chain reaction**, **DNA-** Deoxyribonucleic acid and **NCBI-** National Center for Biotechnology Information.

1.1 Types of Bacterial diseases in White Shrimp

Bacterial Septicaemia

A *Vibrio* infection was verified by normal microbiological and histopathological procedures to isolate bacteria from the shrimp haemolymph. *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, and *Vibrio spp.* It affects shrimps, causing them to get drowsy and swim improperly.

Brown spot or shell disease

Mostly on body surface appendages of the infected animal, there are brownish to black eroded regions. *Vibrio spp.*, *Aeromonas spp.*, and *Flavobacterium spp.* are the bacteria that cause illness. The tips of the shrimp's walking legs, swimmerets, and uropods become necrotic and become brownish or black as a result of this sickness. Broken and melanized setae, antennae, and appendages are common.

Filamentous Bacterial Disease

The affected shrimp larvae show fouling of gills, setae, appendages, and body surface. Mounting of affected shrimps is impaired and may die due to hypoxia. Bacteria such as *Leucothrix mucor*.

Vibriosis - Vibriosis, a disease caused by gram-negative bacteria is one of the major disease problems in the aquaculture of shellfish and finfish (Adams 1991; Chen et al 2000; Lavilla-Pitogo et al 1996; Lavilla-Pitogo et al 1998; Lightner et al 1992; Lightner & Lewis 1975). Some *Vibrio* species identified to cause vibriosis include *V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus*, *V. penaeicida* (Brock and Lightner 1990; Ishimaru et al 1995). *Vibrio* species are among the normal bacterial flora of both natural and cultural populations of shrimp and the culture environment (Jiravanichpaisal et al. 1994, Otta et al. 1999). Vibriosis is caused by *Vibrio spp.* such as *V. fluvalis* and *V. anguillarum*, which induce loss of appetite, sluggish swimming, pale bodies, enlarged cephalothorax with a light yellow to yellowish colour, hepatopancreas, and gills.

Luminescent Bacterial Disease

A major issue in hatcheries, juvenile and adult shrimp may be impacted in grow-out farms as well. *Vibrio harveyi* is the bacterium at fault. Bacteria found by microscopic examination of swimming bacteria within the haemocoel of moribund shrimp larvae would indicate luminous bacterial infections.

I. RESEARCH METHODOLOGY

2.1 Population and Sample

Samples were taken from Aquaculture of Bhavnagar, Gujarat. It includes diseased shrimp with black spots from the aquaculture ponds using the check tray. At least 4-6 trays used in 1 hectare to take a good result of giving feed and also to check the disease causing shrimp counts. There should be accurate estimate in number of shrimp in culture pond for the feed rations and to predict harvest size. If population is underestimated, then the animals will be underfed or overfed. Essential nutrients should be given with the fed, otherwise can cause oxygen depletion and toxic inorganic nitrogen accumulation.

2.2 practical framework

Live infectious shrimp were sampled and transported to the laboratory in a sterile ThermocoolR box within 1 hour, utilising about 40 shrimp approximately 6 times. The water in the ThermocoolR box was emptied after the shrimp were washed in deionized water. The diseased shrimps were taken, and the area with black spots disease portion was diluted till 10-5 times in order to isolate colonies of the responsible disease-causing organisms into the Zobell marine agar medium (for halophiles cultivation), nutrient agar medium, and special medias (TCBS agar, MacConkey's agar, SS agar, King's B agar, TSI agar slant) for identification and characterization and also Gram's staining and 3% KOH procedures were also used for identification. After isolating DNA from the culture and evaluating its quality on 1.0 percent Agarose Gel, a single band of high-molecular-weight DNA was found. PCR was used to amplify a gene fragment. When resolved on Agarose Gel, a single distinct PCR amplicon band was seen. To eliminate impurities, the PCR amplicon was purified using column purification. On the ABI 3730xl Genetic Analyzer, a PCR amplicon DNA sequencing reaction was performed with primer27F using the BDT v3.1 Cycle sequencing kit. The gene sequence was utilized to perform BLAST against the NCBI GenBank database. The first 10 sequences were chosen based on their maximum identity score and aligned using different alignment software packages.

II. RESULTS AND DISCUSSION

3.1 Results

As a result of our detailed experimental study, we observed that 23 pure isolated from which maximum growth was seen in Zobell marine agar media and Nutrient agar medium, which produces a maximum growth yield and is favorable to disease causing and gut flora of shrimp organisms. Identification using 3% KOH is also a simple, rapid, and conclusive gram organism identification approach. The evolutionary history was derived by phylogenetic analysis utilizing the Neighbor-Joining technique. Based on the results of this experiment, we may conclude that, D₂ZW(isolated from diseased shrimp and from Zobell marine agar growth culture) showed high similarity with *Acinetobacter indicus* CIP 110367 strain A648 and from ND₂NAW(Non- diseased shrimp and Nutrient agar growth culture) Showed similarity with *Bacillus subtilis* strain NCDO 1769

Figures and Tables



Fig 1 Diseased and normal shrimps and their dissection procedure.

Phylogenetic Tree:

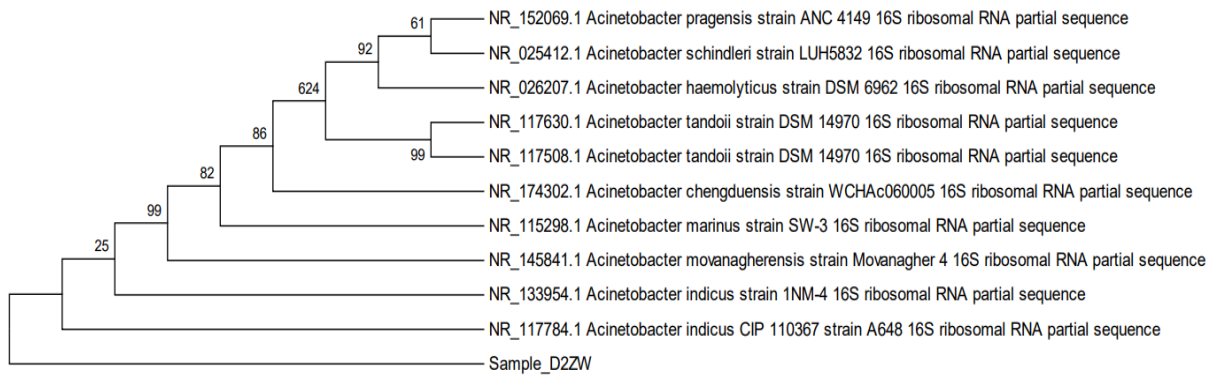


Fig2. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.47522388 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 901 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Phylogenetic Tree:

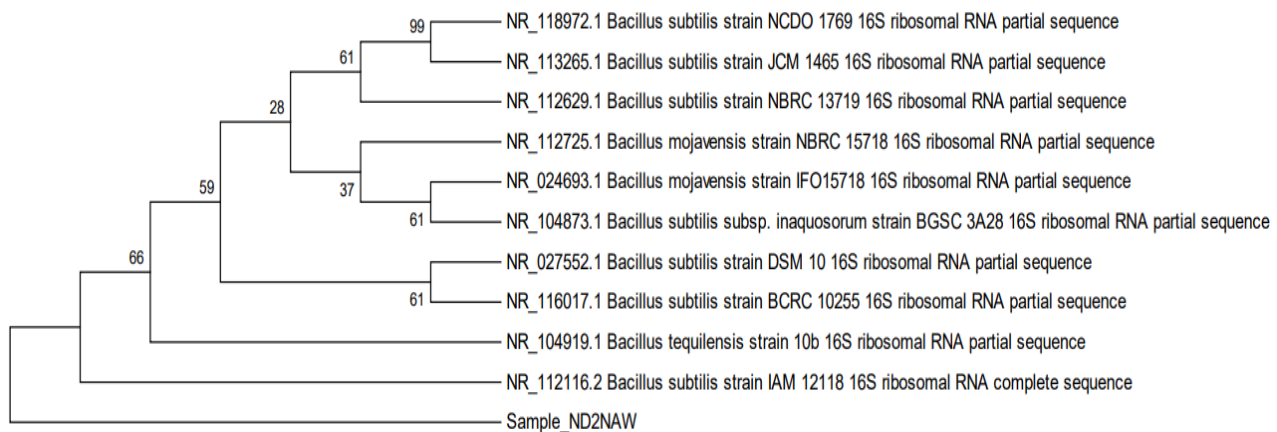


Fig3. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.03281060 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 370 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

IV. ACKNOWLEDGMENT

Greatly acknowledge Atmiya University, Rajkot and Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar.

REFERENCES

- [1] Biju V. N and B. Gunalan, Prevalance of Vibrio infection in penaeus (Litopenaeus) vannamei farms, international journal of science inventions today, ,2016, 5(6), 485-493.
- [2] Vikash Kumar, Suvra Roy, Dharmendra Kumar Meena & Uttam Kumar Sarkar, Application of Probiotics in Shrimp Aquaculture: Importance, Mechanisms of Action, and Methods of Administration, Reviews in Fisheries Science & Aquaculture, 24:4, 342-368, 10.1080/23308249.2016.1193841
- [3] Duan Y, Wang Y, Dong H and Zhang J(2018) Changes in the intestine microbial, digestive and immunerelated genes of Litopenaeus vannamei in response to dietary probiotic Clostridium butyricum supplementation. Front. Microbial. 9:2191, 10.3389/fmicb.2018.02191
- [4] E.H.Pinn, L.A.Nickell A.Rogerson R.J.A . Atkinson, Comparison of gut morphology and gut microflora of seven species of mud shrimp (Crustacea: Decapoda: Thalassinidea), Jan 1999, Marine Biology, 133(1):103-114, 10.1007/s002270050448.
- [5] F.N. Vieira¹ , C.C. Buglione¹ , J.P.L. Mouriño¹ , A. Jatobá¹ , M.L. Martins¹ , D.D. Schleder² , E.R. Andreatta¹ , M.A. Barraco² , L.A. Vinatea¹, Effect of probiotic supplemented diet on marine shrimp survival after challenge with Vibrio harveyi, Arq. Bras. Med. Vet. Zootec., v.62, n3, p.631-638, 2010
- [6] Zheng Z, Aweya J, Wang F *et al*, Acute Hepatopancreatic Necrosis Disease (AHPND) related microRNAs in Litopenaeus vannamei infected with AHPND-causing strain of Vibrio parahemolyticus, BMC Genomics, 2018, 19:1, 10.1186/s12864-018-4728-4.
- [7] Nithya Nand P, Indhumathi T, Ravi A *et al.*, Culture independent characterization of bacteria associated with the mucus of the coral Acropora digitifera from the Gulf of Mannar, World Journal of Microbiology and Biotechnology,2011, 1399-1406, 27(6), 10.1007/s11274-010-0591-4.
- [8] Far H, Saad C, Daud H *et al.*, Isolation and identification of bacteria micro flora of white shrimp, litopenaeus vannamei, with antagonistic properties against Vibrio species, Asian Journal of Animal and Veterinary Advances, 2013, 293-300,8(2), 10.3923/ajava.2013.293.300.
- [9] Farzanfar A, The use of probiotics in shrimp aquaculture, FEMS Immunology and medical microbiology, 2016, 149-158, 48(2), 10.1111/j.1574-695X.2006.00116. x.
- [10] Venkat HK, Sahu NP & Jain KK, Effect of feeding lactobacillus based probiotics on the gut micro flora, growth and survival of post larvae of Macrobrachium rosenbergii (deman), 2014, Aquaculture Res35: 501–507.
- [11] Verschuere L, Rombout G, Sorgeloos P & Verstraete W, Probiotic bacteria as biological control agents in aquaculture. Microbiol, 2000, Mol Biol Rev 64: 655–671.
- [12] Meunpol O, Loponyosiri K & Menasveta P, The effects of ozone and probiotics on the survival of black tiger shrimp (Penaeus monodon), 2003, Aquaculture 220: 437–448.
- [13] Kumar V, Roy S, Meena D *et al.*, Application of Probiotics in Shrimp Aquaculture: Importance, Mechanisms of Action, and Methods of Administration, Reviews in Fishies Sciences and Aquaculture, 2016, 342-368, 24(4), 10.1080/23308249.2016.1193841.
- [14] Senok, A. C., A. Y. Ismeel, and G. A. Botta. Probiotics: facts and myths. Clin. Microbiol. Infect. 11: 958–960 .
- [15] Sharma, P., C. Ram, C. R. Sihag, and S. K. Gahlawat. Relative efficacy of two probiotics in controlling the Epizootic Ulcerative Syndrome disease in Mrigal (Cirrhinus mrigala Ham). J, 2013, Fish. Aquat. Sci., 8: 305–322.
- [16] Sherman, P. M., J. C. Ossa, and H. K. Johnson. Unravelling mechanisms of action of probiotics, (2009), Nutr. Clin. Pract., 24(1): 10–14.
- [17] Sihag, R. C. and P. Sharma. Probiotics: the new eco-friendly alternative measures of disease control for sustainable aquaculture, (2009), J. Fish. Aquat. Sci., 7: 72–103.
- [18] Skjermo, J. and O. Vadstein. Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture, (1999), 177: 333–343.
- [19] Song, Z., T. Wu, L. Cai, L. Zhang, and X. Zheng. Effects of dietary supplementation with Clostridium butyricum on the growth performance and humoral immune response in Miichthys miiuy. J. Zhejiang Univ. Sci. B, (2006), 7: 596–602.
- [20] Sorroza, L., D. Padilla, F. Acosta, L. Roman, V. Grasso, J. Vega, and F. Real. Characterization of the probiotic strain Vagococcus fluvialis in the protection of European sea bass (Dicentrarchus labrax) against vibriosis by Vibrio anguillarum. Vet. Microbiol., (2012), 155: 369–373
- [21] Sotomayor, M. A. and J. L. Balcazar. Inhibition of shrimp pathogenic vibrios by mixture of probiotic strain. Rev, (2003), Aquat. Sci., 19: 9–15.
- [22] Soundarapandian, P. and S. Sankar. Effect of probiotics on the survival and production of black tiger shrimp (Penaeus monodon, Fabricius), (2008), Int. J. Zool. Res., 4: 35–41.