Distribution of *Vibrio vulnificus* in Sea Water of Kwangan beach Pusan, Korea

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To establish an appropriate measure for the prevention of food poisoning from raw fish flesh (sashimi), distribution and population density of *Vibrio vulnificus* were investigated as environmental factors were changed. The bacteria were detected only in August during the study period from February to November, 1989. The number of bacteria was 2.0~6.0/100 *ml*. In August, salinity and pH were low, but water temperature and COD were high as compared to the rest of months.

Introduction

Only 10 species of *Vibrio* were reported to affect human health. *Vibrio vulnificus*, in particular, was first reported as a cause of necrosis by penetrating through external wounds of the swimmer (Roland, 1970). More recently, Tison and Kelly(1980) suggested that *V. vulnificus* caused septicemia by consuming either contaminated marine products or the food contacted with sea water; in addition, these bacteria could penetrate through the external wound.

Distribution of *V. vulnificus* was investigated by many researchers. Kelly and Avery (1980) reported that 38% of the samples from 21 locations around the Gulf of Mexico and U. S. A. was found to be *V. vulnificus* positive. Moreover, *V. vulnificus* was detected around the coast of Senegal, Japan, Florida, North Carolina, Miami, and Portland (Kelly, 1982; Oliver et al., 1982 and 1983; Tamplin, 1982; Schandvyl et al., 1984).

In Korea, the first report about septicemia was produced in 1972, but the exact cause was not determined until Ku et al. (1982) suggested that the disease was caused by *V. vulnificus*. Various studies were carried out to determine the distribution of *V. vulnificus* (Kim and Kim, 1985; Chang et al., 1986; Kim et al., 1986). Most recently, according to the report by Kim et al. (1987), *V. vulnificus* was detected around the Kwangan beach in April, May, August and September. Their results indicate that the constant presence of the bacteria might be possible, but more specific distribution study has to be undertaken.

Kwangan beach, one of the most famous resort area, draws a lot of people during summer. Along the beach line, many restaurants specialized in sashimi were also attracting a number of diner during off-season. Because these restaurants use sea water from Kwangan beach area to keep fish alive, the distribution of *V. vulnificus* should be investigated for the good of public health. Thus, this study was focused on determining the relation between distribution of *V. vulnificus* and environmental conditions of sea water such as water temperature, salinity, pH, and COD. The result of the study will provide an essential basis to establish an appropriate measure for sanitation.

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Materials and Methods

Sampling Procedures

Kwangan beach area was divided into three geographical sections from which surface water was taken one to three times from Febuary, 1989 to November, 1989. As shown in Fig. 1, each section was designated as follows: A for Samick apartment area, B for central part of Kwangan beach, and C for Minrack-dong area. Sea water was taken from each section using 250 *ml* wide-mouth bottle. These samples were immediately moved to laboratory in ice packed container to maintain temperature of 10℃ or lower.

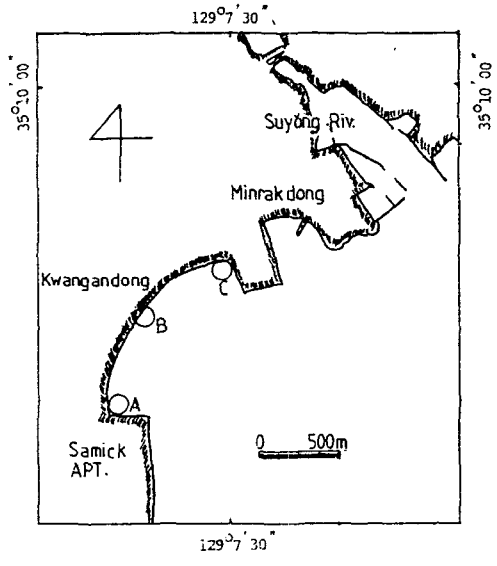

Fig. 1. Location of sampling site in Kwangandong Pusan, Korea.

Determination of Numbers of Bacteria

After serially diluting samples to 10^0~10^{-5}, each dilution was inoculated into peptone water containing 1.5% NaCl. Numbers of bacteria were counted by most probable number (MPN) method after incubating at 35± 0.5℃ for 18~24 hours. Separation of a pure culture of bacteria was performed by streaking each preincubated culture on thiosulfate citrate bile salts sucrose (TCBS) agar plate followed by incubating at 35℃ for 18~24 hours. Green colonies were picked and inoculated on triple sugar iron (TSI) semi slant. At the end of incubation, bacteria exhibiting a typical reaction was transferred to brain heart infusion (BHI) agar slant to evaluate Gram staining, salt-resistance, physiological and biochemical characteristics. To this end, experimental items and methods were followed by the method of Hollis et al. (1976), U. S. A. FDA standard method (1978), and the method of Tison and Kelly (1986). As shown in Fig. 2, *V. vulnificus* was confirmed by the classification method of Krieg and Holt (1984).

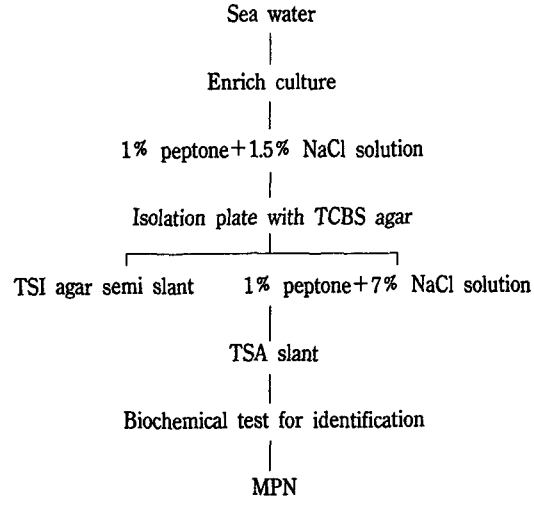

Fig. 2. Isolation scheme for *V. vulnificus*.

Determination of Water Temperature, Salinity, and pH

Water temperature and salinity were determined using conductivity meter (YSI Co., Japen); pH was measured by pH meter with glass electrode (Orion Co., U. S. A.).

COD Measurement

COD was determined by the method of water pollution measurement techniques.

Lethal Toxicity of Isolated Bacteria

Isolated bacteria were incubated with heart infusion (HI) at 35℃ for 16 hours. To instistitute cancer research mouse (ICR mouse), 1 *ml* of culture solution was injected through intra-peritoneal route. Toxicity was determined whether

or not the mouse survivied for 12 hours. Numbers and weight of ICR mouse were 5~10 and 18~20 *g*, repectively.

Results and Discussion

Sampling was carried in between 9:40 and 14:26. Temperatures of surface water were below 20℃ for February~May, October, and November. Above 20 ℃ was recorded in between June and September; especially, 24~26℃ was the tempera-ture during August in which Vibrio septicemia causing bacteria were isolated. At above 20℃, *V. vulnificus* was most likely to be detected according to Kelly (1980). In this case, however it was not detected in June, July, and September.

Salinity was 15~32%, indicating the close relation to the amount of precipitation before sampling. Interestingly, salinity of Samick apartment area (section A) was always higher than any other section. Salinity of section B was almost identical to that of section C. pH was somewhat proportional to temperature. When temperature was high, pH was high, and vice versa. On the contrary, when salinity was low, COD was recorded high value and vice versa (Table 1).

Typical green colonies were isolated from TCBS medium totaling to 180 species: 1 species in February, 8 in January and March, 15 in April, 10 in May, 15 in June, 20 in July, 66 in August, 27 in September, 10 in October, and 8 in November. Among them, 12 species were identified as *V. vulnificus*.

As shown in Table 1, only in August, *V. vulnificus* was detected and the number was 2.0~6.1/100 *ml*. Unlike this result, Kim (1987) detected the bacteria in April, May, August, and September; especially, 1,200/100 *ml* was the number of bacteria in August. In this study, the number and detection ratio were lower than theirs. In August, water temperature was high and pH and salinity were low as compared to the other months; however, high COD value was recorded.

All three samples from section A and B proved to be positive, whereas one out of three showed positive result in section C. Considering section B as buffering region between section A and C, the differences of section A to C were low salinity and pH but high COD. These results suggest that appearance of *V. vulnificus* would have something to do with not only high water temperature but also large influx of organic materials due to the precipitation. However, further research will be required to prove the above conclusion.

This study confirmed the previous report (Kim, 1987) that indicated the detection of *V. vulnificus* from sea water of Kwangan beach. Therefore, if optimal environmental conditions are provided, *V. vulnificus* will appear. More specifically, *V. vulnificus* will be most likely to appear during rainy season from July to September. Thus, proper measures to prevent Vibrio septicemia should be established for the sashimi restaurants around Kwangan beach during rainy season.

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Table 1. Environmental data and MPN's of *V. vulnificus* of sea water in Kwangan beach Pusan, Korea

Sampling date	Sampling time			Salinity(‰)			Tem.(℃)			pH			COD(*ppm*)			MPN/100*ml*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Feb. 15	11:05	11:20	11:40	23	26	25	12.0	11.5	10.0	7.83	7.88	8.02	8.0	1.5	1.3	<1.8	<1.8	<1.8
Mar. 14	10:15	10:37	10:57	28	29	30	12.0	13.0	12.5	7.55	7.62	8.10	2.3	0.8	1.0	<1.8	<1.8	<1.8
27	09:57	10:13	10:30	29	30	30	12.0	12.8	13.1	8.02	8.10	8.05	1.0	0.8	1.0	<1.8	<1.8	<1.8
Apr. 13	10:55	10:10	10:25	26	28	28	15.0	16.0	16.0	7.74	7.74	7.75	1.9	3.1	1.4	<1.8	<1.8	<1.8
24	10:15	10:30	10:40	24	29	31	15.0	14.0	14.0	7.67	8.02	7.99	7.1	2.9	1.4	<1.8	<1.8	<1.8
May 18	11:55	12:15	12:30	31	31	31	16.5	16.5	16.1	7.90	8.05	8.10	1.0	1.0	1.0	<1.8	<1.8	<1.8
Jun. 13	13:10	13:35	13:50	30	31	32	20.0	21.5	22.6	8.10	8.05	8.35	1.6	1.6	1.5	<1.8	<1.8	<1.8
26	11:30	11:40	12:00	30	31	31	23.0	22.5	22.5	8.10	8.10	8.15	1.6	1.4	1.5	<1.8	<1.8	<1.8
Jul. 11	09:40	10:05	10:25	28	29	29	21.5	21.7	23.2	7.70	8.01	7.80	5.1	2.2	3.4	<1.8	<1.8	<1.8
27	12:50	13:35	13:45	26	31	27	23.1	24.5	23.7	7.70	8.20	7.70	2.0	1.8	1.8	<1.8	<1.8	<1.8
Aug. 11	09:35	09:55	10:25	24	26	25	25.0	25.0	25.0	7.96	8.10	8.00	4.1	3.1	3.6	4.0	6.1	4.0
23	10:22	10:50	11:16	15	25	31	24.0	24.0	24.0	7.80	8.20	8.49	5.3	2.6	1.6	2.0	2.0	<1.8
30	10:05	10:20	10:20	24	29	29	26.0	26.0	26.0	7.75	8.00	7.95	7.0	3.2	3.2	<1.8	<1.8	<1.8
Sep. 5	10:15	10:40	11:05	30	30	30	23.0	23.0	23.0	8.83	8.80	8.75	1.2	1.0	1.0	<1.8	<1.8	<1.8
29	13:32	14:02	14:26	30	30	30	22.0	22.0	22.0	8.31	8.30	8.31	1.0	0.7	0.4	<1.8	<1.8	<1.8
Oct. 18	11:00	11:20	11:40	22	31	30	17.0	17.0	17.0	7.27	8.38	8.37	9.9	1.1	1.3	<1.8	<1.8	<1.8
Nov. 10	10:50	11:00	11:20	30	31	31	15.5	15.5	15.5	8.65	8.70	8.80	0.7	0.5	0.4	<1.8	<1.8	<1.8

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부산 광안 해수욕장 해수의 *Vibrio vulnificus* 분포

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광안 해수욕장 해수에 대한 Vibrio 패혈증 균의 분포와 해수 환경의 변화에 따른 Vibrio 패혈증 균의 오염도 변화를 측정하여 생선회 식중독의 위생대책 수립에 필요한 자료를 얻고저 1989년 2월부터 11월까지 실험한 결과 8월에만 Vibrio 패혈증 균이 검출되었고, 균수는 2.0~6.1/100*ml*이었다. 이때 해수는 다른 달에 비하여 염도와 pH가 낮았으며 수온과 COD가 높게 나타났다.