JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 37, No. 4
December, 2006

Characterization of *Vibrio* spp. Associated with Diseased Shrimp from Culture Ponds of Andhra Pradesh (India)

L. JAYASREE,[1] P. JANAKIRAM, AND R. MADHAVI

Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh 530003 India

Abstract.—Surveys undertaken on diseases caused by *Vibrio* spp. in *Penaeus monodon* from culture ponds of coastal Andhra Pradesh recorded the occurrence of five types of diseases: tail necrosis, shell disease, red disease, loose shell syndrome (LSS), and white gut disease (WGD). Among these, LSS, WGD, and red disease caused mass mortalities in shrimp culture ponds. Six species of *Vibrio*—*V. harveyi*, *V. parahaemolyticus, V. alginolyticus, V. anguillarum*, *V. vulnificus*, and *V. splendidus*—are associated with the diseased shrimp. The number of *Vibrio* spp. associated with each disease ranged from two to five. Additionally, shrimp with red disease had concurrent infections with white spot syndrome virus. *Vibrio harveyi* in the case of LSS and WGD, *V. parahaemolyticus* for red disease, and *V. alginolyticus* for shell disease are the major etiologcal agents. Differences occur in the degree of virulence of different species of *Vibrio* and also different isolates of the same species. *Vibrio harveyi* isolated from LSS shrimp is the most virulent. In general, all the *Vibrio* isolates from LSS shrimp tend to be more virulent as compared to their counterparts from other diseased shrimp. It is apparent that the degree of virulence of various *Vibrio* isolates depends on its source and the pond environmental conditions. Most of the *Vibrio* isolates showed susceptibility to oxytetracycline, norfloxacin, and ciprofloxacin. The luminous *V. harveyi* exhibited resistance to many antibiotics and susceptibility to only three drugs. Considering the emergence of antimicrobial resistant strains of *Vibrio*, the need for using probiotics in place of antibiotics for disease control is stressed.

Vibriosis has been identified as a serious disease problem in shrimp culture ponds all over the world. Mass mortalities in hatcheries and grow-out ponds of shrimp attributed to outbreaks of vibriosis have been recorded from many regions (Couch 1978; Overstreet 1978; Lightner 1983, 1985, 1988, 1996; Sindermann 1990; Ruangpan and Kitao 1991, 1992; Chen et al. 1992; Yang et al. 1992; de la Pena et al. 1993; Jiravanichpaisal et al. 1994; Mohney et al. 1994; Lavilla-Pitogo and de la Pena 1998; Lavilla-Pitogo et al. 1998). Over a dozen species of *Vibrio* have been identified as having implication in the disease process (Overstreet 1978; Lightner 1988, 1996; Sindermann 1990). The severity of infection depends on the species and strain of *Vibrio* involved, the stage of development and age of shrimp, and the ambient environmental conditions.

Vibriosis is also rampant in the Indian region where brackish water shrimp farming is the main aquaculture activity. The disease problem is particularly severe in hatcheries, and in the past few years many units were shut down due to invasion by luminous vibrios (Karunasagar et al. 1994; Hameed et al. 1996; Shome et al. 1999). The situation is aggravated by the emergence of antibiotic-resistant strains (Karunasagar et al. 1994) and the ability of *Vibrio* spp. to form biofilms (Karunasagar et al. 1996). Vibriosis is also having its impact in grow-out ponds and is frequently responsible for the morbidity and mortalities of shrimp (Hameed 1994; Abraham and Manley 1995; Jayasree et al. 2000). Considering that bacterial disease has received little attention, an investigation on the bacterial diseases of shrimp from culture ponds of coastal Andhra Pradesh was undertaken during the period 2001–2003. This article gives an account of the *Vibrio* spp. isolated and identified from the diseased shrimp and also deals with their pathogenicity and sensitivity toward antibiotics.

Materials and Methods

Shrimp samples collected from culture ponds at Kakinada, Bhimavaram, and Visakhapatnam regions during 2001–2003 were screened for the presence of bacterial diseases.

[1] Corresponding author.

The diseased shrimp showing external symptoms of bacterial infections were brought to the laboratory alive and subjected to detailed bacteriological analysis including estimation of bacterial loads and isolation and identification of *Vibrio* spp. involved. The pathogenicity and drug sensitivity of the various *Vibrio* spp. isolated were also studied. Altogether 145 diseased shrimp were subjected to bacteriological analysis.

Isolation and Identification of Bacteria

Hemolyph was drawn from the various diseased shrimp and 0.5 mL each was inoculated on Tryptone Soya Agar containing 2% NaCl and Thiosulphate Citrate Bile Sucrose Agar (TCBS). Total bacterial and *Vibrio* loads were estimated following standard methods (APHA 1992). Predominant and morphologically different colonies were selected and streaked on nutrient agar to obtain pure cultures. The isolated bacteria were identified based on morphological, physiological, and biochemical characteristics as given in Cowan and Steel's manual (Barrow and Feltham 1993) and *Bergey's Manual of Systematic Bacteriology* (Baumann and Schubert 1984).

Detection of White Spot Syndrome Virus

The gills and hemolymph of all the diseased shrimp were subjected to polymerase chain reaction (PCR) tests (two step) using kits supplied by Bangalore Genei Pvt. Ltd., Bangalore, India, to determine whether they carried infections with white spot syndrome virus (WSSV).

Pathogenicity Studies

To study the virulence of different *Vibrio* spp., experiments were conducted by injecting intramuscularly into healthy juvenile shrimp (4 g), the bacterial densities ranging from 1×10^1 to 1×10^7 cfu/g, prepared in sterile normal saline solution. The experiments were set with three replicates, each replicate containing 20 healthy juvenile shrimp. Shrimp inoculated with sterile normal saline alone served as control. The shrimp were maintained in glass troughs (20-L capacity) containing sterile seawater, and mortalities were recorded to 48 h postinoculation. The experimentally inoculated bacteria *Vibrio* spp. were reisolated and identified from the moribund shrimp to fulfill Koch's postulates. From the data collected on mortality rates, the LC_{50} values at 48 h postinoculation were calculated.

Drug Sensitivity

Tests were conducted to study the sensitivity of the most virulent and pathogenic bacteria toward 22 antimicrobial drugs. The bacteria were inoculated on Muller Hinton Agar (with 2% NaCl) and the discs (obtained commercially from Himedia Pvt. Ltd, Mumbai, India) were placed 2 cm apart. After 24 h growth, the diameter of inhibition zone around the discs was measured, and based on the width of the inhibitory zone, the sensitivity was graded into three ranks, resistant (1–5 mm diameter), intermediate (5–10 mm diameter), and sensitive (10–15 mm diameter).

Results

Isolation and Identification of Bacteria

The survey undertaken during 2001–2003 revealed the occurrence of five types of bacterial diseases in shrimp, designated, based on external symptoms, as tail necrosis, shell disease, red disease, loose shell syndrome (LSS), and white gut disease (WGD). Mass mortalities occurred in the ponds affected by LSS, WGD, and red disease while mortality rates were low in ponds affected by tail necrosis and shell disease. Shrimp that had red disease showed concurrent infections with WSSV. The following account furnishes details of external symptoms of the various diseased shrimp and the bacteria involved in the manifestation of the disease.

Tail Necrosis

The disease was encountered in *Penaeus monodon* from culture ponds of Visakhapatnam, Kakinada, and Bhimavaram. The symptoms include necrosis of uropods and pleopods. In acute case of infection the uropods were completely lost, the muscle in the distal portion of the abdomen became completely necrotic and began to decompose. The diseased shrimp lost their swimming ability and exhibited erratic gliding movements near the edge of ponds. Mortalities were observed in chronic cases of infection, but the mortality rate was low (5%). Ten shrimp affected by tail necrosis were subjected to bacteriological analysis. Two species of *Vibrio* were isolated from the hemolymph of these shrimp and identified as *Vibrio parahaemolyticus* and *Vibrio anguillarum* (Table 1).

Shell Disease

This disease was recorded occasionally in shrimp from culture ponds and the disease was identified by the presence of black spots and lesions on the exoskeleton and appendages. In chronic conditions, erosion of the appendages and rostrum was observed. The lesions extended deep into the muscle in severely infected individuals. Mortality due to this disease was low (8%) and observed only in shrimp that are in chronic stage of infection. Fifteen shrimp showing acute infection were subjected to bacteriological analysis and two types of bacterial colonies were isolated, identified as *V. anguillarum* and *Vibrio alginolyticus* (Table 1).

Red Disease

Shrimp with red coloration all over the body were collected from culture ponds at Kakinada and Amalapuram during 2001–2002. The disease was found to cause 80% mortality. Two distinct morphotypes of diseased shrimp were found in the same pond, some were deep red in color without any white spots on the exoskeleton while the others showed typical symptoms of white spot syndrome (WSS), that is, characteristic white spots on carapace and exoskeleton in addition to the pinkish body coloration. Both the morphotypes responded positively to WSSV (PCR test). Thirty moribund shrimp affected by red disease were subjected to bacteriological study. Four species of *Vibrio, V. parahaemolyticus, Vibrio harveyi, V. alginolyticus,* and *V. anguillarum*, were identified from the diseased shrimp (Table 1).

Loose Shell Syndrome

LSS was noticed in many farms situated in East Godavari and Visakhapatnam districts. The disease was observed in all the ages of shrimp and was more predominant at 30 d of culture. Diseased shrimp appeared sluggish and showed symptoms such as soft and loose muscle and condensed and melanized hepatopancreas. Mortality in the affected ponds was very high and reached 100% within 10 d of onset of infection. Microbiological analysis of 70 LSS-affected shrimp revealed heavy *Vibrio* loads with an average of 0.1×10^6 cfu/mL. Six species of *Vibrio* were isolated from these shrimp, identified as *V. harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. anguillarum*, *Vibrio vulnificus*, and *Vibrio splendidus* (Table 1). The number of *Vibrio* spp. in each of the diseased shrimp varied from area to area. The shrimp from Kakinada region harbored all six species of *Vibrio* identified during the present study, whereas only four *Vibrio* spp., that is, *V. harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, and *V. anguillarum*, were isolated from shrimp collected from Amalapuram area and three species (*V. harveyi*, *V. alginolyticus*, and *V. anguillarum*) were isolated from shrimp at Visakhapatnam. The luminous bacteria *V. harveyi* was uniformly present in all the LSS shrimp.

White Gut Disease

This disease was observed in culture ponds of Rambilli area (Visakhapatnam district) during summer of 2000–2001. The diseased shrimp showed symptoms of stunted growth and opaque white gut visible through the transparent cuticle as a white streak. The shrimp consumed feed in large quantities and released fecal matter in the form of a white fluid material. Mortality rate in affected ponds was approximately 80%. Twenty individuals in acute phase of infection and in moribund state were collected and subjected to bacteriological analysis. The total *Vibrio* loads in hemolymph were very high with an average of 0.2×10^5 cfu/mL and three *Vibrio* spp., *V. harveyi, V. alginolyticus*, and *V. anguillarum*, were identified (Table 1).

Detection of WSSV

Gill tissues of all the diseased shrimp subjected to PCR studies gave positive result to WSSV only in case of red diseased shrimp.

TABLE 1. *Morphological and biochemical characteristics of* Vibrio *spp. isolated from diseased shrimp.*

Bacteria isolated	*Vibrio harveyi*			*Vibrio parahaemolyticus*		*Vibrio alginolyticus*		
Tests	LSS	WGD	RD	RD	TN	LSS	WGD	RD
Gram stain	–	–	–	–	–	–	–	–
Growth on TCBS	Y	Y	Y	G	G	Y	G	Y
Motility	+	+	+	+	+	+	+	+
Oxidase reduction	+	+	+	+	+	+	+	+
Catalase reduction	+	+	+	+	+	+	+	+
O/F test	F	F	F	F	F	F	F	F
Arginine dihydrolase	–	–	–	–	–	–	–	–
Lysine decarboxylase	+	+	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+
Methyl red test	+	–	–	+	+	+	+	+
Voges proskauer	–	–	–	–	–	+	+	+
Indole production	+	+	+	–	–	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+	+	+
Esculin hydrolysis	–	–	–	–	–	+	+	+
Sensitivity to O/129 (10 μg)	+	+	+	–	–	+	+	+
O/129 (150 μg)	+	+	+	+	+	+	+	+
Growth at 4 C	–	–	–	–	–	–	–	–
Growth at 42 C	–	–	–	–	–	–	–	–
Growth NaCl (%)								
0	–	–	–	–	–	–	–	–
0.5	+	+	–	+	–	+	–	+
1	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
10	–	–	–	–	–	–	–	–
Acid production from								
Arabinose	–	–	–	–	–	–	–	–
Cellobiose	+	–	–	+	+	–	–	–
Galactose	+	+	+	–	–	+	+	–
Glucose	+	+	+	+	+	+	+	+
Inositol	–	–	–	–	+/–	–	–	–
Lactose	–	–	–	–	–	–	–	–
Mannitol	+	–	–	+	+/–	+	+	+
Sorbitol	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Urease	–	–	–	–	–	+	+	+
H_2S production	+	+	+	–	–	–	–	–

+ = Positive; – = negative; F = fermentative; G = green; Y = yellow; LSS = loose shell syndrome; WGD = white gut disease; RD = red disease; TN = tail necrosis; SD = shell disease.

Pathogenicity Studies

Results of pathogenicity experiments showed considerable differences in the virulence of various species of *Vibrio* as well as different isolates of the same species (Table 2). Of all the species of *Vibrio, V. harveyi* from shrimp affected by LSS proved to be the most virulent, with LC_{50} value of 1×10^3 cfu/g for 48 h. *Vibrio harveyi* isolated from shrimp affected by red disease and WGD were also found to be virulent with LC_{50} values of 2.5×10^4 and 1.0×10^4 cfu/g, respectively. *Vibrio parahaemolyticus* was found to be equally pathogenic with LC_{50} value ranging from 1.5×10^4 to 3×10^5 cfu/g.

TABLE 1. *Extended.*

Vibrio anguillarum						*Vibrio vulnificus*	*Vibrio splendidus*
SD	LSS	WGD	RD	SD	TN	LSS	LSS
−	−	−	−	−	−	−	−
G	Y	Y	Y	Y	Y	Y	Y
+	+	+	+	+	+	+	−
+	+	+	+	+	+	+	−
+	+	+	+	+	+	+	−
F	F	F	F	F	F	F	F
−	+	+	+	+	+	−	+
+	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−
+/−	+	−	+	+/−	+	−	−
+	+	+	+	+	+	−	−
+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+
+	+	+	−	+	+	+	+
+	+	+	+	+	+	+	+
+	−	−	−	−	−	+	−
+	−	−	−	−	−	+	+
+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+
−	−	−	−	−	−	−	−
+	−	−	+	+	+	+	+
+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+
+	−	−	−	−	−	+	−
−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−
−	+	+	+	+	+	−	−
+	+	+	−	+	+	−	−
+	+	+	+	+	+	+	+
−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+
+	+	+	+	+	+	−	−
+	+	+	+	+	+	−	−
+	+	+	+	+	+	−	+
+	−	−	+	−	−	+	+
−	−	−	−	−	−	−	−

The isolates of *V. alginolyticus* from different diseased shrimp showed a great deal of variation in virulence, with lowest LC_{50} value of 4.5×10^4 cfu/g from LSS-affected shrimp and highest value of 2.5×10^5 cfu/g from shrimp with shell disease. It is also noted that isolates of different species of *Vibrio* from LSS-affected shrimp were more virulent compared to those from other diseased shrimp. Isolates of *V. anguillarum* from all the diseased shrimp proved to be least pathogenic with LC_{50} value ranging from 5×10^5 to 1×10^9 cfu/g.

Drug Sensitivity Tests

Drug sensitivity studies revealed all the bacterial isolates to be sensitive to oxytetracycline (OTC), norfloxacin, and ciprofloxacin and resistant to penicillin-G and cloxacillin

TABLE 2. *LC_{50} values (48 h) of* Vibrio *spp. Isolated from diseased shrimp.*

Bacterial isolates	Disease	LC_{50} concentration (cfu/g)
Vibrio harveyi	Loose shell syndrome	1.0×10^3
	Red disease	2.5×10^4
	White gut syndrome	1.0×10^4
Vibrio parahaemolyticus	Red disease	1.5×10^4
	Loose shell syndrome	4.0×10^4
	Tail necrosis	3.0×10^5
Vibrio alginolyticus	Loose shell syndrome	4.5×10^4
	Red disease	6.0×10^4
	White gut syndrome	2.0×10^4
	Shell disease	2.5×10^5
Vibrio agnuillarum	Loose shell syndrome	5.0×10^5
	Red disease	1.0×10^6
	White gut syndrome	1.5×10^6
	Shell disease	6.0×10^6
	Tail necrosis	1.0×10^9

(Table 3). *Vibrio parahaemolyticus* showed maximum sensitivity to OTC and ciprofloxacin, while *V. anguillarum* was highly sensitive to ciprofloxacin.

Discussion

The present study shows that diseases caused by *Vibrio* spp. are quite prevalent in shrimp from culture ponds of coastal Andhra Pradesh, and the disease-induced morbidity/mortalities are causing considerable damage to the culture system. Five types of bacterial diseases are encountered in the cultured shrimp, of which tail necrosis, shell disease, and red disease were recorded earlier from many regions (Lightner 1988; Sindermann 1990; Nash et al. 1992; Yang et al. 1992), including India (Hameed 1994; Abraham and Manley 1995; Hameed et al. 1996). The other two diseases, LSS and WGD, constitute new emerging diseases causing severe loss to the shrimp industry in India (Mayavu et al. 2003; Gopalakrishnan and Parida 2005). LSS is characterized by loose exoskeleton, soft muscle, and condensed and melanized hepatopancreas. It affected shrimp in all ponds selected for the study, while WGD with symptoms, such as stunted growth and gut appearing as white streak and releasing fluidy fecal matter, was recorded only during summer months from ponds at Rambilli village of Visakhapatnam. Red disease occurred sporadically in ponds at Kakinada. Tail necrosis and shell disease, which caused morbidity and limited mortality (5–8%), could be controlled by antibiotic treatment or as a safer measure, through environmental management. On the other hand, LSS, WGD, and red disease have caused mass mortalities in the affected ponds, leading to total failure of the crop. Shrimp affected by red disease showed the presence of WSSV, which probably is responsible for the observed mass mortality. WSSV could not be detected in LSS- and WGD-affected shrimp. Special focused attention is therefore needed for controlling these diseases.

In all the diseased shrimp subjected to the study, the bacterial flora comprised largely *Vibrio* species. Altogether six species of *Vibrio* were isolated and identified: *V. harveyi, V. alginolyticus, V. parahaemolyticus, V. anguillarum, V. splendidus*, and *V. vulnificus.* The number of *Vibrio* species associated with each type of disease varied from two to six, with maximum number in shrimp affected with LSS and minimum in those affected with tail necrosis. However, the LC_{50} values (48 h) of various *Vibrio* species associated with each disease suggested *V. harveyi* as the major etiological agent for LSS and WGD, while the remaining *Vibrio* species isolated from these diseased shrimp might represent secondary invasions being less virulent. Earlier workers also reported *V. harveyi* to be the major cause of the disease in shrimp culture ponds (Abraham and Manley 1995; Liu et al. 1996a; Abraham et al. 1997; Lavilla-Pitogo et al. 1998; Ruangpan 1998).

Vibrio parahaemolyticus was the dominant species in shrimp affected by red disease and tail necrosis, while *V. alginolyticus* is predominant in shell diseased shrimp. *Vibrio alginolyticus* had earlier been isolated from shrimp affected by shell disease and WSS (Hameed 1994; Lee et al. 1996; Jayasree et al. 1999, 2000). Mass

TABLE 3. *Drug sensitivity of various bacteria isolated from diseased shrimp.*

Antibiotic	*Vibrio harveyi*	*Vibrio alginolyticus*	*Vibrio parahaemolyticus*	*Vibrio anguillarum*
Amoxicillin	R	R	R	R
Ampicillin	R	R	R	R
Cephadroxil	R	S	I	R
Cefazolin	R	R	R	R
Chloramphenicol	R	S	S	S
Ciprofloxacin	S	S	S	S
Clotrimazole	R	I	R	R
Cloxacillin	R	R	R	R
Co-trimoxazole	R	R	S	S
Erythromycin	R	I	R	R
Furazolidin	I	R	I	R
Gentamycin	I	I	R	I
Metronidazole	R	R	R	R
Nitrofurazone	R	R	S	R
Norfloxacin	S	S	S	S
Oxytetracycline	S	I	I	I
Pefloxacin	R	S	S	I
Penicillin-G	R	R	R	R
Rifampacin	R	R	R	R
Streptomycin	R	R	R	R
Tetracycline	I	I	I	S
Trimethoprim	R	I	S	I

R = resistant; I = intermediate; S = sensitive.

mortalities due to red disease from *V. parahaemolyticus* along with other *Vibrio* spp. were isolated by Tendencia and Dureza (1997) from ponds in the Philippines.

All the isolates of *V. anguillarum* were found to be less pathogenic compared to other *Vibrio* spp. Earlier studies showed *V. anguillarum* as the main cause of acute infectious disease in penaeid shrimp (Lewis 1973; Lightner 1975; Lightner and Lewis 1975; Zheng et al. 1990). Two species, *V. splendidus* and *V. vulnificus*, occurred with low prevalence; hence, their contribution to disease is limited.

The data collected on LC_{50} values indicated differences in the degree of pathogenicity of different isolates of the same species of *Vibrio*. In general, *Vibrio* species isolated from LSS-affected shrimp proved to be more virulent than their counterparts from other diseased shrimp. The LC_{50} value of *V. harveyi* isolated from shrimp with LSS was lower when compared to those isolated from shrimp with red disease and WGD, indicating the isolates from shrimp with LSS to be more virulent. In accordance with this observation, higher mortalities were observed in culture ponds affected by LSS when compared to those affected by WGD. Earlier workers also noted differences in the virulence of different isolates of *V. harveyi.* Liu et al. (1996b) reported differences in virulence between isolates of *V. harveyi* from penaeid and nonpenaeid shrimp. It was reported that strains of *V. harveyi* originally isolated from diseased penaeids were more virulent to *P. monodon* than the reference strains isolated from seawater or diseased amphipod *Talorchestia*. Karunasagar et al. (1994) also reported that *V. harveyi* isolates from larval tanks showed much lower LC_{50} values than those isolated from natural seawater, thus indicating their higher virulence. The above reports indicate that pathogenicity and virulence of *V. harveyi* are dependent on the source of the isolate and the culture conditions. Under stressful environmental conditions, the *Vibrio* spp. tend to become more virulent. It is also necessary to subject the virulent and nonvirulent strains to detailed analysis in order to determine the virulence factors.

Drug sensitivity studies undertaken during the present study showed all the bacterial isolates to be sensitive to OTC, norfloxacin, and ciprofloxacin. OTC is used most frequently both in hatcheries and in the grow-out ponds, and all the bacterial isolates in the present study exhibited sensitivity to OTC though their degree of sensitivity varied from strain to strain. A similar trend of sensitivity to these antibiotics has been reported by other researchers (Corliss et al. 1977; Mohney et al. 1992; Ruangpan and Kitao 1992; Charantchakool et al. 1995). Hameed (1995) reported that chloramphenicol and OTC adversely affected the growth of *Chaetoceros* sp., a diatom used as feed for protozoeae and mysis stages. On the other hand, de la Pena et al. (1993) observed that chloramphenicol was the most effective drug against *Vibrio* bacteria isolated from *Penaeus japonicus* with OTC less sensitive. In the present study *V. harveyi* isolated from LSS-affected shrimp was resistant to chloramphenicol and highly susceptible to only three drugs (ciprofloxacin, norfloxacin, OTC). Karunasagar et al. (1994) observed that luminous *V. harveyi* developed resistance to drugs such as streptomycin, chloramphenicol, and cotrimazole and concluded that antibiotic-resistant *V. harveyi* were responsible for the observed mortality of postlarvae. Similar results were also observed by Ramamurthy et al. (1992). Multiple antibiotic resistance of *V. harveyi* to at least two antimicrobials was observed by Tendencia and de la Pena (2001). From this it can be concluded that the use of antibiotics in shrimp culture systems may lead to the development of resistance. Hence, use of some therapeutants (such as OTC, tetracycline, gentamycin, streptomycin, etc.) in the culture system has been banned by the Government of India and judicious use of recommended antibiotics is essential. In order to minimize the use of antibiotics, application of probiotics is suggested in both grow-out culture ponds and hatcheries (Garrieques and Areval 1995; Moriarty 1998; Rengpipat et al. 1998). Uma et al. (1999) also suggested that in aquaculture systems the use of probiotics has the ability to manipulate the bacterial flora and ecology of ponds and presumed that the inoculation of beneficial bacteria (probiotics) into the culture systems is a viable alternative for shrimp culture to continue as a sustainable animal husbandry. More investigations on the use of probiotics and immunostimulants in rendering protection against vibriosis are needed.

Acknowledgments

We thank the shrimp farmers who gave us access to their farms. We are thankful to Department of Science and Technology and Department of Ocean Development, Government of India, for providing financial assistance to carry out this work.

Literature Cited

Abraham, T. J. and R. Manley. 1995. Luminous and nonluminous *Vibrio harveyi* associated with shell disease in cultured *Penaeus indicus*. Journal of Aquaculture in Tropics 10:273–276.

Abraham, T. J., R. Manley, R. Papanippan, and K. Dhevendran. 1997. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimp. Journal of Aquaculture in Tropics 12:1–8.

APHA (American Public Health Association). 1992. Standard methods for the examination of water and waste water, 18th edition. American Public Health Association, Washington, DC, USA.

Barrow, G. I. and R. K. A. Feltham. 1993. Cowan and Steel's manual for identification of medical bacteria. Cambridge University Press.

Baumann, P. and R. H. W. Schubert. 1984. Vibrionaceae Veron. Pages 516–550 *in* N. R. Krieg and J. G. Holt, editors. Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, Maryland, USA.

Charantchakool, P., M. Pearson, C. Limsuwan, and R. J. Roberts. 1995. Oxytetracycline sensitivity of *Vibrio spp*. isolated from diseased black tiger shrimp, *Penaeus monodon* Fabricius. Journal of Fish Diseases 18:79–82.

Chen, S. N., S. L. Huang, and G. H. Kou. 1992. Studies on the epizootiology and pathogenicity of bacterial infections in cultured giant tiger prawns, *Penaeus monodon*, in Taiwan. Pages 195–205 *in* W. Fulkas and K. L. Main, editors. Diseases of cultured penaeid shrimp in Asia and the United States. Hawaii Oceanic Institute.

Corliss, J., D. V. Lightner, and P. Zein-Eldin. 1977. Some effects of oral doses of oxytetracycline on growth, survival and disease in *Penaeus aztecus*. Aquaculture 11:355–362.

Couch, J. A. 1978. Diseases, Parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North American. Fishery Bulletin 76: 1–44.

de la Pena, L. D., T. Tamaki, K. Momoyama, Nakai, and K. Muroga. 1993. Characteristics of the causative bacterium for vibriosis in the kuruma prawn, *Penaeus japonocus*. Aquaculture 115:1–12.

Garriques, D. and G. Areval. 1995. An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vannamei* post larvae in Equador. Pages 53–59 *in* C.L. Browd and J. S. Hopkins, editors. Proceedings of special session on shrimp farming. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Gopalakrishnan, A. and A. Parida. 2005. Incidence of loose shell syndrome disease of the shrimp *Penaeus monodon* and its impact in the grow-out culture. Current Science 88:1148–1154.

Hameed, A. S. S. 1994. Experimental transmission and histopathology of brown spot disease in shrimp *(Penaeus indicus)* and lobster (*Panulirus homarus*). Journal of Aquaculture in tropics 9:311–322.

Hameed, A. S. S. 1995. Susceptibility of three *Penaeus* species to a *Vibrio campbelli* like bacterium. Journal of World Aquaculture Society 26:315–319.

Hameed, A. S. S., J. J. Farmer, F. W. Hickmann-Brenner, and G. R. Farming. 1996. Characteristics and pathogenicity of a *Vibrio campbelli* like bacterium affecting hatchery reared *P. indicus* (Milne Edwards 1837) larvae. Aquaculture Research 27:853–863.

Jayasree, L., P. Janakiram, and R. Madhavi. 1999. Shell disease in freshwater prawn *Macrobrachium rosenbergii* (de Man) aetiology, pathogenicity and antibiotic sensitivity. Journal of Aquaculture in Tropics 14:289–298.

Jayasree, L., P. Janakiram, and R. Madhavi. 2000. Characteristics, pathogenicity and antibiotic sensitivity of bacterial isolates from white spot diseased shrimps. Asian Fisheries Science 13:327–334.

Jiravanichpaisal, P., T. Miyazaki, and C. Limsuwan. 1994. Histopathology, biochemistry and pathogenicity of *Vibrio harveyi* infecting black tiger prawn, *Penaeus monodon*. Journal of Aquatic Animal Health 6:27–35.

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

Karunasagar, I., S. K. Otta, and I. Karunasagar. 1996. Biofilm formation by Vibrio harveyi on surfaces. Aquaculture 140:241–245.

Lavilla-Pitogo, C. R. and L. D. de la Pena. 1998. Bacterial diseases in shrimp *(Penaeus monodon)* culture in Philippines. Fish Pathology 33:405–411.

Lavilla-Pitago, C. R., E. M. Leano, and M. G. Paner. 1998. Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent Virbios, in the rearing environment. Aquaculture 164:337–349.

Lee, K. K., S. R. Yu, F. R. Chen, T. I. Yang, and P. C. Liu. 1996. Virulence of *Vibrio alginolyticus* isolated from diseased tiger prawn, *P. monodon*. Current Microbiology 32:229–231.

Lewis, D. H. 1973. Response of brown shrimp to infection with *Vibrio* spp. Pages 333–440 *in* J.W. Avault and E. Boudreux, editors. Proceedings of the fourth Annual Workshop. World Mariculture Society Texas A & M. University.

Liu, P. C., K. K. Lee, K. C. Yi, G. H. Kou, and S. N. Chen. 1996a. Isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicus*. Current Microbiology 33:129–132.

Liu, P. C., K. K. Lee, K. C. Yi, G. H. Kou, and S. N. Chen. 1996b. Pathogenicity of different isolates of *V. harveyi* in tiger prawn, *P. monodon*. Letters in Applied Microbiology 22:413–416.

Lightner, D. V. 1975. Some potentially serious disease problems in the culture of penaeid shrimp. Pages 179–183 *in* Proceedings of the US Japan Natural Resources Program. Symposium on Aquaculture Diseases, Tokyo.

Lightner, D. V. 1983. Diseases of cultured penaeid prawn. Pages 289–320 *in* J.P. Mcvey, editor. CRC Hand Book of Mariculture, Vol. 1. Crustacean Aquaculture. CRC Press, Boca, Raton, Florida, USA.

Lightner, D. V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. Pages 79–103 *in* Proceedings of the first International Conference. Culture of penaeid prawns and shrimps. Illio, Philippines, 1984.

Lightner, D. V. 1988. *Vibrio* disease of penaeid shrimp. Pages 42–47 *in* C.J. Sindermann and D. V. Lightner, editors. Disease diagnosis and control in North American Marine Aquaculture, 2nd edition. Elsevier, New York, New York, USA.

Lightner, D. V. 1996. Handbook of shrimp pathology and diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Lightner, D. V. and D. H. Lewis. 1975. A septicemic bacterial disease syndrome of penaeid shrimps. Marine Fisheries Review 3:25–28.

Mayavu, P., A. Purushothaman, and K. Kathiresan. 2003. Histology of loose-shell affected *Penaeus monodon*. Current Science 85:1629–1634.

Mohney, L., T. A. Bell, and D. V. Lightner. 1992. Shrimp antimicrobial testing-1. In vitro susceptibility of thirteen gram negative bacteria to twelve antibiotics. Journal of Aquatic Animal Health 4:257–261.

Mohney, L., T. A. Bell, and D. V. Lightner. 1994. An epizootic of vibriosis in Equadorian pond-reared *Penaeus vannamei* Boone (Crustacea:Decapoda). Journal of World Aquaculture Society 25:116–125.

Moriarty, D. J. W. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164:351–358.

Nash, G., C. Nithimathachoke, C. Tungmandi, A. Arhajamorn, P. Prathanpipat, and P. Rumathaveesub. 1992. Vibriosis and its control in pond reared *P. monodon* in Thailand. Diseases in Asian Aquaculture. 1. *In* Proceedings of the first symposium on diseases in Asian Aquaculture. 26–29 November 1990.

Overstreet, R. M. 1978. Marine maladies. Worms, germs and other symbionts from the Northern Gulf of Mexico. Mississippi Alabama Sea Grant Consortium, Ocean Springs, Mississippi, USA.

Ramamurthy, T., A. Pal, S. C. Pal, and C. B. Nair. 1992. Taxonomic implications of the emergence of high frequency of occurrence of 2-4-doamiono, 6-7, diisopropylpteridine resistant strains of *Vibrio cholera* from clinical cases of cholera in Calcutta, India. Journal of Clinical Microbiology 30:742–743.

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

Ruangpan, L. 1998. Luminous bacteria associated with shrimp mortality. Pages 205–211 *in* T.W. Flegel, editor. Advances in shrimp biotechnology. National Centre for Genetic Engineering and Biotechnology, Bangkok, Thailand.

Ruangpan, L. and J. Kitao. 1991. *Vibrio* bacteria isolated from diseased tiger prawn, *P. monodon*. Journal of Fish Diseases 14:283–288.

Ruangpan, L. and J. Kitao. 1992. Minimal inhibitory concentrations of 19 chemotherapeutants against *Vibrio* bacteria of shrimp *Penaeus monodon*. Pages 135–142 *in* M. Shariff et al., editors. Diseases of Asian aquaculture. I. Fish Health Section. Asian Fisheries Society, Manila, Philippines.

Shome, R., B. R. Shome, and R. Soundararajan. 1999. Studies on luminous *Vibrio harveyi* isolated from *Penaeus monodon* larvae reared in hatcheries in Andamans. Indian Journal of Fisheries 46:141–147.

Sindermann, C. J. 1990. Principal diseases of marine fish and shell fish, volume 2. Diseases of marine shell fish, 2nd edition. Academic Press, Inc.

Tendencia, E. A. and L. D. de la pena. 2001. Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195:193–204.

Tendencia, A. E. V. and L. A. Dureza. 1997. Isolation of *Vibrio* spp. from *Penaeus mondon* (Fabricus) with red disease syndrome. Aquaculture 154:107–114.

Uma, T., T. J. Abraham, M. J. D. Jayaseelan, and V. Sundararaj. 1999. Effect of probiotic feed supplement on performance on disease resistance of Indian white shrimp *Penaeus indicus* H. Milne Edwards. Journal of Aquaculture in Tropics 14:159–164.

Yang, J., X. Wu, and S. Zu. 1992. Observations on black spot on shell disease of cultivated penaeid shrimp by SEM. Donghai Marine Science 11:34–39.

Zheng, G. Y. Shen, and H. Li. 1990. *Vibrio anguillarum* as a cause of disease in *Penaeus orientalis* Kishinouye. Journal of Fisheries of China 14:1–7.