MBB02

DETECTION OF *Vibrio parahaemolyticus* VIRULENT STRAINS FROM BLACK TIGER PRAWN (*Penaeus monodon*) AND ITS ENVIRONMENT

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INTRODUCTION

In terms of trade, the fish industry is one of the major export and income source of Malaysia and the industry contributed RM1.3 billion (1.5 million ton/year) or 20% to the total food export from Malaysia (Radu et al. 2005). The demand for Malaysian tiger prawn is increasing from 22,000 tonne metric in 1999 up to 27,000 tonne metric in year 2000 and it is expecting to reach 150,000 tonne metric by the year of 2010. In year 1997, most of the detentions of the frozen prawns by the European countries came from Malaysia, Bangladesh, India, Thailand and other Asean countries (Rama & Vijaya 1999; Ostergrad 2000; Soponpong 2000). The rejection due to the presence of *V. parahaemolyticus* had increased from 4 in 2000, 4 in 2001, 10 in 2002 and 15 in 2003 (Radu et al. 2005).

V. parahaemolyticus is a marine bacterium that occurs naturally in aquatic environments and seawater and is part of the natural flora of zooplankton, coastal fish and shellfish (Colwell 1984). *V. parahaemolyticus* strains possess a regulatory gene, *toxR*-gene, which is

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present in all the strains irrespective of their ability to produce *tdh* (thermostable direct haemolysin) or *trh* (tdh-related haemolysin) (Kim et al. 1999). Only members of this species that produce virulence factors, the *tdh* and/or the *trh*, are considered to be pathogenic (Nishibuchi & Kaper 1995) and can cause acute gastroenteritis. The aim of this study was to detect the presence of *V. parahaemolyticus* virulent strains isolated from the black tiger prawn and its environment in Malaysia.

MATERIALS AND METHODS

Samples

Samples included fresh black tiger prawn, pond water and sediments. Water was collected using sterile polypropylene bottles about one foot below the water surface. Sediments and prawns were collected into sterile polythene pouches. All the samples were brought to the laboratory in an icebox for further analysis. Samples were collected during the months of Dec. 2005 to March 2006 from two different ponds situated in Klang and Sungai Besar, Selangor.

Sample preparation, enrichment and detection of *V. parahaemolyticus*

Briefly, 25 g of sample was homogenised in 225 ml of alkaline salt peptone water (ASPW pH 8.6) and serial dilutions were made using 0.1% peptone water (PW) containing 3% NaCl and spread plated on thiosulphate citrate bile-salt agar (TCBS). The agar was incubated at 37°C for 18-24 h (Anon 1990). The typical round, green or bluish with 2-3 mm in diameter colonies (Speck 1984) on the TCBS agar were picked and identified by biochemical tests. Identification was done by using analytical profile index (Mini API 20E, BioMerieux). The colonies were grown onto NA containing 3% NaCl and incubated at 37°C for 18 h. A growth assay was carried out in 0.85% NaCl solution and their turbidity were tested using densitometer to reach 0.5 McFarland. ID 32E diagnostic strips were used to study the biochemical properties of the strains.

Detection of *toxR-gene, tdh-gene* and *trh-gene* using PCR technique

The PCR for *toxR-*, *tdh-* and *trh-* genes of *V. parahaemolyticus* was preceded as described by Kim et al. (1999) and Tada et al. (1992). *V. parahaemolyticus* (clinical O3:K6 strain which is *tdh* positive and *trh* negative) was used as control culture.

RESULTS AND DISCUSSION

Based on results obtained (Table 1), all the samples tested were highly contaminated with *V. parahaemolyticus* with 95% and 70% from sediments, 90% and 45% from pond water and only 43% and 55% from the prawns of Klang and Sungai Besar respectively. From this study and by conventional methods, *V. parahaemolyticus* can be detected from all the samples taken, which were in disagreement with Norrakiah et al. (2005). It has been reported that the *V. parahaemolyticus* was detected from pond water, prawns and sediments of up to 5.3%, 14.3% and 5% respectively (Anon 2002; Gopal et al. 2004). The results obtained were in agreement with those obtained in the previous studies. It was assumed that the environments in the prawn farm will always be infected with *V. parahaemolyticus* (Radu et al. 2005). This assumption was made since it has been reported that *V. parahaemolyticus* is a marine bacterium that occurs naturally in aquatic environments worldwide and its density is influenced by temperature, salinity, existence of plankton, tide and others (Colwell 1984).

The presence of *toxR-gene* is always considered as current markers of pathogenicity in *V. parahaemolyticus*. The *toxR-gene* is found only in *V. parahaemolyticus* and not in other bacteria (Kim et al. 1999; Radu et al. 2005). PCR was applied to amplify the *toxR-gene* where the size is 368 bp (Kim et al. 1999) as shown in Fig. 1. In this study, all the samples from two different places were contaminated with pathogenic *V. parahaemolyticus* (Table 1) and sediments contributed the high percentage (63% and 35%) among others.

The PCR amplifying the *tdh-* and *trh-genes* were used for the detection of *V. parahaemolyticus* virulent strains with size of 251 bp and 250 bp respectively (Tada et al. 1992), as shown in Fig. 2 and Fig. 3. The *tdh-* or *trh*-genes are only present in virulent strain of *V. parahaemolyticus* (Kim et al. 1999). The result showed that 5% and 10% *tdh-gene* positive came from prawns while 13% and 15% came from the sediments of Klang and Sungai Besar respectively, but the pond water was found to be absent from virulent genes. This is in agreement with Robert-Pillot et al. (2004), that most of *V. parahaemolyticus* virulent strain can be isolated from environment especially from sediment in France.

TABLE 1 Occurrence of *V. parahaemolyticus* virulent strain from fresh black tiger prawn (*P. monodon*), water pond and sediments

Location	Sample	No. of samples taken	No. of positive samples (%)			
			V. para-haemolyticus	Pathogenic *V. para-haemolyticus*	*V. parahaemolyticus* virulent strain	
				toxR-gene	*tdh-gene*	*trh-gene*
Klang, Selangor	Tiger prawn	40	17 (43)	11 (28)	2 (5)	0
	Pond water	40	36 (90)	16 (40)	0	0
	Sediment	40	38 (95)	25 (63)	5 (13)	0
Sungai Besar, Selangor	Tiger prawn	20	11 (55)	7 (35)	2 (10)	0
	Pond water	20	9 (45)	7 (35)	0	0
	Sediment	20	14 (70)	7 (35)	3 (15)	0

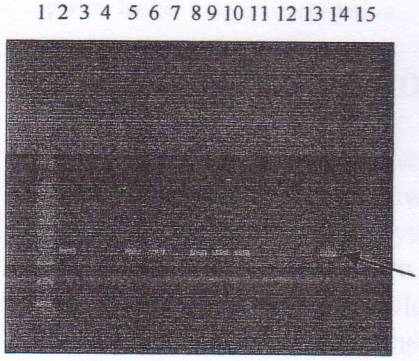

FIGURE 1 The presence of pathogenic *V. parahaemolyticus* by detection of *toxR-gene*. Lane 1: 100-bp molecular size marker (TaKaRa); lane 2: reference strain (*V. parahaemolyticus* with positive *toxR-gene*, 368 bp); lane 3: blank; lanes 4-7: isolates from pond water; lanes 8-11: isolates from prawn; lanes 12-15: isolates from sediment.

The recovery of total and potentially pathogenic *V. parahaemolyticus* was high but the presence of *V. parahaemolyticus* virulent strain was low. According to Alam (2002) probability of isolation of *tdh-* or *trh-* gene-positive strains from environmental samples is very low. This was in agreement with other studies (Nishibuchi & Kaper 1995; Hervio-Health et al. 2002), only 1-5% of environmental *Vibrio* isolates possess the *tdh* or *trh* genes. In this study none of the *V. parahaemolyticus* virulent strains possessed the virulence associated genes with *trh*. It was reported that most strains of *V. parahaemolyticus* associated with human disease produce *tdh-* or/and *trh-genes* (Nishibuchi & Kaper 1985). However, a small proportion of *V. parahaemolyticus* isolates carried neither of the two virulence genes as previously reported (Kelly & Stroh 1989). In contrast, environmental and seafood isolates rarely contained *tdh-* or *trh-genes* (Honda & Lida 1993).

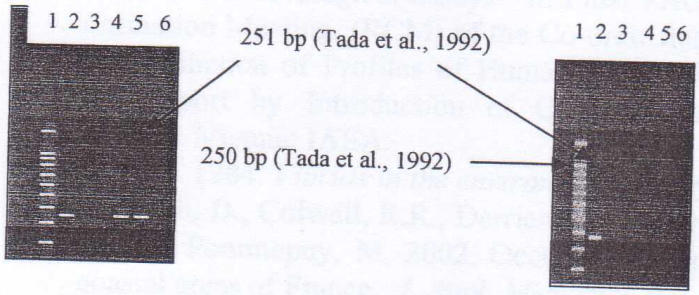

FIGURE 2 The presence of *V. parahaemolyticus* virulent strain by detection of *tdh-gene*. Lane 1: 100-bp molecular size marker (TaKaRa); lane 2: reference strain (*V. parahaemolyticus* with positive *tdh-gene*, 251 bp); lane 3: blank; lane 4: isolate from pond water; lane 5: isolate from prawn; lane 6: isolate from sediment.

FIGURE 3 The detection of *trh-gene*. Lane 1: 100-bp molecular size marker (TaKaRa); lane 2: reference strain (*V. parahaemolyticus* with positive *tdh-gene*, 251 bp); lane 3: blank; lane 4: isolate from pond water; lane 5: isolate from prawn; lane 6: isolate from sediment.

CONCLUSIONS

The detection of the *tdh* gene in 12 strains of *V. parahaemolyticus*, 4 isolated from black tiger prawn and 8 from pond sediment, in prawn culture environment in Malaysia suggests a probable risk for health of people consuming raw prawn. Our results suggest that a long term monitoring programme should be initiated to detect pathogenic *V. parahaemolyticus* isolates in the prawn culture environment as its recovery may affect prawn export industry in Malaysia.

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