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Bacteriological Quality of Oyster (Crassostrea lugubris), Cockle (Anadara granosa) and Their Cultivation Areas in Thailand

KRIENGSAG SAITANU

Deparment of Veterinary Public Health Faculty of Veterinary Science Chulalongkorn University Henri Dunant Road, Bangkok 10330 Thailand

Abstract

Samples of oyster (Crassostrea lugubris) and cockle (Anadara granosa) meat and water and bottom sediments from their growing areas at Ban Don Bay, Surat Thani Province, were collected in March and August 1987. All samples were examined for total heterotrophic bacteria, total marine bacteria, total coliforms, fecal coliforms, *Vibrio parahaemolyticus*, V. alginolyticus, V. cholerae and Salmonella spp. The bacte-riological quality of bivalves collected in March was acceptable while some of the sam-ples in August contained indicator organisms above the recommended wholesale market standard. All the bacterial levels of the samples collected in August were higher than in March.

Introduction

Most of the oysters (*Crassostrea lugubris*) and cockles (*Anadara granosa*) cultured in Thailand are within the boundaries at Ban Don Bay, Surat Thani Province. The Bay also serves as a major recreational area. Because the Bay receives outflows from the Tapi River and many canals which carry domestic waste, bacterial pollution in the surrounding estuary of the Bay was expected. The bacteriological quality of the estuarine water has implications for its uses (Sayler et al. 1975; Goyal et al. 1977; Palpal-Latoc et al. 1986b).

The microbiological criteria for approved growing areas are based on the most probable number of coliforms or $E. \ coli$ (Andrew et al. 1976; Wood 1976; Hunt 1977). Therefore, many studies have

been done to verify the sanitary quality of the cultivation areas for shellfishes in the Upper Gulf of Thailand (Hemachandra and Wisessang 1981; Saitanu et al. 1984a, 1987a, 1987b). However, a limited investigation of mollusc-growing areas in the Ban Don Bay was made (Musig and Ruttanogosrigit 1982).

The present investigation examines the bacteriological quality of oysters, cockles and their growing areas at Ban Don Bay, Surat Thani Province.

Materials and Methods

Samples of cockles, surrounding water and sediment were collected at five and six stations in March and August, respectively, at Ta Chang and one station at Cha-ngoe, Ban Don Bay, Surat Thani Province. Oyster samples including the environmental samples were collected at five and six stations in March and August, respectively, at Amphur Karnchanadit (Fig. 1).

Water samples were collected using a 1-l sterile plastic bottle, approximately 30 cm below the surface. Sediment samples were



Fig. 1. Map of sampling stations at Ban Don Bay, Surat Thani Province. Cockle farms at Ta Chang District, Stations 0-5 (•) and at Cha-ngoe (\blacksquare). Oyster farms at Amphur Karnchanadit, Stations 1-6 (0).

collected using a 500-ml sterile wide-mouth bottle. Bivalves were collected in either plastic bags or sacks. The environmental samples were kept in a styrofoam box with ice. All samples were transported to the laboratory and processed within 24 hours.

Standard bacteriological methods to determine total viable counts of heterotrophic bacteria were used. Plate count agar (ICMSF 1978a) was used for enumeration of total heterotrophic bacteria. The plates were incubated at 37°C for 24 hours, then examined for growth. Colony forming unit per milliliter (CFU·ml⁻¹) was recorded. For evaluation of heterotrophic marine bacteria, marine agar (Difco) was used and incubated at 25°C for 48 hours.

The most probable numbers (MPN) of total coliforms and fecal coliforms per gram of sediment or bivalves and 100 ml of water were established according to methods recommended by ICMSF (1978a) and APHA (1970).

Enumeration of Vibrio parahaemolyticus and V. alginolyticus was by direct plating, using 0.1 ml of 10^{-1} and 10^{-2} of homogenized oyster, cockle and sediment spread on TCBS agar (Eiken). The inoculated plates were incubated at 37°C for 24 hours. Blue-green colonies were recorded as V. parahaemolyticus-like organisms (VPLO) and large moist yellow colonies were recorded as V. alginolyticus-like organisms (VALO) CFU-g⁻¹ was recorded. After the colonies of VPLO and VALO were purified, the organisms were confirmed according to procedures described by ICMSF (1978a) and West and Colwell (1984).

The isolation of V. cholerae and Salmonella spp. from bivalves was determined according to the method recommended by ICMSF (1978a).

Results

Tables 1 and 2 present the occurrence of bacteria in fresh cockles and the growing areas at Ta Chang District and Cha-ngoe District in March and August, respectively. Stations 0 and 1 were located between the cockle cultivation area and the mouth of Ta Chang canal.

In March, most samples were free from coliforms although water from Station 1 contained a high number of coliforms and fecal coli: 2,400 and 430 MPN·100 ml⁻¹, respectively. Water samples from Stations 2 and 5 at the growing area were also contaminated with coliforms, but the level of the organisms was as low as 7.3 and 3.6

Table 1	. Bacter	iological	profile o	f cockle	s and gro	wing an	eas at T	a Chang	and Ch	a-ngoe D)istricts;	samples	collected	l in Mar	ch 1987.		
						Ta (Chang Dist	riet							Cha	-ngoe Dista	iet
Bacteria	Stat Water	ion - 1 Sed.	Static Water	on - 2 Sed.	Cockles*	Water	Station - 3 Sed.	Cockles	Water	Statio Sed.	ur - 4 Cocklea*	Water	Station Sed.	L - 5 Cockles*	Water	Sed.	Cockles*
1HB	4.7x1 02	8.2x10 ⁴	2.02102	1.7±105	3.0x10 ³	4.0210	9.0x10 ⁴	9.0±10 ³	1.8x10 ²	3.3x10 ⁶	6.0x10 ³	3.040	7.0x10 ⁴	1.3±104	5.0×10 ⁴	3.3±102	3.0±1.02
THMB	2.5x103	5.1×10 ⁵	6.0x10 ²	2.8x10 ⁵	2.7×10 ⁵	1.25403	1.1×10 ⁵	8.5x10 ⁶	2.0x10 ²	1.9x10 ⁵	3.4x10 ⁵	1.1±03	1.9±10 ⁵	1.6x10 ⁶	5.7×10 ³	5.3x10 ⁴	2.1×10 ⁵
īC	2,400	·	7.3	•	÷	8		۲		4	•	3.6	E.				·
FC	430	ı.	3.6	•			Ŧ	,	•	4	•		•	×	•	•	
٧P	2.0±10	2.2x1 04	2	4.021 03	2.0x1 03	5.0x10	2.5x10 ³	6.0x1 0 ³	×	1.0x10 ³	4.0x10 ²	2.0410	6.0x10 ³	1.6x10 ⁴	9.0±10	×	2.7xJ04
VA	9.0x10	3.0404		2.0x103	9.0x10 ³	1.0410	8.0x1 02	6.054 04	э	Э	1.8×10 ³	4.0x10	1.0x10 ³	2.5x10 ⁴	5.0x10	1.241 02	3.2x10
VC		9)		a.	٠	•	142	4		(9 2	1240	(1)	,	•	90	a.	
Sal	₽	₽	QN	QN	ē	QN	Ð	æ	Q	Ð	•	Ð	£	•	Ð	Ð	•
E E	Total het	erotrophic l	bacteria (C	FU-m] ⁻¹ or	r,1)) I						

THMB - Total heterotrophic matrine bacteria (CFU:ml⁻¹ or g⁻¹)
TC = Total coliforms (MPN:100 ml or g⁻¹)
VP - V. parabazmolyticus (CFU:ml⁻¹or g⁻¹)
VA - V. advine (CFU:ml⁻¹or g⁻¹)
VC - V. obstras
Not found (negative)

NE = Not done Sed. = Sediment Sal = S*almonello* spp. *pooled samples of 5-10 cockles

Symbols and abbreviations, see Table 1.

MPN·100 ml⁻¹, respectively. The total heterotrophic bacteria in cockles varied among the samples, 3.0×10^2 to 1.3×10^4 CFU·g⁻¹; while the total heterotrophic marine bacteria were 2.7×10^5 to 1.5×10^6 CFU·g⁻¹. Samples of cockle and sediment were positive for V. *parahaemolyticus*: 4×10^2 to 2.7×10^4 CFU·g⁻¹ and 1×10^3 to 6×10^3 CFU·g⁻¹, respectively. All samples were free from V. *cholerae* and *Salmonella* spp. (Table 1).

In August, in contrast to the situation in March, most samples were contaminated with coliforms and fecal coli. Water samples from Stations 0 and 1 were highly contaminated with fecal coli being 750 MPN·100 ml⁻¹. Fecal coliforms in cockle samples varied from 39 to over 1,100 MPN·g⁻¹. Total viable counts of heterotrophic bacteria, heterotrophic marine bacteria, direct quantitative recovering of *V. parahaemolyticus* and *V. alginolyticus* were not significantly changed from the samples of March. All tested samples were free from *V. cholerae* but were positive for *Salmonella* spp. in two samples of cockles (Table 2).

Tables 3 and 4 present the distribution of bacteria in fresh oysters and the growing area at Amphur Karnchanadit which were collected in March and August, respectively.

In March, two of five oyster samples were contaminated with fecal coli at 4 and 15 MPN·g⁻¹. All water samples were positive for coliforms ranging from 7.3 to 11,000 MPN·100 ml⁻¹ (mean 244 MPN·100 ml⁻¹); the levels of fecal coliforms were below 23 MPN·100 ml⁻¹ (3-23 mean 6.64 MPN·100 ml⁻¹). The total heterotrophic bacteria, *V. parahaemolyticus* and *V. alginolyticus*, in oysters ranged from 6.7 x 10³ to 9.1 x 10⁴ CFU·g⁻¹, 6 x 10³ to 8 x 10⁴ CFU·g⁻¹ and 2.8 x 10⁴ to 1.2 x 10⁵ CFU·g⁻¹, respectively. *V. cholerae* and *Salmonella* spp. were not present in the samples (Table 3).

In August, all samples of oysters were found to contain fecal coliforms, ranging from 39 to over 1,100 MPN·g⁻¹. Fecal coliforms in water ranged from 9.1 to 11,000 MPN·100 ml⁻¹. The other bacterial parameters, except total coliforms, were not significantly changed from the samples of March. The important pathogens V. cholerae and Salmonella spp. were not found (Table 4).

Discussion

Although the results were obtained in two months only, March and August, they indicated that the sanitary condition of oysters and cockles and their growing areas were probably related to the

Table 3.	Bacteriol	ogical pr	ofile of oy	sters an	d growin	ig area at	Amphur	r Kancha	madit, sa	mples w	ere colle	cted in M	arch 198	<i>.</i> 7.	
Bacteria		Station -	_		Station - 2			Station - :			Station -	4		Station - f	
	Water	Sed.	Oysters*	Water	Sed.	Oysters*	Water	Sed	Oysters*	Water	Sed.	Oysters*	Water	Sed.	Oysters*
THB	1.0x10 ⁴	1.1x10 ⁶	2.8x10 ⁴	4.0x10	2.2x10 ⁶	2.2x104	4.0x10	2.5x10 ⁶	9.1x10 ⁴	1.6x10 ³	1.9x10 ⁶	3.7x10 ⁴	4.0x10	5.8x10 ⁶	6.7x10 ³
THIMB	6.2x10 ⁴	7.1x10 ⁶	9.2x10 ⁶	1.2x10 ³	1.őx10 ⁶	5.8x10 [€]	1.4x10°	6.6x1 0 ⁶	9.5x10 ⁶	1.5x10 ⁴	4.8x10 ⁵	6.9x10 ⁶	4.0x10 ³	2.1x10 ⁶	5.0x10 ⁶
TC	99 99		40	7.3	•	8	39	1	4	11,000	66	500	36	e	15
FC	3.6	•	ŧ	ი	•	Ą	a	•	3	23	•	15	3.6	•	•
ЧЪ	()	•	1.1x10*	•	•	6.0x10 ³	7.0x10	r	*	5.0x10	1.1x10 ⁴	1.2x10 ⁴			8.0x10 ⁴
VA	2.0x10	1.5x10 ⁴	2.8x10*	٠	8.0x10 ⁴	5.7x10*	1.6x10 ³	1.3x10 ⁴	1.2x10 ⁶	1.6x10 ³	2.6x10 ⁴	2.7x10 ⁴	2.0x10	8.0x10 ³	6.1x10 ⁴
VC	ĩ	•			,	٠	,		(1)		٠		1	t	1
Sal	QN	QN	1	UN	QN	•	QN	QN	x	ΠŊ	UN		UD	ΠŊ	
Symbols a *Pooled se	und abbrevi umples of 3	iations, se -5 oysters	e Table 1.								1				

•

		Station -	1		Station - 2			Station - 3			Station - 4		50	itation - 5			Station - 6
Bacteria	Water	Sed.	Oysters*	Water	Sed.	Oysters*	Water	Sed.	Oysters*	Water	Sed.	Oysters*	Water	Sed.	Oysters*	Water	Sed.
THB	6.4x10 ⁸	6.0x10 ⁴	7.0x1 0 ^s	1.5x103	3.4x10 ⁴	4.8x10 ⁴	1.9x10 ⁵	2.6x10 ⁴	8.0x10 ³	5.4x10 ²	2.5x10 ⁴	8.0x103	3.8x10°	9.0x10°	1.0x10 ³	>10	5.6x10 ⁶
THMB	1.5x104	1.5x10 ⁶	1.5x10 ⁶	8.0x10*	7.8x10*	1.9x10 ⁶	1.4x10 ⁴	2.2x10 ⁴	6.2x10 ⁴	7.0x10 ²	9.0x10	1.4x10 ⁶	2.2x10	2.6x10 ⁴	2.6x10 ⁴	2.3x10	2.4x10 ⁵
J.	930	1	93	23	•	>1,100	4,600	40	8	43	4	>1,100	4,600	•	39	>11,000	500
FC	930	•	93	9.1	ı	>1,100	2,400	4	43	43	4	>1,100	4,600		39	11,000	200
ΥΡ	1.6x10 ²	3.0x10 ^s	2.0x10 ³	2.0x10	•	1.9x10 ⁴	7.0x10	ł	6.0x10 ³	2.0x10	2.0x10 ^a	1.0x103	2.0x10 [*]	2.0x10 ³	3.0x10	4.0x10 ²	7.0x10 ³
VA	2.0x10	2.0x10 ³	3.0x105	1.0x10	1.0x10 ^a	1.0x10 ⁵	۰		2.0x10 ³	1.0x10 ⁴	9.0x10*	ı	1.0x10 ³		1.2x10 ²	3.0x10 ²	1.5x10 ⁴
VC	•	ı	ı	٠	•	•		·	ı	•	'	•	ı	•	'	•	•
Sal	UN	UN	•	<u>(</u> N	QN	•	QN	QN	•	ΠN	QN	•	QN	ΩN	•	UN	QN
Symbols a: *Pooled sa	nd abbrev mples of 3	iations, sí 3-5 oysten	ee Table 1. s.														

season. Most of the samples were contaminated with coliforms and fecal coliforms in August, in the rainy season, while only a few samples were contaminated in March, summer. The numbers of indicator organisms in the samples collected in August were significantly higher than those in March.

It is reasonable to assume that the fecal pollution in the bivalves and the surroundings were influenced by outflow from canals which received domestic wastes. This observation was also observed in the green mussel (Perna viridis) and rock oyster (C. commercialis) growing areas in the Upper Gulf of Thailand. Saitanu et al. (1984a, 1987a) examined the bacteriological quality of 10 stations of growing areas of mussel and rock oyster located at Amphur Bangpra to Bangprakong River estuary in January-December 1982. They found that water and sediment of the stations at the Bangprakong River estuary and those near the town were polluted with coliforms and fecal coli during the second, but not the first half of the year. These indicator organisms were frequently found in larger numbers in the water than in the sediment. Similar variation was observed at the west coast of the Upper Gulf of Thailand (Saitanu et al. 1987b). Studies in 1984-85 examined the musselgrowing areas at Amphoe Ban Laem, Petchaburi Province, and Pran Buri River including its estuary. Most of the stations at Amphoe Ban Laem were far from the town, while the stations in Pran Buri River were surrounded with houses. Fecal contamination was found in all stations year-round at Pran Buri River, while the stations at Amphoe Ban Laem were seldom contaminated. Saitanu et al. (1984b) found that the quality of mussels and rock oysters collected from Amphoe Ang Sila and Bangprakong River estuary was directly influenced by water quality.

The level of V. parahaemolyticus in water and sediment in this study coincided with those in the previous studies in which samples were collected in the Andaman Sea (Saitanu et al. 1981a) and the Upper Gulf of Thailand (Saitanu et al. 1981a, 1981b, 1984a, 1987a, 1987b). However, the level of these bacteria in oysters and cockles was markedly lower than those in a previous report by Poonsuk et al. (1981) who determined the level of V. parahaemolyticus in several bivalves. In their study, 35% from 104 samples were reported positive for V. parahaemolyticus.

Musig and Ruttanogosrigit (1982) studied the bacteriological quality of the same areas in June-December. They found that the bacterial counts, total viable counts, total coliforms and fecal coli in water were 3 x 10^3 -5 x 10^3 CFU·ml⁻¹, 4-220 MPN·100 ml⁻¹, 0-79 MPN·100 ml⁻¹, in oyster; (1.3-4)10⁴ CFU·g⁻¹, 22-130 MPN·100 g⁻¹, 14-49 MPN·100 g⁻¹, in cockle; (3-169)10³ CFU·g⁻¹, 2-4 MPN·100 g⁻¹ and negative for fecal coli, respectively. The present study shows that the bacterial loads of water and of the two molluscs studied were higher than those in their report. However, the concentration of *V. parahaemolyticus*, fecal coli and total plate count in this study was somewhat lower than those reported by Thomson and Vanderzant (1976), Hood et al. (1983), Llobrera et al. (1986) and Palpal-Latoc et al. (1986a, 1986b).

For fresh and frozen bivalve mollucs to be acceptable for human consumption, the aerobic plate count, *E. coli* and *V. parahaemolyticus* must not exceed 5×10^5 , 16 and 10^3 CFU·g⁻¹, respectively, while *Salmonella* should not be found (ICMSF 1978b). Judging from this criteria, the bivalves collected from the studied areas during the summer season were acceptable. The bivalves in the rainy season were contaminated with fecal coliforms. Therefore, during the rainy season, they should be moved or undergo depuration to assure they are safe for consumption.

In Italy, the MPN of *E. coli* in water in approved areas must not exceed 2 per 100 ml in 90% of samples taken in one year, and not more than 10% of samples taken in the same period should contain more than 6 per 100 ml. In the USA, water in approved shellfish-growing areas requires a median coliform MPN lower than 70 per 100 ml and no more than 10% of samples should exceed 230 per 100 ml. In France, shellfish-growing areas are classified in four categories: satisfactory (no *E. coli*), acceptable (1-60 *E. coli* per 100 ml), suspicious (60-120) and unfavorable (over 120) (Wood 1976). By this criterion, the mollusc-growing areas at Surat Thani Province are acceptable during the summer season and unfavorable in the rainy season.

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