BIOTROPIA NO. 15, 2000 : 36 – 47

GENETIC DIVERSITY OF AMPICILLIN-RESISTANT *Vibrio* ISOLATED FROM VARIOUS STAGES OF TIGER SHRIMP LARVAE DEVELOPMENT

WIDANARNI [a] AND ANTONIUS SUWANTO [b,c]

[a] Department of Aquaculture, Faculty of Fisheries and Marine Science
Bogor Agricultural University, Bogor 16680, Indonesia
[b] Department of Biology, Faculty of Science and Mathematics,
and IUC Biotechnology, Bogor Agricultural University, Bogor 16680, Indonesia
[c] SEAMEO-BIOTROP, Jl. Raya Tajur, Km 6 Bogor, Indonesia

ABSTRACT

This research was carried out to study genetic diversity of ampicillin-resistant *Vibrio* from various stages of tiger shrimp larvae (*Penaeus Monodon*) development from Tambak Inti Rakyat hatchery, near Labuan, West Java, Indonesia. A total of 25 ampicillin-resistant *Vibrio* isolates were isolated using thiosulphate citrate bile-salt sucrose. agar (TCBS-Agar) and seawater complete agar (SWC-Agar). Physiological and biochemical characterization showed that the isolates could be grouped into only two species, i.e. *V. harveyi* from the egg stage; and *V. metschnikovii* from larvae and post-larval stage (i.e nauplius, zoea, mysis, PL_1, PL_5, PL_{10}, and PL_{15}). These isolates were also present in their respective rearing water of each stage and some natural feed. Schizotyping analysis employing restriction endonuclease *Not*I (5'-GC↓GGCCGC) indicated that the isolates could be grouped into at least 13 different genotypes. Therefore, schizotyping was more discriminative than physiological characterization. This study showed that particular groups of *Vibrio* colonized all stages of shrimp larvae and demonstrated closed phylogenetic relationship. These groups of *Vibrio* might be the dominant microbiota which could suppress the development of other *Vibrio* including the pathogenic *Vibrio*.

Key words: Shrimp/ampicillin-resistant *Vibrio*/schizotyping

INTRODUCTION

Tiger shrimp (*Penaeus monodon*) culture has developed towards intensive levels due to high demand and significant price of this commodity. However, recently shrimp production has decreased significantly because of diseases and poor environment quality (Anonymous 1994). One of the most serious problems is the disease caused by *Vibrio* (Lavilla-Pitogo *et al.* 1990). The disease greatly influenced the sustainable supply of healthy fry (Lightner *et al.* 1992).

Hameed (1993) observed that the decrease of survival rate of larvae and post-larvae was due to the concomitant increase of the bacteria population. Generally, shrimps at the stadia of zoea, mysis, and the beginning of post-larvae were vulnerable to *Vibrio* infection (Rukyani *et al.* 1992). It was believed that these bacteria contaminated the hatchery through broodstock feces because of their presence in significant number in the midgut (Lavilla-Pitogo *et al.* 1990). Therefore,

*Corresponding author: email address: asuwanto@indo.net.id;Fax: 62-251-315107

contamination of the eggs and nauplius by these bacteria was highly probable. Besides the infected shrimp, these bacteria could also be isolated from seawater used for rearing water in the hatcheries (Tjahjadi *et al.* 1994). Recently, Suwanto *et al.* (1998) demonstrated that *Vibrio harveyi* isolated from different broodstocks were genetically very diverse and different to each other. Therefore, it can be expected that vertical infection from broodstock to eggs is more likely to occur rather than horizontal transfer from larvae to larvae. In order to understand genetic diversity of *Vibrio* at various stages of shrimp larvae development, a study on genetic diversity of *Vibrio* isolated from egg, larvae, and post-larvae of shrimp originated from the same broodstock was carried out.

A number of techniques have been developed to identify bacteria at the subspecies level. These include phage typing (Stringer 1980), analyses of plasmid DNA (Davies *et al.* 1981), ribotyping (Olsen *et al.* 1986), Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland 1990), and schizotyping by pulsed-field gel electrophoresis (PFGE) (Suwanto and Kaplan 1992). PFGE analysis of large sizes of DNA molecules requires a technique to isolate intact genomic DNA as well as the availability of rare-cutting restriction endonucleases and the appropriate mega-base molecular size markers. Under optimized condition, PFGE can efficiently separate DNA fragments of 100 to 10,000 kbp to distinguish strains of microorganisms which otherwise exhibit similar or identical physiological and morphological characteristics (Suwanto 1994).

Genetic diversity analysis based upon genomic DNA profile utilizing PFGE has been performed for various bacteria for various aims, such as *Vibrio anguillarum*, a causative agent of vibriosis in fish (Skov *et al.* 1995), genomic DNA analysis of *Xanthomonas campestris*, a causative agent of root-pustule disease of soybean (Rukayadi 1995) and *Vibrio harveyi*, a causative agent of "luminous-bacterial disease" (Suwanto *et al.* 1998). PFGE genomic DNA analysis used in the experiments above have proved to be more discriminative to visualize phylogenetic diversity rather than analysis based on phenotype characterization.

The purpose of this research was to study the genetic diversity of ampicillin-resistant *Vibrio* from various stages of shrimp larvae development by PFGE analyses.

MATERIALS AND METHODS

Isolation and identification of *Vibrio sp.*

Vibrio were isolated from eggs, larvae (nauplius, zoea, and mysis), and post-larvae (PL_1, PL_5, PL_{10}, and PL_{15}) of tiger shrimp (*P. monodon*), natural shrimp feed (i.e. *Artemia* and *Skeletonema*), and rearing water obtained from Tambak Inti Rakyat hatchery, near Labuan, West Java, Indonesia. Shrimp larvae were collected in the month of March-April, 1998. Five eggs and shrimp larvae were washed gently in sterile seawater and collected into a test tube containing 1 ml sterile seawater, and homogenized by Vortex agitator before it was spread on TCBS agar. Appropriate dilution was performed to obtain single isolated colony on TCBS agar by adding sterile seawater. The culture was incubated at room temperature, (28-31)°C, for 24 hours. Isolated colonies were randomly selected for further study. Biochemical and physiological characterization were conducted with Microbact^R Analysis kit (Medved Science Pty. Ltd. Australia). Identification of *Vibrio* isolates were done as described by Baumann *et al.* (1984). Seawater complete agar (5 g bactopeptone, 1 g yeast extract, 3 ml glycerol, 15 g agar, 750 ml seawater, and 250 ml distilled water) supplemented with ampicillin (50 ug/ml) (SWC-Ap) was employed as a selective media to screen for ampicilin-resistant *Vibrio* isolates. The isolates were subsequently suspended in sterile seawater containing 15% glycerol (v/v) before they were stored in the freezer at –50°C.

Preparation of intact genomic DNA and digestion

Purified isolates of ampicillin-resistant *Vibrio* were grown in SWC agar. One separated colony was regrown in 10 ml LB medium (10 g tryptone, 5 g yeast extract, 25 g NaCl and 1 L distilled water) at 28°C overnight. One ml of bacterial suspension was centrifuged at 5,000 rpm for 30 seconds. The bacterial cell pellet was suspended in sterile PIV solution (10 mM Tris-Cl pH 7.5, 1 M NaCl). Preparation of intact genomic DNA was performed by embedding the bacterial cells in low melting agarose blocks as described previously (Schwartz and Cantor 1984). Digestion of the intact genomic DNA was done using restriction enzyme as described by Suwanto *et al.* (1998) as follows: digestion with 10 units of *Not*I were performed in 150 ml of appropriate restriction buffer (1335 µl distilled water, 150 µl 10x restriction buffer, 15 µl of 10 mg/ml bovine serum albumine). The mixture was incubated at 4°C for 15 minutes followed by incubation in 37°C overnight. Dialysis of the gel plugs were performed by immersing the gel plugs in excess of 1x TE buffer solution (10 mM Tris-Cl pH 8.0, 1 mM EDTA pH 8.0) before placing the gel plugs into the wells of the running gel.

Separation of DNA fragment using PFGE

The running gels of 1% (w/v) agarose (Pharmacia) were prepared in 100 ml of 0.5x TBE (50 mM Tris-Borate buffer, 0.1 mM EDTA pH 8.0). Pulsed-field gel electrophoresis was performed using CHEF-DRII (Bio Rad, Richmond, CA). The gels were run in 0.5 x TBE buffer, 5 volt, 14°C with ramping pulse time from 10-80 seconds for 20 hours. As molecular marker, *Rhodobacter sphaeroides* 2.4.1 genomic digested with *Ase*I was routinely used for PFGE (Suwanto and Kaplan 1989).

DNA visualization

Gels were stained by submerging in ethidium bromide solution (1 µg/ml) for 10 minutes. Destaining were done in distilled water for 20-30 minutes. Transilluminator with UV length of 280 nm (Hoefer Scientific Instrument, San Francisco) was used to visualize the gel. Photographs were taken with fast film polaroid (Type 667, Japan Polaroid Company).

Statistical analysis

A matrix was constructed as the basis for determining the presence or absence of schizotyping bands at a given position over the size range from 30–500 kb. A cluster analysis was carried out using the unweighed pair group method with arithmetic means (UPGMA clustering with simple matching coefficient) of similarity coefficient for all pairs of strain and a dendrogram was generated using a computer-based taxonomy program (Numerical Taxonomy System, NTSYS-PC version 1.60) (Rohlf 1990).

RESULTS AND DISCUSSION

Physiological and biochemical characterization of *Vibrio* isolates

Twenty five ampicillin-resistant isolates have been isolated and selected randomly from either TCBS or SWC-Ap for further study (Table 1). Of these isolates, 20 isolates were found to be associated with eggs, larvae, post-larvae, and the rearing water of each stage; two isolates were isolated from seawater reservoir; and the other isolates were obtained from natural feed (i.e. *Artemia* and *Skeletonema*).

Table 1. Codes and sources of *Vibrio* isolates

No.	Code	Source	No.	Code	Source
1.	E_1	Egg	14	ST	Broodstock tank water
2.	E_2	Egg	15.	ET	Spawning tank water
3.	N	Nauplius	16.	NT	Nauplius tank water
4.	Z	Zoea	17.	ZT	Zoea tank water
5.	M_1	Mysis	18.	MT	Mysis tank water
6.	M_2	Mysis	19.	PL_1T	Post-larvae 1 tank water
7.	M_3	Mysis	20.	Pl_5T	Post-larvae 5 tank water
8.	PL_1	Post-larvae 1	21.	$PL_{10}T$	Post-larvae 10 tank water
9.	PL_5	Post-larvae 5	22.	$PL_{15}T$	Post-larvae 15 tank water
10.	PL_{10}	Post-larvae 10	23.	SKT	*Skeletonema*
11.	PL_{15}	Post-larvae 15	24.	AT_1	*Artemia*
12.	SW_1	Sea water	25.	AT_2	*Artemia*
13.	SW_2	Sea water			

Physiological and biochemical characterization showed that the isolates shared several similar characters. The cell was rod shape, produced lysine decarboxylase and indole, and all were able to ferment glucose (Table 2). The isolates could be classified into three groups. First group, composed of E_1, E_2, M_1, SW_1, SW_2, and MT shared the same characters such as: forming green colonies on TCBS, oxidase-positive, produce protease and chitinase, and able to reduce nitrate. A number of isolates were also able to utilize arabinose as a carbon source. The second group of *Vibrio* composed of only one isolate, AT_2 isolated from *Artemia* had the same characters as first group except that it was luminous. The third group (N, Z, M_2, PL_1, PL_{15}, ET, ZT, PL_5T, $PL_{10}T$, SKT, AT_1), was the dominant group of the isolates found in every larval stage except the eggs possessed completely different characters from the two groups in having non-luminous yellow colonies on TCBS, showed negative oxidase reaction, did not produce protease and chitinase, and were able to use sucrose as a carbon source.

Based upon Baumann *et al.* (1984) identification, the first group was non-luminous *V. harveyi*, the second was luminous *V. harveyi*, and the third was *V. metschnikovii*. Therefore, physiological characterization of *Vibrio* isolated from each stage from Tambak Inti Rakyat hatchery, West Java, showed that two species of *Vibrio* had been found. *V. harveyi* were associated with eggs, while *V. metschnikovii* were found in larvae and post-larvae (nauplius, zoea, mysis, PL_1, PL_5, PL_{10}, and PL_{15}). These isolates were also present in their respective rearing water of each stage and some natural feêd.

Schizotyping analysis

PFGE electrophoresis of the 25 *Vibrio* isolates (obtained in this study) and of *V. harveyi* S14B (Suwanto *et al.* 1998) using *Not*I restriction enzyme (Figure 1) showed discrete profiles of genomic DNA of the isolates based upon the number of fragments and migration distance (Table 3). Restriction with *Not*I produced 10-16 discrete DNA fragments with sizes ranging from 31-910 kb. Genomic DNA analysis of 25 isolates showed 13 different DNA profiles. These DNA profiles could be used as a unique fingerprint of each of those isolates.

Dendrogram of genomic DNA digested with *Not*I (Figure 2) showed genetic relatedness of the isolates, which could be divided into 13 different sub-groups. The sum of horizontal lines connecting the two isolates indicated genetic distance of those isolates. For example, the distance between isolate PL_1 and PL_5 is zero indicated that these isolates are identical. Of these, 13 sub-groups were divided into 2 major groups. The first major group consisted of 20 isolates associated with all stages of shrimp larvae and their rearing water. The second group consisted of 5 isolates, i.e. 2 isolates from seawater reservoir, 1 isolate from broodstock rearing water, and 2 isolates from natural feed (*Skeletonema* and *Artemia*). Some *Vibrio* isolates originated from seawater and natural feed also could not be isolated from all larvae stages and eggs of shrimp. This result suggested that eggs were exposed to bacterial contamination not only from broodstock feces as reported by Lavilla

Table 2. Biochemical and physiological characteristics of *Vibrio* isolates*

Characteristics	Isolates Code																		
	E_1	E_2	N	Z	M_1	M_2	PL_1	PL_{15}	SW_1	SW_2	ET	NT	ZT	MT	PL_5T	$PL_{10}T$	SKT	AT_1	AT_2
Colony colour on TCBS	G	G	Y	Y	G	Y	Y	Y	G	G	G	Y	Y	G	Y	Y	Y	Y	G*
Rod shape	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	+
Protease	+	+	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	+
Chitinase	+	+	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	+
Nitrate reduction	+	+	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose fermentation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	-	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β-galactosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	+	-	-	+	+	+	-	+	-	-	-	-	-	-
Voges proskauer reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malonate utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Continued

Characteristics	Isolates Code																		
	E_1	E_2	N	Z	M_1	M_2	PL_1	PL_{15}	SW_1	SW_2	ET	NT	ZT	MT	PL_5T	$PL_{10}T$	SKT	AT_1	AT_2
Sucrose	-	-	-	+	-	+	+	+	-	-	-	+	+	-	+	+	+	+	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Identification	Vh	Vh	Vm	Vm	Vh	Vm	Vm	Vm	Vh	Vh	Vh	Vm	Vm	Vh	Vm	Vm	Vm	Vm	Vh

Remarks : + = positive Y = yellow G* = luminous Vm = *Vibrio metschnikovii*
- = negative G = green Vh = *Vibrio harveyi* * Only representative isolates of schizotype groups in Table 3 were analysed.

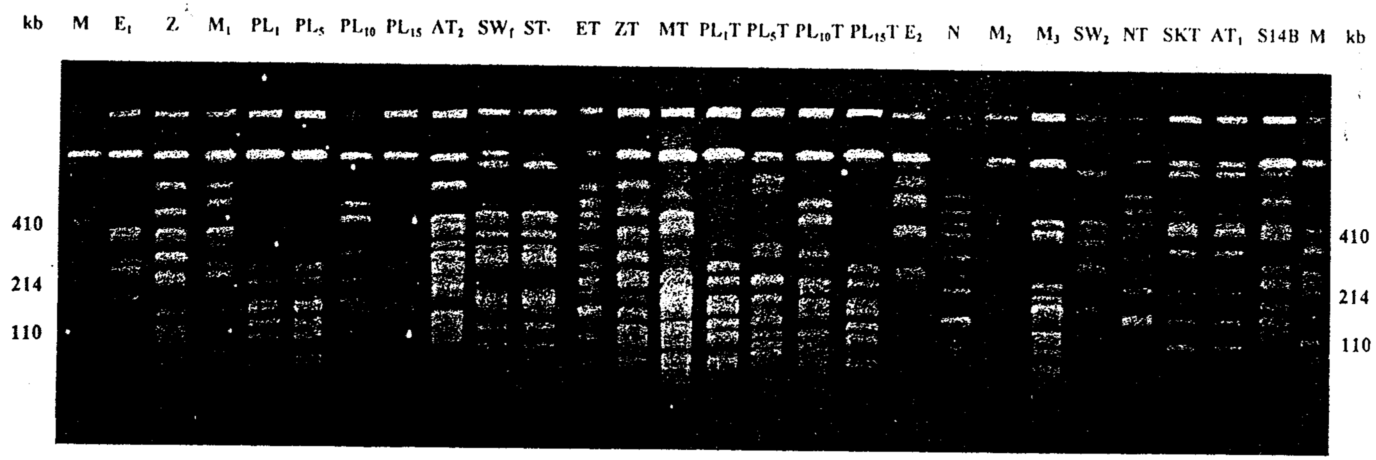

Figure 1. Pulsed-field gel eletrophoresis of *Not*I-digested genomic DNA of *Vibrio* isolates. M is *Ase*I-digested genomic DNA of *Rhodobacter sphaeroides* 2.4.1 as molecular size marker. The numbers in the right and left sides of the figure indicate molecular size of DNA fragment in kilobase pairs (kb). S14B is bacterial isolate identified as *V. orientalis* (Suwanto *et al.* 1998).

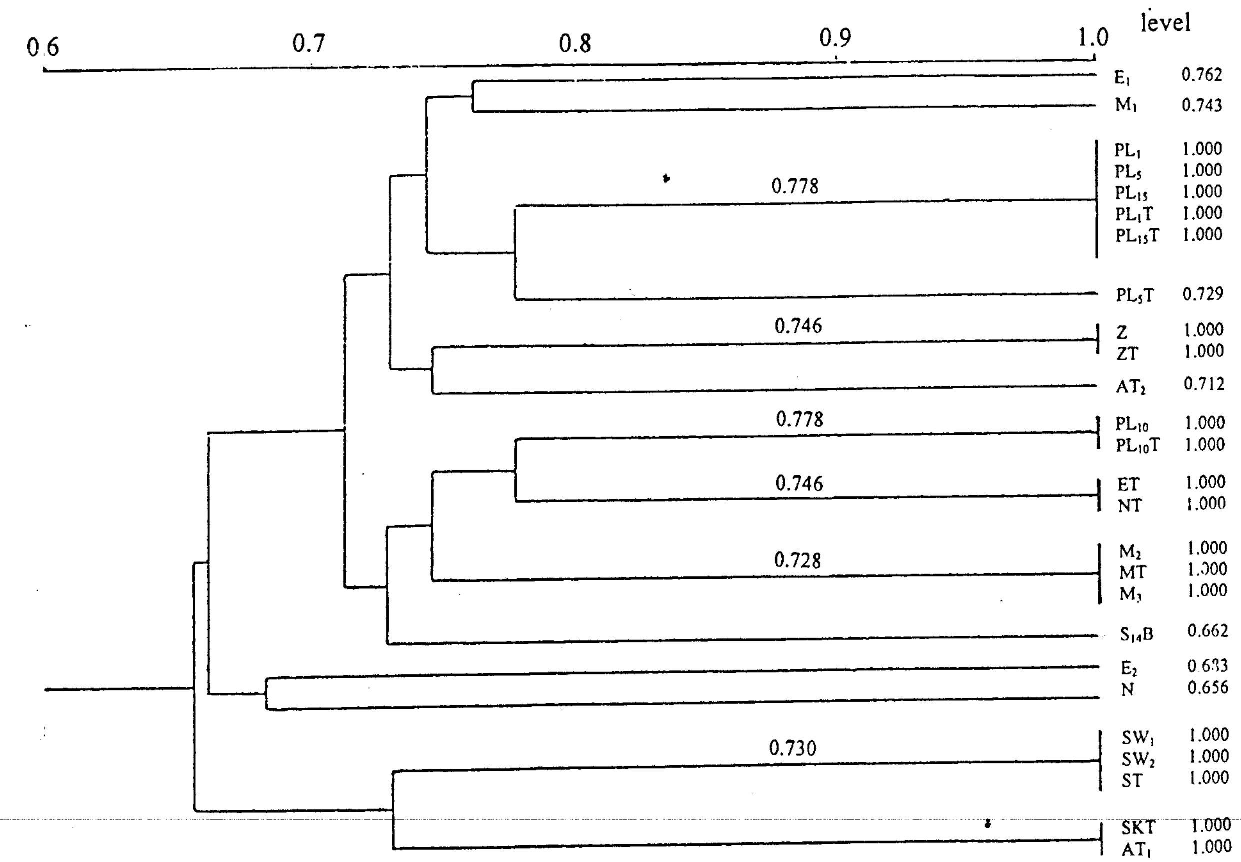

Figure 2. Dendrogram showing relationship amongst *Vibrio* isolates based on PFGE analysis of genomic DNA fragments generated by *Not*I.

Table 3. Grouping of genomic DNA profiles of 25 *Vibrio* isolates generated by *Not*I schizotyping

No.	Schizotype profile	Isolates exhibited similar schizotype
1.	E_1	E_1
2.	M_1	M_1
3.	PL_1	PL_5, PL_{15}, PL_1T, $PL_{15}T$
4.	PL_5T	PL_5T
5.	Z	ZT
6.	AT_2	AT_2
7.	PL_{10}	$PL_{10}T$
8.	ET	NT
9.	M_2	MT, M_3
10.	E_2	E_2
11.	N	N
12.	SW_1	SW_2, ST
13.	SKT	AT_1

Pitogo *et al.* (1990), but also when they were still in the ovary. Our study also demonstrated that *Vibrio* isolates from sea water or natural feed possessed distance genetic relationship to that of *Vibrio* from eggs and larvae, indicating that *Vibrio* from eggs and larvae may be the dominant microbiota and might play a role in suppressing the growth of *Vibrio* from seawater or natural feed.

Compared to the results of Suwanto *et al.* (1998), all of *Vibrio* isolates in this experiment showed relatively similar genetic background. This might be due to the fact that the broodstocks in this study were obtained from the same area (Aceh province), while Suwanto *et al.* (1998) used broodstocks from different areas and provinces.

This study showed that genetic analysis by PFGE was more discriminative than that of physiological and biochemical analysis. From 25 isolates characterized by physiological analysis, they could be grouped into two species, while using schizotyping analysis, there were 13 different groups of genotypes. Furthermore, with this analysis, *V. metschnikovii*, that did not produce protease and chitinase was apparently closely related to *V. harveyi*, a shrimp pathogen. Therefore, the existence of *V.metchnikovii* might influence shrimp larval fitness and survival.

In conclusion, physiological and biochemical characterization were able to classify 25 isolates *Vibrio* (both luminous and non-luminous) into two species, i.e. *V. harveyi* and *V. metchnikovii*. *Not*I schizotyping analysis showed that the isolates consisted of at least 13 different genotypes, which indicated that schizotyping was more discriminative than physiological characterization. The result also demonstrated that *Vibrio* isolates present in all stages of shrimp larvae showed close phylogenetic relationship. These groups of *Vibrio* might be dominant and might play a role in suppressing the development of other *Vibrio* originated from sea water, broodstock feces or natural shrimp feed.

ACKNOWLEDGEMENTS

We express our thanks to Tambak Inti Rakyat Hatchery, Labuan, West Java, for supplying shrimp larvae and to allow us to use the field laboratory facility. We would like also to thank Prof. Dr. Maggy T. Suhartono and Dr. Dwi Andreas Santosa for their invaluable advice during the experiment. This research was supported by Riset Unggulan Kemitraan (RUK) research grant to Antonius Suwanto and Biaya Pendidikan Pasca Sarjana (BPPS) research grant to Widanarni.

REFERENCES

Anonymous. 1994. Problem solution alternatives of shrimp culture in Java. Directorate General of Fisheries. Jakarta.

Baumann P, Furniss AL, Lee JV. 1984. Facultative anaerobic gram negative rods. *In:* Krieg NR (ed) Bergey's Manual of Systematics Bacteriology. Williams and Wilkins, Baltimore, MA. Vol. 1: 1513-1523.

Davies FL, Underwood HM, Gasson MJ. 1981. The value of plasmid profiles for strain identification in lactic streptococci and the relationship between *Streptococcus lactis* 712, ML3 and C2. J Appl Bacteriol 51: 325-337.

Hameed ASS. 1993. A study of the aerobic heterotrophic bacterial flora of hatchery-reared eggs, larvae and post-larvae of *Penaeus indicus*. Aquaculture 117:195-204.

Lavilla-Pitogo CR, Baticados CL, Cruz-Lacierda ER, de la Pena LD. 1990. Occurrence of luminous bacterial diseases of *Penaeus monodon* larvae in the Philippines. Aquaculture 91:1-13.

Lightner DV, Bell TA, Redman RM, Mohney LL, Natividad JM, Rukyani A, Poernomo A. 1992. A review of some major diseases of economic significance in penaeid prawn/shrimp of the Americans and Indopacific. *In*: Shariff M, Subasinghe RP, Arthur JR (eds). Diseases in Asian Aquaculture 1. Fish Health Section. Asean Fisheries Society. Manila, Philippines p.57-80.

Olsen GJ, Lane DJ, Gionvannoni SJ, Pace NR. 1986. Microbial ecology and evolution: a ribosomal RNA approach. Ann Rev Microbiol 40: 337-365.

Rohlf FJ. 1990. NT-Sys-pc, Numerical taxonomy and multivariate analysis system, version 1.60. Exeter Software, New York.

Rukayadi Y. 1995. DNA profile analysis of the genomes of a number of *Xanthomonas campestris* pv. glycines isolates using Pulsed-field gel Electrophoresis. Thesis Pascasarjana Program, IPB. Bogor. 69p.

Rukyani A, Taufik P, Taukhid 1992. *Luminous* vibrios in tiger shrimp hatchery and the control of the disease in shrimp larvae. J. Litbang Pertanian 2:1-17.

Schwartz DC, Cantor CR. 1984. Separation of yeast chromosome-sized DNA by pulsed-field gradient gel electrophoresis. Cell 37:67-75.

Skov MN, Pedersen K, Larsen JA. '995. Comparison of pulsed-field gel electrophoresis, ribotyping, and plasmid profiling for typing of *Vibrio anguillarum* serovar O1. App and Env Microbiol 61:1540-1545.

Stringer J. 1980. The development of a phage typing system for group B streptococci. J Med Microbiol 13:133-143.

Suwanto A, Kaplan S. 1989. Physical and genetic mapping of *Rhodobacter sphaeroides* 2.4.1 genome: genome size, fragment identification and gene localization. J Bacteriol 171:1135-1145.

Suwanto A, Kaplan S. 1992. Chromosome transfer in *Rhodobacter sphaeroides*: Hfr formation and genetic evidence for two unique circular chromosomes. J Bacteriol 174:1135-1145.

Suwanto A. 1994. Pulsed-field gel electrophoresis: a revolution in microbial genetics. As Pac J Mol Biotechnol 2:78-85.

Suwanto A, Yuhana M, Herawaty E, Angka SL. 1998. Genetic diversity of luminous *Vibrio* isolated from shrimp larvae *In*: Flegel TW (ed) Advances in shrimp biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, p 2-9.

Tjahjadi MR, Angka SL, Suwanto A. 1994. Isolation and evaluation of marine bacteria for biocontrol of luminous bacterial disease in tiger shrimp larvae (*Penaeus monodon*, Fab.). As Pac J Mol Biol Biotechnol 2:347-352.

Welsh J, Mc Clelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213-7218.