ISSN: **0976-3031**

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 3, Issue, 3, pp.133 -136, March, 2012

International Journal of Recent Scientific Research

ISOLATION OF BACTERIA FROM VARIOUS TISSUES OF INFECTED AQUAFARM SHRIMP *PENAEUS MONODON*

Maharajothi, P., Kavitha, P and Rajendran, K

Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi – 613 503, Thanjavur District, Tamil Nadu, India.

ARTICLE INFO

Article History:

Received 12th February, 2012
Received in revised form 20th February, 2012
Accepted 28th February, 2012
Published online 25th March, 2012

Key words:

Penaeus monodon, Vibrio spp, aqua farm shrimp.

ABSTRACT

The infected aquafarm Shrimp *Penaeus monodon* were used for bacteriological examinations. Tissues of carapace, alimentary canal and gills of shrimp were cultured in both Nutrient Agar (NA) and Thiosulfate Citrate Bile Sucrose Agar (TCBS) plate after homogenization and existence of bacterial population were observed. Results showed that there was a significant variation in bacterial flora among the tissues. According to the diagnostic scheme for *Vibrio* species described by Larsen and Pedersen (1999), three types *Vibrio spp* such as *V.harveyi, V.alginolyticus, V. parahaemolyticus* and some of the non – *Vibrio* spp (*Bacillus, Pseudomonas, E.coli, Streptococcus, Staphylococcus, Proteus*) were identified. The findings of this study would suggest that there may be a wide range of *Vibrio* spp in the various tissues and some characteristics of these bacteria are given in this report.

INTRODUCTION

Aquaculture is one of the fastest growing food production sectors in the world (Subasinghe *et al.,* 1998) and is estimated itself as high protein resources to fulfill the food demand since the natural resources exhibits over exploitation. But, presently, the biggest problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non – biological agents. Among the groups of microorganisms that cause serious losses in shrimp culture, the best known are bacteria because of the devastating economic effects on affected farms. Bacterial diseases, mainly due to *Vibrio*, have been reported in Penaeid shrimp culture systems. Culture of *Penaeus monodon*, tiger shrimp is one of the most profitable ventures in aquacultures sector in India and in many countries of the world. The total export of processed shrimp from India is estimated to be 11,000 metric tones of which 48. 96% is contributed by cultured shrimps. Commercial culture of shrimp involves stocking of shrimp seeds in the ponds rearing them to a marketable size and harvesting. There are about 226 hatcheries along the coastal region of the country with the production capacity of 1,05,828 lakhs post larvae. Tamilnadu has 72 hatcheries with capacity to produce 29,330 lakhs of post larvae per annum (Anon, 2004). Currently, aquaculture industry in India and other part of the world has been facing serious problems due to microbial diseases. In aquatic environments, diseases in fishes and shrimps are caused by opportunistic pathogens(Lundin,1996) Species from *Vibrio* are the most common bacterial pathogens causing some of the most serious diseases, growth retardation and sporadic mortalities in Penaeid shrimp (Lavilla – Pitago,1998; Vendenberghe et al, 1998). Shrimp production has been affected severely by infectious diseases caused by *Vibrio* spp. (Gomez et al., 2000; Vijayan et al., 2006). In addition, the existence of bacterial pathogens in the marine shrimp have been reported by many investigations (Ruangpan, 1982; Ruangpan and Kitao, 1991; Chandrasekaran et al ., 1987; Dela - cruz et al., 1990; Chen et al., 1990; Suwansonthichai and Rengpipat,2003) Hence we have attempted to isolate and identify the bacterial pathogen which exists in various tissue of infected aquafarm shrimp *Penaeus monodon.*

MATERIALS AND METHODS

Source of bacterial strains

The infected aqua farm shrimp *P. monodon* were collected a modified extensive farm located at kattumavadi which is a coastal village of Thanjavur Dt., Tamil Nadu, South India. This study area has more number of shrimp aquafarm.The specimens were brought to the laboratory in ice stored conditions and tissues of carapace, alimentary canal and gills were taken aspectically.One gram of each samples were homogenized and inoculated at the center of the plate containing nutrient agar(NA) and thiosulfate citrate bile salt sucrose (TCBS) agar medium. After inoculation, the petriplates were incubated at 37^{o}C and 30^{o}C for 24 – 48 hours respectively. Discreate colonies and pure culture

* Corresponding author: +91
E-mail address: rajafayazali@yahoo.co.in

was made before staining. The process of staining was done by gram stain. Isolated pure cultures were sub - cultured on Nutrient Agar,TCBS and stock cultures were maintained at -35^{o}C in 10% glycerol/ brain heart infusion broth medium with 1.5% sodium chloride.

Morphological and physiological study

Colony morphology was observed on TCBS and NA after 24 hrs. of incubation at 30^{o}C and 37^{o}C respectively. Cell morphology was determined from light microscope observations of Gram- stained smear preparations. Motility was tested under light microscope of 100 magnification by using slide with a drop of young bacteria.

Biochemical Characteristics

Selection of biochemical characters is followed the diagnostic scheme for *Vibrio* species associated with fish described by Larsen and Pedersen(1999).The biochemical test such as Indole, MR, VP test, citrate utilization test, catalase test and oxidase tests, etc., were carried out for identification of unknown pathogenic bacteria. The isolates were photographed under Nikon microscope and were identified based on Buchanan and Gibbon(1974).

RESULT AND DISCUSSION

The significant variation of bacterial population in various tissues of ice stored infected aqua farm shrimp *P.monodon* which had showed in Table 1.

Table 1 Existence of bacterial flora in various tissues of infected shrimp *P.monodon*

S.No	Name of the Bacteria	Carapace	Alimentary cananl	Gills
1.	Bacillus sp.	+	+	+
2	Pseudomonas sp.	+	_	_
3.	E. coli sp.	_	+	+
4.	Protease sp.	+	+	_
5.	Streptococcus sp.	-	+	_
6.	Staphylococcus sp.	+	+	+
7.	Vibrio harveyi	_	+	+
8.	V. alginolyticus	+	_	_
9.	V. parahaemolyticus	+	_	+

(+) Present; (-) Absent

There were 9 species of bacteria predominantly isolated and identified. The isolates were *Pseudomonas, Bacillus, E.coli, Proteus, Streptococcus, Staphylococcus* and *Vibrio* spp. Such as *V.harveyi, V. alginolyticus and V. parahaemolyticus.*

Three groups of *Vibrio* spp were the predominant species of bacteria and rare related bacteria were also present in *P. monodon* was confirmed by the standard biochemical tests (Table 2) .

The bacterial species of *Pseudomonas, E.coli, Protease* and *Vibrio* spp., are gram negative bacteria and the species of *Bacillus, Staphylococcus* and *Streptococcus* are also confirmed as gram positive bacteria by these biochemical tests.

Vibrio spp have great phenotypic diversity, therefore it is really complex to identify them. The genes *Vibrio* is gram negative, oxidase reaction positive, grow on TCBS agar, oxidative – fermentative test positive. Besides these characteristics, they also give reaction with indole, citrate, Voges - proskauer, urease, catalase, H2S, growth at temperature 35 and 40^{o}C, etc.

Vibrio harveyi

Vibrio harveyi has been described as a marine pathogenic luminous or non luminous bacterium (Farmer and Hickman – Brenner 1992). Infections of luminescent bacteria can cause diseases leading to weakness and death of the animal host. Occurrences of diseases were recorded from few shrimps at the end of culture operation. Hence the culture organisms were harvested immediately in order to present the further infection to other organisms and avoid economic loss. *V. harveyi* was found to be high during later stages of culture at the time of diseases outbreak. Observations on the higher number of *V.harveyi* at the end of the cultivation period agrees with the previous work of Nash et al.,(1992); Karunasagar et al., (1994). Further they suggested that Vibrios can cause secondary infection in *P.monodon.*

Vibrio alginolyticus

Vibrio alginolyticus, the second most frequently isolated bacterium, is very common in the marine environment and is a normal part of the micro flora of pond and raceway reared shrimp (Lightner 1988). Its effect on the growing larvae is rather unclear. It has been reported to be a pathogen for *P. monodon* larvae (Nash et al., 1992).

Vibrio parahaemolyticus

Vibrio parahaemolyticus is a gram – negative bacterium that occurs naturally in the estuarine environment. This human pathogen is frequently found in seawater, sediments, plankton, finfish and shellfish (Pavia et. al., 1989) and can cause acute gastroenteritis characterized by diarrhea, vomiting and abdominal cramps through consumption of contaminated raw fish or shellfish (Rippey, 1994).It forms part of the indigenous micro flora of aquatic habitats of various salinity (Colwell et. al., 1984) and are the major causative agents for some of most serious diseases in fish, shellfish and Penaeid shrimp (Lee et. al., 1996; Sugumar et. al., 1998).

Vibriosis is a common problem world wide, particularly in India. Itami,(1996) suggested that luminescent Vibriosis may be controlled in the hatchery by washing eggs with iodine and formaldehyde and avoiding contamination. *Vibriosis* is controlled by rigorous water management and sanitation to prevent the entry of Vibrios in the culture.(Baticados, et al.,(1990) and to reduce stress on the shrimps(Lightner,1993). As a result of present work, *V. harveyi, V. alginolyticus, V. parahaemolyticus* are main pathogens that are collected from shrimp pond at kattumavadi village. Therefore, it is very important to pay attention that considerable on the environmental parameters in these ponds, which may promote susceptibility to infection of shrimp.

Table 2 Biochemical test of bacterial species isolated from infected aqua farm shrimp *P.monodon*

Name of the Bacteria	Gram reaction	Motility	Indole	MR	VP	Citrate	Urease	H2S	Carbohydrate fermentation	
									Glucose	Lactose
Bacillus sp.	+	Motile	-	-	+	+	-	-	+	+
Pseudomonas sp.	-	Motile	-	-	-	+	-	-	+	-
E. coli sp.	-	Motile	+	+	-	-	-	-	+	+
Protease sp.	-	Motile	+	+	-	-	+	+	+	-
Streptococcus sp.	+	Non Motile	-	+	-	-	-	-	+	+
Staphylococcus sp.	+	Non Motile	-	+	+	-	-	-	+	-
Vibrio harveyi	-	Non Motile	+	+	-	-	-	-	+	-
V.alginolyticus	-	Motile	+	-	+	-	-	-	+	-
V.parahaemolyticus	-	Motile	+	+	-	-	-	-	+	-

(+) Positive; (-) Negative

The present study reveal that the demonstrates the relationship between the physico – chemical conditions of shrimp culture environment, the abundance of *Vibrio* spp and consequent outbreak of viral diseases in farmed animals. Thus, monitoring the bacterial population especially the *Vibrio* group is crucial for a healthy aquaculture system. This study emphasise the indiscriminate usage of feed and higher stocking density of shrimp will trigger the high BOD and the related stress reactions make the animals more susceptible for the attack of opportunistic pathogens.

References

Anon 2004. An overview of the present state of shrimp aquaculture in India. Fishing chimes, 20(10,11): 11-16.

Baticados M.C.L., Lavilla- pitogo C.R., curz lacierda E.R., de lapena L.D., and sunaz, N.A. 1990. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. Splendides* isolated from diseased *Penaeus monodon* larvae and rearing water. Disease of aquatic organisms 9: 133-139.

Chandrasekaran M, Lakshmana perumalsamy P and Chandramohan D (1987) Spoilage bacteria *P. indicus Fish Technol. Soc. Fish Technol*. Cochin 24, 122-125.

Chen- Hsing-Chan, Moody Mw and Jiang and shann-Tzong, (1990) changes in biochemical and bacteriological quality of grass prawn during transportation by icing and oxygenating., *J. Food Sci*. 55, 670-673.

Colwell, R.R., West, P.A., Maneval, D., Remmers É,F., Ellion. E.L. and Carlson, N.E. (1984) Ecology of pathogenic vibrios in Chesapeake Bay.,In R.R. Colwell (ed)., *Vibrios* in the environment 367-387. John wiley and sons New York.

Dela- cruz A R G,Santos L.M, Agudo F and Dangla É (1990) Microbiology of prawn processing. Indo-Pacific Fishery commission papers.

Farmer J.J., and F.W. Hickman Brenner, (1992). The prokaryotes, second Edition, A. Handbook on the biology of bacteria; Ecophysilogy, Isolation, Identification Application, Volume III the genera *Vibrio* and phytobacteria, Chapter-157.

karunasager, I. R, Pai, G.R Malathi and L. Karunasagar. Mass mortality of *Penaeous monodon* larvae due to antibiotic vesistant. *Vibrio harveyi* infection. Aquaculture. 128. 203-209 (1994)

Nash G. N. Charetana ,T. Cholada, A. Anutra. P. Phusit and R. Pongcham: *Vibriosis* and its control in pond-reared *Penaeus monodon* in Thailand. In: Diseases in Asian aquaculture (Eds: M. Shariff, R. Subasinghe and J.R. Arthur). Fish Health section, Asian Fisheries society, Manila, Phillippiness. pp. 143-145 (1992).

Lavilla- Pitago, C.R., E.M. Leano and M.G. Paner: Mortalities of pond cultured juvenile shrimp *Penaeus monodon* associated with dominance of luminescent *Vibrios* in the rearing environment Aquaculture 164, 337.349 (1998).

Lee, M., Taylor, G.T., Bricelj. V.M., Ford S.E. and Zahn. S, (1996). Evaluation of *Vibrio* spp and micro plankton blooms as causative agents of Juvenile Oyster Disease in *Crassostrea virginica* (Gmelin)., J Shellfish Res., 15: 319-329.

Lighther, D.V., 1988. *Vibrio* disease of *Penaeus* shrimp In: C.J. Sindermann and D.V. Lighther (Editors). Disease Diagnosis and control in North American marine aquaculture. Developments in Aquaculture and Fisheries science, Vol.6, Elsevier Amsterdam, pp. 42-47.

Lightner, D.V. 1993. Diseases of cultured shrimp. INCRC Handbook of Mariculture (e d, p.v. Mcvey), pp. 393-486. CRC press, Boca Raton, Florida, USA) Gomez G.B.A Roque and F.J.Turnbull 2000 the use and selection of probiotic bacteria for use in the culture of Larval aquatic organisms. Aquaculture, 191 (1-3): 259-270.

Lundin, G.G.: Global attempts to address shrimp disease. Marine/Environmental Land, Water and Natural Habitats Division. Environment Department. The world Bank Report. P. 45 (1996).

Pavia, A.T., Bryan, J.A., Maher. K.L, Hester Jr. T.R. and Farmer, J.J. 111. (1989). *Vibrio carchariae* infection after ashark bite. Annual International Medicine III 85-86.

Rippey. S.R (1994). Infectious diseases associated with molluscan shellfish consumption clin. Microbiol, Rev. 7: 419-25.

Ruangpan L (1982) Disease and Parasites of *P. monodon* (Fabricius) *Thai. Fish. Gaz.* 35, 385-387.

Ruangpan L and Kitao T (1991) *Vibrio* bacteria isolated from black tiger shrimp, *P Monodon* (Fabricius). *J. Fish Diseases* 14, 383-388.

Subasinghe, R. D., M. Bartly, S. Mcgladdery and U. Barg: Sustainable shrimp culture development

Biotechnological issues and challenges. In: Advances shrimp biotechnology (Eds.: T.W. Flegel). National Center for Genetic development: Engineering and Biotechnological, Bankok. pp.13-18(1998)

Sugumar, G., Nakai, T., Hirata Y., Matsubara. D. and Muroga K. (1998). *Vibrio Splendidus* biovar II as the causative agent of bacillary necrosis of Japanese oyster *Crassostrea gigas* Larvae. Dis Aquat. Org 33: 111-118.

Suwansonthichai S and Rengpipat S (2003) Enumeration of coliforms and *Escherichia coli* in frozen black tiger shrimp *P. monodon* by conventional and rapid methods. *Int. J. Food Microbiol*. 81. 113-121.

Vijayan, K.K., I.S.B. Singh. N.S. Jayaprakash, S.V. Alavandi, S.S Pai, R. Preetha, J.J.S. Rajan and T.C. Santiago 2006 A brackish water isolate of *Pseudomonas* PS- 102, a potential antagonistic bacterium against pathogenic *Vibrios* in penaeid and non- penaeid rearing systems. Aquaculture, 251(2-4): 192-200.

Vandenberghe, J., Y.Li.L. Verdonck J. Li. H.S. XU and J. swings *Vibrios* associated with *Penaeus chinensis (*crustacea: Decapoda) larvae and post- larvae in chinese shrimp hatcheries, Aquaculture 169, 121-132 (1998).

****