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Bacteria flora associated with different body parts of hatchery reared juvenile *Penaeus monodon*, tanks water and sediment

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Abstract - Bacteria flora of intestine and hepatopancreas, body surface and muscles of juvenile *Penaeus monodon* along with its rearing water and sediment was analyzed. Juvenile shrimp were reared in four tanks in the Hatchery complex, Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia. Water quality parameters were measured every day. Samples were collected aseptically and homogenized before being inoculated in Tryptone Soy agar, Thiosulphate Citrate Bile Salt agar, MacConkey agar and *Pseudomonas*-isolating agar. There was no significant difference between water quality parameters and shrimp body weight of replicate tanks. Total plate count for water and total *Vibrio* count for rearing water and digestive system were within previous reported ranges. Eight different genera were isolated in which 7 genera were identified. Gram negative bacteria were dominant (72%) *Vibrio* was the most dominant genera followed by *Shewanella* and *Burkholderia*. *Clavibacter* followed by *Staphylococcus* were the most dominant gram positive bacteria. No coliform bacteria was detected in the shrimp body parts and rearing environment. Incidence of *Shewanella* in the digestive system was significantly higher than sediment, rearing water and muscles. This may be implied its ability to colonize in the digestive tract of juvenile *P. monodon*.

Key words: shrimp; *Penaeus monodon*; microflora; *Vibrio*; *Shewanella*.

INTRODUCTION

Global shrimp culture production is growing at an annual rate of 16.8% between 1984 and 1995 (Vaseeharan, 2003). Bacteria are known to be associated with endemic and epidemic diseases of shrimp (Lightner, 1996). However, microbial disease outbreaks occur at all stages of shrimps and are responsible for considerable economic losses in several countries (Vaseeharan, 2003). Opportunistic shrimp pathogenic bacteria are common in sea water, and will take advantage of ecological changes introduced when the water is used in aquaculture (Moriarty, 1998). Gram negative bacteria especially *Vibrio* spp. are the predominant bacteria in the marine environment (Brisou *et al.,* 1965; Li *et al*., 2008) and usually constitute the majority in the normal microflora of farmed and wild penaeid shrimp (Vanderzant *et al*., 1971; Costa *et al.,* 1998). With the development of prawn cultivation techniques, bacteriological surveys have received more attention because some species of bacteria associated with the prawn cause disease, while other bacteria seem to be a useful food for prawn larvae in large scale cultivation (Yasuda and Kiato, 1980). Reports of bacterial diseases in penaeid shrimps caused by *Vibrio* spp. have been the most numerous. Virtually all of the species of bacterial pathogens in penaeid shrimp have also been reported to be part of their normal microflora (Costa *et al.,* 1998). Despite the opportunistic nature of most *Vibrio* pathogens, some occurring diseases in penaeid shrimp have been caused by *Vibrio* spp. which behaves more like true pathogens than opportunistic invaders.

Luminous *Vibrio*, the causative organism of the disease, is an opportunistic pathogen. They can invade the host through the hepatopancreas, a common target organ of most bacterial pathogens of shrimps (Leaño *et al.,* 1998). These bacteria proliferate and colonize in the host's digestive tract and become pathogenic (Colorni, 1985). Thus, it is necessary to determine the bacterial load of the hepatopancreas and digestive tract and their relationship with the rearing water tank and sediment to assess the levels of bacteria, specifically *Vibrio* spp., which is represented in healthy shrimp. Along with all researches that have performed on penaeid shrimp microflora especially *Penaeus monodon* there are a little information on microflora of its juvenile.

Therefore, this study was conducted to isolate and identify microflora of digestive system (hepatopancreas and intestine),

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body surface, muscle of juvenile *P. monodon* together with shrimp rearing water and sediment.

MATERIALS AND METHODS

To prevent bias in sampling from commercial shrimp farms and hatcheries which usually utilize commercial probiotic additives, healthy post larvae of *P. monodon* was purchased from local hatchery and reared in the Hatchery complex, Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia. This investigation was conducted from June 2007 to January 2008.

Rearing condition. The seeds were kept in 4 circular fibreglass tanks (2 m, diameter and 1 m height) with conical bottoms equipped with air stones in a static water regime. They were feed with probiotic free commercial pellet for 2 months before being used for bacteria flora sampling. During rearing period, pH, salinity and temperature was monitored daily using a pH meter (YSI, USA) and a hand refractometer (Atago 8808).

Sampling. A total number of 25 juvenile shrimp from each tank were scooped randomly, in the morning before water being exchanged. The collected shrimps were washed gently with sterile sea water to remove loosely adhering particles. Shrimp samples were then blot dried using sterile blotting papers before being weighted and dissected. The same organs from each tanks pooled together to form a unique sample before being weight aseptically. The samples then were homogenized in sterile normal saline using a dry heat sterilized glass tissue homogenizer before being diluted up to 10^{-6} in normal saline.

Shrimp rearing water of each rearing tank was collected in a sterile polyethylene tube and serially diluted up to 10^{-4} in normal saline. Shrimp rearing tank sediments was collected aseptically via a sterile tube and 50 ml sterile disposable syringe. A total amount of 10 ml of sediments were homogenized before being serially diluted up to 10^{-8}.

Twenty five shrimp from each rearing tank was randomly scooped out and used for bacteriological analysis of shrimp body surface. Samples were then pooled in a 250 ml media bottle containing 50 ml of sterile normal saline after their excess water being removed. The bottle was then shacked vigorously for 5 min, and the resultant media was diluted serially up to 10^{-4}.

Inoculation and culture. Four different media were selected for primary isolation and total plate count (TPC). Tryptone Soy agar (TSA) was used for enumeration of total aerobic heterotrophic bacteria or total viable counts. MacConkey agar, which is a selective medium for genus *Entrobacteriaceae*. Thiosulphate Citrate Bile Salt agar (TCBS) is a selective media for genus *Vibrio* and *Aeromonas* and *Pseudomonas*-isolating agar which is a selective media for genus *Pseudomonas*.

Aliquots of 100 µl of different diluted samples of digestive system (intestine and hepatopancreas), abdominal muscles, body surface, Shrimp rearing water tanks and shrimp rearing tanks sediment were spread plated on different mentioned selected media in triplicate.

Isolation and identification of shrimp putative bacteria. Morphological character, pigmentation and representative type of the colonies which were constituted at least 10% of the total number of colonies on the plate were recorded. These isolates were then sub-cultured on separate TSA plate to prepare pure culture. The isolates were then subjected to biochemical tests as exhibited in Table 1, and identified to generic level (MacFaddin, 1980; Buchanan, 1984; Drew *et al.,* 1986). This identification was confirmed with identifying of at least several colony from each group using Biolog GN and GP microplates (Biolog, Hayward, CA, USA) (Sung *et al.,* 1999*,* 2001; Olsson *et al*., 2004).

Statistical analysis. The difference in bacterial counts of replicate tanks was assessed using one way analysis of variance, ANOVA. All statistical analysis was carried out using statistical software (Minitab 15, USA).

RESULTS AND DISCUSSION

Water quality and body weight of shrimp

The possible difference among water quality (physicochemical conditions) of shrimp larval raring tanks was analyzed. There was no significant difference between temperature, pH and salinity of different rearing tanks being 29 ± 0.7 °C, 7.9 ± 0.1, and 25 ± 1‰, respectively. Those were within suitable range for shrimp culture. No mass mortality or disease was observed within rearing period. The resultant statistical analysis between juvenile shrimp body weight of different larval tanks was not exhibited any significant difference ($P < 0.05$), as well.

Bacterial count

Total aerobic heterotrophic microflora of shrimp rearing water tanks, shrimp rearing tanks sediment, shrimp body surface, shrimp digestive system and shrimp abdominal muscles on TSA was determined (Table 1 and Fig. 1). TPC of mentioned samples on TCBS agar (Fig. 2), *Pseudomonas*-isolating agar and MacConkey agar for enumeration of *Vibrio*-like organism, *Psudomonas*-like organism and *Enterobacteriaceae* are exhibited in Table 1.

There was no significant difference between bacteria counts of replicate tanks ($P < 0.05$). TPC of shrimp rearing water on TSA was 2.9×10^4 CFU/ml. TPC for pond water of *Litopenaeus vannamei* was reported 1.11×10^6 and 6.25×10^6 CFU/ml for new and 3 years old pond, respectively (Wang, 2005). Sung and his coworkers (2001) reported a range of 2×10^3 to 3×10^6 CFU/ml for pond water of 3 *P. monodon* grow out ponds These differences may be due to the rearing condition of grow out pond which is

TABLE 1 - Bacterial count on different selective media (mean ± SD, n = 3), from sediments, cultural water, shrimp body surface (CFU x 10^5/ml), digestive system and muscles (CFU x 10^5/g) of *Penaeus monodon*

Media	Sediment	Cultural water	S.B.S.	D.S.	Musle
TSA	530000 ± 50000	0.29 ± 0.03	0.3 ± 0.02	11 ± 0.3	0.061 ± 0.008
MacConkey A	-	-	-	-	-
TCBS A	12.87 ± 0.777	0.121 ± 0.0035	0.0117 ± 0.0035	7.51 ± 0.193	0.014 ± 0.0038
PIA	2.07 ± 0.351	0.012 ± 0.0036	0.0953 ± 0.0055	0.252 ± 0.0125	0.034 ± 0.004

TSA: Tryptone soy agar, MacConkey A: MacConkey agar, TCBS A: Thiosulfate citrate bile salt agar, PIA: *Pseudomonas* isolating agar. S.B.S.: Shrimp body surface, D.S.: Digestive system.

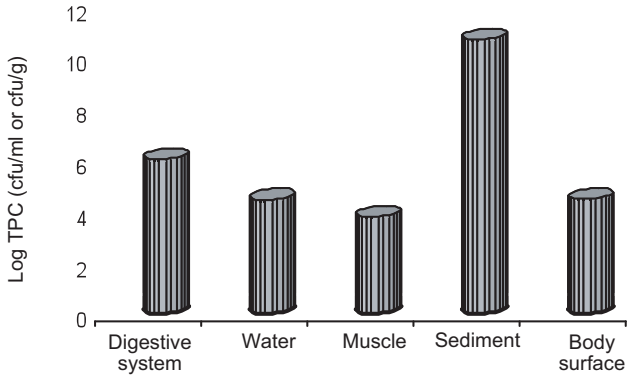

FIG. 1 - Log transferred TPC of shrimp different body part, water and sediment of shrimp rearing water.

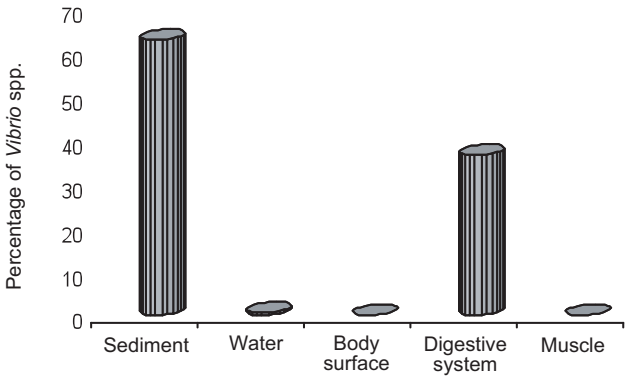

FIG. 2 - Comparison of *Vibrio* spp. associated with different body parts of *Penaeus monodon* and its rearing environment.

contained higher level of organic material comparing to hatchery tanks. This condition provides good environment for higher bacteria production. Total plate count of larval and post larval tanks of several *P. monodon* hatcheries in India was reported in a range of 10^2 to 10^4 and 10^4 to 10^7, respectively (Otta *et al.,* 2001), There were no significant difference between rearing water of juvenile of black sea bream and red sea breams, the mean value were 3.1 x 10^4 (Muroga *et al.,* 1987). The quantitative analysis of total bacteria of juvenile *Penaeus japonicus* rearing water was lower than 10^4 CFU/ml (Yasuda and Kiato, 1980). Total bacteria count of *Macrobrachium rosenbergii* larval rearing tank water ranged from 1.20 x 10^3 to 3.00 x 10^5 CFU/ml (Kennedy *et al.,* 2006). Mean value of total bacterial count of larval rearing tanks from 9 different *P. monodon* hatcheries in India was reported 3.08 ± 0.28 x 10^5 CFU/ml (Vaseeharan, 2003). There are quite varying quantities of total bacterial counts for shrimp rearing water reported for diverge hatcheries and grow out pond for different shrimp species. Total bacteria plate count of juvenile *P. monodon* in this study was fairly placed in the reported ranges. The revealed differences might be due to water source, rearing species, rearing water management and feeding management.

There was no bacterial growth on MacConkey agar which was indicated deficient of members of *Enterobacteriaceae* in the different body parts of shrimp, rearing water and sediment. This is in contrast with coliform analysis of shrimp pond water, sediment and shrimp body in grow out ponds in India (Bhaskar *et al.,* 1995). This difference may be owing to water source, water management and source of fertilizer used for fertilizing grow out pond water.

The highest level of total bacterial count, *Vibrio*-like organisms and *Pseudomonas*-like organism count was occurred in sediments followed by shrimp digestive tract, while those were minimum for shrimp rearing water and body surface for *Pseudomonas*-like bacteria and *Vibrio*-like bacteria, respectively (Table 1).

Bacteria flora

Based on similar morphological and biochemical characteristics of 118 isolates, bacteria flora was demonstrated in 11 different groups (Tables 2 and 3). There were 7 different genera identified and 1 unidentified from shrimp digestive system, muscle and body surface and shrimp rearing tank water and sediment. The isolated bacteria from different body parts of juvenile *P. monodon* and its rearing tanks were predominantly gram negative (72%), which is agreed with previous findings in egg, larvae and post larvae of *Penaeus indicus* (Hameed, 1993) and microflora of *M. rosenbergii* in hatcheries (Anderson *et al.,* 1989; Phatarpekar *et al.,* 2002; Kennedy, 2006). Similar finding was cited for wild *P. japonicus* (Yasuda and Kiato, 1980).

Bacterial compositions of *P. monodon* different body parts and rearing environment in the current study were dominant by *Vibrio* (30.5%), followed by *Shewanella* (23.7%) and *Burkholderia* (17.8%) genera which formed gram negative microflora, but *Clavibacter* (9.3%), *Staphylococcus* (8.5%), *Corynebacterium* (5.9%) and *Brevibacterium* (2.5%) were gram positive identified bacteria flora.

There were 3 identified and 1 unidentified species of genus *Vibrio* in different body parts of shrimp, water and sediment. Those were 30.51% of total identified isolated bacteria. Among those, *Vibrio parahaemolyticus* with 16.1% frequency was the most common species of the genus *Vibrio* within different body parts, water and sediment, while the other three isolates were comprised of 14.4% of total isolate.

In the current study *Shewanella* was the second dominant group in the *P. monodon* body parts and its rearing environment within identified isolate (23.7%). This is the dominant bacteria in the digestive tract of the *P. monodon* (Fig. 3) which was consisted of 9.3% of total identified isolate; although incidence of the *shewanella* in the shrimp rearing water and sediment were much lower than digestive tract. This may imply that digestive tract of *P. monodon* has good condition for colonization of *Shewanella*.

Incidence of *Burkholderia* was 17.8% of total isolated bacteria form *P. monodon* different body parts and rearing environment. Its frequency within rearing water and sediment was 2 times higher than digestive tract, while it was not observed in the muscles at all.

The most abundant gram positive isolated bacterium was *Clavibacter* (9.3%). It was distributed in different body parts, water and sediment but it was more frequently present in body surface (4.2%) than other parts. *Staphylococcus*, *Corynebacterium* and *Bravibacterium* with 8.5, 5.9 and 2.5% frequency were present in different body parts, water and sediment

Presence of *Vibrio*, and *Staphylococcus* agreed with finding of same bacteria in digestive tract and rearing water of larval stages of cultural *P. japonicus* (Yasuda and Kiato, 1980). They reported that the digestive tract of cultural juvenile *P. japonicus* contained *Vibrio*, *Aeromonas* and *Pseudomonas*, while in digestive tract of wild *P. japonicus Staphylococcus* was replaced with *Vibrio*. Existence of *Vibrios* and Gram positive non spore forming rods in this study was similar to incidence of same bacteria flora in *M. rosenbergii* in hatchery which composed of *Aeromonas*, *Pseudomonas*, *Vibrio* spp., *Bacillus* spp. and non spore formers which were reported as dominant Gram positive bacteria (Kennedy *et al.,* 2006). Occurrence of dominant *Vibrio* (25-32%)

TABLE 2 - Biochemical characteristics of bacteria flora in juvenile *Penaeus monodon*

Number	Test	Unidentified *Vibrio*	*Vibrio parahaemolyticus*	*Vibrio alginolyticus*	*Vbriobrio tubiashi*	*Shewanella*	*Burkholderia*	*Clavibacter*	*Staphylococcus*	*Brevibacterium*	*Corynebacterium*	Unidentified
1	Gram	–	–	–	–	–	–	+	+	+	+	+
2	Colony morphology	C. R	C. R	C. R	C. R	R	R	R	C	R	R	R
3	Oxidative	+	+	+	+	D	+	–	+	ND	+	–
4	Fermentive	+	+	+	+	D	–	–	+	ND	+	–
5	Motility	+	+	+	+	+	+	–	–	–	–	–
6	Oxidase	+	+	+	+	+	+	+	–	–	–	+
7	Catalase	+	+	+	+	+	+	+	+	+	+	+
8	H_2S	–	–	–	–	+	–	–	–	–	–	–
9	Indol	+	+	+	+	–	–	–	–	–	–	–
10	Citrate	+	+	+	+	–	–	–	–	–	–	–
11	Mr	+	+	+	+	–	–	–	–	–	–	–
12	Vp	–	–	–	–	–	–	–	–	–	–	–
13	Nitrate reduction	+	+	+	+	+	–	–	+	–	–	D
14	Urease	–	–	–	–	–	–	–	+	–	D	–
15	Gelatinase	+	+	+	+	+	+	–	+	+	–	+
16	LDC	+	+	+	–	–	–	–	–	–	–	–
17	ODC	D	+	–	–	+	V	–	–	–	–	D
18	ADH	–	–	–	+	–	+	–	–	–	–	D
19	Manitol	+	+	+	+	–	+	–	+	–	–	+
20	Glucose	+	+	+	+	–	+	–	+	–	+	+
21	Maltose	+	+	+	+	–	+	–	+	–	–	+
22	Inositol	+	+	–	–	–	–	–	–	–	–	+
23	Sucrose	+	+	+	+	–	+	–	+	–	–	–
24	Lactose	+	+	–	–	–	–	–	–	–	–	–
25	Sorbitol	+	+	–	–	–	–	–	–	–	–	–
26	Arbinose	+	+	+	–	–	+	–	+	+	–	+w
27	6 % NaCl	+	+	+	+	+	+	–	–	+w	+w	–
28	2% NaCl	+	+	+	+	+	+	+	+	+	+	+
29	0129 (150)	+	+	+	+	–	–	–	ND	ND	ND	ND
30	0129 (10)	+	+	+	+	–	–	–	ND	ND	ND	ND
31	MacConeky A	+	+	+	+	+	V	–	ND	–	–	ND
32	TCBS A	+Y	+G	+G	+Y	–	–	–	ND	–	–	ND
33	Grow at 40 °C	+	+	+	–	+	ND		ND	ND	+	ND

D: not determine, C.R: curved rod, R: rod, ND: not done, V: variable, +w: weak positive, LDC: lysine decarboxylase, ODC: ornithine decarboxilase, ADH: arginine dehydrolase.Y: yellow, G: green.

TABLE 3 - Relative frequency of bacteria isolated from shrimp rearing water, sediment, shrimp body surface, digestive tract and muscle

	Bacteria Groups	Body surface		Sediment		Digestive system		Water		Muscle		Total	
		N	%	N	%	N	%	N	%	N	%	N	%
1	Unidentified *Vibrio*	---	---	4	44.4	2	22.2	1	11.1	2	22.2	9	7.6
2	*V. parahaemolyticus*	3	15.8	6	31.6	3	15.8	1	5.3	4	31.6	17	14.4
3	*V. alginolyticus*	---	---	3	50	2	16.7	2	33.3	---	---	7	5.9
4	*V. tubiashi*	---	---	1	33.3	1	33.3	1	33.3	---	---	3	2.5
5	*Shewanella*	3	10.7	5	17.9	11	39.3	4	14.3	5	17.9	28	23.7
6	*Burkholderia*	6	28.6	6	28.6	3	14.3	6	28.6	---	---	21	17.8
7	*Clavibacter*	5	45.5	2	18.2	1	9.1	2	18.2	1	9.1	11	9.3
8	*Staphylococcus*	4	40	---	---	5	50	1	10	---	---	10	8.5
9	*Brevibacterium*	1	33.3	---	---	2	66.7	---	---	---	---	3	2.5
10	*Corynebacterium*	1	14.3	2	28.6	---	----	4	57.1	---	---	7	5.9
11	Unidentified	---	---	---	---	2	100	---	---	---	---	2	1.7
	Total	23	19.5	29	24.6	30	25.4	22	18.6	14	11.9		118

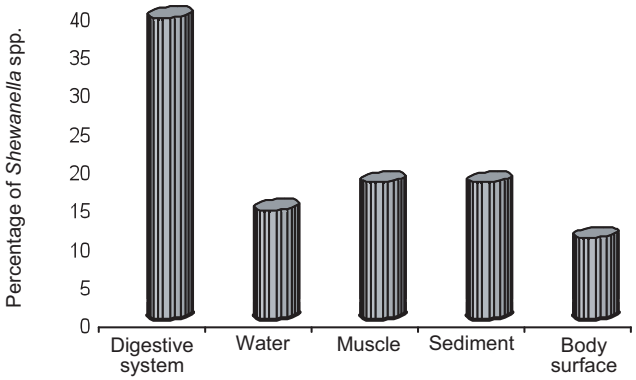

FIG. 3 - Comparison of *Shewanella* spp. associated with different body parts of *Penaeus monodon* and its rearing environment.

in the eggs, larvae and post larvae of *P. indicus* (Hameed, 1993) was comparable with presence of *Vibrio* genera in the different body part and cultural environment of juvenile *P. monodon* in this study (30.5%). But this is in contrast with the rest of his finding on bacteria flora of eggs, larvae and post larvae in the same shrimp species which was followed by *Pseudomonas* (18-21%), *Alcaligenes* (10-13%), *Aeromonas* (8-10%) *Fluvobucterium* (6-8%) and others. In the tanks water, however, he was indicated *Alcaligenes* (19-24%) as predominant bacteria, followed by *Vibrio* (16-20%) *Pseudomonas* (10-13%), *Aeromonas* (7-11%), *Flavobacterium* (6-12%) and others.

In this study, the incident of *Vibrio* spp. in the shrimp rearing water was 0.6% of total water bacterial count which was comprised of *V. parahaemolyticus*, *V. alginolyticus* and *V. tubiashi* with relatively equal proportion. The occurrence of the *Vibrios* in the shrimp body parts (36.7%) was dominant by *V. parahaemolyticus*. Sediment comprised of highest level of *Vibrio* counts (62.5%) followed by digestive tract (36.6%), while body surface and muscles exhibited 0.06 and 0.07% of total *Vibrio* count, respectively. Presence of *Vibrio* in marine shrimp aquaculture is a common experience, in a *M. rosenbergii* hatchery in which *Vibrio* count was reported from 10^2 to 10^4 CFU/10 larvae, while it ranged 10^1 to 10^3 CFU/ml in larvae rearing tanks (Kennedy *et al.,* 2006). Presence of 1×10^1 to 1×10^4 CFU/ml *Vibrio* in three *P. monodon* cultural ponds water was stated by Sung *et al*. (2001), as well.

Total *Vibrio* count in post larvae rearing water of *P. monodon* hatchery was $5.2 \pm 0.53 \times 10^2$, while total *Vibrio* count for post larvae was $1.55 \pm 0.35 \times 10^3$ CFU/larvae which was composed of *V. harveyi*, *V. anguillarum* and *V. vulnificus* (Vaseeharan, 2003). *Vibrio* was found to be the dominant (25-32%) genera in *P. indicus* eggs, larvae and post larvae, whilst in the rearing water *Alcaligenes* (19-24%) was predominant, followed by *Vibrio* (16-20%) (Hameed, 1993). Incidence of *Vibro* in marine aquaculture of other species was also cited. Bacteria flora of intestine larvae and juvenile of red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*) were 7.4×10^4 and 3.4×10^4 CFU/fish, respectively. Those consisted of 45% *Vibrio* and 30% *Pseudomonas* (Muroga *et al.*, 1987).

Similar microflora pattern was observed in gut microflora of deep water marine crustaceans from the Gulf of Mexico. It revealed the high population of *Vibrio* spp. in their gut content ranging from 10^5 to 7×10^6 CFU/g, while relatively low population of *Vibrio* spp. were reported in water column and sediments (Dilmore, 1986).

In this study the dominant populations of bacteria flora in digestive tract is alike to those of shrimp rearing water and sediment, which is consisted of *Vibrio*, *Shewanella* and *Burkholderia*. This can be anticipated because of shrimp feeding behavior and habitat. Shrimp feed submerges in the rearing water and sucks water due to leisurely chewing habit of shrimp which usually takes place on the bottom of the rearing tanks. Therefore the ingesta are commonly harboured of fecal bacteria and rearing tank water, as well. Incidence of *Vibrio* bacteria flora in digestive system was higher than that of the rearing water tank. This agreed with finding of Hameed (1993) for *P. indicus*, Kennedy *et al.* (2006) and Vaseeharan (2003) for *M. rosenbergii*. The higher total *Vibro* count in the digestive system of shrimp can be explained by the contamination of the ingested feed with sediment which had the highest *Vibrio* count. However, higher *Vibrio* count in digestive tract of marine crustaceans of deep water compare to the column water and sediment (Dilmore, 1986) is in contrast with the mentioned hypothesis. Furthermore, Muraga and his colleagues explained same concept for total *Vibro* count of digestive tract of larvae and juvenile of red sea bream and black sea bream, It implied that the prevalence of *Vibrio* was much more smaller value in the rearing water compared to intestinal flora (Muroga *et al.,* 1987). According to feeding behavior of these fishes which is grabbing the feed within water column, the feed pellets does not have any chance to be in contact with the sediment or enough time to suck in water from the tank. Therefore it may be concluded that digestive system of the shrimp has provided *Vibrio* genera a good condition to be colonized there.

Bacteria flora of *P. monodon* digestive system consisted of 7 genera and 6 of them were common to those of the water. In 1989, Anderson and his coworkers found 14 out of 15 genera isolated from washed larval tissue slurries which were common to the water rearing tanks. Although Yasuda and Kiato (1980) quoted same result for intestine of the *P. japonicus* larvae and their rearing tanks but it is in contrast with Phatarpekar and his colleagues (2002). Their report on *M. rosenbergii* larvae micro flora was 5 out of nine genera of isolated bacteria from digestive tract that were common in the rearing water.

This out come is much more agreed with Chahill (1990). He insisted that in aquaculture the intestinal microbiata have more interaction with ambient environment.

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