PAIR/T.140/1985

THE PRESENCE OF *VIBRIO PARAHAEMOLYTICUS* AROUND THE FISHING AREAS OF INDONESIAN WATERS AND THEIR RADIATION SENSITIVITY

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K.A. 381

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ABSTRACT

THE PRESENCE OF *VIBRIO PARAHAEMOLYTICUS* AROUND THE FISHING AREAS OF INDONESIAN WATERS AND THEIR RADIATION SENSITIVITY. The isolation and identification of *Vibrio parahaemolyticus* from sediment and seafood around fishing areas of the eastern coast of Sumatra and the north coastral areas of Java were investigated. The results showed that 79 out of 2,434 sediment and seafood samples analyzed (3.3 %) were positive for *V.parahaemolyticus*. Shellfish samples relatively showed higher positiveness for *V.parahaemolyticus* (9.0 %), when compared with the sediment (4.7 %) and fish (2.5 %). Based on the sampling areas, the highest incidences of *V.parahaemolyticus* was found in samples collected from the Riau area (5.4 %). *V.parahaemolyticus* is a fairly radiation sensitive marine microorganism. D_{10} value of nine strains tested were found to be around 150 to 275 Gy. The reduction at doses of 500 and 750 Gy were found to be 1.8 - 4.3 and 3.7 - 6.3 log cycles respectively.

ABSTRAK

VIBRIO PARAHAEMOLYTICUS DI LINGKUNGAN DAERAH PERIKANAN INDONESIA DAN SIFAT KEPEKAANNYA TERHADAP RADIASI. Dalam makalah ini dilaporkan isolasi dan identifikasi *Vibrio parahaemolyticus* pada lumpur dan hasil laut yang berasal dari daerah perikanan pantai timur Sumatra dan daerah pantai utara pulau Java. Hasil penelitian menunjukkan bahwa dari 2.434 contoh lumpur dan hasil laut yang dianalisis, 79 contoh (3,3 %) positif mengandung *V.parahaemolyticus*. Contoh kerang yang positif mengandung *V.parahaemolyticus* relatif lebih tinggi (9,0 %), bila dibandingkan dengan lumpur (4,7 %) dan ikan (2,5 %). Berdasarkan lokasi pengambilan contoh, daerah Riau menunjukkan persentase positif *V.parahaemolyticus* paling tinggi (5,4 %). *V.parahaemolyticus* termasuk mikroorganisme laut yang sensitif radiasi. Nilai D_{10} sembilan strain yang diuji menunjukkan kisaran antara 150 dan 275 Gy. Reduksi akibat iradiasi dalam suspensi ikan dengan dosis 500 dan 750 Gy berturut-turut menunjukkan nilai sekitar 1,8 - 3,4 log dan 3,7 - 6,3 log.

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* This work was supported in part by the IAEA under Research Contract No.1281/RB.

** Centre for the Application of Isotopes and Radiation, BATAN.

INTRODUCTION

V.parahaemolyticus is an enteropathogenic, facultative halophilic bacteria which usually contaminates seafood (1, 2). The distribution of *V.parahaemolyticus* in nature appears to be limited to the coastal areas (3 - 6). This organism was first isolated during an outbreak of food poisoning in Japan, and called *Pasteurella parahaemolytica* (7). TAKIKAWA (8) proposed a name *Pseudomonas enteritis* for this organism. In 1961 MIYAMOTO et al.(9) introduced a new genus Oceanomonas for halophilic vibrios (including *V.parahaemolyticus*), which consisted of three species namely *O.parahaemolyticus*, *O.enteritidis*, and *O.alginolytica*.

Based on morphological, cultural and biochemical characteristics of the enteropathogenic facultative halophilic bacteria , SAKAZIKI et al.(10) proposed a name *V.parahaemolyticus* for this bacteria which included 3 biotypes, i.e. biotype I (*V.parahaemolyticus*), biotype II *(V.alginolyticus)*, and biotype III *(V.anguillarum)*. Although the genus Oceanomonas proposed could not be accepted by SAKAZAKI et al.(10), it was obvious that *V.alginolyticus* (biotype II) was identical to *O.alginolytica* in culture. Separation of biotype II from *V.parahemolyticus* was suggested by ZEN-YOJI et al.(11), based on differences in their morphological characteristics and some biochemical reactions between the two biotypes. SAKAZAKI (12) then decided to adopt the name *V.alginolyticus* for the biotype II of *V.parahemolyticus*.

Due to the habit of eating raw fish or other uncooked seafood, over 50% of the major seafood poisoning outbreaks in Japan were caused by *V.parahaemolyticus* (10, 13). As an effective means of prolonging

the storage life of food, irradiation must be sufficient to free the food products from pathogens which might be present and grow due to a possible mishandling of the product (14). This paper reports the presence and distribution of *V.parahaemolyticus* in sediment and seafood collected from the fishing areas of Indonesian waters, as well as radiation sensitivity of some isolated strains.

MATERIAL AND METHODS

Sampling. Samples were collected during the dry season from 70 sites around the fishing areas of the eastern coast of Sumatra and the north coastal areas of Java (Fig. 1). Sediment speciments were taken by graph-sampler from the sea at 1 to 2 km from the shore at water depth of 3 to 25 m. Seafood samples, i.e. fish, shellfish (clam,oyster, snail), shrimp, and crab, were directly bought from the local fishermen. The sample were separately packed in plastic bags and cooled with ice for transportation to Jakarta. A total of 2,434 samples were collected consisting of 467 sediments, 1,650 various fish, 133 shellfish, and 123 shrimps, and 61 crabs.

Isolation and Purification. TCBS agar (Difco) and Salt Trypticase agar (containing 3% sodium chloride, 0.4% starch, and 2 units penicillin per ml medium) were used for isolation. Individual homogenized samples were streaked onto TCBS agar and Salt Trypticase agar plates, and then incubated at 30°C for 1-2 days. Small colonies with bluish - green centre were suspected to be *V.parahaemolyticus* on TCBS agar plates, while on Salt Trypticase agar plates the colonies were smooth and hydrolyzed starch. The colonies were then cultured on Salt Trypticase agar slants. The pure cultures were stained using Gram staining for

for their morphological characteristics. Mortality and growth of the microorganisms were tested in one set of Salt Trypticase broth containing 0, 3, 7, and 10% sodium chloride.

Biochemical Characteristics. In this investigation, biochemical tests conducted included the reaction against Voges-Proskauer, methyl - red test, hydrogen sulfide production in TSI, nitrate reduction, blood hemolysis, gelatin liquefaction, indole test and some carbohydrates fermentations (arabinose, fructose, glucose, inositol, lactose,maltose, mannitol, rafinose, rhamnose, sucrose and xylose).

Strains. Seven local strains (V21, V31, V62, V91, V102, V142 and V201) and two standard strains (EB101 and ATCC 17802) .were used for growth tests after irradiation treatment. The local strains were originally isolated from sediment and seafood samples collected around fishing areas of Indonesian waters. The two standard strains were obtained from Microbiology Department, Faculty of Medicine, University of Indonesia.

Irradiation. An overnight culture of *V. parahaemolyticus* strains were harvested under continuous shaking and suspended in 3% brine fish homogenate. Culture tubes containing 10 ml of the suspension were then irradiated to ^{60}Co gamma rays at a dose rate of about 48 Gy per minute with air bubbling. The dose used were 0, 250, 500, 750, and 1,000 Gy. The culture tube were held in ice during irradiation. The suspension to be irradiated contained about 10^7 cells of bacteria per ml.

Determination of Total Bacterial Counts. Irradiated and unirradiated bacterial suspensions were spreaded on TCBS agar plates (0.5% yeast

extract , 1% protease peptone , 1% sodium citrate , 1% sodium thiosulphate, 0.1% ferric citrate, 0.004% brom thymol blue, 0.004% thymol blue, and 1.5% agar) after serial dilution in 3% brine. After incubation at 32°C for 24-48 h, colonies formed on agar plates were counted.

RESULTS

The presence of *V.parahaemolyticus* in samples of sediment and seafood is shown in Table 1. The result showed that from 2,434 sediment and seafood samples analyzed, 79 samples (3.3%) were found to be positive for *V.parahemolyticus* . It appeared that the incidence of *V.parahaemolyticus* contamination in the samples of sediment, fish,shellfish, shrimp, and crab were about 4.7 , 2.5 , 9.0 , 1.6, and 3.3% respecti - vely. The average percent positive for all raw seafood samples was about 3.3% and for seafood other than fish (shellfish , shrimp , and crab) was 5.0 %. Although the presence *V.parahaemolyticus* was not restricted to specific types of sediment or seafood species, it appeared that the incidence of vibrios in shellfish was rather higher (9.0%).

The distribution of *V.parahaemolyticus* related to the sampling areas in shown in Table 2. It shows that among the sampling areas,Riau gave the highest positive samples for *V.parahaemolyticus*.

Table 3 presents the pyisological and biochemical characteristics of 79 isolated strain of *V.parahaemolyticus*. All of the strains were found as smooth colonies on Trypticase Soy agar plates after incubation at 30°C for 24 hours, motile in SIM medium, rod shaped , grew well in Salt Trypticase broth containing 3 and 7% sodium chloride, and capable to hydrolyze starch.

All of the isolated strains were Gram negative, nonsporeforming , did not grow in Salt Trypticase broth containing 0 and 10% sodium chloride, negative to Voges-Proskauer, and negative to fermentation of rafinose, rhamnose, and sucrose.

However, for some other biochemical tests, the 79 isolated strains showed different reaction as shown in Table 3.

Fifure 2 present the survival curves of nine strains of *V.parahaemolyticus* after irradiation in fish homogenate in 3% brine. The D_{10} value were found to be around 150 to 275 Gy. The log reduction of *V.parahaemolyticus* caused by the dose of 500 and 750 Gy were around 1.8 - 3.4 and 3.0 - 6.3 , respectively (Table 4).

DISCUSSION

Starch Agar medium was first demonstrated by BAROS and LISTON (4) for the isolation of *V.parahaemolyticus*. To inhibit the growth of other spoilage organisms, 2 units penicillin per ml medium were added. Following overnight incubation of the culture at 30°C, and after flooding the plate with lugol solution a clear zone of starch hydrolysis surrounding the colony was found. TCBS agar was developed by NAKANISHI (15) and modified by KOBAYASHI et al.(16). It was used for isolation and selective cultivation of *V.cholerae* and other enteropathogenic vibrios, e.g. *V.parahaemolyticus*. According to KAMPELMACHER et al. (17), TCBS agar has been found useful in the detection of *V. parahaemolyticus* in fish. The colonies of *V.parahaemolyticus* on TCBS agar plates are small, with bluish-green centre.

The 3.3 % contamination level of *V. parahaemolyticus* in sediment and seafood around the fishing areas of the eastern coast of Sumatra

and the north coastal areas of Java is lower than that reported for some other areas. BAROSS and LISTON (15) reported that the incidence rates of organisms related to *V.parahaemolyticus* in water, sediment, oyster, and clam around to oyster areas were 78, 100, 100, and 100 % of samples analyzed, respectively; while in water, sediment, fish, and crab intestines around the Puget Sound areas were about 18, 100 , 52, and 100%, respectively. According to SAKAZAKI et al.(10) the 79 isolated strains in this investigation belonged to biotype I (*V.parahaemolyticus*), which were able to grow at 3 and 7% sodium chloride , but negative at 0 and 10% sodium chloride. The higher incidence of *V.parahaemolyticus* contamination found by some investigators might include the biotypes II and III.

On the other hand, the result of this investigation is in agreement with the data of some investigators. SAYLER et al. (18) reported that 10.4 % of a total of 86 water and sediment samples from the Upper Chesapeake Bay were positive of *V.parahaemolyticus*. BROEK et al. (19) found that from 79 tissue samples of Dutch mussels originating from the East Schelde Estuary, the Netherland, 3 samples (3.8 %) were positive *V.parahaemolyticus* . In the second survey, 6 out of 23 bags of mussels (26%) contained one or more strains of *V.parahaemolyticus* in 5 g tissue samples.

It has been reported that the growth of *V.parahaemolyticus* depends on the kind of medium, growth temperature, and salt concentrations. Optimum growth for halophilic vibrios requires a salt concentration of about 2-3 %, but for *Aeromonas* sp. and other vibrios is about 0 - 2 % (10). LISTON and MATCHES (20) found that *V.parahaemolyticus* grew well at 22°C in medium containing 0.1 - 7% sodium chloride, but did not grow

in salt concentration of 8-10 %. On the other hand, COLWELL (21) reported that *V.parahaemolyticus* could grow in salt concentration ranges from 0.5 to 10%, although a variable growth was found at 10% salt concentration. TWEDT et al.(22) isolated *V.parahaemolyticus* which was able to grow at 10% sodium chloride, while FISHBEIN et al.(23) found 4 strains which were able to grow at 10 % salt concentration. SON and FLEET (24) studied the behaviour of *V.parahaemolyticus* during depuration, re-laying, and storage. They reported that after oyster were contaminated with *V.parahaemolyticus* 1,000 cells per g, during the first 4 days of storage the count increased, and afterwards a decrease occured.

The outbreak of gastroenteritis in Japan caused by *V.parahaemolyticus* was mainly occured in the summer season (25, 26). According to AISO (1960 and 1967) as reported by KANEKO and COLWELL (27) there were more than 20% incidences of organisms related to *V. parahaemolyticus* in coastal seawater throughout the year. Distribution of *V.parahaemolyticus* and related organisms in the Atlantic Ocean was determined during the summer in samples collected from the coastal areas of South Carolina and Georgia. No *V.parahaemolyticus* strains were found in samples of seawater, sediment, and plankton collected (27). BAROSS and LISTON (5) reported the correlation between the seasonal variation of the total count levels of this organisms in oyster,and the temperature of the overlaying water. The high incidence of food poisoning caused by the vibrios in Japan was to the custom of eating raw seafood.

Different characteristics among the 79 isolated strains were found in some biochemical reactions, such as : methyl-red reaction, ability of blood hemolysis , hydrogen sulfide production, nitrate

reduction, gelatine liquefaction, indole reaction, and fermentation of arabinose, fructose, glucose, inositol, maltose, mannitol, and xylose. Such a fact had also been known and reported by previous investigators (5, 10, 12, 21, 22, 23, 26, 28).

V.parahaemolyticus is a fairly cold-, heat sensitive (29), and radiation sensitive marine microrganism. Radiation sensitivity varied among the strains as reported in this investigation, and appeared depend upon the suspending medium. MATCHES and LISTON (17) reported that *V.parahaemolyticus* in seawater pepton media can be reduced by as much as 4-7 logs at 300 Gy irradiation treatment. This organism was found to be more resistant in fish homogenate in seawater, i.e. only about 2.7 - 6.1 logs reduction due to the 300 Gy irradiation treatment. In aqueous fish homogenate, a dose of 100 Gy could reduce the bacterial number by 2.3 - 4.6 log cycles.

CONCLUSION

Distribution of *V.parahaemolyticus* in western part of Indonesian waters are not found is any particular species of organism, types of sediment, or location of samples. Shellfish samples were relatively higher positive of *V.parahaemolyticus* when compared to sediment or other organism samples. The characteristic of organisms related to *V.parahaemolyticus* were variable in reactions to some physiological and biochemical tests. *V. parahaemolyticus* belongs to the radiation sensitive group bacteria. This organism can be controlled easily in seafoods by treatments of radurization doses.

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Tabel 1. The presence of *Vibrio parahaemolyticus* in samples of sediment and seafood around fishing areas of the eastern coast of Sumatra and north coastal areas of Java.

Samples	No. of samples	No. of positive samples (%)
Sediment	467	22 (4.7)
Raw seafood :		
Fish	1,650	41 (2.5)
Shellfish	133	12 (9.0)
Shrimp	123	2 (1.6)
Crab	61	2 (3.3)
Total	2,434	79 (3.3)

Table 2. The presence of *Vibrio parahaemolyticus* related to sampling areas around the eastern coast of Sumatra and the north coastal areas of Java.

Sampling areas	No. of sites	No. of samples	No.of positive samples (%)
Aceh	5	300	5 (1.7)
North Sumatra	7	281	9 (3.2)
Riau	9	486	26 (5.4)
South Sumatra	9	451	14 (3.1)
West Java	15	350	14 (4.0)
Central Java	8	304	10 (3.3)
East Java	14	262	1 (0.4)
Total	70	2,434	79 (3.3)

Table 3. Physiological and biochemical characteristics of 79 isolated strains of *Vibrio parahaemolyticus*.

Characteristics	No. of positive and negative strains	
	Positive	Negative
TCBS agar or STA plates	79	0
Colony morphology (smooth)	79	0
Gram staining	0	79
Motility	79	0
Spore-forming	0	79
Rod shape	79	0
Growth in 0% NaCl	0	79
Growth in 3% NaCl	79	0
Growth in 7% NaCl	79	0
Growth in 10% NaCl	0	79
Starch hydrolysis	79	0
Voges-Proskauer	0	79
Methyl red test	41	38
Hydrogen sulfide production (TSI)	74	5
Nitrate reduction	68	11
Blood hemolysis	58	21
Gelatin liquefaction	78	1
Indole test	43	36
Arabinose fermentation	4	75
Fructose fermentation	69	10
Glucose fermentation	72	7
Inositol fermentation	2	77
Lactose fermentation	1	78
Maltose fermentation	72	7
Mannitol fermentation	65	14
Rafinose fermentation	0	79
Rhamnose fermentation	0	79
Sucrose fermentation	0	79
Xylose fermentation	2	79

Table 4. D_{10} value of *Vibrio parahaemolyticus* and reduction after irradiation in 3% brine fish homogenate.

Strain	D_{10} (Gy)	Log reduction at doses (Gy)	
		500	750
V-21	225	2.6	5.0
V-31	210	2.7	6.0
V-62	170	3.3	-
V-91	275	1.8	4.7
V-102	185	2.9	6.3
V-142	150	4.3	-
V-201	260	1.9	3.7
EB 101	200	2.6	4.4
ATCC 17802	250	1.9	4.0

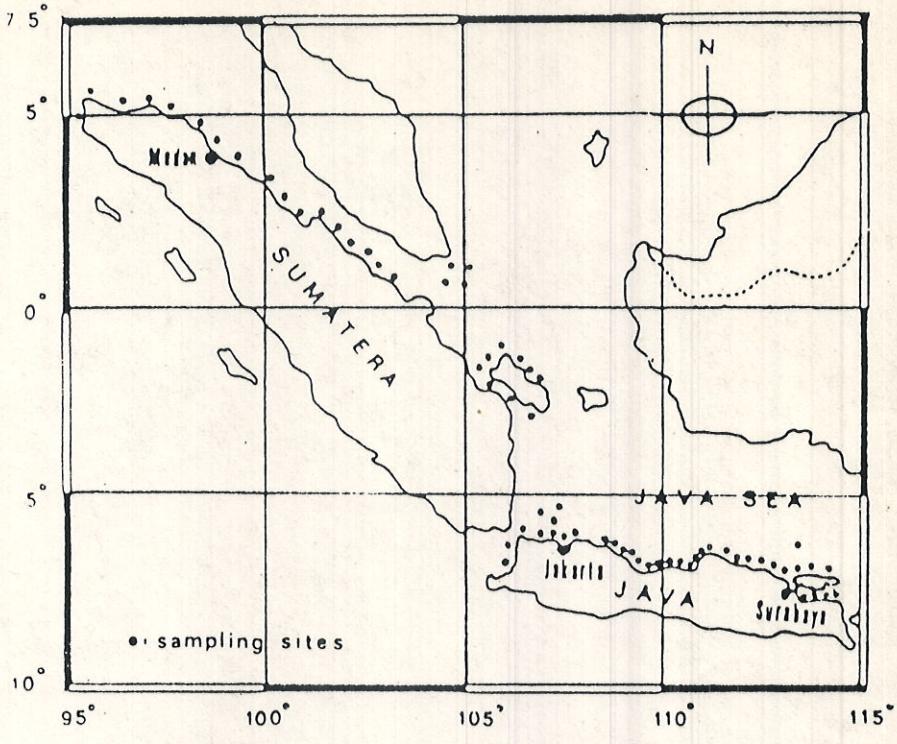

Fig. 1. Sampling sites of sediment and seafoods around the eastern coast of Sumatra and north coastal areas of Java.

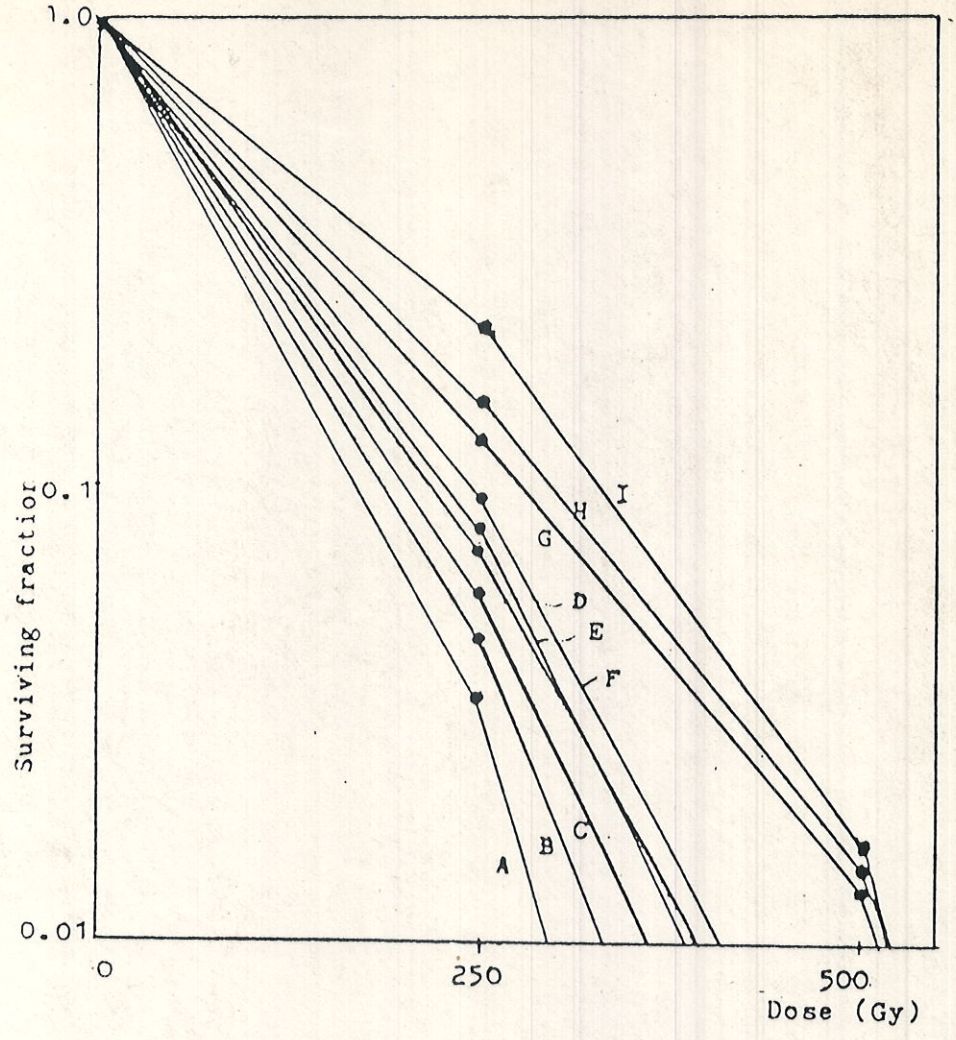

Fig. 2. Survival curves of *Vibrio parahaemolyticus* after irradiation in 3% brine solution fish homogenate.

A (strain V-142), B (strain V-62), C (strain V-102), D (strain EB 101), E (strain V-31), F (strain V-21), G (strain ATCC 17802), H (strain V-201), and I (strain V-91).