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Review Article

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VIBRIOSIS - DETECTION AND PATHOLOGY: REVIEW

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Abstract

Aquaculture has been considered as tradition in several parts of Asia. At present aquaculture is the world's fastest growing food production sector, with cultured shrimp and prawn to be considered as sub sector. Diseases in hatcheries and farms are being increasingly recognized as major hurdles to successful and profitable industry. In majority of the diseases, Problems can be traced to water quality and poor management. Intensive culture systems offer an ideal environment for disease outbreak, because such systems have stressed the host and virulent pathogen. Based on the disease condition, which may result in mass mortality of the affected population in a short time leads to small scale mortality, reduced growth make the cultured shrimp unsuitable for human consumption.

Key Words: Vibrio, pathogen, histology, shrimp, detection.

INTRODUCTION

Aquaculture remains a growing vibrant and important production sector for high-protein animal food. The contribution of aquaculture to the total production of capture fisheries raising from 34.5% in 2006 to 36.9% in 2008, in the period 1970 – 2008. According to the state of world fisheries and aquaculture 2010,the production of food fish from aquaculture increased at an average annual rate of 8.3%. However, a major setback in aquaculture is the sudden outbreak of disease, especially those caused by vibrio spp, which are considered a significant problem to the development of a sector with severe economic losses worldwide, Global estimation of disease losses by the world bank in 1997,was approximatelyUS$3 billion per year. *Vibrio harveyi* and *Vibrio anguillaum* are most frequently isolated marine vibrio species. Which are associated with large scale losses of larval and juvenile penaids.

Vibriosis is one of the major disease problems in shellfish and finfish aquaculture. Vibriosis is a bacterial disease responsible for mortality of cultured shrimp worldwide (Lightner and Lewis, 1975: Adams, 1991: Lightner *et.al.*, 1992: Lavilla Pitago *et.al.*,2000). Vibrio species are widely distributed in cultured *Penaeus* and act as opportunistic pathogen and autothonous flora. . Facilitates throughout the world.vibrio related infections frequently occur in hateheries. 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 & Miyazaki,1994:Alday-Sanz *et.al*,2002).

Vibriosis is also rampant in the Indian region where brackish water shrimp farming is the main aquculture activity.

The disease problem is particularly severe in hatcherics ,and in the past few years many units were shutdown due to invasion by luminous vibrios (Karunasagar *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 growout pounds and is frequently responsible for the 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 disease of shrimp from culture ponds of coastal Andhra Pradesh was undertaken during the period 2001-2003.

Vibrosis species are a normal part of the bacterial flora in aquatic environments and formerly considered to be mostly opportunistic pathogens. (Lightner,1996: Myers,*et.al.*, 2006: Thompson,*et.al.*,2003) However.Some work occurring disease syndromes of penaied shrimp have been caused by vibrio species which behave more like true pathogens than opportunistic invaders (Gomez-Gil,*et.al.*, 2004: Kannaripan,*et.al.*,2008).Vibriosis causes mortality in larvae postlarvae,juveniles,sub-adults and also adults of shrimps. Outbreak of the disease cause mortality up to nearly 100% of affected population (Sunaryanto and Mariyam,1987).

The gross signs of localized infection in the cuticle (or) sub cuticle are called shell disease (or) black (or) brown spot disease and these superficial infection under some circumstances.It is the systemic infection that cause mortality (Chen *et.al.*, 1992: Myers:*et.al.*,2003 Suddesh and Xu,2000).

Isolation And Identification of Bacterial Isolates

Vibrio sp are quite prevalent in shrimp from culture ponds of costal area and the disease – induced

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morbidity / mortalities are causing considerable damage to the culture system. Bacterial disease are encountered in the cultured shrimp of which tail necrosis, shell disease, and red disease were recorded earlier from many region (Lighter 1988; Sindermann 1990; Nash *et al*. 1992; yang *et.al* 1992) including India (Hameed 1994 ; Abraham and Manley 1995; Hameed *et.al* 1996) other disease, constitute new emerging disease causing server loss to the shrimp industry in India , (Mayavo *et al.,* 2003; Gopalakrishnan and Parida 2005).Chandrakala and Ayyavoo(2006) isolated Vibrio sp such as V.*cholerae, V.parahaemolyticus ,V.splendidus, V.harveyi(NL), V.harveyi(L)* from diseased cultured and wild *Penaeus monodon*. Chandrakala *et al*., (2008) isolated vibrio protein from diseased *P.monodon*. Chandrakala et al (2005) prepared antibiogram for vibrio sp isolated from diseased *Penaeus monodon*. *Vibrio parahanmolyticus* 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 by shell disease and VSS (Hameed 1994; Lee *et.al* 1996; Jayasree *et al*1999,2000). Mass Mortalities due to red disease from *V.parahaemolyticus* along with other vibrio spp were isolated by Tendencies and Dureza (1997) from ponds in the Philippines.Chandrakala and Ayyavoo(2010)studied the WSSV infected wild and cultured *P.monodon* along the South East coast of India. Chandrakala et al (2010) made survey on the pathogenic microbes in ice stored *Penaeus monodon*.

Adult shrimps suffering from vibriosis may appear hypoxie, show reddening of the body with red to brown gills, reduce feeding and may be observed swimming lethargically at the edges and surface of ponds (Anderson *et al*., 1988; Nash *et al.,* 1992). Vibrio spp also cause red-leg disease, characterized by red colouration of the pleopods, periopods and gills, in juvenile to adult shrimps and may cause mortality of upto 95% during the warm season (Chen, 1992). Eyeball necrosis disease is caused by *V.cholerae*, The eyeballs of infected shrimps turn brown and fall away and mortality occurs within a few days (Chen, 1992).Sankar ganesh et al(2011) isolated the Vibrio sp from the culture ponds.Chandrakala et al(2010) made the survey on the pathogenic microbes in ice stored *Penaeus monodon* (Fab).

Molecular Basis of Identification

An array of molecular techniques is gaining popularity for the identification of different aquaculture related bacterial pathogens. DNA sequence based identification, analysis of 16s rRNA and other housekeeping gene sequence are the most popular and precises method currently used to identify closely related vibrio among other methods, ribo typing and PCR based techniques, eg.Amplified fragment length polymorphism (AFLP) fluorescence Insights Hybridization, (FISH), Random Amplified polymorphic DNA (RAPD), repetitive palindrome PCR (rep-PCR), and Restriction fragment length polymorphism (RFLP) have yield the most valuable information and new insights into the identification of closely related marine bacteria.Chandrakala *et al*(2008) made detection of Vibrio proteins from diseased *P.monodon*(Fab). Chandrakala et al (2013)Salt passage analysis of vibrio species isolated from diseased *Penaeus monodon* (Fab).

PCR-Based Identification: The majority of reported work has been to identify V.harveyi – related marine bacteria using PCR, because V.harveyi is the major causative organism of luminous vibriosis, causing potential devastation to diverse ranges of marine invertebrates over a wide geographical area, these microgranisms, however, are extremely difficult to identify because they are phenotypically diverse

Bramhachari and Dubey (2006) developed PCR-based identification methods for *V.harveyi* targeting a partial 16s rRNA gene (2006) . Fukui and Sawbe (2007) modified the method by developing a one step colony PCR targeting the same 16s rRNA gene to identify pathogenic *V.harveyi* from aquaculture setting .Similarly Conejero and Hedreyda (2003) developed haemolysin gene based multiplex PCR for simultaneous detection of *V.campbelli,V.harveyi, and V.parahamolyticus*.The for R gene for identification of *V. harveyi* from aqualture system Conejero and Hedreyda 2003).However the most precise methods to identify *V.harveyi* the *V.campebellii* and *V.parahaemolyticus* was developed by Haldal *etal*(2010).

16sRNA and housekeeping gene based identification

16sRNA gene sequencing is considered by many authors to be a very reliable method for indetification of any bacteria including marine vibrio(Gomez Gill *et al.,*2004;Maugeri *et al.,* 2006:Chatterjee *et al*., 2008:Gugliandolo *et al.,* 2010;Manmadan *et al.,* 2006;Haldar et al;2011b).The 16sRNA gene (about 1,500bp in length) consist of highly conserved regions and is present in almost all bacteria which may reveal deep-branching relationships, while variable regions may be demonstrated to be useful in discriminating species with the same genus, This feature has enable to use 16s RNA both as a phylogenetic marker and as an identification tool.

Fluroescent insitu Hybridization (FISH) provides a powerful tool for identifying the location of cloned DNA sequence.It use fluorescent probes to bind to those parts of chromosomes will which they show high degree of similarity and is often used in the field of microbial ecology.

Recently a one-step multi-probe FISH method has been developed. In short, the FISH method was combined with

micro-colony formation culture and is known as FISH following cultivation (FISHFC). It has the advantage of increasing its applicability. A probe reacting to the micro colonices in selective media with in a short time, increasing its applicability. A probe reacting to the micro colonies generates stronger

Restriction Fragment length polymorphism or RFLP is a technique that exploits variations in homologous DNA sequences.It refers to differences between samples of homologous of restriction enzyme sites. A simple and rapid RFLP method was developed by Sha *et-al* in 2006 based on the chromosomal ori sequence of *V.cholerae*. It was effective to delineate between two closely related biotypes of pathogenic vibrio stains. In recent study Chowdhmy *et.al.(77)* has developed an RFLP method targeting sections of the super integron region of the *V.cholerae* genome, and demonstrated good delineation between different biotypes of *V.cholerae* strains.

Amplified Fragment Length Polymorphism (AFLP)

ALFP another successful PCR-based method for differentiating closely related vibrio species. The AFLP method consists of three main step (i) digestion of total genomic DNA with two restriction enzymes and subseqvent ligation of the restriction half-site specific adaptors to all restriction fragment.(ii) selective amplification of these fragment withtwo PCR primes that have corresponding adaptor and restriction site sequvances as their target sites and. (iii) electrophoretic sepaaretion of the PCR products on polyacrylamide gels with selective detection of fragment which contrin the fluorescently labeled primes and computa-assisted numerical analysics of the band patterns the original method described in 1995 by Vos *etal* (28) used radio labeled primers, but it has since been modified to utilize fluoresecent labels.

Random Amplifed Polymophic Dna (RAPD)

RAPD is a rapid powerful and inexpensive PCR method using arbitrary primes to defect a segment of DNA in the genome. No knowledge of the DNA sequvance of the targeted gene is required, as the primers will bind somewhere in the sequence . In recent years, RADP has been used to characterize nd trace the phylogency of diverse plant and animal species. In *V.harveyi*. It has been used to differentiate pathogenic and non-pathogenic strains and has been used in diversity studies of the vibrios(Chandrakala and Ayyavoo,2006).

HISTOPATHOLOGY

Systemic vibroisis typically results in the formation of septic haemocyte nodules in the lymphoid organ, heart and connective tissues of the gills,hepatopancreas, antennal gland, nerve card, telson and muscle (Anderson *et al.,* 1988; Mohney *et al* 1991; Jiravanichapaisal *et al* 1994; Infected hepatopancreocytes may appear poorly vacuolated, indicating low lipid and glycogen reserves (Anderson et al 1988) vibriosis in *P.monodon* is associated with the formation of "speroids" in the lymphoid organ (Naush et al 1992). Chandrakala et al (2006) performed the PCR detection and Histopathology of WSBV infected *Penaeus monodon* (Fab).

Reference

Abraham,T.J. and R.Manley.1995. Luminous and nonluminous *vibrioharveyi* Associated with shell disease in cultured *Penaeus indicus*. *Journal of Aquaculture in Tropics* 10:273-276.

Adams,A.1991. Response of Penaeid shrimp to exposure to vibrio species. Fish shellfish Immunol 1:59-70.

Alday –Sanz. V., Roque,A., Turnbull, JF.2002. Clearing mechanisms Adams, A.1991 Response of *Penaeid* Shrimp to exposure to Vibrio *Penaeusmonodon*. Dis Aquat org 48:91-99.

Anderson,I.G., Shamsudin,M.N. and Shariff, m.1988. Bacterial septicemia in Juvenile tiger shrimp, *Penaeus monodon*, cultured in Malaysian brackish waterponds. AsianFis.Sci 2:93-108.

Bramhachari PV, Dubey SK [2006] Rapid and specific detection of luminous and non-luminous *vibrio harveyi* isolates by PCR for amplification. *Curr sci* 90:1105-1108.

Chandrakala N,S.Sarala and Prabakaran M (2010). Survey on the pathogenic microbes in ice stored *Penaeus monodon* (fab) hamdard medicens, 53(2) 88-97.

Chandrakala, N. Ayyavoo. M. Comparative studies on the WSSV infected wild an cultured *P.monodon* along the South East coast of india. *Indian hydrobiology*. 13(1) 19-25;2010.

Chandrakala, N. Sampathkumar, G. P.Samuvel, P.Ganesan, U Prabakaran M and Ayyavoo.M (2006). PCR detection and Histopathology of WSBV infected *Penaeus monodon* (fab) Hamdard medicous,XLIX (4) 100-105.

Chandrakala, N.Ganesan. U. Screening of MDR Bacterial strains hospitals in Trichirapalli. I.J. Appl. Microbiol.,12(1) 13-15; 2010.

Chandrakala,N,Sankar Ganesh,P , Prabakaran, M, and Sampathkumar,G, [2013] preliminary screening of *curcuma longa [Linn] against Penaeus monodon pathogenic* Vibrio sp. Ind. J. Applied Microbiology 16(1)21-29

Chandrakala,N and Ayyavoo, M [2006] an intensive study on the existence of microbial pathogens in the tiger shrimp *Penaeus monodon[Fab]*. *PhD* Theses submitted to Bharathidasan University, Tiruchirappalli.

Chandrakala,N. Sampathkumar,G, Ganeshan,U ,Prabakaran.M. and Ayyavoo.M(2005). Invitro studies on the susceptibility of bacteria isolated from multispeciality hospitals of Thanjavur. *I. J.Appl. Microbiol*. 87-89.

Chandrakala. N, Sampathkumar, G, Rebekka, P ,Kirubajasmine, G Prabakaran M and Ayyavoo.M (2008). Detection of *Vibrio* proteins from diseased *P.monodon* (fab) by ELISA and western blotting. *Proc. Nat. Acad. Sci. India. Sec.* B; Vol 78 pt II 133-136.

Chandrakala.N, Sampathkumar,G, Sankar ganesh, P M.Prabhudevan (2006).Studies on the role of VAM fungi in plant growth. AD. plant.sci. 19(1)167-170.

Chatterjee S , Haldar S, Asakura M ,Yamasaki S, Balasubramanian T [2008] Molecular identification and phylogenetic status of marine *Bacillus* associated with coral sediment, showing antibacterial effects against human pathogens *Annal Microbiol* 58:309-312.

Chen, S.N.,S.N.L.Huang, and G.H.Kou. 1992. Studies on the epizootiology and pathogenicity of bacterial infection in cultured giand tiger prawns *Penaeus monodon*, in Taiwan. pages 195-205 in w.Fulkas and K.L.Main editors. diseases of clutured *Penaeid* shrimp in Asia and the United states. Hawaii Oceanic Institue.

Chen,D.1992.An overview of the disease situation,diagnostic techniques,treatments and preventatives used on shrimp farms in China. In:W.Fuls and K.L.main(eds.) Diseases of cultured Penaeid shrimp in Asia and the Unites states. *The Oceanic Institute*,Hawaii.pp,47-55.

Chen,S.C.,S.L.Huang and G.H.KOU.1992 studies on the Epizoology and Bacterial Infection in cultured Giant Tiger Prawns,*Penaeusmonodon* in Taiwan.In Disease of cultured *Penaeid* shrimp in Asia and the united states pp:195-205.

Chowdhury N, Asakura M, Neogi SB, Hinenoya A, Haldar S, et al [2010] Development of simple and rapid PCR -fingerprinting method for *Vibrio cholerae* on the basis of genetic diversity of the superintegron. *J Appl Microbial* 109:304-312 .

Conejero MJU, Hedreyda CT [2003] Isolation of partial toxR-targeted PCR primers for spcies detection. J Appl microbiol 95:602-611.

Cook, DW., Lofton,SR.1973.Chitinoelastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab, Jwild-Dis 9:154-159.

Fukui Y, Sawabe T [2007] Improved one step colony PCR for detection of *Vibrio harveyi*. *Microbes* Environ 22: 1-10.

Geomez-Gil,B.,S.Soto-Rodriquez,A.Garcia-Garca,R.Vazquez-Juarez,F.L.Thompson, and J. Swings.2004. Moleculer identification of *Vibrio harveyi* related isolates Associated with Disease Aquatic Organisum microbiol.,150:1709-1777.

Gomez-Gil B, Soto-Rodri guez S , Garci`a-Gasca A, Roque A,Vazquezjuarez R, et al.[2004] molecular identification of *Vibrio harveyi*-related isolates associated with diseased aquatic organisms. *microbiology* 150:1769-1777.

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.

Gugliandolo C, Lentini V, Spano A, Mauguri TL(2010) conventional and molecular methods to detect bacterial pathogens in mussels let Appl microbiol 52;15-21.

Haldar S, Mody KH, jha B [2011b] Abundance, diversity and antibiotic resistance pattern of *Vibrio spp*. In coral ecosystem of Kurusadai island. *J Basic Microbial* 51:153-162

Haldar S, Neogi SB, Kogure K, Chatterjee S Chowdhury N, et al [2010] Development of a haemolysin gene-based multiplex PCR for simultaneous detection of *Vibrio campbellii,Vibrio harveyi* and *vibrio parahaemolyticus*. *Lett Appl microbiol* 50:146-152.

Hameed, A.SS., J.J. Farmer, F.W.Hickmann Brenner, and G.R.Farming.1996. characteristics and Pathogenity of a *Vibrio camplelli* like bacterium affecting hatchery reared *P.indicus* (Milne Edwards 1837) larvae. *Aquaculture Research* 27:85.

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

Jayasree,L., P.Janakiram, and R.madhavi.2000 charateristics, pathogenicity and antibiotic sensitivity of bacterial isolates from white spot diseased shrimps. *Asian Fisheries Science* 13:327-334.

Jayasree,L.,P.Janakiram, and R. Madhavi. 1999. Shell disease in freshwater prawn *Macrobachium rosenbergii* [de Man] aetiology, pathogenicity and antibiotic sensitivity. *Journal of Aquaculture in Tropics* 14:289-298.

Jiravanichapaisal,P and Miyazaki,T.1994. Histopathology, biochemistry and pathogenicity of *Vibrio harveyi* infecting black tiger shrimp *Penaeus monodon J .aquat.An.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.,, otta,S.K. and karunasagar, I.1996.Effect of chorination on shrimp Pathogenic *Vibrio harveyi*.World Aquaculture.96,book of abstracts the world Aquaculture society, Baton Rouge,La.p.193.

Kunnaripan,E,Ravindran,J.R.Chandrasekar and A.Kalaiarasi 2008.studies on luminous, *Vibrio harveyi* Associated with shrimp culture system searing *Penaeus monodon* Triveni Enterprise Lucknow, India.

Lavilla–Pitogo,CR., Leano,Em., Paner,MG,1998. Mortalities of pond-cultured jevenile shrimp *Penaeus monodon* associated with dominance of luminescent *Vibriosis* in the searing 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.

Lightner, D.V and Lewis ,D.H.1975.A septicemic bacterial disease syndrome of *Penaeid* shrimp marine. *Fisheries.Rev*.37(56):25-28.

Lightner,D.V.(ed.).1996.A Handbook of shrimp pathology and Diagnostic Procedures far Disease of cultured *Penaeid* shrimp world Aquaculture sociaty,Baton Rouge,LA,USA.

Lightner,D.V., Bell,T.A., Redman,R.m., Mohney,L.L., Natividad,J.m., Rukyani,A. and Poernomo, A.1992.A review of some major disease of economic significance in Penaeid shrimps/shrimps of the Americas and Indo-pacific.In:M.shariff,R.subasinghe and J.R Arthur (eds.)proceedings 1st symposium on Disease in Asian Aquaculture Fish Health section, Asian Fisheries Society, manila, philippines.PP.59-80.

Lightner,D.V.1988. *Vibrio* disease of *Penaeid* shrimp pages. 42-7 in C.JSindermann and D.V.Lightner in North American marine aquaculture, 2ndedition. Elsevier. Newyork. Newyork,USA.

Manmadan K,Sasaki H,Haldar S,Yamaski S,Nagata S(2006) Antibacterial activities of marine epibiotic bacteria isolated from brown algae of japan *Annal Microbiol* 56:167-173.

Maugeri TL,Carbone M ,Fera MT,Gugliantolo C[2006] Dection and differentiation of *Vibrio vulnificus* in seawater and plankton of a coastal zone of the mediterranean sea. *Res microbiol* 157:194-200.

Mayavu, P., A.Purushothaman, and k.karthiresan.2003. Histology of loose-shell affected *Penaeus monodon, current science* 85:162-1634.

Mohney, LL. Bell T.A and Lightner, D U 1992 Shrimp .antimicrobial testing In Invitro susceptibility of thirteen gram negative bacteria to twelve antimicrobials *J.Aqua. Animal health* 4 pp 257-261.

Myers M.LPanicker G. and Bej.A.K.2003. PCR detection of a newly emerged Pandemic *Vibrio parahaemolyticus* 03:k6 pathogen in pure culture and seeded waters from the Gulf of mexico. *Appl Environ microbiol*.,69():2194-2200

Myers.M.L., Panicker G. and Bej, A.K.2006. Detection of Pandemic *Vibrio parahaemolyticus* 03:k6 serovar in Gulf of mexicounter and shellfish using realtimePCR with Taqmanflourescent Probes FEM Smicnobiollett., 262(2):185-192.

Nash,G.Nithimathachoke,C.,Tungmandi,C.,Arkarjamorn, A., prathanpipat, p. and Ruamthaveesub, P.1992.*Vibriosis* and its control in pond-reared *Penaeusmonodon* in Tailand.Disease in Asian aquculture.1.Inproceeding of the first symposium on diseases in Asian Aquaculture. 26-29 November 1990.

Saha A, Deb R, Shah S, Ramamurthy T ,Shinoda S,et al [2006] PCR -based identification of *Vibrio cholerae* and the closely related species *Vibrio mimicus* using chromosomal ori sequence of *Vibrio cholerae*. *FEMS Microbial Lett* 257: 84-91.

SankarGanesh. P. Saravanan J, Krishnamoorthy., P,Chandrakala.N, & Rajendran. K.2011. Isolation and identification of *Vibrio spp.* in diseased *Channa punctatus* from aquaculture fish farm. *Indian journal of Geo-Marine sciences*.pp.159-163.

Sindermann, C. J. 1990. Principal disease of marine fish and shell fish.volume 2. disease of marine shell fish 2nd edition. Academic press.Inc.

Suddesh, P.S., and H.S.XU-2001.pathogenicity of *Vibrio paraheamolyticus* in Tiger prawn *Penaeus monodon* Fabricus :possible Role of Extracellular Protease. *Aquaculture*,196:37-46.

Sunaryanto A.and A.mariyam.1987.occuraence of a pathogenic bacteria causing luminescene in *Penaeid* larvae in Indonesia hatcheries. *Bull.Brackish.Wat Agua*., 8:6-70.

Tendencia,A.E.V. and L.A Dureza.1997.isolation of *Vibrio spp.*from *Penaeus monodon*.(Fabricus)with red disease syndrome, *Aquaculture* 15:107-114.

Thompson,F.L.,C.C.Thompson,B.Hoster.K.Vandermeulebroecke,Guillan,and J.Swings 2003,*Vibrio* vortis sp.nov. and *Vibrio hepatorius* sp.nov. isolated from aquatic animal and the marine environment *Int.J.syst.Evol.micribiol*,53:1495-1501.

Vos p ,Hogers R, Bleeker M, Reijans M, Vandelee T,et al.[1995] AFLP: a new technique for DNA- finger printing. *Nucleic Acids Res* 23:4407-4414.

Yung J.,X.WU, and S.ZU. 1992 observation on black spot on shell disease of cultivated *Penaeid* shrimp by SEM. *Donghai marine science* 11:3-39.

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