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Effect of a Commercial Probiotic and *Cassia auriculata* Leaf Powder on Vibriosis Induced Freshwater Prawn, *Macrobrachium rosenbergii*

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ABSTRACT

A study was carried out to determine the influence of a commercial probiotic ('Aqualact') and dried leaf powder of *Cassia auriculata* against *Vibrio parahaemolyticus* infection in the freshwater prawn, *Macrobrachium rosenbergii*. Histological studies in the hepatopancreas and gills of prawns, during infectivity trials, showed bacterial invasion and multiplication in their lumens. Oedema and cell necrosis were the major pathological changes observed in gills and hepatopancreas. Degenerative changes in the electrophoretic pattern of muscle proteins were proportional to the concentration of vibrios infected. Infected prawns fed on probiotic supplemented feed could show a regaining trend towards normal protein bands. Marked reduction in mortality could be observed in infected prawns fed on feed supplemented with probiotic as well as with powdered *Cassia auriculata* leaves.

Key words: Disease resistance, *Cassia auriculata*, probiotics, *Macrobrachium rosenbergii*, *Vibrio parahaemolyticus*

INTRODUCTION

Vibriosis is a major problem in prawn culture, causing heavy mortality and severe economic loss. Though they are members of the normal microbiota of prawns, *Vibrio* species often act as opportunistic pathogens or secondary invaders and many induce mortality even up to 100% in the affected population (Bell and Lightner, 1988; Rosemark and Fisher, 1988). Major species causing vibriosis in prawns are *Vibrio alginolyticus*, *V. harveyi*, *V. anguillarum* and *V. parahaemolyticus* (Bell and Lightner, 1988; Ruangpan and Kitao, 1991; Nash *et al.*, 1992; Xu *et al.*, 1994; Chanratchakool *et al.*, 1995; Alapide-Tendencia and Dareza, 1997). Bacterial septicemia is a common symptom of vibriosis (Mermoud *et al.*, 1998).

These moribund prawns display a wide spectrum of clinical signs, like disoriented swimming, lethargy, weakness and abnormal colouration of body and appendages. Infections due to *Vibrio* species are prevalent in hatcheries and grow-out facilities, causing mass mortalities and shell deformities (Lavilla-Pitogo *et al.*, 1990).

Generally farmers apply antibacterial compounds to combat microbial infection. A wide range of antimicrobial drugs (oxytetracyclin, ciprofloxacin, chloramphenicol) are used in hatcheries and farms of freshwater prawns and marine shrimps in India (Karunasagar *et al.*, 1994; Hameed and Balasubramanian, 2000). Indiscriminate use, or the large scale prophylactic application of antimicrobial compounds leads to the development of antibiotic resistant microorganisms which may cause multiple antibiotic resistance-transfer to other pathogenic bacteria (Frappalo and Guest, 1986; Moriarty, 1997). Transfer of resistance to human pathogens and gut bacteria is also a major concern (Salyers, 1995).

Increased concern about the emergence of antibiotic resistant microorganisms has led to alternative options for disease prevention, such as the use of non-pathogenic bacteria such as probiotic biocontrol agents (Austin and Allen, 1982; Murthy, 1997). Probiotics are widely referred to as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microfloral balance" (Fuller, 1989). The use of probiotics in aquaculture is not restricted to feed alone. It has also been used to modify the living environment of cultured-prawns (Verschuere *et al.*, 1997; Robles *et al.*, 1998).

Probiotics generally include bacteria, cyanobacteria, microalgae and fungi. The possible mode of probiotic-action is by the production of inhibitory compounds, siderophores, hydrogen peroxide, protease or organic acids (Vandenbergh, 1993; Sugita *et al.*, 1997). Competition for chemicals and available energy and competition for adhesion sites are the other possible modes of antagonism (Verschuere *et al.*, 2000). Enhancement of immune response and enzymatic contribution to digestion may also be the ways by which probiotics act (Balcazar *et al.*, 2006).

Another alternative disease prevention method is the use of medicinal plant extracts. Besides many others, members of the genus *Cassia auriculata* are noteworthy for their diverse medicinal properties. The leaves and petals are traditionally used for their antihyperglycaemic action, antihaemorrhaging properties and antimicrobial activity (Chythanya *et al.*, 2002). Studies on the effect of medicinal plants against Yellow Head Virus (YHV) and Systemic Ectodermal and Mesodermal Baculovirus (SEMBV) in *Penaeus monodon* are available (Ruangpan, 1998).

About 25 species of *Macrobrachium* were found in India, mostly inhabit in freshwater (Soundarapandian and Kannan, 2008). *M. rosenbergii* culture is spreading fast to all Indian states due to its large size attainment, tolerance water quality changes, stress and ability to feed on unconventional feeds (Yathavamoorthi *et al.*, 2010). Vibriosis in *Macrobrachium rosenbergii* is a less explored area and disease management through biocontrol agents is yet to emerge. Hence, this investigation is intended to assess the inhibitory activity of a commercial probiotic ('Aqualact') and dried leaf powder of *Cassia auriculata* against *Vibrio parahaemolyticus* infection in *M. rosenbergii*.

MATERIAL AND METHODS

Pathogenic bacterial strains: Bacterial strains of *V. parahaemolyticus* were collected from *P. monodon* with black gill disease. *V. parahaemolyticus* used in this study were identified using standard morphological, physiological and biochemical tests (Holt *et al.*, 1994). Optimal growth was noticed between 28-35°C in Zobell's marine agar, Citrate agar and MacConkey agar media (Himedia, Bangalore). Up to 8% sodium chloride concentration in nutrient agar medium favored the growth of bacteria. *V. parahaemolyticus* were maintained in TCBS agar medium (Himedia, Bangalore).

Diet formulation: Pelleted feed was formulated for prawns following the guidelines of Boonyaratpalin and New (1993) and its percentage composition by weight is given below: Trash fish (28.35), Soyabean meal (11.34), fish meal (2.84), Cornmeal (22.68), Di-Calcium phosphate (0.57), Vitamin and mineral mix (0.20), Broken rice-boiled (8.5), Chicken feed (21.6), Shrimp shell meal (4.25).

Probiotic: A commercial shrimp-farm probiotic, 'Aqualact' manufactured by Biostadt Agrisciences (Wockhardt, India) was used in the present study. The composition of the probiotic per kilogram of the substance is given below: *Lactobacillus sporogenus* (45,000 million CFU), *Lactobacillus acidophilus* (45,000 million CFU), *Bacillus licheniformis* (30,000 million CFU), *Bacillus subtilis* (30,000 million CFU), *Saccharomyces cerevisiae* (1,25,000 million CFU), sea weed extract (100 g), enzymes: amylase (24,000 IU), phytase (22,00,000 IU), protease (4,00,000 IU), cellulase (150-250 IU), beta-galactosidase (800-1000 IU), lipase (50-100 IU); Vitamin C, Vitamin B1 and B6. The regular feed of prawns was enriched by the probiotic at 1 and 3% of feed weight using agar as the binding agent. Serial dilution and pour plate methods of bacterial enumeration could confirm the viability and density of the component microflora of 'Aqualact'.

Plant material: *Cassia auriculata* is a perennial shrub growing wild, reaching up to 60 cm height. Leaves were collected, dried in shade, ground into fine powder and stored in closed containers. The leaf powder was added at 1 and 3% by weight as supplement to the feed at its formulative stage using agar as binding agent.

Experimental animal and experimental design: Healthy, freshwater prawns *M. rosenbergii* were collected from a private farm at Perambalur district in Tamil Nadu, India which had no prior history of vibriosis. Before acclimatization they were washed for few a seconds in 0.1% benzalkonium hydrochloride to clear the adhering bacteria. Animals were washed three times in sterilized water, after benzalkonium chloride treatment. They were acclimatized in 60 L PVC troughs for a week, where optimum hydrological conditions were maintained. The prawns were fed daily at three intervals with formulated feed at 3% body weight.

The experimental animals were divided into six sets, each set in two groups comprising of 10 animals each. First set was kept as control set, fed only with normal formulated feed and instead of injecting pathogenic bacteria, appropriate quantities of physiological saline were injected to them. Second set of animals, fed on normal formulated diet, were injected with pathogenic bacterial suspension as detailed later. Third and fourth sets of animals were fed with 1 and 3% bacteria-incorporated feeds, respectively. These groups were challenged with pathogenic bacteria through injections. Fifth and sixth sets of animals were fed respectively with 1 and 3% *Cassia auriculata* leaf powder incorporated feeds and challenged with vibrio. Before the pathogen challenge, prawn sets were kept on their respective diets for 14 days.

LD_{50} test: *V. parahaemolyticus* stock suspension was diluted at 1:100000, 1:1000, 1:100, 1:10 with sterile physiological saline. Prawns were injected with 0.1 mL of each dilution of bacterial suspension. Mortality was recorded for two weeks and the LD_{50} was determined by employing probit analysis (Statistical package SPSS-10). Enumeration of vibrios at the LD_{50} concentration was carried out by standard pour plate technique using Nutrient Agar medium, with 8% NaCl.

Pathogen challenge test: After feeding the animals for two weeks with the probiotic (3rd and 4th set) and leaf powder supplemented food (5th and 6th sets), prawns in each group were challenged with *V. parahaemolyticus*. All the prawns were injected with 0.1 mL *V. parahaemolyticus* suspension in physiological saline intramuscularly at the LD_{50} dosage between their 3rd and 4th abdominal segments. The number of bacteria in the suspension was standardized by adjusting its absorbance in a spectrophotometer at 600 nm. The injected animals were observed for behavioural changes. The mortality/infectivity percentage was estimated after Sung *et al.* (1994):

$$\text{Mortality (\%)} = \frac{\text{(No. of dead /infected prawns-A)}}{\text{(Total No. of prawns-A)}} \times 100$$

where, A is the number of prawns dead in first day after injection due to administration stress.

Randomly selected animals from control and from experimental groups of animals were dissected using sterilized instruments. For histological studies, tissues were fixed in Davidson's fixative (Bell and Lightner, 1988), embedded in paraffin wax, sectioned at 5 micron thickness and stained with haematoxylin and eosin.

Electrophoresis of muscle proteins: The 12% Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) modified protocol (Laemmli, 1970) was used at present. Muscle proteins were identified by running molecular mass reference standards (Bangalore Genei Cat No: PMW-M) containing Phosphorylase 97.4, Bovine Serum Albumin 66, Ovalbumin 43, Carbonic Anhydrase 29, Soyabean Trypsin Inhibitor 20 and lysozyme 14.4 kDa). Electrophoresis was carried out on a constant voltage (50 V) at room temperature for 4 h.

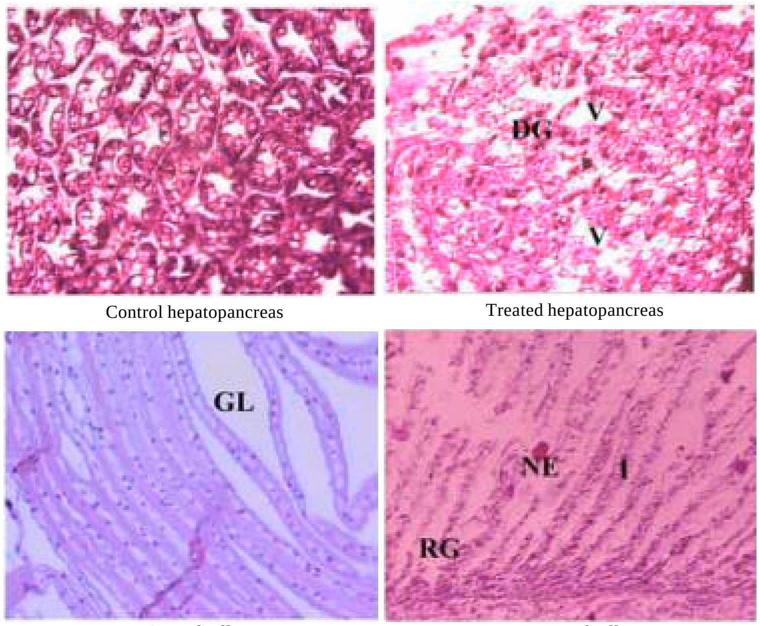
Protein detection by Coomassie Brilliant Blue (CBB) stain: Once the gel electrophoresis was completed the gel was rinsed with distilled water for 2 min and stained with 0.5% CBB R-250 in a solution of 40% methanol and 10% acetic acid at room temperature, for 2 h. The stained gel was destained in a solution containing 40% methanol and 10% acetic acid, until appropriate background was obtained. The gel was washed with distilled water and stored in refrigerator. The lanes selected for electrophoresis are explained in the legends for electrophorogram (Fig. 2).

RESULTS

Histopathology of the hepatopancreas of infected animals showed an invasion of its tubular lumens by vibrio and a reduction in lipid vacoules. Hepatopancreatic cells lining the tubules were walled off by haemocytes around the thickened basal lamina. The interstitial tissues around these invaded tubules were swollen, owing to oedema. Moreover, granulomatous lesions were formed; the thickened basal lamina underwent coagulation and a small number of bacteria remained among cellular debris (Fig. 1).

Gills of infected animals showed multifocal lamellar fusion, hyperplasia of epithelial cells and adhesion of the lamellar tips. Cyst like structures formed between lamellae became necrotic. Multifocal epithelial cell-lifting due to oedema of the lamellar tissue could be observed with separation of epithelial cells extending the length of lamellae. Necrotic tissue was characterized by loss of cellular integrity, karyolysis and infiltration by leucocytes (Fig. 1).

The biochemical characteristics of *V. parahaemolyticus* isolated from diseased *P. monodon* are presented in Table 1. After feeding the prawns, with supplemented feeds of 1 and 3% probiotic and *Cassia* leaf powder for fourteen days, they were challenged with LD_{50} dosage of *V. parahaemolyticus* (7.2×10^5 CFU). After three weeks of experiment about 59% mortality could be observed in vibrio challenged prawns. 33.2% mortality was observed in prawns supplemented with



Control hepatopancreas | Treated hepatopancreas

Control gill | Treated gill

DG: Degangulation
V: Vacuole
I: Invaded tublues
NE: Necrosis
GI: Gill lamellae
RG: Rupture of gills

Fig. 1: Pathological effects of Vibriosis in the hepatopancreas and gills of *Macrobrachium rosenbergii*

Table 1: Main phenotypic characteristics of the strain *Vibrio parahaemolyticus* isolated from black gill diseased *Penaeus monodon*

Characteristics	*Vibrio parahaemolyticus*
Gram	-
Motility	+
Oxidase	+
Catalase	+
TCBS	+
Swarming on solid media	+
Methyl red	+
Voges-Proskauer	-
Simmons Citrate	-
H_2S production	-
Gelatinase	+
Nitrate reduction	+
Acid from	
Glucose	+
Mannitol	+
Sucrose	-
Arabinose	+

Table 1: Continued

Characteristics	*Vibrio parahaemolyticus*
Ornithine decarboxylase	+
Arginine dihydrolase	-
Lysine decarboxylase	+
Growth on NaCl	
0% NaCl	-
1% NaCl	+
6% NaCl	+
8% NaCl	+
Resistance/Sensitivity to	
O/129	S
Ampicillin	R
Tetracycline	S
Chloramphenicol	S

-: Absent, +: Present

Table 2: Mortality percentage of *M. rosenbergii* following *V. parahaemolyticus* challenge by intramuscular injection after supplementation with probiotic and *Cassia auriculata* leaf powder for three weeks

Treatment	Mortality (%)
Control	Nil
Vibrio infected prawn	59.0±1.0
1% Probiotic supplementation	33.2±0.2
3% Probiotic supplementation	13.1±0.2
1% *Cassia* leaf powder supplementation	46.3±0.4
3% *Cassia* leaf powder supplementation	23.1±0.2

1% concentration of probiotic, whereas the mortality was 46.3 in 1% leaf powder supplemented group. A mortality rate of 13.1% was observed in 3% probiotic supplemented group and in 3% leaf powder supplemented group, the mortality was 23.1% (Table 2).

Electrophoretic pattern of various polypeptide fractions of the muscle tissue of the control prawn and muscle tissues of the animals treated with probiotics and infected with vibrios are shown in Fig. 2. More than 16 polypeptide fractions were seen electrophoresed in control tissue (Fig. 2, lane 2). Major fractions could be seen at 68.0, 57, 36, 32, 29, 22 and 14.3 kDa regions. Lane 3 shows the nature of polypeptide fractions of muscle tissue of prawns injected with a stock suspension of vibrio. In these prawns, muscle proteins were fused together and did not show any separation of fractions. Lane 4 shows the polypeptide fractions of muscle tissue from prawns injected with sublethal concentration of vibrio. In this lane, disappearance of some of the fractions and the decrease in intensity of few fractions (36 kDa) could be observed. In lane 5, where the muscle tissue of prawns injected with LD_{50} concentration of vibrio colonies, further reduction in the intensity of polypeptide fractions, could be observed. Lane 6 shows the recovery of prawns treated with 3% concentration of probiotic. This lane showed the reappearance of polypeptide fractions that disappeared in lane 5. Prominent fractions could be seen between polypeptide such as 14.3 and 68 kDa. Almost similar results were observed in lane 7 also, where the muscle tissue was from prawns treated with 1% concentration of probiotic. When compared to lane-6, bands at 18 and 20 kDa were less intense. Basically all the animals infected with vibrios showed the disappearance of polypeptide fractions at 14.3 kDa and formation of a new fraction at 82.3 kDa, irrespective of the probiotic treatment.

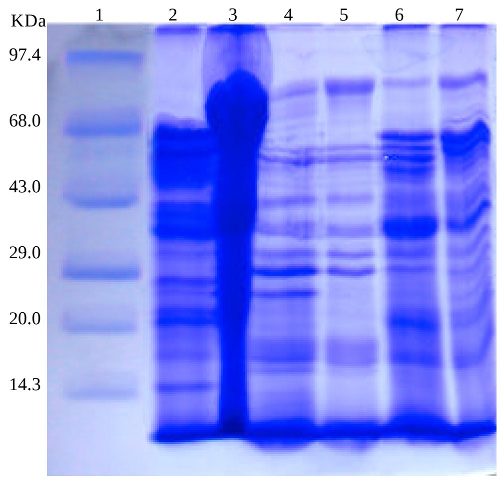


Fig. 2: Electrophorogram of proteins in the muscle of *M. rosenbergii* fed on probiotic incroporated diet. Lanes (1): Marker, (2): Central diet fed prawns. Vibrie injected prawns (3): Vibrie injected at stock concentration, (4): Vibrie at sublethal concentration, (5): Vibrie at LD50 concentration, (6): Vibrie challenge at prawns kept on 30% probiotic, (7): Vibrie challenge prawns kept on 1% probiotic supplemented diet

DISCUSSION

Most bacterial diseases like vibriosis, strike when the culture organisms are in a weakened or stressed condition. Vibriosis is a serious problem in the hatcheries and farms of marine shrimps and freshwater prawns (Takahashi *et al.*, 1985; Hameed *et al.*, 1996). Like many other species of vibrio, like *V. harveyi*, *V. anguillarum* and *V. ordalii* (Austin *et al.*, 1995), *V. parahaemolyticus* has also been recognized to be pathogenic to prawn larvae in saltwater and freshwater hatcheries (Sae-Qui *et al.*, 1987; Lavilla-Pitogo *et al.*, 1992; Tonguthai, 1992).

In the present study, histopathological manifestations of vibrios in the gills and hepatopancreas of *M. rosenbergii*, could be observed and the pathological changes observed were comparable with earlier studies. Numerous tubules of the hepatopancreas had reduced number of stored lipid vacuoles and many were dilated and devoid of epithelial cells. These changes suggest that the prawns under pathological stress, were probably not eating well or metabolizing feeds normally (Fig. 1). Villalon (1993) also made similar suggestion in his work on *Vibrio alginolyticus* infestation of *Artemia*. Jiravanichpaisal *et al.* (1994) and Esteve and Herrera (2000) could demonstrate the granulomatous appearance of hepatopancreatic tubules in vibrio-infected *P. monodon*. They suggested that, the host was producing inflammatory response in an attempt to destroy or wall off the injured tissues. A similar inflammatory response to vibriosis in the hepatopancreas of *M. rosenbergii* could be observed at present. Hepatopancreas important site of vitelline synthesis and total protein content fluctuation during the reproductive cycle in *M. rosenbergii* was studied by Shanju and Geraldine (2011).

Detachment and thickening of basal lamina from hepatopancreatic tubule, reduced lumen, proliferation of blood cells into the lumen and degeneration of tubules appear to be the basic response of shrimps to toxicants (Nagesh *et al.*, 1999). Similar observations were made in *P. monodon* exposed to Gusathion A (Baticados and Tendencia, 1991) and Endosulfan (Pillai, 1991) and in *P. indicus* exposed to Phosphamidon (Renukaprasad, 1993). Detachment of basal lamina, loss of structural integrity and reduced lumen could also be noticed in the hepatopancreas of *P. monodon*, exposed to Perfekthion (Vogt, 1987). The degenerative changes in the hepatopancreas of *M. rosenbergii* observed at present, under vibrio infestation were similar to those general changes effected by pesticides in penaeids.

Gills of vibrio-infected *M. rosenbergii* also showed various degenerative changes like necrosis, vacoulation of nuclei leading to karyolysis, oedema and cellular disintegration. Observations similar to those in *M. rosenbergii* could be recorded by Karunasagar *et al.* (1997) in virus-affected shrimps from the west coast of India. Egusa *et al.* (1988) and Mohney *et al.* (1994) have reported that gills of many vibriosis affected penaeids may also show evidence of bacteria and amorphous debris accumulated to the surface of the secondary gill filaments. This might be due to decreased cleaning activity by the animals or increased suspended material in the water column (Bauer, 1977, 2002; Martin *et al.*, 2000). Loss of the regular surface structure of gill may result in gill fouling as well (Bauer, 1979).

Vibriosis has generally been considered indicative of the unhealthy status of a culture system. Antimicrobial compounds are used prophylactically and as medicines in aquaculture. There exists a possibility of transmittance of drug resistant pathogens to human beings either through consumption of seafood or via farm effluents. Although, the use of probiotics in aquaculture is not as popular as in livestock management, their use can be an effective strategy in combating diseases in aquaculture (Gatesoupe, 1999; Gram *et al.*, 1999; Rengpipat *et al.*, 1998; Rengpipat *et al.*, 2000). The use of probiotic as biological control agents could be like a risk insurance under normal conditions and also highly effective during disease outbreaks. Several possible mechanisms have been proposed to explain the health-promoting action of probiotics such as competitive exclusion, supplementing the enzyme production of the host, water quality improvement, immuno-modulation and production of inhibitory compounds (Balcazar *et al.*, 2006). Several investigations have shown that the manipulation of gut microbiota through feed supplementation by probiotics bacteria can bring about increased disease resistance and survival rate (Villamil *et al.*, 2003; Balcazar and Rojas-Luna, 2007). Many of these beneficial strains also show immunostimulatory effect and increased phagocytic activity (Gullian *et al.*, 2004). *Lactobacillus acidophilus* isolated from the gut of marine prawn *P. monodon* exhibited bacteriocin activity can be used as probiotic strain (Karthikeyan and Santhosh, 2009). In the present study, a commercial probiotic with *Lactobacillus* and *Bacillus* as dominant components, was administered as feed-supplement (1 and 3%) to *M. rosenbergii*. Although lactobacilli are widely used probiotic for terrestrial mammals, their effectiveness against vibriosis in aquatic environment has also been established (Gatesoupe, 1994; Sugita *et al.*, 1998). Rengpipat *et al.* (1998, 2000) and Balcazar and Rojas-Luna (2007) have recorded the probiotic prospects *Bacillus* strains in the health and immunity enhancement of aquatic organisms such as fish and shrimps. In probiotic administered *M. rosenbergii*, a noticeable reduction in mortality could be observed (Table 2). Survival percentage of prawns were directly proportional to the dosage of probiotic supplemented. This might be indicative of the need for maintaining an ideal level of health promoting gut microbiota, through high level probiotic supplementation on a regular basis. The present results were comparable to those of Vaseeharan and Ramasamy (2003) for their study on the antagonistic effect of *Bacillus subtilis* against *V. harveyi* infected *P. monodon*. Enhanced survival rate, protein content with supplemented feed was observed in *M. rosenbergii* (Davassi, 2011). Inoculation of *Bacillus* S11 strain and a mixture of *Lactobacillus* spp. also have showed improved survival rate in the juveniles of *P. monodon*

(Gatesoupe, 1994; Rengpipat *et al.*, 1998; Rengpipat *et al.*, 2000; Otta and Karunasagar, 1999). Ziaei-Nejad *et al.* (2006) also reported the use of a commercial probiotic to increase the survival and enhance the growth in *Fenneropenaeus indicus*.

The devastating effect of vibriosis was evident in the electrophorogram of the muscle proteins of *M. rosenbergii* (Fig. 2, Lane 3). A structural disintegration was evident due to the heavy dosage of *V. parahaemolyticus*. With LD_{50} and sublethal dosages of *Vibrio*, the effect of pathogens on the structural assembly of polypeptides was not so intense. However, when the prawns were maintained on a probiotic supplemented diet, the effect of vibriosis on the polypeptide fractions was less evident. As there was no difference was exhibited in food and feeding habits of two species of *Macrobrachium* the formulated feed supplemented with probiotic can be used for all commercially important species (Bello-Olusoji *et al.*, 2006). In general, it may be concluded that probiotics help host animals to resist pathogen-induced biochemical changes, although, its specific mechanism needs further investigation.

Another effective alternative for chemotherapeutic control of microbes could be the herbal products which enhance the growth and also elicit antimicrobial activity (Karunasagar *et al.*, 1994; Hameed and Balasubramanian, 2000). Herbal products are presumed to be devoid of toxic chemical substances and hence, considered environment-friendly. Antimicrobial activity of two medicinal plants against common pathogens were reported in our earlier studies (Balasundaram *et al.*, 2011). Enhanced growth and increased survival rate against vibriosis have been reported in prawns when supplemented with the extracts of terrestrial plants and seaweeds (Ramesh *et al.*, 2002; Immanuel *et al.*, 2004). Vibriosis resistance has also been observed when Chinese medicinal herbs and the extracts of several seaweeds have been used as feed supplements (Jian and Wu, 2003; Selvin and Lipton, 2004; Huang *et al.*, 2006). Impact of three medicinal plants on biochemical parameters and combat against *Aeromonas hydrophila* in *Labeo rohita* was observed in our earlier studies (John *et al.*, 2011). In *Tilapia rendalli* fed with plant diets enhance growth performance and higher protein efficiency in turn resist diseases (Hlophe and Moyo, 2011). Pharmacological properties of *Cassia nigricans* Vahl, against human and veterinary diseases was reported by Attitalla (2011). Use of *Cassia auriculata* leaf powder as feed supplement in the present study also confirmed the health promoting property of the herbal extract, expressed as increased survival rate against *V. parahaemolyticus* infection (Table 2), although not to the extent as effected by the probiotic. However, as seen in the case of probiotic-use, increased supplementation (3%) with *Cassia auriculata* showed a proportionate reduction in mortality. Disease resistance potential of *Cassia auriculata* leaf powder, with reference to shrimps has already been demonstrated (Supamattaya *et al.*, 2005). Several medicinal plants were studied for their curative potential against vibriosis (Selvin and Lipton, 2004; Immanuel *et al.*, 2004). Experiments conducted so far using medicinal plants and their extracts, including the present study, underscore the effectiveness of many phytic principles in the mitigation of diseases common in cultured crustaceans and fishes.

CONCLUSION

In the present study, even though both probiotic and leaf powder supplemented prawns showed increased survival rate, leaf powder has been used in a crude form. Thus, it could effect only reduced survival rate compared to that of probiotic supplemented group. If the active compound from the plant is identified and used in the purified form, its effect on vibriosis may be further impressive.

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REFERENCES

Alapide-Tendencia, E.V. and L.A. Dureza, 1997. Isolation of *Vibrio* spp. from *Penaeus monodon* (Fabricius) with red leg disease syndrome. Aquaculture, 154: 107-114.

Attitalla, I.H., 2011. Pharmacological properties of *Cassia nigricans* Vahl against human and veterinary diseases. Asian J. Anim. Vet. Adv., 6: 886-887.

Austin, B. and D.A. Allen, 1982. Microbiology of laboratory hatched brine shrimp (Artemia). Aquaculture, 26: 369-383.

Austin, B., L.F. Stuckey, P.A.W. Robertson, I. Effendi and D.R.W. Griffith, 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseased caused by *Aeromonas salmonicida Vibrio anguillarum* and *Vibrio ordalii*. J. Fish Dis., 18: 93-96.

Balasundaram, A., P.R. Kumari, G. John and B.N. Selvakumar, 2011. Antimicrobial activity of leaf extracts of two medicinal plants against MRSA (Methicillin resistant *Staphylococcus aureus*) from human urinary tract pathogens. Res. J. Microbiol., 6: 625-631.

Balcazar, J.L. and T. Rojas-Luna, 2007. Inhibitory activity of probiotic *Bacillus subtilis* UTM 126 against *Vibrio* species confers protection against vibriosis in juvenile shrimp (*Litopenaeus vannamei*). Curr. Microbiol., 55: 409-412.

Balcazar, J.L., I. de Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell and J.L. Muzquiz, 2006. The role of probiotics in aquacultue. Vet. Microbiol., 114: 173-186.

Baticados, M.C.L. and E.A. Tendencia, 1991. Effects of Gusathion A on the survival and shell quality of juvenile *Penaeus monodon*. Aquaculture, 93: 9-19.

Bauer, R.T., 1977. Antifouling adaptations of marine shrimp (Crustacea: Decapoda: Caridea): Functional morphology and adaptive significance of antennular preening by the third maxillipeds. Mar. Biol., 40: 261-276.

Bauer, R.T., 1979. Antifouling adaptations of marine shrimp (Decapoda: Caridea): Gill cleaning mechanisms and grooming of brooded embryos. Zool. J. Linn. Soc., 65: 281-303.

Bauer, R.T., 2002. The ineffectiveness of grooming in prevention of body fouling in the red swamp crayfish, *Procambarus clarkii*. Aquaculture, 208: 39-49.

Bell, T.A. and D.V. Lightner, 1988. A Handbook of Normal Penaeid Shrimp Histology. 1st Edn., Word Aquaculture Society, Baton Rouge, Louisiana, ISBN: 0-935868-37-2.

Bello-Olusoji O.A., M. Bankole, A. Sheu and F.B. Oyekanmi, 2006. Availability, diet composition and feeding behaviours of some commercially important *Palemonaeidae* prawns in fresh and brackish waters of Nigeria. J. Biol. Sci., 6: 15-21.

Boonyaratpalin, M. and M.B. New, 1993. On farm feed preparation and feeding strategies for marine shrimps and freshwater prawns. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-made Aquafeed, December 14-18, 1992, Bangkok, Thailand, pp: 434.

Chanratchakool, P., M. Pearson, C. Limsuwan and R.J. Roberts, 1995. Oxytetracycline sensitivity of *Vibrio* species isolated from diseased black tiger shrimp, *Penaeus monodon* Fabricius. J. Fish Dis., 18: 79-82.

Chythanya, R., I. Karunasagar and I. Karunasagar, 2002. Inhibition of shrimp pathogenic vibrios by a marine *Pseudomonas* I-2 strain. Aquaculture, 208: 1-10.

Davassi, L.A., 2011. Survival and growth of the freshwater prawn *Macrobrachium rosenbergii* in relation to different nutrients composition. J. Fish. Aquat. Sci., 6: 649-654.

Egusa, S., Y. Takahashi, T. Itami and K. Momoyama, 1988. Histopathology of vibriosis in the Kuruma prawn, *Penaeus japonicus* Bate. Fish Pathol., 23: 59-65.

Esteve, M. and F.C. Herrera, 2000. Hepatopancreatic alterations in *Litopenaeus vannamei* (Boone, 1939) (Crustacea: Decapoda: Penaeidae) experimentally infected with a *Vibrio alginolyticus* strain. J. Invertebr. Pathol., 76: 1-5.

Frappalo, P.J. and G.B. Guest, 1986. Regulatory status of tetracycline, penicillin and other antimicrobial microbial drugs in animals feeds. J. Anim. Sci., 62: 86-92.

Fuller, R., 1989. Probiotics in man and animals. J. Applied Bacterol., 66: 365-378.

Gatesoupe, F.J., 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic *Vibrio*. Aquat. Living Res., 7: 277-282.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture, 180: 147-165.

Gram, L., J. Melchiorsen, B. Spanggaard, I. Huber and T.F. Nielsen, 1999. Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH 2, a possible probiotic treatment of fish. Applied Environ. Microbiol., 65: 969-973.

Gulhan, M., F. Thompson and J. Rodriguez, 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture, 233: 1-14.

Hameed, A.S.S. and G. Balasubramanian, 2000. Antibiotic resistance in bacteria isolated from *Artemia nauplii* and efficacy of formaldehyde to control bacterial load. Aquaculture, 183: 195-205.

Hameed, A.S.S., P.V. Rao, J.J. Farmer, F.W. Hickman-Brenner and G.R. Fanning, 1996. Characteristic and pathogenecity of *Vibrio Campbelli*-like bacterium affecting hatchery reared *Peneaus indicus* larvae. Aquat. Res., 27: 853-863.

Hlophe, S.N. and N.A.G. Moyo, 2011. The effects of different plant diets on the growth performance, gastric evacuation rate and caracass composition of *Tilapia rendalii*. Asian J. Anim. Vet. Adv., 6: 1001-1009.

Holt, J.G., N.R. Kreig, P.H.A. Sneath and J.T. Staley, 1994. Facultatively Anaerobic Gram Negative Rods. In: Bergey's Manual of Determinative Bacteriology, Baltimore, M.D. (Ed.). 9th Edn., Wilkins and Wilkins, USA., pp: 255-273.

Huang, X., H. Zhou and H. Zhang, 2006. The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, *Fenneropenaeus chinensis*. Fish Shellfish Immunol., 20: 750-757.

Immanuel, G., V.C. Vincybai, V. Sivaram, A. Palavesam and M.P. Marian, 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquaculture, 236: 53-65.

Jian, J. and Z. Wu, 2003. Effect of traditional Chinese medicine on non specific immunity and disease resistance of large yellow coraker, *Pseudosciaene crocea* (Richardson). Aquaculture, 236: 53-56.

Jiravanichpaisal, P., T. Miyazaki and C. Limsuwan, 1994. Histopatholagy, biochemistry and pathogenicity of *Vibrio harveyi* infecting black tiger shrimp *Penaeus monodon*. J. Aquat. Anim. Health, 6: 27-35.

John, G., P.R. Kumari and A. Balasundaram, 2011. Health promoting effects of three medicinal plants on normal and *Aeromonas hydrophila* infected *Labeo rohita*. J. Fish. Aquat. Sci., 6: 633-641.

Karthikeyan, V. and S.W. Santhosh, 2009. Study of bacteriocin as a food preservative and the *L. acidophilus* strain as probiotic. Pak. J. Nutr., 8: 335-340.

Karunasagar, I., R. Pai, G.R. Malathi and I. Karunasagar, 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture, 128: 203-209.

Karunasagar, I., S.K. Otta and I. Karunasagar, 1997. Histopathological and bacteriological study of white spot syndrome of *Penaeus monodon* along the west coast of India. Aquaculture, 153: 9-13.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.

Lavilla-Pitogo, C.R., L.J. Albright, M.G. Paner and N.A. Sunaz, 1992. Studies on the Sources of Luminescent *Vibrio harveyi* in *Penaeus monodon* Hatcheries. In: Diseases in Asian Aquaculture 1. Fish Health Section, Shariff, M., R.P. Subasinghe and J.R. Authur (Eds.). Asian Fisheries Society, Manila, Philippines, pp: 157-164.

Lavilla-Pitogo, C.R., M.C. L. Baticados, E.R. Cruz-Lacierda and L.D. De-la-Pena, 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture, 91: 1-13.

Lightner, D.V., 1998. Vibrio Disease in Penaeid Shrimp. In: Disease diagnosis and control in North American Marine Aquaculture, Sindermann, C.J. and D.V. Lightner (Eds.). Development in Aquaculture and Fisheries Science Publishing, New York, USA., pp: 42-47.

Martin, G.G., M. Quintero, M. Quigley and H. Khosrovian, 2000. Elimination of sequestered material from the gills of decapod crustaceans. J. Crustac. Biol., 20: 209-217.

Mermoud, I., R. Costa, O. Ferre, C. Goarant and P. Haffner, 1998. Syndrome 93 in new Caledonian outdoor rearing ponds of *Penaeus stylirostris*: History and description of the three major outbreaks. Aquaculture, 164: 323-335.

Mohney, L.L., D.V. Lightner and T.A. Bell, 1994. An epizootic of vibriosis in Ecuadorian pond reared *Penaeus vannamei* Boone (Crustacea: Decapoda). J. World Aquacult. Soc., 25: 116-125.

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture, 151: 333-349.

Murthy, H.S., 1997. The collapse of shrimp farming in India: An analysis. Infofish Int., 1: 38-40.

Nagesh, T.S., N. Jayabalan, C.V. Mohan, T.S. Annappaswamy and T.M. Anil, 1999. Survival and histological alterations in juvenile tiger shrimp exposed to saponin. Aquacult. Int., 7: 159-167.

Nash, G., C. Nithimathachoke, C. Tungmandi, A. Arkarjamorn, P. Prathanpipat and P. Ruamthaveesub, 1992. Vibriosis and its Control in Pond-Reared *Penaeus mondon* in Thailand. In: Diseases in Asian Aquaculture I, Fish Health Section. Shariff, M., R.P. Subasinghe and J.R. Arthur, Asian Fisheries Society, Manila, Philippines, pp: 143-155.

Otta, S.K. and I. Karunasagar, 1999. Bacterial flora associated with Shrimp culture ponds growing *Penaeus monodon* in India. J. Aqua. Trop., 14: 309-318.

Pillai, D., 1991. Responses of larval *Penaeus monodon* to Endosulfan and larval *Penaeus indicus* to *Vibrio harveyi*. M.F.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Ramesh, N., M.B. Viswanathan, A. Sarasawathy, K. Balakrishna, P. Brindha and P. Lakshmanaperumalsamy, 2002. Phytochemical and antimicrobial studies of *Begonia malabarica*. J. Ethnopharmocol., 79: 129-132.

Rengpipat, S., S. Rukpratanporn, S. Piyatiratitivorakul and P. Menasaveta, 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiotic bacterium (*Bacillus* S11). Aquaculture, 191: 271-288.

Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul and P. Menasveta, 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture, 167: 301-313.

Renukaprasad, G.L., 1993. Stress induced soft-shelling, its reversal and histopathology in juvenile *Penaeus indicus*. H. Milne Edwards. M.F.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Robles, R., P. Sorgeloos, H.V. Duffel and H. Nelis, 1998. Progress in bioremediation using live food. J. Appl. Icthyol., 14: 207-212.

Rosemark, R. and W.S. Fisher, 1988. Vibriosis of Lobster. In: Disease Diagnosis and Control in North American Marine Aquaculture, Sindermann, C.J. and D.V. Lightner (Eds.). Development in Aquaculture and Fisheries Science Publishing, New York, USA., pp: 240-242.

Ruangpan, L. and T. Kitao, 1991. *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. J. Fish Dis., 14: 383-388.

Ruangpan, L., 1998. Luminous Bacteria Associated with Shrimp Mortality. In: Advances in Shrimp Biotechnology, Flegel, T.W. (Ed.). National Center for Genetic Engineering and Biotechnology, Bangkok, pp: 205-211.

Sae-Qui, D., A. Transtapanich and L. Ruangpan, 1987. *Penaeus meruguiensis* department of fisheries, brackish water fisheries division. Technical Paper 6, Songkhla, Thailand (In Thai).

Salyers, A.A., 1995. Antibiotic Resistance Transfer in the Mammalian Intestinal Tract: Implications for Human Health, Food Safety and Biotechnology. Springer-Verlag, New York, pp: 109-136.

Selvin, J. and A.P. Lipton, 2004. *Dendrilla niger*, a marine sponge, as a potential source of antibacterial substances for managing shrimp diseases. Aquaculture, 236: 277-283.

Shanju, S. and P. Geraldine, 2011. Quantitative protein profile of three *Macrobrachium* species during reproductive cycle. Asian J. Anim. Vet. Adv., 6: 731-737.

Soundarapandian, P. and A. Kannan, 2008. South Indian technology of nursery farming for better survival and production of *Macrobrachium rosenbergii* (De Man). J. Fish. Aquatic Sci., 3: 137-144.

Sugita, H., N. Matsuo, Y. Hirose, M. Iwato and Y. Deguchi, 1997. *Vibrio* sp. strain NMIO, isolated from the intestine of the Japanese coastal fish has an inhibitory effect against *Pasteurella piscida*. Applied Environ. Microbiol., 63: 4986-4989.

Sugita, H., Y. Hirose, N. Matsuo and Y. Deguchi, 1998. Production of the antibacterial substance by *Bacillus* sp. strain NM 12, an intestinal bacterium of Japanese coastal fish. Aquaculture, 165: 269-280.

Sung, H.H., G.H. Kou and Y.L. Song, 1994. Vibriosis resistance induced by glucan treatment in tiger shrimp (*Penaeus monodon*). Fish Pathol., 29: 11-17.

Supamattaya, K., N. Sukrakanchana, M. Boonyaratpalin, D. Schatzmayr and V. Chittiwan, 2005. Effects of ochratoxin a and deoxynivalenol on growth performance and Immuno-physiological parameters in black tiger shrimp (*Panaeus monodon*). Songklanakarin J. Sci. Technol., 27: 91-99.

Takahashi, Y., Y. Shimoyama and K. Momoyoma, 1985. Pathogenecity and characteristics of Vibrio spp. isolated from culture Kuruma shrimp *Penaeus japonicas*. Bate. Bull. Jpn. Soc. Sci. Fish, 51: 721-730.

Tonguthai, K., 1992. Disease of the Fresh Water Prawn *Macrobrachium rosen bergii* in Thailand. In: Disease in Asian Aquaculture, Shariff, M., R.P. Subasinghe and J.R. Arthur (Eds.). Vol. 1., Asian Fisheries Society, Manila, pp: 89-95.

Vandenbergh, P.A., 1993. Lactic acid bacteria, their metabolite products and interference with microbial growth. FEMS Microbiol. Rev., 12: 221-237.

Vaseeharan, B. and D. Ramasamy, 2003. Control of pathogenic *Vibrio* spp. By *Bacillus subtilis* BT 23, a possible probiotic treatment for black tiger shrimp *Penaeus mondon*. Lett. Applied Microbiol., 36: 83-87.

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev., 64: 655-671.

Verschuere, L., J. Dhont, P. Sorgeloos and W. Verstraete, 1997. Monitoring Biolog patterns and r/K-strategists in the intensive culture *Artemia* juveniles. J. Appl. Microbiol., 83: 603-612.

Villalon, J.R., 1993. Commercial Semi-Intensive Penaeid Growout Techniques in Ecuador. In: CRC Handbook of Mariculture: Crustacean Aquaculture, McVey, J.P. (Ed.). Vol. 1., 2nd Edn., CRC Press, Boca Raton, pp: 237-274.

Villamil, L., A. Figueras, M. Planas and B. Novoa, 2003. Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. Aquaculture, 219: 43-56.

Vogt, G., 1987. Monitoring of environmental pollutants such as pesticides in prawn aquaculture by histological diagnosis. Aquaculture, 67: 157-164.

Xu, B., H.S. Xu, W.S. Ji and J. Shi, 1994. Pathogens and pathogenecity to *Penaeus orientalis* kishinouye. Acta Oceanol. Sin., 13: 297-304.

Yathavamoorthi, R., A. Surendraraj and K.H.S. Farvin, 2010. Enteric bacteria and water quality of freshwater prawn *Macrobrachium rosenbergii* (De Man) in culture environment from Kerala, India. J. Fish. Aquat. Sci., 5: 282-292.

Ziaei-Nejad, S., M.H. Rezaei, G.A. Takami, D.L. Lovett, A.R. Mirvaghefi and M. Shakouri, 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture, 252: 516-524.