African Journal of Microbiology Research Vol. 6(20), pp. 4270-4276, 30 May, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.731
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Characterization of *Vibrio alginolyticus and Vibrio parahaemolyticus* isolated from *Penaeus monodon*: Antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis

Nilima Priyadarshini Marhual, Basanta Kumar Das* and Surya Kanta Samal

Fish Health Management Division, Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-2, Orissa, India.

Accepted 3 February, 2012

***Vibrio alginolyticus* and *Vibrio parahaemolyticus* are important pathogens which cause serious episode to shrimp. We characterize the suitability of randomly amplified polymorphic DNA (RAPD), and plasmid profiling for rapid molecular characterization of pathogenic bacteria for epidemiological and antibiogram study to find out the relationship between plasmids and bacterial sensitivity to antibiotics. Nine primers, belonging to OPA and OPY series were selected for the RAPD study. Primer OPA3 and OPY2 amplified 3 to 7 bands ranging from 0.306 to 3.94 Kb in *V. alginolyticus*. In *V. parahaemolyticus*, primer OPA 3 and OPY 2 amplified 3 to 7 bands of molecular wt 0.306 to 4.11 Kb. *V. alginolyticus* possess only one plasmid each with molecular weight 10. 23 Kb. *V. parahaemolyticus* strains contained 1 to 3 plasmids with molecular weight 7.12 to 10.23 Kb. The strains were mostly resistant to Ampicillin and Cefuroxime. Among the strains 90 and 70% were resistant Bacitracin and Co-trimazole, respectively. All strains of *V. alginolyticus* and *V. parahaemolyticus* were sensitive to Amoxycillin, Chlorotetracycline and Ciprofloxacin. Unique distinct amplicons generated by RAPD polymerase chain reaction (PCR) and plasmid profiling will be used for several diagnostic markers for identification of these pathogenic microorganisms and in epidemiological study.**

Key words: *Vibrio parahaemolyticus, Vibrio alginolyticus*, RAPD, antibiogram, plasmid profiling.

INTRODUCTION

Diseases are the major cause of losses in the aquaculture industry. Among them Vibriosis is one of the most frequent diseases affecting fishes, molluscs and crustaceans (Manivasagan et al., 2010). The *Vibrionaceae* are gram negative γ-proteobacteria that occurs in temperate to tropical, coastal and estuarine marine systems (Thompson et al., 2004). *Vibrio* spp. occupies a diverse range of ecological niches including sediments, water column, and in association with organisms either as symbionts (Ruby et al., 2005) or pathogens (Tracy et al., 2007). It is an economically important disease of fish, marine invertebrates (particularly penaeid shrimps) and large marine mammals and is responsible for high mortality rates in aquaculture worldwide (Marhual et al., 2010). *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are important and halophilic Gram-negative pathogen, which cause serious episode to marine fish and shellfish including shrimp (Zorrilla et al., 2003; Du et al., 2007; Caburlotto et al., 2008; Marhual and Das, 2009; Marhual et al., 2010).

Subtyping methods are important invaluable techniques

*Corresponding author. E-mail: basantadas@yahoo.com. Tel: +9437080411. Fax: +91 674 246 5407.

for studying microbial epidemiology and ecology. Random amplification of polymorphic deoxyribonucleic acid (DNA) RAPD is a rapid subtyping method to characterize genetic differences and has been used to fingerprint a variety of bacterial species including *V. alginolyticus* and *V. parahaemolyticus* (Welsh and McClelland, 1993; Ripabelli, 2003; Marhual and Das, 2009; Marhual et al., 2010). The earliest DNA-based method applied to epidemiological studies reported by molecular biologists is plasmid profiles determination (Meyer, 1988; Radu et al., 1998; Zulkifli et al., 2009). The profile identifications are useful to determine the serotype-specific reference patterns that are responsible for detecting certain strain with possible variation in plasmid content. The profiling is also a relatively quick and easy to characterize, but carriage can be unstable during *in vitro* cultivation. The earlier workers have been reported that plasmids are present in Vibrios including *V. alginolyticus* and *V. parahaemolyticus* (Molina-Aja et al., 2002; Li et al., 2003; Zulkifli et al., 2009). High frequency of plasmid in *Vibrio* may be ecologically important for the survival of these bacteria in the environment (GU and Zhang, 2005). It is also reported that there is a correlation between possessions of the plasmid with antibiotic resistance; this genus including *V. alginolyticus* and *V. parahaemolyticus* (Li et al., 1999; Kagiko et al., 2001; Zulkifli et al., 2009) and in some cases, their involvement in resistance to many antibiotics has been proven (Zhao et al., 1992).

As Vibriosis is a dominant pathogen in shrimp industry causing significant loss to the export market and foreign earning of major producing country, the present study was aimed at to characterize the strains of *V. alginolyticus* and *V. parahaemolyticus* obtained from *Penaeus monodon* by antimicrobial susceptibility, plasmid profiling and RAPD fingerprint pattern in order to provide a basis for epidemiological study and drug sensitivity pattern of this two species.

MATERIALS AND METHODS

Bacterial strain isolation and identification

Vibrio alginolyticus and *V. parahaemolyticus* strains previously isolated from diseased penaeid prawn, *P. monodon* were maintained in the Fish Health Management Division were used for the present study. The strains of the *V. alginolyticus* were CPVA2, CPVA10, CPVA18, *V. alginolyticus* (ATCC-17749) and *V. parahaemolyticus* CPVP7, CPVP15, CPVP16 and *V. parahaemolyticus* (ATCC17802).

DNA isolation

Isolation of chromosomal DNA was made by kit method using MB505 HipurA^{TM} Bacterial and Yeast Genomic DNA purification Spin Kit (Himedia Pvt.Ltd, Mumbai). The bacteria was inoculated in Nutrient broth (NB) and incubated at 37°C for 24 h. 1.5 ml of the bacterial culture was centrifuged at 10,000 rpm for 5 min. The DNA was extracted as following the recommendations of the manufacturer (Himedia). Isolation of plasmid DNA was made by kit method using HipurA^{TM} Endotoxin Free Plasmid miniprep Purification spin kit (MB513-20 PR, Himedia Pvt.Ltd, Mumbai).

Random amplified polymorphic DNA (RAPD) Amplification

The RAPD technique was standardized using OPA and OPY series of 10-mer oligonucleotide primers (Operon Technologies, INC., Alameda, USA). Out of 40 primers of OPA and OPY series tested 2 primers, OPA3 (5'AGTCAGCCAC3') and OPY2 (CATCGCCGCA) were selected for RAPD fingerprinting. Prior to amplification DNA sample were diluted to a concentration of 25 ng/µl. DNA sample was mixed with 2.5 µl 10X Buffer (Genei Pvt.Ltd Banglore, India), 1 µl of a deoxynucloside triphosphate (dNTP) mixture (Genei Pvt. Ltd Banglore, India), 5 pmol primer (Operon Technologies, INC., Alameda, USA). 1 U Taq DNA polymerase (Genei Pvt. Ltd Banglore, India). The final volume was 25 µl was adjusted with sterilized double distilled water. The tube were then placed in the thermal cycler with programme being set as follows: one cycle of initial denaturation step at 94°C for 4 min followed by 45 cycles of 45 s for 94°C (denaturing temp), 36°C (annealing temp) for 45 s and 72°C (extension temp) for 1.30 min. The cycling was concluded by an additional final extension at 72°C for 7 min and the reaction products were stored at 4°C until further analysis.

Analysis of RAPD fingeprint patterns

The PCR product was resolved on 1.2% agarose at 100 V for 1 h. The gel was stained with ethidium bromide and photographed on gel documentation (Sambrook et al., 1989). The molecular weight of the PCR product was determined by using software, Alpha Innotech. Each reproducible band was considered to be an independent character and the RAPD banding profiles generated by each primer were recorded as binary data of "1" or "0" (presence or absence of bands) The similarity matrix was calculated by using the Dice coefficient (SD). SD = [2A/(B +C)], where A is the number of fragments common to both patterns, B is the number of fragments in one pattern, and C is the number of fragments present in the other pattern. Genetic diversity =1 - DC, where DC is the similarity value obtained from Dice coefficient equation.

Antimicrobial susceptibility tests

Antimicrobial susceptibility test was performed using standard antibiotics disc and Disc diffusion technique (Bauer et al., 1966). The antimicrobial agents tested are Amoxycillin (10 µg); Cefuroxime (30 µg); Ampicillin (10 µg); Co-trimoxazole (25 µg); Erythromycin (15 µg); Chlorotetracycline (30 µg); Bacitracin (10 µg); Gentamicin (10 µg); Cephalexin (30 µg); Penicillin-G (10 µg); Tetracycline (30 µg) and Ciprofloxacin (5 µg) (Himedia pvt. Ltd Mumbai,India). *In vitro* antimicrobial activity was screened by using Mueller Hinton agar (MHA) obtained from Himedia (Mumbai, India). The MHA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The standard antibiotics disc was placed on the surface of medium and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

RESULTS

In this study we characterize the suitability of RAPD,

Table 1. Primers used for RAPD fingerprint study.

Sl no	primer	Sequence (5'-3')
1	OPA3	AGTCAGCCAC
2	OPA9	GGGTAACGCC
3	OPA13	CAGCACCCAC
4	OPA14	TCTGTGCTGG
5	OPA18	AGGTGACCGT
6	OPY1	GTGGCATCTC
7	OPY2	CATCGCCGCA
8	OPY7	AGAGCCGTCA
9	OPY15	AGTCGCCCTT

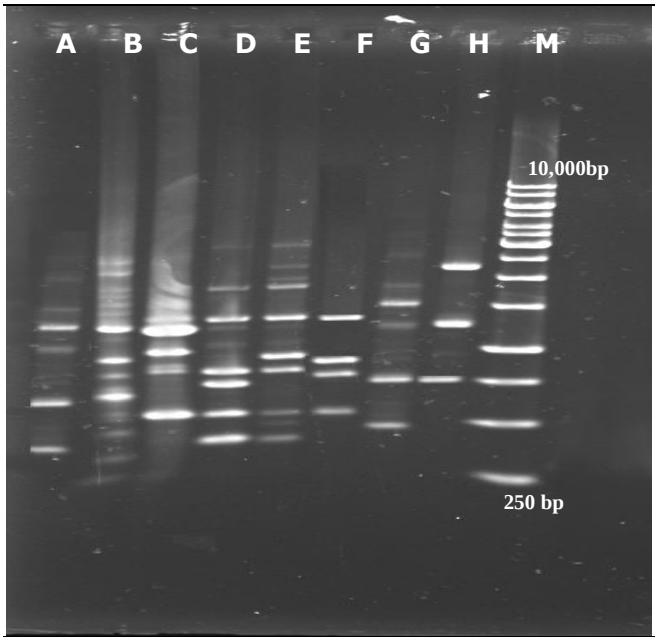

Figure 1. RAPD patterns obtained with OPA 3 primer (A-CPVA2, B- CPVA10, C- CPVA18, D- CPVP7, E- CPVP15, F- CPVP16,G-ATCC 17749, H- ATCC-17802), M- 1kb DNA ladder.

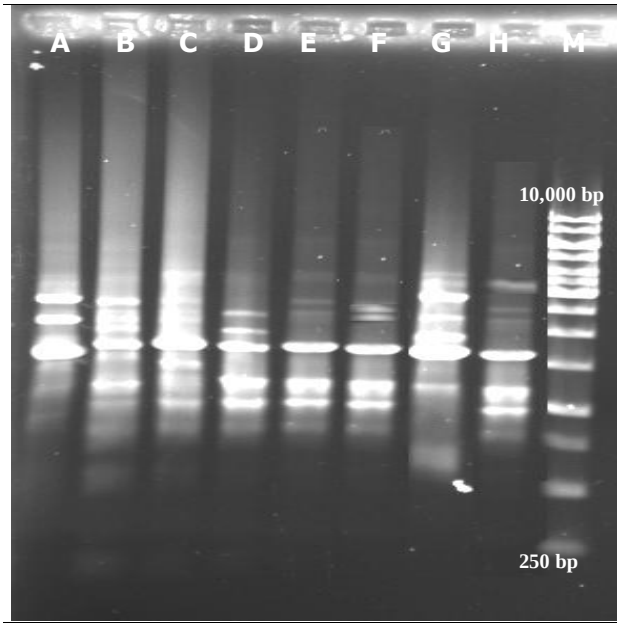

Figure 2. RAPD patterns obtained with OPY 2 primer (A-CPVA2, B- CPVA10, C- CPVA18, D- CPVP7, E- CPVP15, F-CPVP16,G-ATCC 17749, H-ATCC-17802), M- 1kb DNAladder.

Antibiogram and plasmid profiling technique for rapid molecular characterization of pathogenic bacteria, especially at strain level. These techniques become increasingly important to be able to discriminate between strains of *V. alginolyticus* and *V. parahaemolyticus* to trace the routes of infection and implement of suitable control measure.

The RAPD-PCR profiles of the four strains each of *V. alginolyticus* and *V. parahaemolyticus* including two ATCC strain showed genetic variation. Out of nine primers, OPA3 and OPY2 were selected for further tests because of the number of clearly discernible distinct bands, the reproducibility and optimal discrimination for both species after amplification (Table1). The remaining primers produced either poor quality amplification, or could not discriminate well. Primer OPA3 amplified 5 to 7 bands with band sizes ranging from 0.306 to 2.8 Kb where as Primer OPY2 amplified 3-6 bands with molecular wt. ranging from 0.830 to 3.94 Kb in *V. alginolyticus*. Similarly incase of *V. parahaemolyticus*, Primer OPA3 amplified 3 to 6 bands with band sizes ranging from 0.306 to 4.04 Kb where as Primer OPY2 amplified 6 to 7 amplicons with molecular wt. ranging from 0.830 to 4.11 Kb. *V. alginolyticus* yielded 3 different profiles by RAPD analysis when amplified by OPA3 and 4 different profiles when amplified by OPY2 primers. Similarly *V. parahaemolyticus* yielded 4 different fingerprint profiles when amplified by OPA3 and 3 different RAPD profiles when amplified by OPY2 primer (Figures 1 and 2, Table 2). The unique bands those were

Table 2. RAPD profile of *V. alginolyticus* and *V. parahameolyticus*.

Strain	RAPD profile		Molecular weight range (Kb)	
	OPA3	OPY2	OPA3	OPY2
CPVA2	A	a	0.306 - 1.79	1.9 - 3.9
CPVA10	B	b	0.366 - 2.8	1.0 - 3.9
CPVA18	C	c	0.480 - 1.79	0.83 - 3.9
CPVP7	D	d	0.306 - 2.39	0.83 - 3.9
CPVP15	E	e	0.306 - 4.04	0.83 - 3.9
CPVP16	F	e	0.480 - 4.04	0.83 - 3.9
ATCCVA	A	f	0.306 - 2.39	1.3 - 4.1
ATCC VP	G	g	0.797 - 2.81	0.83 - 3.9

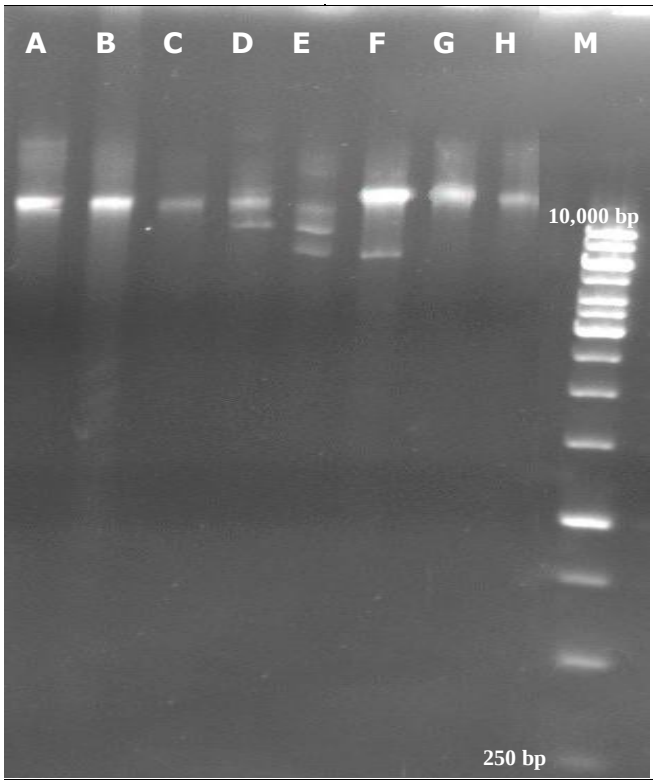

Figure 3. Plasmid profile of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* (A-CPVA2, B- CPVA10, C- CPVA18, D-CPVP7, E- CPVP15, F-CPVP16, G-ATCC 17749, H-ATCC-17802), M- 1kb DNA ladder.

present in all the strains for the particular primer was found out. Primer OPA3 amplified 1.44 and 0.797 Kb unique bands in all the strains. Similarly 1.9 Kb was a common unique band when amplified by the primer OPY2.

In all strains of *V. alginolyticus* and *V. parahaemolyticus* plasmids were detected. Four strains of *V. alginolyticus* including reference strain possess only one plasmid each with molecular weight 10. 232 Kb. But in *V. parahaemolyticus* 1 to 3 plasmids were detected with molecular weights ranged from 7.121 to 10.232 Kb (Figure 3 and Table 1). A common band of 10.232 Kb plasmid was common in all the strains including reference strain. Four different plasmid profiles was obtained from these two species (Table 3).

V. alginolyticus and *V. parahaemolyticus* cultures were resistant to the 10 µg O/129 disks but susceptible to the 150 µg O/129 disks (Tables 3). All strains showed resistance to two or more of the antibiotics tested. The strains were mostly resistant to Ampicillin and Cefuroxime. Among the strains 90 and 70% were resistant Bacitracin and Co-trimazole respectively. All strains of *V. alginolyticus* and *V. parahaemolyticus* were demonstrated to be sensitive to Amoxycillin and Chlorotetracycline and Ciprofloxacin. Sensitive results were also available for Tetracycline (90%), Co-Trimazole and Cephalexin (75%).

DISCUSSION

Bacterial typing systems are used to distinguish genera, species or strains by detecting differences in their characteristics. The Phenotypic identification of genera and species of Vibrionaceae is problematic, mainly because of great variability of diagnostic phenotypic feature such as arginine dihydrolase, indole production, and carbon utilization (Akayli et al., 2008; 2010). In our earlier report we have reported the different isolates of *V. alginolyticus* and *V. parahaemolyticus* were grouped in to strains based on their biochemical characteristics (Marhual and Das, 2009; Marhual et al., 2010). In this study, both RAPD and plasmid profiling along with antibiogram study proved to be reproducible and generated information on intraspecific differences and disease diagnosis for both the species.

RAPD is a useful technique of analyzing genetic diversity among strains of *Vibrio* species (Silva-Rubio et al., 2008; Akayli, 2010; Marhual and Das, 2009; Marhual et al., 2010) also, it is used to characterize the bacteria to the genotype level (Akaylli et al., 2008; Marhual et al., 2010). It is noteworthy to mention that prior sequence

Table 3. Resistance and susceptible of the strains of *V. alginolyticus* and *V. parahaemolyticus* employed in this study to different antibiotics and plasmid profiles.

Strain	Resistance to 0/129 disc		Antimicrobial susceptible	Antimicrobial resistance	Plasmids		
	10 µg	150 µg			Size (Kb)	Number	Profile
CPVA2	R	S	Am, E, Ct, G, Cp, T, CF	Cu, A, Co, B, P	10.232	1	1
CPVA10	R	S	Am, Co, Ct, G, Cp, T, CF	Cu, A, E, B, P	10.232	1	1
CPVA18	R	S	Am, Ct, G, Cp, CF	Cu, A, Co, E, B, P,T	10.232	1	1
CPVP7	R	S	Am, Ct, B, G, T, CF	Cu, A, Co, E,CP, P	10.232, 8.723	2	2
CPVP15	R	S	Am, Ct, G, Cp, T, CF	Cu, A, E, B, P	10.232, 8.352, 7.121	3	3
CPVP16	R	S	Am, Co, Ct, Cp, T, CF	Cu, A, E, B, G, P	10.232, 7.121	2	4
ATCCVA	R	S	Am, E, Ct G, T, CF	Cu, A, Co, B, P	10.232	1	1
ATCC VP	R	S	Am, Ct, G, Cp, T, CF	Cu, A, Co, E, B, P	10.232	1	1

Am- Amoxycillin 10 µg/disk; Cu -cefuroxime 30 µg/disk; A- Ampicillin 10 µg/disk; Co -Co-Trimoxazole 25 µg/disk; E- erythromycin 15 µg/disk; Ct-Chlorotetracycline 30 µg/disk; B-Bacitracin 10 µg/disk, G-Gentamicin 10 µg/disk; Cp-Cephalexin 30 µg/disk; P- penicillin-G 10 µg/disk, T- tetracycline 30 µg/disk; CF- Ciprofloxacin 5 µg/disk
ATCC VA- *V. alginolyticus* (ATCC-17749), ATCCVP- *V. parahaemolyticus* (ATCC17802).

information of isolated genomic DNA is not needed for this type of fingerprint study. While revealing the molecular differentiation studies with random Operon primers between the two Vibrios belonging to several strain each of it has been shown that there are genetic variation between the two species. The unique bands presented in the RAPD-PCR can then be used in the recognition of genera and species. In case of OPA 3 primer similar RAPD profile was obtained between reference strain of *V. alginolyticus* and field strain (CPVA2) where as other strains possess different RAPD profiles. Similarly the same profile was obtained between the two strains of *V. parahaemolyticus* when amplified by the primer OPY 2. Analysis of *V. alginolyticus and V. parahaemolyticus* indicated that this species is also genetically heterogeneous and there was a high level of genetic diversity among isolates (Son et al., 1998; Ripabelli et al., 2003).

All the strains of *V. alginolyticus* and *V. parahaemolyticus* isolated from *P. monodon* harbour plasmid between 1 to 3 Plasmid DNA bands. The plasmid profiles of *V. parahaemolyticus* has been reported with sizes from 9 to 123 kb (Li et al., 2003; Kagiko et al., 2001; Kaufman et al., 2002; Zulkifli et al., 2009). The plasmid profile of other *Vibrios* such as *Vibrios vulnificus Vibrios ordalli* and *Vibrios salmonicida* have been studied (Hanninen et al., 1995; Tiainen et al., 1994; Son et al., 1998). In our study we found that all the strains of *V. alginolyticus* and *V. parahaemolyticus* yielded plasmids and the percentage was 100% as compared to the other findings (Son et al., 1995; Li et al., 2003; Zulkifli et al., 2009). In our study, plasmids only exist with low copy number and with sizes ranging from 8.352 to 10.232 Kb. These findings are also similar to those of Li et al. (2003) who reported similar plasmid profile profiles for *V. parahaemolyticus*. Zulkifli et al., 2009 reported 14 plasmid profiles in *V. parahaemolyticus* and opined that plasmid profiling can be used as an epidemiological tool for typing this species. Son et al. (1998) stated that epidemiologically unrelated isolates contain different plasmid profile where as related isolates could also display variation in plasmid profile and this could be used as a marker for the isolates. Interestingly we have noticed a single plasmid in all the four strains of *V. alginolyticus* and 1 to 3 plasmids in *V. parahaemolyticus*. This one plasmid could be used as a specific marker for *V. alginolyticus* and the variability in *V. parahaemolyticus* as reported by others and it could be further differentiated for specific markers and epidemiological study. However, *V. parahaemolyticus* ATCC (17802) and all the plasmid-bearing *Vibrio* isolates showed multiple-resistance to antibiotics test in this study. The presence of both resistance of antibiotics and the large plasmid in *Vibrio* isolates may have significant ecological and public health implications (Gu and Zhang, 2005). Bacterial resistance is usually associated with the presence of plasmids and the ability of plasmids for transconjugation. In general, plasmids which could be transconjugated usually possess a high molecular weight (Li et al., 1999). There are other factors like mutation in cellular DNA it could

modify the antibiotic target site or transport mechanism causing to a decreased action of the antibiotic in a cell. Furthermore, an extra gene factor could be active against antibiotics. Extensive use of antibiotics has resulted antibiotics resistance bacteria (Saitanu et al., 1994; Zulkifli et al., 2009). The multiple resistance pattern of *V. alginolyticus* and *V. parahaemolyticus* showed that resistance to Ampicillin was observed in these and they are similar to other studies that have been reported, which was ranging from 44.4 to 98% (Son et al., 1998; Lesmana et al., 2001). These results are also similar to those of French et al. (1989) who reported similar antibiotic susceptibility profiles for *V. parahaemolyticus*. In their study, most isolates were resistant to Ampicillin but most were susceptible to chloramphenicol. Generally, the susceptibility to tetracyclines of the *V. parahaemolyticus* examined in this study is similar to those in *Vibrio*s reported elsewhere (Zanetti et al., 2001; Zulkifli et al., 2009).

Conclusion

Specific, sensitive and rapid methods for detecting and identifying pathogenic microorganisms are needed to control bacterial infections in Aquaculture. Unique distinct amplicons generated by RAPD PCR will be used for several diagnostic markers for identification of these pathogenic microorganisms. Further development of a sequence characterized amplified region would be more useful in epidemiological studies and disease diagnosis. The plasmid profiling is used as serotype-specific reference patterns for detecting certain strain with variation in plasmid content which is important in epi-demiological study. The resistant antibiotics compound in this study can be used for control of infectious disease in aquaculture industry. Resistance to antibiotics is widespread in these two species and their relationship with plasmids should be further studied.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Council of Scientific and Industrial Research (CSIR) for granting them Senior Research Fellowship and the Director of the Central Institute of Freshwater Aquaculture for providing the necessary facilities used to carry out this work.

REFERENCES

Akayli T, Timur G, Albayrak G, Aydemir B (2010). Identification and Genotyping of *Vibrio ordalii*: A Comparison of Different Methods. The Israeli J. Aquacult. – Bamidgeh, 62: 9-18.

Akayli T, Timur G, Albayrak G, Aydemir B, Kiziler AR, Coskun O, Albayrak G, Arican E (2008). Characterization of *Vibrio alginolyticus* isolates from Diseased Cultured Gilthead Sea Bream, *Sparus aurata.* The Israeli J. Aquacult. - Bamidgeh 60: 89-94.

Bauer RW, Kirby MDK, Sherris JC, Turck M (1996). Antibiotic susceptibility testing by standard single disc diffusion method. Am. J. Clin. Pathol. 45: 493-496.

Caburlotto G, Ghidini V, Gennari M, Tafi MC, Lleo MM (2008). Isolation of *Vibrio parahaemolyticus* pandemic strain from a marine water sample obtained in the norten Adriatic. Eurosurveillance, p. 13.

Du M, Chen J, Zhang X, Li A, Li Y (2007). Characterization and resuscitation of viable but nonculturable *Vibrio alginolyticus* VIB283. Archial. Microbiol. 88: 283–288.

French GL, Woo ML, Hui YW, Chan KY (1989). Antimicrobial susceptibilities of halophilic *vibrio*s. J. Antimicrob. Chemother., 24: 183-194.

Gu J, Zhang R (2005). Abundance of Environmental *Vibrio* Species in Marine Environment and Characterization of Cryptic Plasmids. Seminar on Microbiology in Food and Water, Analytical Technique and Applications, Ecology and Biodiversity: Conference papers, pp. 10.

Hanninen ML (1995). Occurrence of *Aeromonas* spp. In samples of ground meat and chicken. Int. J. Food Microbiol., 18: 339-342.

Kagiko MM, Damiano WA, Kayihura MM (2001). Characterization of *Vibrio parahaemolyticus* isolated from fish in Kenya. East Afr. Med. J. 78: 124-127.

Lesmana M, Subekti D, Simanjuntak CH, Tjaniadi P, Campbell JR, Ofoyo BA (2001). *Vibrio parahaemolyticus* associated with cholera-like diarrhea among patients in North Jakarta,Indonesia. Diagn. Microbiol. Infect. Dis., 39: 71-75.

Li J, Yie J, Foo RWT, Ling JML, Xu H, Woo NYS (1999). Antibiotic Resistance and Plasmid Profiles of Vibrio Isolates from Cultured Silver Sea Bream, *Sparus sarba.* Mar. Pollut. Bull., 39: 245-249.

Manivasagan P, Ramesh S, Sivakumar K, Thangaradjou T, Vijayalakshmi S, Balasubramanian T (2010). Antibiotic resistance and plasmid profiles of *Vibrio* isolates from Muthupettai mangrove environment, Southeast coast of India. J. Int. Dent. Med. Res., 3: 38-44.

Marhual NP, Das BK, Sadique M, Swain AK, Mishra BK, Maiti NK, Eknath AE (2010). Molecular identification and typing of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* strains isolated from black tiger shrimp *Penaeus monodon.* J. Aquaculture in Trop*.,* 25: 25-33.

Marhual NP, Das BK (2009). A Use of Random Amplified Polymorphic DNA and Restriction fragment lengthpolymorphism for typing of *Vibrio alginolyticus* isolated from black tiger black tiger shrimp *Penaeus monodon.* J. Pure Appl. Microbiol., 3:75-82.

Marhual NP, Samal SK, Das BK (2009). Random Amplified Polymorphic DNA-PCR typing of *Vibrioparahaemolyticus* isolated from black tiger shrimp *Penaeus monodon* e- planet., 7: 1-6.

Meyer LW (1988). Use of plasmid profiles in epidemiologic surveillance of disease outbreaks and in tracing the transmission of antibiotic resistance. Clin. Microbiol. Rev., 1: 228-243.

Molina-Aja A, Garcia-Gasca A, Abreu-Grobois A, Bolan Mejia C, Roque A, Gomez-Gil B (2002). Plasimid Profiling and antibiotic resistance of Vibrio strain isolated from cultured Penaeid shrimp. FEMS Microbiol. Lett., 213: 7-12.

Ripabelli G, Sammarco M, McLauchlin LJ, Fanelli I (2003) Molecular Characterisation and Antimicrobial Resistance of *Vibrio vulnificus* and *Vibrio alginolyticus* isolated from Mussels *(Mytilus galloprovincialis)* Syst. Appl. Microbiol., 26: 119–126.

Ruby EG, Urbanowski M, Campbell J, Dunn A, Faini M, Gunsalus R, Lostroh P, Lup C, McCann J, Millikan D, Schaefer A, Stabb E, Stevens A, Visick K, Whistler C, Greenberg EP (2005). Complete genome sequence of *Vibrio fischeri*: a symbiotic bacterium with pathogenic congeners., 102: 3004-3009.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, UDA, ISBN 0-87969309-6.

Saitanu K, Chongthaleong A, Endo M, Umeda T, Takami K, Aoki T, Kitao T (1994). Antimicrobial susceptibility and detection of transferable R-Plasmids from *Aeromonas hydrophila* in Thailand. Asian Fish. Sci., 7: 41-46.

Silva-Rubio A, Acevedo C, Magarinos B, Jaureguiberry B, Toranzo AE, Avendaño-Herrera R (2008). Antigenic and molecular characterization of Vibrio ordalii strains isolated from Atlantic salmon *Salmo salar* in Chile. Dis. Aqu. Org., 79: 27-35.

Son R, Nasreldin EH, Zaiton H, Samuel L, Rusul G, Nimita F (1998). Use of Randomly Amplified Polymorphic DNA analysis to differentiate

isolates of *Vibrio parahaemolyticus* from cockles (*Anadara granosa*) World J. Microbiol. Biotechnol., 14: 895-901.
Thompson JR, Randa MA, Marcelino LA, Tomita-Mitchell A, Lim E, Polz MF (2004). Diversity and dynamics of a north Atlantic coastal *Vibrio* community. Appl. Environ. Microbiol., 70: 4103-4110.
Tiainen T, Pedersen K, Larsen JL (1995). Ribotyping and plasmid profiling of *Vibrio anguillarum* serovar O2 and *Vibrio ordalii*. J. Appl. Bacteriol., 79: 384-392.
Tracy HH, Dongying Wu, Jonathan AE, Patricia AS (2007). Sequence Characterization and Comparative Analysis of Three Plasmids Isolated From Environmental *Vibrio* spp. Appl. Environ. Microbiol., 73: 7703–7710.
Welsh J, McClelland M (1993). The characterization of pathogenic micro-organisms by genomic fingerprinting using arbitrarily primed polymerase chain reaction. (AP-PCR), In: Diagnostic molecular microbiology (D. H. Persing, T. F. Smith, F. C. Tenover, T. J. White eds.) Washington, D.C., ASM Press, pp. 595–602.
Zanetti S, Spanu T, Deriu A, Romano L, Sechi LA, Fadda G (2001). *In vitro* susceptibility of *Vibrio* spp. isolated from the environment. Int. J. Antimicrob. Agents, 17: 407-409.
Zhao J, Kim EH, Kobayashi T, Aoki T (1992). Drug resistance of *Vibrio anguillarum* isolated from Ayu between 1989 and 1991. Nippon Suisan Gakkaishi/Bull. Jpn. Soc. Sci. Fish., 58: 1523-1527.
Zorrilla I, Arijo S, Chabrillon M, Diaz P (2003). *Vibrio* species isolated from diseased farmed sole (*Solea senegalensis, Kaup*) and evaluation of the potential virulence role of their extracellular products. J. Fish Dis., 26:103–108.
Zulkifli Y, Alitheen NB, Son R, Raha AR, Samuel L, Yeap SK, Nishibuchi M (2009). Random amplified polymorphic DNA-PCR and ERIC PCR analysis on *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia. Int. J. Food Res., 16: 141-150.