Journal of Aquatic Biology and Fisheries Vol. 2/2014/ pp. 534 to 538

ANTIBIOTIC SENSITIVITY AND ENZYME PRODUCTION OF BIOLUMINESCENT VIBRIO SPECIES ISOLATED FROM SQUID AND PENAEID SHRIMP OF PONNANI ESTUARY, KERALA

Ramina, P.P*, Sabira, K.M, and Razia Beevi, M.
Department of Aquaculture and Fishery Microbiology, MES Ponnani College, Malappuram
**Email: mail2remie@gmail.com*

Received on: 10 October 2013, accepted on: 12 December 2013

Abstract: Luminous bacteria are the most abundant and widely distributed of the light emitting organisms and are found on marine fresh water and terrestrial environments. The review of literature reveals that most of the microbiological work from Ponnani estuary emphasized on the coliform load in the area. However a detailed study on the bioluminescent bacteria, its abundance and distribution is lacking. During the present work occurrence of bioluminescent bacteria in squid (*Loligo duvauceli*) and Penaeid shrimp (*Metapenaeus dobsoni*) from Ponnani estuary were studied. Objectives of the present study include Isolation and Identification of bioluminescent bacteria, antibiotic sensitivity studies and its enzyme production. The bioluminescent bacteria were isolated by using Sea water complex agar. The isolated strains were subjected to antibiotic sensitivity test (Kirby Bauer disc diffusion method) against nine different antibiotics. Isolates obtained from the squid were resistant to all nine antibiotics used for the test. Strains isolated from shrimp were highly sensitive only to two antibiotics gentamycin and chloramphenicol and are resistant to Amoxicillin, vancomycin and cefotaxime. The enzyme lipase produced by the *Vibrio* spp. were also isolated and purified.

Key words: Bioluminescent bacteria, Sea water complex agar, Antibiotic sensitivity, Lipase

INTRODUCTION

Luminous bacteria are most abundant and widely distributed of the light emitting organisms and are found in marine, freshwater and terrestrial environments. The most common habitats are free living species in the ocean as saprophytes growing on dead fish or meat as gut symbionts in the digestive tracks of marine fish and as light organ symbionts in the squid (Hasting *et al*., 1986).These bacteria are all gram negative motile rods and can function as facultative anaerobes (Bauman *et al*., 1983). Almost all luminous bacteria have been classified into three genera, Vibrio, Photobacterium and Xenorhabdus with most of the species being marine in nature (Campbell *et al*., 1989). Literature survey reveals that antibiotic sensitivity studies of luminous *Vibrio* species from hatcheries all over the world were sufficient, but the information regarding antibiotic sensitivity studies of luminescent bacteria from estuarine environment is lacking. So the present study focus about the antibiotic sensitivity studies of *Vibrio* species isolated from squid (*Loligo duvauceli*) and shrimp (*Metapenaeus dobsoni*) of Ponnani estuary. Antibiotics have been used to control luminous vibriosis in grow out ponds with little success. The principle drawback of antibiotic application is the development and spread of antibiotic resistant organisms. There are no universally acceptable pharmaceutical agents that are approved by FDA for treating infections in shrimp aquaculture, although studies are underway to improve disease, control and treatment (Reed *et al*., 2004) .Hence there is need to develop suitable chemotherapy to control luminous vibriosis.

Marine microorganisms which are halotolerant produce many enzymes to meet our therapeutic requirements. Bioluminescent *Vibrio* species produces many enzymes including lipase, esterase, chitinase, and asparaginase. It is believed that sea water ,which is saline in nature and chemically closer to the human blood plasma could provide microbial products, in particular the enzymes, that could be safer having no or less toxicity or side effects when used for

therapeutic applications to humans (Sabu, 2003).The bioluminescent bacterium *Vibrio fischeri* produces lipase enzyme under specific substrate concentration. Lipases have a dual role in catalyzing hydrolysis and synthesis of esters formed from glycerol and long chain fatty acids. These enzymes exhibit broad substrate specificity and degrade tweens and phospholipids often with positional, stereo and chain length selectivity (Jaeger *et al*., 1994). Lipases have been recognized as very useful biocatalysts because of their wide ranging versatility in industrial applications such as food technology, detergent, chemical industry, biomedical sciences (Jaeger and Reetz, 1998; Jaeger *et al*., 1999; Ghanem *et al*., 2000; Gupta *et al*., 2004). Information of lipolytic enzymes produced by marine *Vibrio* species from estuarine environment is scanty. Therefore, this research article also focuses about the production of lipase enzyme of *Vibrio* species isolated from squid and shrimp.

MATERIALS AND METHODS

Samples of squid and shrimp were collected aseptically from Ponnani estuary and brought to the laboratory for analysis. Presence of bioluminescent bacteria was detected by streak plate technique. The plates were then incubated for 24 hours at 37°C and at every six hours the appearance of luminescent colonies were observed. The distinct isolated luminescent colonies were marked while observing for luminescence and were further purified by sub-culturing in Sea Water Complex Agar plates. Species identification done with the help of Bergey's manual of Determinative Bacteriology (Holt *et al*., 1994).

Vibrio species isolated from estuarine squid and shrimp were subjected to antibiotic sensitivity testing against commonly used antibiotics with the help of Agar disc diffusion method of Baur *et al*., (1966). Different antibiotic discs (Chloramphenicol, Ciprofloxacin, Gentamycin, Azithromycin,Tobramycin,Erythromycin, Amoxicillin, Cefotaxime, vancomycin) with effective concentrations were placed over the plates swabbed with isolated *Vibrio* species in Muller Hinton Agar medium and incubated at 37°C for 24 hours and the zone of inhibition were measured.

Isolation, Purification and enzyme assay

Purification and enzyme assay for lipase was done by following the procedures of Ranjitha *et al.*, (2009). All strains were pre cultivated on Sea Water Complex Agar medium. For detection of lipolytic activity Zobell 2216E modified media was used. After inoculation, incubated at 30°C for 10 days. The total diameter, minus the diameter of the colony was considered to be proportional to the lipolytic activity rate. After 1-10 day incubation the halos or clear zones were measured. Cell free extract was made by inoculating 150 µl of culture into 200ml of sea water medium with substrate and incubated at 30°C for 3 days. The portion was centrifuged at 4°C for 30 min. The supernatant was filtered and the obtained cell free filtrates were used as the crude enzyme in the purification experiments and were purified by ammonium sulphate fractionation.

RESULTS AND DISCUSSION

During the present study *Vibrio* species were isolated from estuarine squid and shrimp of Ponnani. Phenotypic studies of bacterial isolates revealed that all the strains isolated were Gram negative, motile, rod shaped catalase and oxidase positive and halophilic. Sugar fermentation tests including Mannitol,Glucose and Sucrose were tested for bacterial isolates and only positive result was obtained for mannitol. Srinivasan and Ramaswamy(2009) reported positive result for D-Glucose without gas production .Many studies on the taxonomy of Marine luminous bacteria both free living and symbiotic have been carried out (Hendrie *et al.*,1970; Reichelt and Baumann ,1973). Lee and Ruby, (1994) reported that squid receives a significant input of cells of symbiotic *V. fischeri* from the habitat.

Sensitivity pattern of luminous bacteria from squid and shrimp to selected antibiotics

Antibiogram pattern showing varying degrees of susceptibility were given in Table 1.

Sensitivity pattern for luminous bacteria from squid and shrimp

Isolates obtained from squid were resistant to all nine antibiotics which were used for the test.

Table 1. Antibiogram pattern showing varying degrees of susceptibility

Antibiotics	Squid		Shrimp			
			Vibrio spp 1		*Vibrio* spp 2	
	Zonedia	S,R or I	Zonedia	S,R or I	Zonedia	S,R or I
Chloramphenicol	12	R	22	S	22	S
Ciprofloxacin	15	R	23	S	21	S
Gentamycin	12	R	16	S	15	S
Azithromycin	15	R	23	S	23	S
Tobramycin	12	R	16	S	14	I
Erythromycin	13	R	19	I	16	I
Amoxicillin	12	R	12	R	12	R
Cefotaxime	14	R	14	R	14	R
Vancomycin	9	R	9	R	9	R

Although the antibiotic sensitivity studies of bioluminescent bacteria from shrimp and shrimp hatcheries were abundant, related studies on antibiotic resistant pattern of bioluminescent *Vibrio* spp isolated from squid was lacking.

The luminescent *Vibrio* species isolated from shrimp were highly sensitive to only two antibiotics namely gentamycin and chloramphenicol. Studies related to antibiotic resistance pattern obtained from bioluminescent *Vibrio* spp from estuarine shrimp is lacking. But studies from shrimp hatcheries were sufficient. Devika and Jayabalan (1996) revealed a similar result to that of the present observation. Ciprofloxacin and azithromycin are also sensitive in border lines for *Vibrio* species 1. Tobromycin and erythromycin show intermediate level for *Vibrio* species 1 while tobromycin were sensitive in border line for the *Vibrio* species 2. Similar result were shown by Srinivasan and Ramaswamy, (2009). Amoxicillin, Vanco-mycin and Cefotaxime are resistant to both the *Vibrio* species isolated from shrimp. The incidence of antibiotic resistance was higher in amoxicillin, vancomycin and cefataxamine than other antibiotics used in this study. Resistance of amoxicillin was also focused by Srinivasan and Ramaswamy (2009). Adeleye, *et al.*, (2008) and Jun *et al.*, (2003) while studying the antimicrobial susceptibility test showed that all the *Vibrio* isolates (100%) were resistant to amoxicillin, augmentin, chloramphenicol and nitroforantoin. Manjusha *et al.,* (2008) focused multiple resistance patterns of *Vibrio* species to gentamycin, nitrofurantoin, tetracycline, augmentin, chloramphenicol, amoxicillin, ofloxacin, cotrimozazole, ceftriazone, and ciprofloxacin.

Screening of Lipase Enzyme

In ZoBell modified media, the isolated *Vibrio* spp.Showed 27 mm, 29 mm, 25 mm of halos respectively for one strain isolated from squid and two from shrimp. These halos indicate little lipase activity. Similar study were done by Ranjitha *et al.*, (2009) with *V. fischeri* in ZoBell modified media and showed halos of 40mm indicating significant lipase activity than the present work. Bruni *et al.*, (1982) reported that most strains of *Pseudomonas* sp. NCMB 1082 split all tweens, tributyrin, but not triolein of the growth media. Lipase production at the end of log phase was reported (Sarkar *et al.,* 1998; Kiran *et al.*, 2008; Ramani *et al.*, 2010). Kouker and Jaeger (1987) detected a plate assay for bacterial lipase in a medium containing triacylglycerol and the fluorescent dye rhodamine B. The low lipase activity obtained in the present study may be due to difference in substrate ingredients.

Purification of lipase enzyme

After 3 day incubation at 30^{0}C in sea water containing glycerol, highest yield of enzyme was obtained. The purification procedures were performed at 4^{0}C in order to reduce the loss of enzyme activity. After dialysis, the specific activity of lipase containing dialysate was increased than that in culture supernatant fluid. Nawani and Kaur (2000) used phenyl sepharose chromatography as a single step process that yielded a high active and pure lipase. (Ranjitha *et al*., 2009 and Khunt *et al*., 2012) reported similar result for *Vibrio* species. In the present study the quantity of enzyme obtained by enzyme activity assay is too low. The minimum amount may be due to enzyme loss during purification procedures.

REFERENCES

Adeleye, A., Vivian, E., Rita N., Stella, S. and Emmanuel, O. 2008. *African Journal of Biotechnology*, 7(20), 3791-3794. *Aquacult. Dev. Cent*., 8: 64-70.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. 1966. *American Journal of Clinical Pathology*, 45: 493-496.

Baumann, P., Baumann, M. Woolkalis and Bang, S. 1983. Evolutionary relationship in *Vibrio* and *Photobacterium*. A basis for a natural classification. *Annu Rev. Microbial*., 37: 369-398.

Bruni, V., Maugeri, T and Alonzo, V. 1982. Lipolytic Activity of Marine Bacteria Influence of Nacl and $MgCl_2$. *Marine Biology.*, 67: 113-119.

Campbell, A.K. 1989. Living Light: Biochemistry, Function andBiomedical Applications Essays in Biochem. 24: 41-81

Devika Pillai and Jayabalan, N. 1996. Sensitivity of luminous bacteria *Vibrio harveyi* to nine selected antibiotics. *Indian J. Fish.,* 43(4): 399-402

Ghanem, E.H., Al-Sayeed, H.A. and Saleh, K.M. 2000. An alkalophilic thermostable lipase produced by new isolate of *Bacillus alcalophilus*. *World Journal of Microbiology and Biotechnology*, 16: 459-464.

Gupta, R., Gupta, N. and Rathi, P. 2004. Bacterial lipases: an overview of production, Purification and biochemical properties. *Appl. Microbiol. Biotechnol.,* 64: 763-781

Hastings, J.W., Potrikas, C.J., Gupta, S.C., Kurfurst, M., and Makemson, J.C 1986. Biochemistry and physiology of bioluminescent bacteria. *Ado. Microbiot Physiol.*,26: 235-291

Hendrie, M.S., Hodgkiss, W. and Shewan, J.M. 1970. The identification, taxonomy and Classification of luminous bacteria. *J. Gen. Microbiol.*, 64: 151-169.

Holt, J.H., Krieg, N.R., Sneath, P.H.A. and Staley, J.T. 1994. *Bergey's Manual of Determinative Bacteriology, Ninth edition*, pp. 255-273

Jaeger, K.E. and Reetz, M.T. 1998. Microbial lipases form versatile tools for biotechnology. *Trends in Biotechno.,* pp. 396-403.

Jaeger, K.E., Dijkstra, B.W. and Reetz, M.T. 1999. Bacterial biocatalysts: molecular biology, Three-dimensional structure and biotechnological applications of lipases. *Ann. Rev. Microbiol.,* 53: 315-35.

Jaeger, K.E., Ransac, S. Dijkstra, B.W. Colson, C. Van Henvel and Misset M.O. 1994. Bacterial lipases. *FEMS Microbiol. Rev.,* 15: 29-63.

Jun, L., Jun Y., Foo, R., Julia, L., Huaishu, X. and Norman, Y. 2003. *Mar. Poll. Bull*., 39(1- 12), 245- 249.

Kiran, G.S., Shanmughapriya, S., Jayalakshmi, J., Selvin, J., Gandhimathi, R., Sivaramakrishnan, S., Arunkumar M., Thangavelu, T. and Natarajaseenivasan, K. 2008. Optimization of extracellular psychrophilic alkaline lipase produced by marine *Pseudomonas* sp. (MSI057), *Bioprocess. Biosyst. Eng.,* 31: 483–492

Lee, K.H. and Ruby, E.G. 1994. Effect of squid host on the abundance and distribution of symbiotic *V. fischeri* in nature. *App. Environ. Microbiolog.,* 60: 1565-1571

Manjusha, S., Sarita, G.B., Elyas, K.K and Chandrasekaran, M. 2008. American *Journal of Biochemistry and Biotechnology*, 1(4): 201-206

Mrugesh Khunt and Neepa Pandhi. 2012. Purification and characterization of lipase from extreme halophiles isolated from little rann of kutch,*international journal of life science and pharma science* 2 (1)

Nawani, N. and Kaur, J. 2000. Purification, characterization and thermo stability of lipase from a thermophilic *Bacillus* sp. *Molecular and Cellular Biochemistry,* 206: 91-96.

Ranjitha, P., Karthy, E.S. and Mohankumar, A. 2009. Purification And Characterization of The Lipase From Marine *Vibrio fischeri, Inter. Jour. of Microbio.* 1(2)

Ramani, K., Kennedy, L.J., Ramakrishnan, M. and Sekaran, G. 2010. Purification, characterization and application of acidic lipase from *Pseudomonas gessardii* using beef tallow as a substrate for fats and oil hydrolysis, *Process Biochemistry,* 45: 1683–1691

Reed, L.A., Siewicki, T.C and Shah, J.C. 2004. *Aquaculture*, 232: 11-28

Reichelt, J.L. and Baumann, P. 1973. Taxonomy of the marine, luminous bacteria. *Arch. Microbiol*. 94: 283-330.

Sabu, A. 2003. Sources, properties and applications of microbial therapeutic enzymes. *Indian J. Biotechnol*., 2: 334-341.

Sarkar, S., Sreekanth, B., Kant, S., Banerjee, R. and Bhattacharyya, B.C. 1998. Production and optimization of microbial lipase, *Bioprocess Engineering*, 19: 29-32

Srinivasan, P. and Ramaswamy, P. 2009. Occurance, Distribution and Antibiotic Resistance Patterns of *Vibrio* species associated with viral diseased Shrimp of South Indian Aquaculture Environment. *International Journal of Agriculture Sciences*, ISSN: 0795-3710, 1(2): 01-10