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Analisis Mikrobiologi Lingkungan Budidaya Kerang Hijau (*Perna Viridis*) di Perairan Tanjung Kait, Tangerang, Banten

(Analysis of Environmental Microbiology of the Green Mussels (Perna viridis) Culture in the Waters of Tanjung Kait, Tangerang, Banten)

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ABSTRAK

Parameter mikrobiologi adalah salah satu faktor penting dalam keberhasilan budidaya kerang hijau. Ciri khas kerang hijau sebagai *filter feeder* mudah terkontaminasi oleh racun yang terlarut di air maupun menjadi pembawa penyakit. Penelitian ini bertujuan untuk menentukan jumlah kontaminasi bakteri patogen pada area budidaya dan kerang hijaunya. Sample air dan kerang hijau diambil dari perairan teluk Tanjung kait, Tangerang, Banten dan diidentifikasi di Loka Pemeriksaan Penyakit Ikaqn dan Lingkungan di Pasauran, Cinangka, Serang. Parameter bakteriologi yang dianalisis ialah total *Coliform*, species of *E coli*, *Salmonella* sp., total of bakteri aerobik dan *Vibrio*. Hasil analisis kualitas air dan kerang hijau menunjukkan bahwa total *Coliform* < 3 sampai 240 MPN/ml di air dan sekitar 8,3 hingga >1600 APN/g pada kerang hijau, *E. coli* < 3 hingga 43 APM/ml di air dan 0,18 hingga 130 APM/g pada kerang hijau, hasil negatif untuk *Salmonella* sp. Baik di air maupun kerang hijau. Total nakteri aerob antara 34-880 $x10^2$ CFU/ml di air, 24-980 $x10^4$ CFU/ml di sedimen dan 12-100 $x10^7$ CFU/ml pad kerang hijaul. *Vibrio* di air berkisar 50-340 CFU/ml, dan 4,4-88 $x10^2$ CFU/g di sedimen dan 0,082-25 $x10^4$ CFU/ml pada kerang hijau. *Salmonella* tidak ditemukan pada penelitian ini, ini sesuai dengan SNI untuk budidaya kerang hijau baik kualitas air dan parameter biologi bagi batas kontaminasi mikrobia pada makanan menurut SNI 7388-2009 sedangkan parameter bakteri patogen lainnya masih. dalam standar yang dibolehkan.

Kata kunci: bakteria aerob, *Coliform*, *Perna viridis*, *Salmonella* sp., *Vibrio* sp.

ABSTRACT

Microbiology parameter is one important factor for the success of green mussels culture. Characteristic of green mussels as the filter feeder might be contaminated easily by the diluted toxin as well as the carrier of pathogens. This research aims to find out the amount of contamination of pathogenic bacteria. Samples of water and the green mussels were taken from the waters of Tanjung Kait, Tangerang, Banten and were then identified in the Laboratory of Fish Diseases Investigation and Environment in Pasauran, Serang. Analysed bacteriological parameters were total Coliform, as well as the species of E coli, Salmonella sp., a total of aerobic bacteria and Vibrio. The result of seawater quality and

green mussel showed that the total abundance of Coliform cell was lower than <3 until 240 MPN/ml in water and about 8,3 until >1600 MPN/g in green mussel, E. coli was < 3 until 43 MPM/ml in water and 0,18 until 130 MPM/g in green mussel, negative result for Salmonella sp. neither in water nor in green mussels. Total aerobic bacteria was found about 34-880 x10^2 CFU/ml in water, 24-980 x10^4 CFU/ml in the sediment and 12-100 x10^7 CFU/ml in green mussel. The result of Vibrio in water was about 50-340 CFU/ml, 4,4-88 x10^2 CFU/g in sediment and 0,082-25 x10^4 CFU/ml in the green mussels. Salmonella was found the negative test results in accordance with predefined standards, both with the seawater quality standard based on biological parameters and SNI 7388-2009 regarding the limit of microbial contamination of foods, while for other pathogenic bacteria parameters still above the predefined standard.

Keywords: *aerobic bacteria, Coliform, Perna viridis, Salmonella sp., Vibrio sp.*

INTRODUCTION

Tanjung Anom village is a coastal area about 3620 km^2 width with about 9,03% of their area include to the Mauk district, Tangerang regency, directly bordered with Java Sea in the north and Sukadiri district in the east. The population of Tanjung anom village in 2014 was about 7253 peoples, as the seventh populated area among 11 other villages in the Mauk District (SCA Tangerang 2015). The populated areas would commonly have an adverse effect on domestic waste management. The most wastes product was produced by the domestic wastes. It also as the source of wastes in the waters (Effendi 2003). The waters with abandon organic wastes would increase the population of decomposing bacteria including the pathogenic bacteria in that areas. (Effendi 2003). There are 100 tonnes of green mussels *Perna viridis* cultured in the area approximately two square miles of Tanjung kait waters which was the coastal area of Tanjung Anom village (MFA, 2015). Accordingly, one of the parameters that supports the success in aquaculture is the bacteriological condition in the water environment (Kastoro 1988). The most common bacteria for water quality parameter were the *E. coli* group and pathogenic bacteria (Sutiknowati and Ruyitno 2008). However, the green mussels among the filter feeders found were resistant with polluted water conditions (Kastoro, 1988). Therefore, the green mussels susceptible for bacterial contamination and most probably suffering the accumulated pathogenic bacteria (Retyoadhi *et al.* (2005). One of the danger is the emergence of *foodborne disease* that is the disease caused by foodstuff which has been contaminated by microorganisms as well as by the toxin. Salmonellosis is the most common cause of *foodborne disease* in the USA (Wanke 1988 *in* Eppy 2009). The criteria of water quality for the green mussels *Perna viridis* culture were almost the same as the other cultured marine organisms (Kastoro 1988). The purpose of this study is to determine the level of polluted bacteria of *Coliform, E. coli* and *Salmonella* sp. in water and in the cultivated green mussels areas of Tanjung Kait waters.

METHODOLOGY

The study was carried out in May-June 2016 by taking the water sample from Tanjung Kait waters, while the green mussel was found out from the green mussels culturist in the Tanjung Kait waters. Detection and calculation of the bacteria were done in the laboratory of microbiology Station of Fish Diseases Investigation and Environment, Pasauran, Serang, Banten to know the occurrence of *Coliform*, *E. coli*, *Salmonella* sp., total aerobic bacteria and the total *Vibrio* sp. Furthermore, the observation of water quality such as pH, DO, salinity and water clearness was done in situ by using apparatus for water quality checker (pH meter, Do meter, salinometer and TDS Meter Generic).

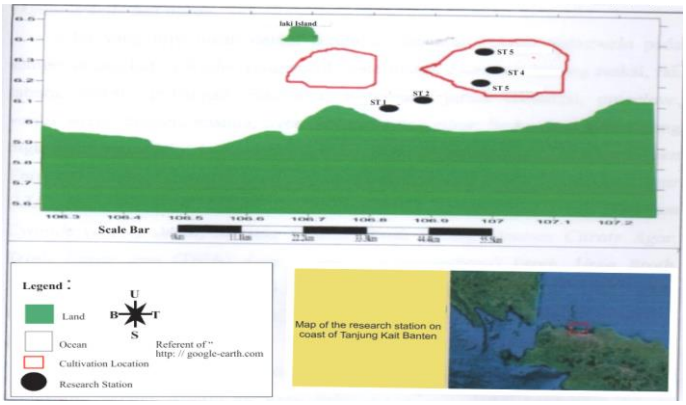

Figure 1 Map of the Research Station of Coast of Tanjung Kait Tangerang

Materials and Apparatus

The material and apparatus used for the study were *sterilized blender*, *Petridishes*, *flasks* provided with its racks, balance, incubator, *water bath*, *inoculators needle*, *autoclave*, *vortex mixer*, *bunsen*, spatula, oven, *hot plate* dan *stirrer*.

Furthermore, the materials used were *Lactose Broth* (LB), *Bismuth sulfite Agar* (BSA), *Hectoen Enteric* (HE) *Agar*, *Lactose Broth*, *Lysine Decarboxylase Broth*, *Lysine Iron Agar* (LIA), *Malonate Broth*, *MR-VP Broth*, *Phenol red Carbohydrate Broth*, *Potasium Cyanide* (KCN) *Broth*, *Selenite Cystine Broth* (SCB), *Simmon Citrate Agar*, *Triple Sugar Iron* (TSIA) *Agar*, *Tryptone* (*Tryptophane*) *Broth*, *Urea Broth*, *Xylose Lysine Desoxycholate* (XLD) *Agar*, *Plate Count Agar* (PCA L), *Aquadest*, *Ethanol* 70%, *Reagen Kovac's*, Indikator *Methyl Red*, dan *Reagen Voges-Proskaue*, *Brilliant Green Lactose Bile* (BGLB), *Lauryl Tryptose Broth* (LTB), EC *Broth*, *Levine's Eosin Methylen Blue* (L-EMB) agar, *Butterfield's Phosphate Buffered* (BPB) solution, Gram stain, *Trisalt* and *Thiosuphate Citrate Bile Salt Sucrose* (TCBS) solution.

Detection of *Salmonella* (NSA 2006a)

Samples were prepared aseptically and then weigh 25 g of sample and diluted in 225 ml Lactose Broth solution and incubated one day. Furthermore the sample were then inoculated to the medium of HE, XLD, BSA and the colony observed in the next day. And then take out the *typhical* colony and inoculated into the medium of TSIA and LIA, incubated in the next day (24 hours ± 2 hours). The suspected colony were then transferred to the media urea broth and then

incubated again for 24 hours. If the result showed negative urease then the biochemical test is necessary to confirm that the *Salmonella* sp. was not available, however if the result is positive that it has confirmed that the *Salmonella* sp. was existed in the sample.

Detection of *Coliform* and *Escherichia coli* (NSA 2006b)

Preparation of 25 g of samples aseptically diluted with 225 ml BPB solution (dilution of 10^{-1}), The dilution were then carried out according to the necessary dilution, and then 1 ml of each diluted samples were then transferred to the LTB tube which contain the Durham tube and incubated for 48 hours. The positive samples were inoculated to the bacterial medium of Brilla and EC Broth, and were then incubated for 48 hours. For the number of coliform could be seen in the positive Brilla tubes while that of *E. coli* must be further tested by inoculating from the EC Broth positive to the EMBA media and incubated for 24 hours. If the thypical colony found then continued with the biochemical test.

Total Plate Number (TPN) of Bacteria (NSA 2006c)

By using the aseptic technique 25 g sample were prepared then diluted 225 ml Butterfield's Phosphate Buffered (BPB) solution. diluted further with 9 ml of BPB solution and then make the solution according to the necessary dilution, then pipet each 1 ml from each dilution to the steril petri dishes added with PCAL and then stir the petri dish in the shape of number eight direction and wait until the agar solid and then incubated for 48 hours and analyse the result when the colony have been formed.

Total Plate Number (TPN) of The Vibrio (NSA 2013)

By using the aseptic technique 1 g sample were prepared then diluted with 9 ml Tri-salt and then diluted further with 9 ml steril trisalt and make the solution according to the necessary dilution. Pipette 100 µl into the media Thiosulphate Citrate Bile Salt Sucrose Agar (TCBSA) and spread evently by using spreading rod then incubated for 18 hours, and count the colony.

Calculation Total Coliform and *E coli*

Calculation of *Coliform and E coli* bacteria was following the method of Most Probable Number (MPN). This method calculating the microbe with liquid medium in the flasks by using 3 to 5 series of dilutions and it was calculated by statistical approach.

RESULTS AND DISCUSSION

***Coliform* and *Eschericia coli* in Water**

Distribution of *Coliform and E coli* in waters of Tanjung Kait from March to May showed in Table 1. The *Coliform* were in the range of < 3 MPN/ml to 240 MPN/ml. The highest density of *Coliform* was found in the station 1 with the average value 89,13 MPN/ml. This is due to the station 1 is the nearest point to the terrestrial areas, where the human activity is in very high rate. While at station 2 the value of *Coliform* is lower, however, the *Coliform* value increase again in station 3, 4 and 5. The same trend has been reported in the research of Sutiknowati and Ruyitno (2008) in *Klabat Bay* waters. Whereas the bacterial

content in waters with higher sewage tends to be higher tahan the normal water areas.

The density of *E. coli* in water is lower than the value of *Coliform.* The highest density in station 1 was 16,33 TPN/ml. The abundance occurrence of *E. coli* in water indicated that the waters has been contaminated with human faeces and might be also those of pathogenic bacteria. According to Suriawiria (2008) in about 100-150 gram of faeces at least $3x10^{11}$ (or 300 billion) cells of *E. coli*. The same condition also has been reported by Thayyib *et al*. (1978) in cockle and oyster *Crassostrea* sp.

Tabel 1. The density of *Colliform* and *E. coli* (MPN/ml) in water samples

		March	April	May	Average
Station 1	*Coliform*	7,4	20	240	89,13
	E.coli	< 3	< 3	43	16,33
Station 2	*Coliform*	< 3	9,2	< 3	5,07
	E. coli	< 3	3,6	< 3	3,2
Station 3	*Coliform*	< 3	< 3	23	9,7
	E. coli	< 3	< 3	3,6	3,2
Station 4	*Coliform*	< 3	23	6,2	10,73
	E. coli	< 3	< 3	< 3	< 3
Station 5	*Coliform*	< 3	3,6	43	16,53
	E. coli	< 3	3,6	< 3	3,2

Salmonella sp. in Water

Test on pathogenic bacteria of *Salmonella* in water samples indicated negative results in all samples. It was might be caused the water condition in Tanjung kait has higher salinities about 24-31 ppt. In this condition *Salmonella* could not survived. According to Jay *et al.* (2005) the salinity range for *Salmonella* in water habitat is in range about 0-9 ppt.

Total Aerobic Bacteria and *Vibrio* sp.

Total number of aerobic bacteria and *Vibrio* in water sample and sediment is presented in Table 2. Results of analysis on total aerobic bacteria showed in a range of value $54x10^2$ CFU/ml to $88x10^3$ CFU/ml in water sample and $24x10^4$ CFU/g to $16x10^6$ CFU/g on soil samples. The highest total bacterial density was found at the sampling station one at average $55x10^3$ CFU/g. It was noted that this sampling station was the first area where various wastes from the domestic waste from the surrounding areas, waters from the post harvest fishes as well as the wastes from the food stall were assembled. Moreover, at the time of sample collection in April was conducted after the rain in the previous day, therefore the abundant of food wastes increased in the sampling area which supposedly increased the amount total bacteria than sample in March and May. The same situation was also fund during the sample collection in May. Rahayu (1993) pointed out that the presence of food wastes would increase the metabolisms to form of new bacterial cells and energy which finally increase the density of bacteria. Anyhow, as more further go in to the green mussels culture areas the density of soil bacteria was decreased. It was suggested that there were different

sediment composition between that in the coastal water and that of the areas of green mussels culture. The composition of sediment in the coastal areas contained more mud and less sand compound, therefore its particle size is more refined, while that of the green mussels culture areas was in the contrary. According to Retyoadhi *et al* (2005), the different in particle size would significantly correlate to the bacterial abundant, where the refined mud particles and mud compound would have much more bacterial abundant compared to the rough sediment compound.

Tabel 2. Total number of aerobic bacteria and *Vibrio* in water sample (CFU/ml) and sediment (CFU/g).

Sampling Station	Replication	Water		Soil	
		TPN B	TPN V	TPN B	TPN V
Station 1	22 March	$7x10^3$	71	$66x10^4$	2500
	22 April	$88x10^3$	260	$98x10^5$	2000
	22 May	$71x10^3$	74	$16x10^6$	3000
Average		**$55x10^3$**	**135**	**$88x10^5$**	**2500**
Station 2	22 March	$67x10^2$	50	$44x10^4$	3800
	22 April	$86x10^2$	190	$28x10^4$	920
	22 May	$14x10^3$	82	$49x10^5$	440
Average		**$98x10^2$**	**107**	**$19x10^5$**	**1720**
Station 3	22 March	$51x10^2$	130	$40x10^4$	3300
	22 April	$56x10^3$	270	$89x10^4$	8800
	22 May	$12x10^3$	180	$26x10^5$	1100
Average		**$24x10^3$**	**193**	**$13x10^5$**	**4400**
Station 4	22 March	$34x10^2$	130	$44x10^4$	3600
	22 April	$34x10^3$	280	$53x10^4$	1800
	22 May	$66x10^3$	340	$46x10^4$	460
Average		**$34x10^3$**	**250**	**$48x10^4$**	**1953**
Station 5	22 March	$54x10^2$	140	$40x10^4$	1000
	22 April	$42x10^3$	120	$60x10^4$	4200
	22 May	$14x10^3$	98	$24x10^4$	950
Average		**$20x10^3$**	**119**	**$41x10^4$**	**2050**

Microbial Content in Green Mussels

Analysis of microbial content in green mussel showed in Tabel 3. It was indicated that the bacterial density was increased in each sampling station. Content of *E. coli* on first sampling was about 0,18-2 MPN/g and was under the threshold standard decided by Indonesian National Standard (INS) that was < 3 MPN, however, in the second and third sampling the bacterial content have been over the INS standard. Common bacterial content was found at $5x10^5$ CFU/g higher than that of the INS standard. During carried out this study, some time raining has fallen. It was supposed that the rain also affected the samples taken, where the sewage from the household much more abundant in the sampling area. This indicated in the bacterial TPN number in green mussels were increased into the value of $12x10^7$ - $10x10^8$ CFU/g. TPN value of the *Vibrio* also have over the INS standard where the fresh green mussels should be free from *Vibrio.*

However, the green mussels in this study did not indicate the availability of *Salmonella* and was in accordance to the INS standard.

Tabel 3. Results of observation on microbial content in green mussels in this study

Sampling Location	Time	*Coliform*	*E.coli*	*Salmonella*	TPN Bacteria	TPN *Vibrio*
Station 3	22 Maret	120	0,18	Negative	$24x10^7$	3500
	22 April	920	13	negative	$24x10^7$	3000
	02 Mei	>1600	130	negative	$62x10^7$	250000
Average		**880**	**47,8**	**negative**	**$37x10^7$**	**85.500**
Station 4	22 Maret	47	< 2	negative	$10x10^8$	24000
	22 April	240	7,8	negative	$12x10^7$	7400
	02 Mei	>1600	23	negative	$70x10^7$	94000
Average		**629**	**10,93**	**negative**	**$61x10^7$**	**41800**
Station 5	22 Maret	8,3	2	negative	$78x10^7$	12000
	22 April	140	33	negative	$12x10^7$	820
	02 Mei	>1600	6,1	negative	$43x10^7$	40000
Average		**582,8**	**13,7**	**negative**	**$43x10^7$**	**17607**

CONCLUSION

There were no *Salmonella* sp. in water, sediment as well as in the green mussels samples. Density of *Coliform* were at range between < 3 to 240 MPN/ml in water, and 8,3 to >1600 MPN/g at green mussels. Density of *E. coli* were at range between < 3 to 43 MPN/ml at water and 0,18 to 130 MPN/g in green mussels. Total aerobic bacteria in water about 34 - 880 $X10^2$ CFU/ml, 24 - 980 $X10^4$ CFU/g in sediment, while in green mussels were between 12-100 $X10^7$ CFU/g. Moreover, for *Vibrio* in water were in range between 50-340 CFU/ml, 4,4-88 $X10^2$ CFU/g in sediment and green mussels in range 0,082-25 $X10^4$ CFU/g.

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