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Research Article

VIBRIOSIS IN FARM REARED WHITE SHRIMP, *LITOPENAEUS VANNAMEI* IN ANDHRA PRADESH-NATURAL OCCURRENCE AND ARTIFICIAL CHALLENGE

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Abstract

In the present study, a total of five species of Vibrio bacteria were isolated from diseased shrimp, *Litopenaeus vannamei*, collected from commercial shrimp cultured ponds of Eethamukkala, Chinaganjam and Pedaganjam areas, Prakasam district, Andhra Pradesh. The isolated bacterial species were identified as *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio mimicus* and *Vibrio vulnificus.* The symptoms shown by diseased shrimps include loss of appetite, red coloration of the body and pleopods, gills often appear red to brown in colour, reduced feeding, empty gut and general septicemia. In diseased shrimp, hepatopancrocytes may appear poorly vacuolated, indicating low lipid and glycogen reserve. In affected shrimps, localized lesions were also observed in the cuticle. Experimental infection trials reveals that *V. parahaemolyticus* is highly pathogenic to *L. vannamei* while *V. harveyi* found to be moderate pathogenic to challenged shrimp and remaining three bacterial species namely *V.alginolyticus*, *V.mimicus* and *V.vulnificus* were less pathogenic in nature.

Keywords: Shrimp; infection; vibriosis; bacteria

Introduction

The intensification of the shrimp culture and the transfer of aquatic organisms worldwide have been accompanied over the last twenty years by an increased incidence of microbial infectious pathogens. In this regard, bacterial diseases due to *Vibrio* species are often associated with low survival rates in hatchery or grow out conditions (Denis Saulnier *et al.*, 2000). Larval mortalities associated with the presence of *V. harveyi* have been reported in *P. monodon* and *P. vannamei* in Indonesia (Sunaryanto and Mariam, 1986), Thailand (Jiravanichpaisal *et al.*, 1994), India (Karunasagar *et al.*, 1994), Philippines (Baticados *et al.*, 1990; Lavilla-Pitogo *et al.*, 1990), Australia (Pizzutto and Hirst, 1995), Taiwan (Song and Lee, 1993; Liu *et al.*, 1996) and Ecuador (Robertson *et al.*, 1998). Disease outbreaks attributed to other *Vibrio* species such as *V. alginolyticus*, *V. damsela*, *V. parahaemolyticus*, *V. vulnificus* and *V. penaeicida* have been observed in nursery or grow out ponds of *P. vannamei*, *P. monodon*, *P. japonicus* and *P. stylirostris* in Ecuador (Lightner, 1992) Malaysia (Anderson *et al.*, 1988) Taiwan (Song *et al.*, 1993; Lee *et al.*, 1996), Philippines (Alapide-Tendencia and Dureza, 1997), Japan (de la Pena *et al.*, 1993) and New Caledonia (Costa *et al.*, 1998).

Vibriosis is a bacterial infection responsible for mortality of commercial shrimp culture system worldwide (Lightner and Lewis, 1975; Overstreet, 1978; Sidermann, 1990; Lightner *et al.*, 1992; Lavilla-Pitogo *et al.*, 1996; Lavilla-Pitogo *et al.*, 1998; Chen *et al.*, 2000). Vibrio species are widely distributed in aquaculture facilitates throughout the world. Vibrio-related infections also occur in hatcheries, but epizootics also commonly occur in pond reared shrimp species. This disease is caused by gram-negative bacteria of the family Vibrionaceae. Outbreaks may occur when environmental factors trigger the rapid multiplication of bacteria already tolerated at low levels within shrimp body (Sizemore and Davis, 1985) or by bacterial penetration of host barriers. *Vibrio* spp., are among the chitinoclastic bacteria associated with shell disease (Cook and Lofton 1973) and may enter through wounds in the exoskeleton or pores (Jiravanichpaisal and Miyazaki, 1994). The gills may appear susceptible to bacterial penetration because they are covered by a thin exoskeleton (Taylor and Taylor, 1992), but their surfaces are cleaned by the seto-branchs (Bauer, 1998). The mid gut, composed of the digestive gland (DG) and the mid gut trunk (MGT, often referred to as the intestine, (Lovett and Felder, 1990), is not lined by an exoskeleton and therefore seems to be a likely site for penetration of pathogens carried in the water, food and sediment (Ruby *et al.*, 1980; Denis Saulnier *et al.*, 2000 and Jayasree *et al.*, 2006). The present paper communicates incidences of Vibriosis in farm reared shrimp, *L. vannamei*

in Andhra Pradesh-Natural occurrence and artificial challenges.

Materials and Methods

Collection of Diseased Shrimp Samples

In the present study, a total of 250 diseased alive shrimp (weight between 16-18 gm) samples were collected from commercial cultured ponds of Eethamukkala, Chinaganjam and Pedaganjam areas of Prakasam district, Andhra Pradesh. The diseased shrimp samples were brought to laboratory under sterilized conditions. Affected shrimps were observed for gross symptoms by keeping them in glass aquaria. Morphological and behavioral symptoms of affected shrimps were recorded. For the isolation of bacteria from affected shrimps, standard methods described by Lightner (1995) were fallowed. Haemolymph drawn from affected shrimp and plated onto Trytone Soy Agar (TSA) and Thiosulphate Citrate Bile Sucrose (TCBS). Samples were taken aseptically from different tissues such as hepatopancreas, intestine and haemolymph and inoculated onto the surface of TSA and TCBS agar plates. Inoculated plates were incubated at 30±2ºC for 24h. Bacterial colonies were observed in incubated plated from 24 to 96 h. The purification of bacteria was done by subsequent culturing of bacterial cultures. Identification and characterization of bacteria was done on the basis of their biochemical tests as per the methods of Buchanan and Gibbons (1974). Biochemical tests such as Gram's staining, Catalase, Oxidase, MR-VP test, Urase, Oxidative/Fermentative test, and Citrate utilization tests were carried out in the laboratory condition.

Artificial Infection Trials

In order to confirm the pathogenicity of isolated bacterial species from diseased shrimp and to verify the Koch's Postulates, pathogenicity experiments have been conducted in the laboratory condition. For this purpose a total of 500 alive healthy, diseased free shrimp were procured from local shrimp farm (Average weigh 8-10 gm and Length 5-8cm) used in this study and were acclimatized in the laboratory condition for one week. In each group 20 animal were used and kept in glass aquaria and filled with 20 litre of freshwater. The isolated bacterial species were cultured in TS broth and purified. The purified cultures were used to prepare the bacterial cell suspension in 0.85% saline solution to get appropriate cell number in the suspension. Then the suspension contains cell number from 10^4-10^6cfu/shrimp were injected intramuscularly (IM) to the challenged shrimp. Each experiment was conducted in triplicates.

Bacterial Count

Strains were grown for 24h in TS broth to count the colony forming units (CFU). Bacteria were centrifuged at 10,000g during 20min at room temperature and the cellular pellet was washed two times with sterile saline water (0.85% NaCl) and resuspended in 1mL of the same water. The bacterial suspension was then adjusted to an optical density of one in a Thermo Spectronic Genesys 2 Spectrophotometer at 580nm. To determine the CFU/mL of bacterial suspension, serial dilution method was adopted.

Results and Discussion

In this study, five species of bacteria were isolated from diseased shrimps of different cultured ponds of *L. vannamei* in Prakasam district. Andhra Pradesh. The diseased animals showed signs like localized cuticular lesions, red coloration of the body and pleopods, reduced feeding, empty gut. In some cases, red colour animals appeared in the corners of ponds. Similar symptoms were also reported by number of workers (Lightner, 1995; Jayasree *et al.*, 2006; Denis Saulneir *et al.*, 2000).The isolated bacteria were identified on the basis morphological and biochemical characters. The isolated bacteria were identified as *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio mimicus* and *Vibrio vulnificus.* Among the five bacterial species *V. parahaemolyticus* and *V. harveyi* has dominated in all diseased shrimp samples. The morphological and biochemical characters were given in Table-1. All the species of bacteria isolated in the present study are gram native, rod shaped and fermentative bacteria. On agar plates *V. parahaemolyticus* cultures appear as smooth, motile, circular, opaque colonies with entire margins. It showed oxidative positive while catalase negative. While *V. alginolyticus*, *V.fluvialis* and *V. mimicus* showed oxidative, catalase positive and fermentative bacteria. By virtue of biochemical test the isolated bacteria were identified *as V. parahaemolyticus, V. alginolyticus, V. fluvialis* and *V. mimicus.* The same characters were described by Buchanan and Gibbons (1974). Vibrio species are part of the natural microflora of wild and cultured shrimps (Sinderman, 1990) and become opportunistic pathogens when natural defence mechanisms are suppressed (Brock and Lightner, 1990). They are usually associated with multiple etiological agents. However, some of species of Vibrio have been identified as primary pathogens (Owens and Hall-Mendelin, 1989; Owens et al, 1992; Lavilla-Pitogo *et al.*, 1990; de la Peñaa *et al.*, 1995). Some of the pathogens like *V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus* have causes serious disease problems in Thailand (Nash *et al.*, 1992) and the Philippines (Lavilla-Pitogo *et al.*, 1990). In the present study; it has been observed that same species of Vibrio bacteria have associated with diseased shrimp. Harris (1995) reported that luminescent *V. harveyi* appears to release exotoxins (Liu *et al.*, 1996) and may cause 80-100% mortality in *P. monodon* hatcheries. Species like *V. anguillarum*, V. *campbelli*, *V. nereis*, *V. cholerae* and *V. splendidus* have also been reported their association with disease outbreaks in shrimp culture systems by various workers in India and abroad (Chen, 1992; Lavilla-Pitoga, 1990; Esteve and Quijada, 1993; Sahul-Hameed *et al.*, 1996).

Table 1: Biochemical characters of *Vibrio* spp., isolated from the diseased shrimps

S.N.	Character	Response				
		V. parahae	***V. harveyi***	***V. algi***	***V. mimi***	***V. vulni***
1	Colour of colony	Green	Green	Yellow	Yellow	Yellow
2	Shape	R	R	R	R	R
3	Gram's staining test	_	_	_	_	_
4	Motility	+	+	+	+	+
5	Catalse	+	+	+	+	+
6	Oxidase	+	+	+	+	+
7	Oxidative/Fermentative	F	F	F	F	F
8	Acid production from glucose	+	+	+	+	+
9	Nacl tolerance					
	5%	+	+	+	+	+
	8%	+	+	+	+	+
	10%	+	+	+	+	+
10	Decarboxylation of Amino acids					
	Arginine	_	_	_	_	_
	Ornithine	+	+	+	+	+
	Lysine	_	_	+	+	+
11	Methyl red test	+	+	+	+	+
12	VP test	_	_	+	_	_
13	Indole test	+	+	_	+	+
14	Starch hydrolysis	_	_	+	+	_
15	Urase hydrolysis	_	+	+	+	+
16	Gelatin liquefaction	+	+	_	+	+
17	Utilization of carbohydrates					
	L-Arbinose	+	_	+	_	_
a	Dextrose	+	+	+	+	+
b	Fructose	+	+	+	+	+
c	Lactose	+	_	_	_	_
d	Mannose	_	_	+	+	_
e	Galactose	+	+	+	+	_
f	Sucrose	+	+	+	_	+
g	Trehelose	+	+	_	+	+
h	Salicin	+	+	+	+	_
i	Xylose	_	_	_	_	_
18	Citrate utilization	_	+	+	+	+
19	Nitrate reduction	_	+	+	+	+

-: Negative, +: Positive, R: Rods, F: Fermentative, O: Oxidative, V. parahae: *V. parahaemolyticus, V.harveyi, V. alginolyticus, V.mimi: V.mimicus, V.vulni: V.vulnificus*

Table 2: Results of artificial infection trials with *Vibrio* spp. bacteria isolated from diseased shrimp

S.N.	Bacterial species	Shrimp	Route of injection	Dose	Symptoms Observed in challenged shrimp
1	*V. parahaemolyticus*	*L.vannamei*	IM	10^5cfu/shrimp	Localized lesions and red colorations of body and pleopods
2	*V. harveyi*	*L.vannamei*	IM	10^5cfu/shrimp	Localized lesions and red colorations of body and pleopods
3	*V. alginolyticus*	*L.vannamei*	IM	10^6cfu/shrimp	No symptoms
4	*V. mimicus*	*L.vannamei*	IM	10^6cfu/shrimp	No symptoms
5	*V. vulnificus*	*L.vannamei*	IM	10^6cfu/shrimp	No symptoms

IM: intra-muscular; CFU: Colony forming unit

Jayasree *et al.* (2006) have reported occurrence of five types of diseases in shrimp culture systems, such as tail necrosis, shell disease, red disease, loose shell syndrome (LSS) and white gut disease (WGD) and association of *Vibrio* spp. in *P. monodon* from culture ponds of coastal Andhra Pradesh. Among these, LSS, WGD, and red disease caused mass mortalities in commercial shrimp culture ponds. They have isolated six species of *Vibrio* species like *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* and V. *splendidus* were isolated from diseased shrimp. Jawahar Abraham (2004) reported the distribution and species composition of luminous bacteria in commercial shrimp hatcheries.

In this study, an experimental infection trial indicates that all the bacteria isolated from diseased shrimps are pathogenic in nature. *V. parahaemolyticus* is highly pathogenic and it produced disease symptoms within 24 h after injection. While *V. harveyi* is moderately pathogenic to challenged animals. In most cases, a high inoculum was needed to reproduce the disease and to reisolate the inoculated bacteria from the experimentally infected shrimp (Lightner, 1988). However, pathogenic *Vibrio* isolates have also been detected in apparently healthy shrimp (Nakai *et al.*, 1997; Vandenberghe *et al.*, 1998) and in seawater samples from near-shore and estuary areas, where shrimp farms rearing water is pumped and from affected farms, (Lightner, 1992; Lavilla-Pitogo *et al.*, 1990, 1998; Moriarty, 1998), as well as in sediment (de la Pena *et al.*, 1992). These observations lead researchers to consider Vibrio diseases as secondary infections due to opportunistic pathogens and occurring only in immunologically compromised shrimps. Primary causes could encompass other infectious agents, nutritional deficiencies or intoxication, environmental and management practices and induced stress.

Conclusion

In the present study, five species of bacterial viz., *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio mimicus* and *Vibrio vulnificus* were isolated from Vibriosis affected diseased shrimp. All the species of bacteria were pathogenic in nature. Among the five species of bacteria, *Vibrio parahaemolyticus* was highly pathogenic in nature while V. harveyi was moderately pathogenic to challenged shrimps.

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