BACTERIA IN GREEN MUSSEL *PERNA VIRIDIS* (L.) AND ITS ENVIRONMENT

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ABSTRACT

Pathogenic bacteria and bacterial indicators were isolated from green mussel and sea water collected at Muara Kamal, Jakarta Bay.
Samples were collected at five lift-nets on two occasions in 1997. Two size classes of mussels (< and > 5 cm shell length) were analysed. Higher number of *Shigella* sp. and *Escherichia coli* but not *Salmonella* sp. and *Vibrio* sp. were found in large mussels. Counts of *E. coli* were higher in large mussels ($24 \cdot 10^3$ MPN 100 g^{-1}) compared to small mussel ($16 \cdot 10^3$ MPN 100 g^{-1}) and sea water ($13 \cdot 10^3$ MPN 100 ml^{-1}). The number of *Salmonella* in small and large mussel were $11.6 \cdot 10^{12}$ and $0.2 \cdot 10^{12}$ cfu 100 g^{-1} respectively. *Salmonella* sp. occurred in 10 % of the sea water samples. The number of *Shigella* sp. in small and large mussel were $4.2 \cdot 10^{13}$ cfu 100 g^{-1} and $2.6 \cdot 10^{13}$ cfu 100 g^{-1} respectively. *Shigella* was recorded in sea water at concentrations of $14.9 \cdot 10^2$ cfu 100 ml^{-1}. *Vibrio* sp. were rare in mussels, less than 30 cfu 100 g^{-1}. Sea water contained $1.0 \cdot 10^3$ cfu 100 ml^{-1}.

INTRODUCTION

Jakarta Bay is the recipient of discharges from 13 rivers, which have caused a decrease in water quality and biodiversity because of the discharge of sewage (Anonymous, 1998). The decrease in the water quality would also affect the quality of green mussel, which are cultured in net-pouches hung in lift nets, especially in the western part of the bay. Mussels are usually sold or consumed by local people in spite of the fact that they are likely to serve as vectors of any water borne disease or contaminant they can take up from polluted water.

Sewage discharges typically contain pathogenic bacteria, which are concentrated by marine filter feeders. Shumway in Gosling (1992) mention that *Mytilus edulis* may be the vector of bacterial and viral contaminants, such as *Vibrio haemolyticus*, *Klebsiella pneumonia*, *Enterobacter* spp., *E. coli*, *Shigella dysenteriae*, *Yersinia enterocalitica*, *Hepatitis* virus A, *Citrobacter*, *Salmonella*, *Proteus*, *Streptococcus faecalis*, *Salmonella anatum*, *Bacillus subtilis*, *Serratia marcescens*, *Aeromonas hydrophila*. Furthermore, the mussel *Perna canaliculus* may bear viral contaminants including Coxsachie virus BY, CBS virus, and polio viruses 1, 2, and 3.

Hasan *et al.* (1996) studied bacterial content of various marine species but in general very little is known about this problem in Indonesian waters. We have studied green mussel from Jakarta Bay to detect if they contain bacteria, which could be related to discharge of sewage. The result may assist authorities working with potential health problems.

MATERIAL AND METHODS

This research was conducted at 5 lift nets located at Muara Kamal, Jakarta Bay (106° 42, - 106° 46 E and 04° 08, - 06° 08 S). The distance between the nets was 60 m. The nets were located 4 km from the beach and adjacent to the estuary of Cikamal river (Figure 1). Sea water and mollusc were sampled on 26 October and 15 November

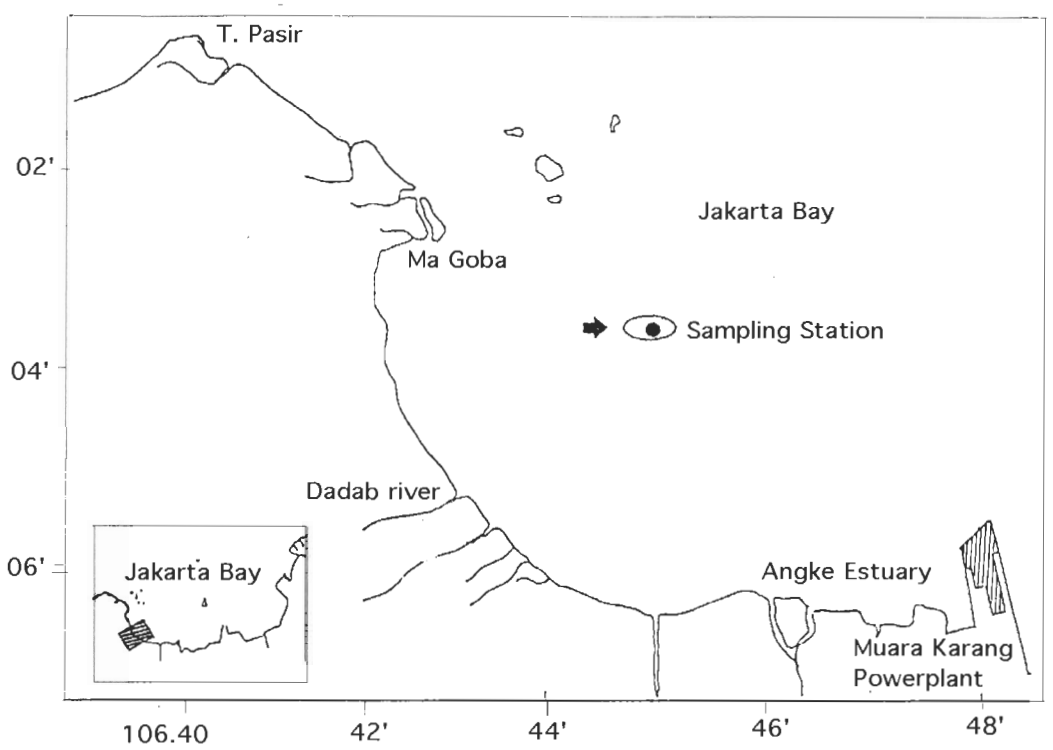

Figure 1. Sampling station in Jakarta Bay.

1997. The bacteria were isolated and identified in the laboratory of Fish's Health Department of Aquaculture Faculty of Fisheries and Marine Sciences, Bogor Agricultural University.

Water and mussel sampling

One green mussel < 5 cm and one mussel > 5 cm were taken at random from each lift net containing mussel at sizes ranging from 3-9 cm length.

Mussels were washed in sterilised physiological solution and kept in a closed sterilised container. Temperature, salinity, pH and soluble oxygen were measured in sea water, which was sampled in a 60-ml sterilised bottle. The samples were transported to the laboratory in a Styrofoam box filled with ice. All flesh of the mussel, except the mantle, was separated from the shell and weighed. It was ground in a sterilised mortar after addition of distilled water (flesh: water ratio 1:10). This suspension was stored in a sterilised bottle and kept at 1 °C. Sea water samples were stored the same way.

Identification of bacteria

E. coli was isolated using the liquid media, Lauryl Tryptose Broth (LTB) and Eosin Methylene Blue-Agar (EMB) (SNI, 1991). *Salmonella* and *Shigella* were cultured in Selenite Cystine Broth (SCB) and in Salmonella Shigella-Agar (SS-Agar) (SNI, 1991). *Vibrio* spp were isolated using Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS Agar) (AOAC, 1975).Bacteria identification was based on morphological characteristics, gram staining, shape of bacteria, and biochemical testing according

to Cowan and Steel.

Enumeration of bacteria

The number of *E coli* was calculated using the Most Probable Number MPN (APHA, 1976). The number of *Salmonella* sp., *Shigella* sp., and *Vibrio* sp., was calculated using the spreading bowl method (Hadioetomo, 1993).

Data analysis

Two-way non-parametric statistics was used to identify the difference in bacterial concentration (Barnard *et al.* 1993). Bacterial numbers were analysed with respect to sampling time, size of mussels, and different concentrations in mussels and water sample. Comparison were made of bacterial concentration in mussels < 5 cm shell length to mussels > 5 cm; bacterial concentration in mussels < 5 cm shell length to bacterial concentration in sea water; bacterial concentration in mussels >5 cm shell length to bacterial concentration in sea water.

RESULTS

Water quality was normal with temperatures between 29.2-32.0 °C, salinity 31.0-33.0 ‰, pH 7.4-8.0, and dissolved oxygen 6.2 -7.0 ppm.

Escherichia coli, *Salmonella* sp., *Shigella* sp., and *Vibrio* spp.were isolated and identified. The average concentrations of the bacteria are shown on Table 1.

E. coli were found in all mussel and sea water samples. Green mussels concentrate bacteria as a result of their filter-feeding. The average concentration in mussels <5 cm and >5 cm were $16 \cdot 10^3$ and $24 \cdot 10^3$ MPN $100\ g^{-1}$ respectively. The concentration in sea water was $13 \cdot 10^3$ MPN $100\ ml^{-1}$ The concentration of *E. coli* in mussel < 5 cm was lower than in mussels > 5 cm (P<00.1). The concentration of *E. coli* did not increase significantly with time in the mussels (P> 0.05). *E. coli* in water and mussels < 5 cm were not significantly different (P>0.05) but significantly diferent for mussels > 5 cm (P<0.05).

Salmonella sp. was present in all mussel samples but only in half of the water samples. The concentration in mussels >5 cm was not significant different from the concentration in mussels <5 cm (P>0.05). Counts of *Salmonella* in mussels < 5 cm were higher than in sea water (P<0.001). The concentrations of *Salmonella* in October and November were not significantly different (P>0.05).

Shigella sp was found in 95% of the mussels and 80% of the water samples. The concentration of *Shigella* sp was higher in mussels >5 cm than in mussels <5 cm (P<0.05) and both concentrations were significantly higher than in sea water (P<0.0001). The values in October and November did not differ significantly

Table 1. Average bacterial concentrations (MPN $100\ g^{-1}$) in mussel and sea water (MPN $100\ ml^{-1}$) at Muara Kamal, Jakarta Bay (n=5)

Sampling	Bakteria	Sample		
		Size < 5 cm	Size > 5 sm	Water
1	*E. coli*	2.3E+04	2.4 E+04	9.4 E+02
	Salmonella sp.	2.3 E+13	3.1 E+11	0.0 E+oo
	Shigella sp.	8.2 E+12	1.8 E+12	2.5 E+03
	Vibrio sp.	0.0 E+00	00 E+00	4.7 E+02
2	*E. coli*	8.4 E+03	2.4 E+04	2.4 E+04
	Salmonella Sp	1.7 E+11	5.0 E+10	2.5 E02
	Shigella sp.	1.2 E+11	5.2 E+13	4.7 E+02
	Vibrio sp.	0.0 E+00	0.0 E+00	1.6 E+03

Table 2. Results of a two-way non-parametric rank testing of the bacterial data.

Comparison	H_0	*E. coli*	*Salmonella*	*Shigella sp.*	*Vibrio sp.*
MA[1] - MB[2]	$O_2 > 0_1$	p >0.05	p >0.05	p >0.05	3)
	MB > MA	p < 0.01*	p >0.05	p <0.05*	
MA - water	$O_2 > O_1$	p < 0.01*	p >0.05	p >0.05	
	MA > water	p >0.05	p < 0.001*	p < 0.001*	
MB - water	$O_2 > O_1$	p <0.05*	p >0.05	p >0.05	
	MB > water	p <0.05*	p < 0.001*	p < 0.001*	

All the sea water samples and half of the mussels contained few *Vibrio*. Both *Vibrio cholerae* and *V. parahaemolyticus* were isolated (morphological characteristics and biochemical tests). However, the counts of the two types of *Vibrio* were combined because the concentrations were very low (Table 1). The concentrations in October did not differ significantly from November (P>0.05).

DISCUSSION

Bonadonna *et al.* (1990)in Gosling (1992).Thayib and Suhadi (1976) in Thayib and Listiawati (1977) states that high numbers of coliform bacteria are found in water close to the beaches, estuaries and islands of Jakarta Bay. However, *E. coli* can also be found in places far away from beaches. The rivers carry domestic waste with suspended feces containing *E. coli* from of a big number of people living in the subdistrict of Penjaringan,North Jakarta (7259 people km^{-2}, Anonymous, 1998). Yates (1992) found that coliform bacteria are released through human feces at an average concentration of 107 cells g^{-1}. Accordingly a positive correlation has been found between suspended particulate mattter and coliform bacteria (for example Ruyitno and Thayib 1994).

This study indicates a lower concentration of *E. coli* in sea water (Table 1) compared with findings of Thayib and Suhadi (1976) in Thayib and Listiawati (1977). They recorded an average concentration of $33 \cdot 10^3$ MPN 100 ml^{-1}. The concentration of *E. coli* in JakartaBay should be expected to decrease because of the implementation of the "Program KaliBersih" aiming at getting clean water of rivers in Indonesia.The establishment of Waste Treatment Installation in many industries located along the watershed area has at least lessened the input of pollutants coming to the estuaries by rivers. However, the concentration of *E. coli* in sea water is still too high for fish culture in accordance with the Decree of Minister of Bureau for Population and the Environment the Republic of Indonesia. According to WHO it is also too high for water provided to recreational activities. Concentrations of *E. coli* should not be higher than 10^3 MPN 100 ml^{-1}. Italian regulations require no more than $3.9 \cdot 10^3$ MPN 100 ml^{-1} (Bonadonna *et al.* 1990 in Gosling 1992).

The concentrations of *Salmonella* was lower in Jakarta Bay than found by Hasan & Parlindungan (1996) in the water of Riau. The average concentration of *Salmonella* sp in Rupat Strait was $9.3 \cdot 10^3$ cfu 100 ml^{-1}. *Salmonella* cannot survive long in sea water (Highsmith and Crow 1992; McKee and Wolf 1963 in Al Massawi *et al.* 1983). Our samples were taken 4 km from the nearest shore and the concentration of *Salmonella* in most mussel samples passed the standard of the Italian Regulation stating that content in fish product must be nil (Bonadonna *et al.*,1990 in Gosling, 1992). It also passed the standard of nil *Salmonella* set by the

Bureau for Population and the Environment, the Republic of Indonesia. However, more intensive sampling is needed to ascertain the situation in Jakarta Bay. Three out of five mussels contained *Salmonella* sp; while none of the 5 water samples contained *Salmonella* in October. Buttiaux and Leurs 1953 (in Thayib 1991) found adaptation of *Salmonella* to environmental conditions. The growth curve of *Salmonella thypi* in sea water decreased drastically to begin with but after a period of 11 hours the bacteria started to increase again. According to Cook 1991 and Rowse & Fleet 1982 (in Cook 1991), most of the microorganism filtered by mussels can survive for as long as 28 days (Highsmith & Crow, 1992).

V. cholerae can quickly adapt and survive in sea water and estuaries (Liston 1973 in Barrow & Miller 1976; Pallitzer 1990 in Rodrich 1991). In addition, *Vibrio* can attach to broken corals, algae, and lime stone which in turn may be a source of *Vibrio* in the water (Rodrich, 1991). According to Barrow and Miller (1976) *V. parahaemolyticus* are a found more frequent in low coastal waters and estuaries than in deep water. The concentration of *Vibrio* in Jakarta Bay was lower than in waters off Riau (Hasan & Parlindungan 1996). These authors found an average concentration of $3.9 \cdot 10^6$ cfu 100 ml^{-1}.*Vibrio* spp. in Rupat Strait. WHO allows 300 MPN 100 g^{-1} (Gosling, 1992),and Italian regulations prohibit concentration of more than 34 MPN 100 g^{-1}.

ACKNOWLEDGEMENTS

We wish to thank the people who assisted us during the laboratory and field work, and DANIDA for supporting the research through the TMMP.

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