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Species of *Vibrio* isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeus vannamei*

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Abstract

The number and species of *Vibrio* spp. bacteria that may be present in normal healthy *Penaeus vannamei* juveniles are described. The hepatopancreas, stomach, intestine and haemolymph of *P. vannamei* juveniles were sampled. All three areas of the digestive tract contained a diverse population of *Vibrio* spp. but the haemolymph contained bacteria in only 14.3% of the animals sampled, with counts of *Vibrio* spp. ranging from 2×10^2 to 3×10^3 CFU/ml. The *Vibrio* spp. isolated from the digestive tract included both sucrose and non-sucrose fermentors whereas the haemolymph contained only non-sucrose fermentors. The findings of this study would suggest that there may be a wide range of *Vibrio* spp. in the hepatopancreas of normal healthy *P. vannamei*.

Keywords: Shrimp; *Penaeus vannamei*; Bacteria; *Vibrio*

1. Introduction

Although there have been several studies of the bacteria associated with disease in shrimp (Takahashi et al., 1985; Lightner, 1993), there have been relatively few reports describing the normal bacterial flora of healthy farmed shrimp. The bacterial flora of

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fish and other aquatic species is related to the environment in which they live (Karthiayani and Iyer, 1975; Scott and Thune, 1986) and it is reasonable to assume that such a relationship exists in shrimp. It is, however, necessary to have information relating to the numbers and species of bacteria colonising healthy shrimps, in order to interpret abnormal findings and assist in diagnosis of clinical disease.

Bacteria of the genus *Vibrio* are ubiquitous in the marine and estuarine aquatic ecosystems in which shrimp occur naturally and are farmed (Vanderzant et al., 1971; Ruangpan and Kitao, 1991). These bacteria are also associated with the majority of bacterial infections in shrimp (Lightner, 1993, Jiravanichpaisal et al., 1993; Ruangpan and Kitao, 1991; Lavilla-Pitogo, 1993) and are generally agreed to be opportunistic pathogens causing disease when the shrimp are compromised. It has also been shown that bacteria can be isolated from the haemolymph of apparently healthy crustaceans, such as *Procambarus clarkii* (Scott and Thune, 1986), *Homarus americanus* (Cornick and Steward, 1966), *Callinectes sapidus* (Haskell et al., 1975) and from *Penaeus monodon*. [1] It is difficult, therefore, to interpret the clinical significance of their isolation from shrimp.

The objective of this study was to examine the *Vibrio* spp. component of the bacterial flora of a population of healthy *P. vannamei* juveniles risen under laboratory conditions and, to provide a basis for comparison with findings during outbreaks of disease.

2. Materials and methods

Juvenile *P. vannamei* shrimp were obtained as late post larvae (pl) and maintained in a wet laboratory for 12 weeks prior to sampling. They were kept at a density of 0.2 shrimp per litre in 500-l tanks containing 150 l of water (Salinity 35‰, 25–28°C, 7.5–8.5 pH, 4.5 mg/l minimum dissolved oxygen). The tanks were run on a recirculating system including sedimentation tank and biofilter. Approximately 20% of the water was changed per week and the sedimentation tank was siphoned once every 3–4 days. The water was taken from the sea through a sand filter, a 25 μm cartridge filter and an ultra violet steriliser. The *P. vannamei* were fed ad libitum twice daily with commercial shrimp pellets (CP-Aquaculture, Bangkok, Thailand). They were sampled 1 h after feeding when the gut was most likely to be full.

2.1. Health status of P. vannamei

The general condition of the shrimp, including feeding, swimming, moulting and other behaviour was monitored daily. A total of 30 *P. vannamei* were sampled over a period of 3 weeks, at a rate of two or three per day. After gross external examination each *P. vannamei* was weighed, measured and inspected for macroscopic signs of

[1] Issarasak, N., Tangtrongpiros, J., Koeypudsa, W., Ponpornpisit, A., unpublished. Bacterial flora in normal *Penaeus monodon* broodstock. Veterinary Medical Aquatic Animal Research Center, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.

disease. The haemolymph was examined to determine turbidity, clotting time and the presence of any pathogens. Fresh preparations of the hepatopancreas, intestine and gills were examined by microscopy for the presence of any abnormalities or parasites.

2.2. Sampling for bacteriology

The tissues examined were the stomach, the mid- and hindgut (intestine), the hepatopancreas (digestive gland) and the haemolymph. First an area of carapace lateral to the heart was sterilised with 96% ethanol, the haemolymph was extracted with an insulin syringe and plated on to Thiosulphate Citrate Bile Sucrose agar (TCBS agar) (Difco Laboratories, Detroit, MI, USA). The hepatopancreas, stomach and intestine were then dissected and the hepatopancreas separated from the rest of the gut taking care to avoid cross contamination. The hepatopancreas, stomach and the intestine were weighed separately and homogenised by vortexing in sterile saline (2.5% NaCl). The supernatants were diluted serially, 0.1 ml aliquots plated onto TCBS and the plates incubated at 30°C for 24–48 h.

The morphology and the number of yellow and green colonies on TCBS were recorded for all the organs from all the *P. vannamei*. A representative of each colony type was subcultured on TCBS and Tryptone Soya Agar (TSA) + 2.0% NaCl (Difco Laboratories) and identified with the BIOLOG-GN system (BIOLOG, Hayward, CA, USA). The test was carried out in 96-well microtitre plates using an inoculum prepared in 2.5% NaCl. Each well provides a different single carbon source. The pattern of substrate utilisation allowed identification of some of the *Vibrio* spp. The colour change was recorded and analysed using Microlog 2™ software, which compares the observed reaction pattern with the ones stored in its database.

Some isolates could not be identified by this system and these were analysed further by a series of phenotypic tests (Oxidase production, motility, fermentation of glucose and lactose, gas production from glucose, nitrate reduction and sensitivity to 0/129, 10 μg and 150 μg). These results helped to identify some but not all of the additional strains according to Bergey's manual of determinative bacteriology (9th edn.).

3. Results

The general health of the *P. vannamei* sampled was good. The only signs of disease were occasional small melanised lesions on the external carapace, which were invariably lost during moulting. Other than bacteria, no potentially pathogenic organisms were detected and the haemolymph clotted in less than one min. The hepatopancreata of all the animals were reddish brown in colour and contained abundant lipids.

The 30 *P. vannamei* sampled had a mean weight of 10.66 g (standard deviation: 1.224, Max.: 13.54 g, Min.: 7.93 g) and a length of 10.73 cm (standard deviation: 0.553, Max.: 11.60 cm, Min.: 9.50 cm).

The mean numbers of *Vibrio* spp. found in the hepatopancreas was 4.30×10^4 CFU/g (median 1.32×10^4, Max. 2.67×10^5, Min. 1.11×10^2, $n = 26$), in the intestine 2.10×10^6 CFU/g (median 5.32×10^5, Max. 1.03×10^7, Min. 1.01×10^4, $n = 25$),

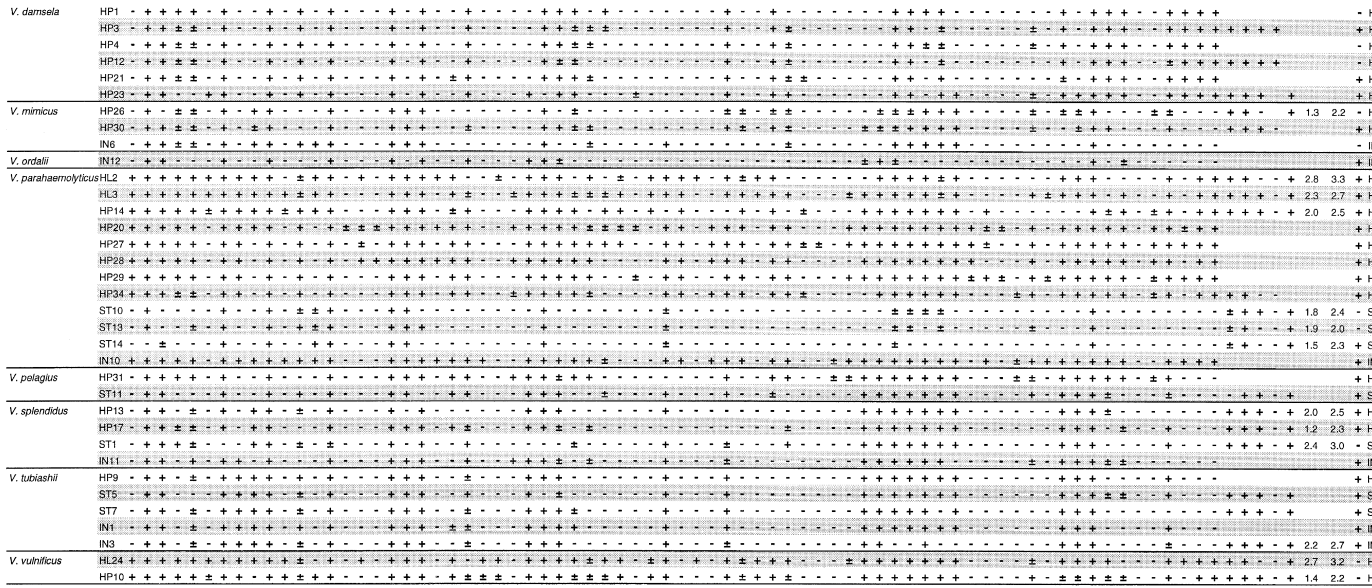
Table 1
Diagnostic table of strains isolated from *P. vannamei* juveniles

Species	Code	Alpha-cyclodextrin	Dextrin	Glycogen	Tween 40	Tween 80	N-acetyl-D-galactosamine	N-acetyl-D-glucosamine	L-arabinose	Cellobiose	D-fructose	L-fucose	D-galactose	Gentiobiose	Alpha-D-glucose	m-inositol	Alpha-lactose	Alpha-D-lactose lactulose	Maltose	D-mannitol	D-mannose
unidentified	ST6	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	ST9	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ST12																				
P. phosphoreum	HP5	-	+	+	-	-	-	+	-	-	+	-	-	+	+	-	-	-	+	-	+
	IN8	-	+	+	+	±	-	+	-	-	+	-	-	-	+	-	+	-	+	+	+
Vibrio sp.	HL1	+	+	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-	+	+	+
	HL4	-	+	+	-	±	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+
	HL5	-	+	-	-	-	-	+	-	+	+	-	-	-	+	-	-	-	+	-	-
	HP11	-	+	-	-	±	-	+	-	-	-	-	-	-	+	-	-	-	+	+	+
	HP15	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	HP18	-	+	-	±	-	-	+	-	+	+	-	-	-	+	-	-	-	+	+	+
	HP32	-	+	-	±	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
	HP33	+	+	+	±	+	-	+	-	+	-	-	+	+	+	-	±	-	+	-	-
	ST3	-	+	-	-	-	-	+	-	±	+	-	-	-	+	-	-	-	+	±	±
	ST15	-	±	±	-	±	-	±	-	-	-	-	-	-	+	-	-	-	+	-	-
	ST16	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	IN5	-	±	-	±	±	-	-	-	-	-	-	-	-	±	-	-	-	±	-	-
V. alginolyticus	HP35	+	+	+	+	+	-	+	-	+	+	±	+	±	+	±	±	±	+	+	+
	IN2	-	+	±	-	±	-	+	-	-	+	-	+	+	+	-	-	-	+	+	-

Species	Code	D-melibiose	Beta-methyl D-glucoside	Psicose	D-raffinose	L-rhamnose	D-sorbitol	Sucrose	D-trehalose	Methyl pyruvate	Mono methyl succinate	Acetic acid	Cis-aconitic acid	Citric acid	Formic acid	D-galacturonic acid	D-gluconic acid	D-glucuronic acid	P-hydroxy phenylacetic acid
unidentified	ST6	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+
	ST9	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	-
	ST12																		
P. phosphoreum	HP5	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	IN8	-	-	±	-	-	-	-	+	-	-	-	-	-	-	-	±	-	-
Vibrio sp.	HL1	±	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
	HL4	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
	HL5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HP11	-	-	±	-	±	-	+	±	-	-	-	-	-	-	-	+	-	-
	HP15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HP18	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
	HP32	-	-	-	-	-	-	-	-	+	±	-	-	-	-	-	-	-	-
	HP33	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
	ST3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ST15	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	ST16	-	-	-	-	-	-	-	+	±	-	-	-	-	-	-	-	-	-
	IN5	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-
V. alginolyticus	HP35	-	-	+	-	-	+	+	+	+	+	+	±	±	±	-	+	-	-
	IN2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	±	-	-

Species	Code	Alpha-Keto glutaric acid	D,L-lactic acid	Propionic acid	D-saccharic acid	Succinic acid	Bromo succinic acid	Succinamic acid	Glucuronamide	Alaninamide	D-alanine	L-alanine	L-alanyl-glycine	L-asparagine	L-aspartic acid	L-glutamic acid	Glycyl-L-aspartic acid	Glycyl-L-glutamic acid	L-histidine
unidentified	ST6	-	+	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-	-
	ST9	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	ST12																		
P. phosphoreum	HP5	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	IN8	-	±	±	-	-	-	-	-	-	-	-	-	-	-	±	±	-	-
Vibrio sp.	HL1	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-
	HL4	-	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-
	HL5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HP11	-	-	-	-	-	-	-	±	-	-	-	+	±	-	±	+	±	-
	HP15	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	HP18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HP32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HP33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	ST3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ST15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ST16	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-
	IN5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. alginolyticus	HP35	+	+	+	-	+	+	±	-	-	+	+	+	+	+	+	+	+	+
	IN2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Species	Code	Hydroxy L-proline	L-leucine	L-ornitine	L-proline	D-serine	L-serine	L-threonine	Inosine	Uridine	Thymidine	Phenyl ethylamine	Putrescine	Glycerol	D,L-alpha-glycerol phosphate	Glucose-1-phosphate	Glucose-6-phosphate	Motility*	Fermentation of glucose*	Fermentation of lactose*	Production of gas from glucose*	Nitrite reduction*	0/129 (10mg)*	0/129 (150mg)*	Yellow colony in TCBS agar*	Code
unidentified	ST6	-	-	+	-	-	+	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	3.4	4.0	-	ST6
	ST9	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	±	+	+	-	+	0.0	1.4	+	ST9
	ST12																	+	+	+	-	+			+	ST12
P. phosphoreum	HP5	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	±	+	+	-	-	0.0	1.5	+	HP5
	IN8	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	+	+	+	-	+	1.7	2.5	-	IN8
Vibrio sp.	HL1	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+								-	HL1
	HL4	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	+	-	+	0.0	1.2	+	HL4
	HL5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	2.1	3.1	-	HL5
	HP11	-	-	-	-	-	-	-	+	±	-	-	-	-	-	-	-	+	+	+	-	+	1.9	2.4	+	HP11
	HP15	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	+		+	2.3	2.9	+	HP15
	HP18	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-	+	2.0	2.5	+	HP18
	HP32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	2.4	2.9	-	HP32
	HP33	-	-	-	-	-	-	+	+	±	-	-	-	-	-	-	-	+	+	+	-	+	2.3	2.8	-	HP33
	ST3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	2.5	3.8	-	ST3
	ST15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	+	+	-	+	1.2	2.0	+	ST15
	ST16	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	3.8	4.4	-	ST16
	IN5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	0.0	2.0	+	IN5
V. alginolyticus	HP35	±	±	±	+	-	+	+	+	+	+	-	-	+	+	+	+								-	HP35
	IN2	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	2.0	2.8	-	IN2



Reaction performed with the BIOLOG-GN system and additional test (∗).
HL = haemolymph, HP = hepatopancreas, ST = stomach, IN = intestine.
+ = Positive reaction, − = negative reaction, ± = dubious reaction.
A negative reaction of all strains to the following compounds was observed: water; adonitol; D-arabitol (Exp. HL3 ±); *i*-erythritol (HP14 ±); turanose (ST9 +, HP23 ±) xylitol (Exp. ST9, Pos.); D-galactonic acid lactone; D-glucosaminic acid (HP17 +, ST9 +); alpha-hydroxy butyric acid (HL1 +, HP35 ±); beta-hydroxy butyric acid (HL2 ±, HL24 +); Gamma-hydroxy butyric acid (HL1 +); itaconic acid; alpha-keto butyric acid (HL2 ±, HP35 ±); alpha-keto-valeric acid; malonic acid (HP14 ±, HP17 +); quinic acid; sebacic acid; L-phenyl alanine; L-pyro glutamic acid; D,L-camitine; Gamma-amino-butyric acid (HL1 ±); urocanic acid (ST9 +); phenylethylamine; 2-amino ethanol and 2,3-buanediol; oxidase (HP21 −).

and for the stomach 1.29×10^6 CFU/g (median 7.43×10^5, Max. 4.40×10^6, Min. 3.33×10^4, $n = 28$). The numbers of bacteria in the stomach and intestine were not significantly different ($p > 0.05$, *t*-test). The hepatopancreata had significantly fewer bacteria than the stomach or the intestine ($p < 0.01$, *t*-test). The numbers of bacteria in the intestine and the weight of the intestine were not significantly correlated (-0.259, Spearman rank correlation, $p > 0.05$). Bacteria were only recorded from the haemolymph of four (14.3%) of the *P. vannamei*, the counts were as follows: 3.00×10^3, 1.60×10^3, 3.00×10^2 and 2.00×10^2 CFU/ml.

The number of isolates capable of utilising sucrose (yellow colonies on TCBS) varied greatly between tissues and individuals. In the hepatopancreata the number of sucrose fermentors varied between 0% and 100% with a mean of 38.28%. In the stomach, from 0.25% to 100% were sucrose fermentors with a mean of 49.58% and in the intestine the percentage varied from 0.85% to 100% with a mean of 48.38%. None of the isolates from the haemolymph were able to utilise sucrose.

The colony types isolated from the *P. vannamei* yielded a total of 54 phenotypically distinct strains (Table 1). These included nine identified species of *Vibrio*, one *Photobacterium phosphoreum*, 12 unspeciated *Vibrio* strains and three unidentified Gram negative bacteria. While there was little difference between the number of species recovered from the stomachs, intestines and hepatopancreata, there were more phenotypically distinct isolates from the hepatopancreata. The haemolymph contained relatively few bacteria of a limited number of species.

4. Discussion

Issarasak et al. [1] found the hepatopancreata of *P. monodon* contained at least four species of bacteria including *V. alginolyticus*, *V. parahaemolyticus* and *V. cholerae*. In this study, the hepatopancreata of apparently healthy *P. vannamei* contained several *Vibrio* species, including *V. alginolyticus*, *V. damsela* and other *Vibrio* spp. (Table 2). Other authors have claimed that bacteria are not commonly found in the hepatopancreas because they are prevented from entering by the gastric sieve which excludes particles larger than 0.1 mm (Hopkin and Nott, 1980). It has been suggested that the sieve may combine with the digestive enzymes to prevent bacteria gaining access to or colonising the hepatopancreas and therefore the presence of bacteria in the hepatopancreas may represent a failure of these mechanisms (Alday-Sanz, 1994). However, it may be possible for bacteria to enter the hepatopancreas by other routes. It is possible that the *P. vannamei* in this study, in common with all farmed shrimp suffered damage to the gastric sieve as a result of feeding on commercial pellets. Additional work is required to determine the relationship between diet and bacterial content of the hepatopancreas.

There were fewer bacteria and a wider range of distinct isolates recovered from the hepatopancreas compared to the stomach and intestine, however, from these data it is not possible to conclude that the bacterial population in these portions of the digestive tract were significantly different.

The findings presented here suggest that the presence of bacteria in the hepatopancreas is not necessarily indicative of disease and diagnosticians should expect to find a

Table 2
The bacterial strains isolated from *P. vannamei* juveniles obtained on TCBS agar

Species	Haemolymph	Hepatopancreas	Intestine	Stomach
Vibrio spp.	3	5	1	3
V. alginolyticus	–	7	1	–
V. damsela	–	6	–	–
V. mimicus	–	2	1	–
V. ordalli	–	–	1	–
V. parahaemolyticus	2	–	1	3
V. pelagius	–	1	–	1
V. splendidus	–	2	1	1
V. tubiashii	–	1	2	2
V. vulnificus	1	1	–	–
P. phosphoreum	–	1	1	–
Unidentified	–	–	–	3
Total no. of strains	6	26	9	13
Total no. of species[a]	2	8	7	4

[a] Total number of positively identified, excluding unidentified and *Vibrio* spp.

wide range of *Vibrio* spp. isolates in the hepatopancreata of healthy *P. vannamei*. This differs from the findings from diseased shrimp, where one or two species predominate (Song et al., 1993; de la Peña et al., 1993; Yang et al., 1992). In two cases of high mortality, bacteria were isolated from the hepatopancreata of moribund *P. monodon* in Taiwan (Chen et al., 1992). The proportions of *Vibrio* spp. isolated were 73.4% and 84.6%. The majority of these isolates were of two species, *V. damsela* 16.6% and 22.4%, and *V. harveyi* 43.1% and 26.9%. The remaining isolates included a large number of *Vibrio* spp.

The variation in the weight of the intestine was largely due to the differing amounts of ingesta, despite attempts to ensure that all the *P. vannamei* sampled had full intestinal tracts. There was no significant positive correlation between the weight of the intestine and the number of CFU recovered. Therefore the amount of ingesta in the tract did not significantly affect the number of bacteria. This suggests that the majority of the bacteria are associated with the tract itself and not with the food.

It has been suggested that the presence of bacteria in the haemolymph is indicative of septicaemia (Lightner, 1977) and a common sequelae to stress (Lightner, 1988). Other authors have recovered bacteria from the haemolymph of apparently healthy farmed shrimp, for example, from 1×10^1 to 1×10^2 bacteria were isolated from the haemolymph of *P. monodon.* [2] More than 50% of these bacteria were *Vibrio* spp. , the remainder were *Pseudomonas* spp. and *Aeromonas* spp. Bacteria have also been isolated from the haemolymph of other species of apparently healthy crustacea such as *H. americanus* (Cornick and Steward, 1966), *C. sapidus* (Haskell et al., 1975), *P. clarkii*

[2] Ruangpan, L., Rangsichai, T., Sangrungruang, K., unpublished. Bacterial flora of intensive cultured Black Tiger Shrimp, *Penaeus monodon*. Coastal Aquaculture Division, Dept. Of Fisheries, Kasetsart University, Bangkhen, Bangkok 10900, Thailand.

(Scott and Thune, 1986) and *Machrobrachium rosenbergii* (Brady and Lasso-de la Vega, 1992). This study indicates that bacteria may be present in the haemolymph of healthy shrimp but the numbers detected were low and the range of species limited to *V. parahaemolyticus*, *V. vulnificus* and other three unidentified species of *Vibrio*. Every attempt was made to optimise the environment for the *P. vannamei* used in this study. However, it is possible that the presence of bacteria in the haemolymph may have been an indication that the animals were compromised. It is still reasonable to assume that the haemolymph of apparently healthy farmed shrimps may also contain low numbers of a limited range of bacterial species.

Normal *P. vannamei* may have a diverse population of bacteria within their tissues including sucrose fermentors in the intestinal tract. This differs from the situation in diseased animals where one or two species of bacteria predominate. Since there is still no reliable experimental model for inducing vibriosis in the laboratory, it is not possible to determine if clinical vibriosis occurs as a result of multiplication of bacteria already present in the tissues or if it occurs following invasion of additional bacteria from the surrounding environment. Further work is required on this and other aspects of the aetiology of vibriosis, including the relative importance of the host defences and bacterial virulence.

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