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The Effect of Environmental Factor on the Survival of Marine Vibrio vulnificus

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This study was conducted to investigate the effects of environmental factors such as temperature, salinity, pH, and UV light on the survival of life-threatening Vibrio vulnificus. In the temperature range of 15 to 25℃, the numbers of V. vulnificus increased during the 6-day incubation, but outside this range, the survival of V. vulnificus was poor. Incubation between 1 and 10℃ showed that V. vulnificus survived poorly below 10℃. At salinities between 5 and 25ppt, the numbers of V. vulnificus increased or remained unchanged for 6-day. At salinities above this range, the numbers of V. vulnificus decreased. The optimal pH range was 6.5 to 8.0 and outside this range, the survival ratio of V. vulnificus was small. At 15 and 25℃, UV radiation was bactericidal for cultures of V. vulnificus. The counts of V. vulnificus were reduced approximately 10,000-fold after 2h of UV light treatment at both temperatures. Above results showed that environmental factors were effective on the survival of V. vulnificus in the environment.

Key words : environmental factor, temperature, salinity, pH, UV light

1. Introduction

Environmental pollution in marine water is increasing and seafood exists in many regions of the world including Korea. Marinal bacteria are affected by many factors such as salinity and temperature, et al. Among these, Vibrio vulnificus is a common bacterium in estuarine waters in temperate and tropical climate. It is distributed widely in waters where it is found in seawater and sediment and various marine life forms (Tamplin et al., 1983), particularly when seawater temperature is elevated. It, previously designated as a lactose-positive vibrio, is a slightly basophilic bacterium and inhabits in the marine and estuarine areas. This vibrio causes wound infection, septicema, and other types of infections, after contact with seawater or consumption of seafood contaminated with the vibrio (Blake et al., 1979, Blake et al., 1980). Occasionally, V. vulnificus causes invasive disease and life-threatening infections in humans who suffer from preexisting illness (Klontz et al., 1988). Many studies show that warm water temperatures and low-to-moderate salinities are associated with the presence of V. vulnificus (Tamplin et al., 1983). Other studies suggest that temperature and salinity are controlling factors in its distribution. Low temperatures are shown to be detrimental to survive and induce formation of viable but nonculturable cells (Oliver et al., 1991).

This study was performed to further elucidate and define the survival properties of V. vulnificus by the environmental factors such as temperature, salinity, pH, and UV light. Natural seawater samples were used to determine the effects of temperature, salinity, and et al.

2. Materials and Methods

2.1 Organism and culture condition

The strain used in this study was Vibrio vulnificus. It was cultured in tryptic soy broth (TSB) at 37℃ for 24h which contained additional 5 g/l of NaCl. It was obtained from the microbiology laboratory of the Paik Hospital in Pusan, Korea.

2.2 Seawater preparation

Samples were prepared by using subsurface seawater, aseptically collected from the Gulf of Suyoung. It was diluted with deionized water to the appropriate salinity and sterilized at 12℃ for 15 min. Salinity was determined by measuring the refractive index. A standardized bacterial suspension was added to sterilized seawater to reach a final concentration of around 2.0×10^4 colony-forming unit/ml (CFU/ml).

2.3 Effect of temperature on survival

To investigate the effect of temperature on the survival of V. vulnificus, triplicate tubes containing 25 ml of the V. vulnificus suspension in 10 ppt (noninhibitory salinity) sterile seawater were incubated with agitation in a temperature gradient incubator. The temperature gradient was monitored daily in blank tubes at each end of the incubation by using a thermometer to measure seawater temperature. Temperatures did not vary by more than 1℃.

2.4 Effect of salinity on survival

Likewise, the effect of salinity on the survival was determined in triplicate tubes with agitation at 15 and 25℃, noninhibitory temperatures. The remainder of the samples were set up in triplicate by using 500 ml screw cap flasks containing 300 ml of sterile seawater at the appropriate salinity.

2.5 Effect of pH on survival

The effect of pH on the survival of V. vulnificus was investigated in triplicate tubes incubated at the various pH conditions (pH 2 to 11).

2.6 Effect of UV light on survival

The bactericidal effect of UV light systems was determined. Environmental strain, V. vulnificus was suspended in equal numbers in phosphate-buffered saline (PBS), washed with PBS by centrifugation at $5{,}000 \times g$, and added to 500 ml of sterile seawater (salinity, 15 ppt) at 105 CFU/ml. After 2 days of incubation, the bacterial suspensions were added to two aquaria filled with 50 l of seawater at 104 CFU/ml. Seawater was recirculated through 60W of UV light. The control aquarium did not have UV light. The UV light chambers were standard watertight hollow chambers, each containing a 30 W bulb.

2.7 Bacterial enumeration

At each time point, a sample was removed from each seawater sample and a portion was plated directly or following dilution in 0.1% peptone. The numbers of CFU/ml were determined after incubation at room temperature for 20 to 24h. Mean values for replicate samples were determined. Survival (percent CFU/ml) was calculated by using the numbers of CFU/ml immediately following inoculation as 100%.

3. Results and Discussion

3.1 Effect of temperature on survival

Survival of V. vulnificus was optimal at temperatures between 15 and 25℃ in 10ppt (noninhibitory salinity) sterile seawater (Table 1, Fig. 1). On day 2 numbers of V. vulnificus increased in the temperature range of 15 to 30℃, but on day 6 the greatest numbers were found in the temperature range of 15 to 25℃. Temperatures outside this range reduced the time of V. vulnificus survival. At these restrictive temperatures, V. vulnificus numbers were reduced by 90% after 6 days of incubation.

To further define the sensitivity of V. vulnificus to low temperatures, a more detailed study was conducted with samples held at temperatures from 1 to 10℃ and at a salinity of 10 ppt. A decrease in temperature correlated with a decrease in the time of survival (Table 2, Fig. 2). In samples incubated at temperatures between 1 and 6℃, the numbers of V. vulnificus were 30 to 50% of the initial con-

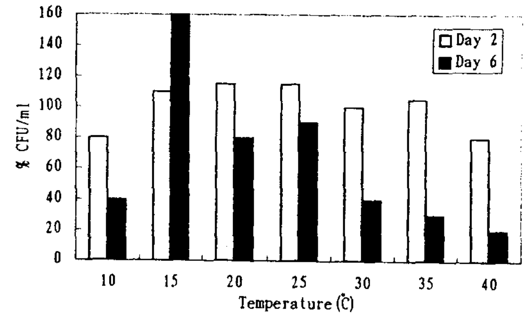

Fig. 1. Effect of temperature on the survival of V. vulnificus in 10ppt sterile seawater. Mean percentages and the mode temperatures for three tubes are shown. Percentages were calculated by using the numbers of CFU/ml following inoculation as 100%.

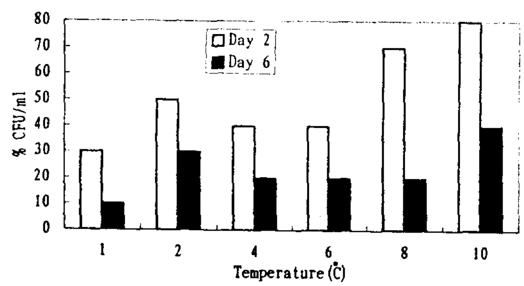

Fig. 2. Survival of V. vulnificus in 10 ppt sterile seawater at temperatures from 1 to 10℃. The data are mean values from triplicate tubes.

Table 1. Effect of temperature on the survival of V. vulnificus

Temperature (℃)	% CFU/ml on Day 2	% CFU/ml on Day 6
10	80	41
15	112	157
20	115	82
25	113	91
30	98	42
35	105	28
40	81	20

Table 2. Survival of V. vulnificus in 10 ppt sterile seawater at temperatures from 1 to 10℃

Temperature (℃)	% CFU/ml on Day 2	% CFU/ml on Day 6
1	28	9
2	49	29
4	38	20
6	42	21
8	71	22
10	80	41

Table 3. Effect of salinity on the survival of V. vulnificus in sterile seawater

Salinity (ppt)	% CFU/ml on Day 2	% CFU/ml on Day 6
0	0	0
5	81	118
10	137	86
15	108	169
20	159	121
25	168	148
30	70	48
35	40	12

Table 4. Effect of pH on the survival of V. vulnificus after 2 days.

pH	2	3	4	5	6	6.5	7	8	8.5	9	10	11
% CFU/ml	14	21	62	85	91	122	149	138	94	88	69	38

centration after 3 days of incubation, 10 to 30% after 7 days, and 0 to 8% after 13 days. At temperatures of 8℃ and higher, the numbers of V. vulnificus increased and remained higher for the duration of the experiment compared with the initial concentration of cells. The sensitivity of V. vulnificus to low temperature is consistent with the results of environmental surveys and studies of other vibrios (Colwell, 1984).

3.2 Effect of salinity on survival

The survival of V. vulnificus was determined in seawater samples with salinities that were incubated at noninhibitory temperature. In samples with salinities between 5 and 25ppt, V. vulnificus numbers increased during the 6 days of incubation with the exception of samples containing 10 ppt seawater, in which numbers decreased by 14% (Table 3, Fig. 3). In comparison, at salinities of 30 and 35 ppt, numbers of V. vulnificus decreased by 30 and 60%, respectively. Although V. vulnificus numbers in all samples were reduced by>87% after 16 days of incubation, samples with salinities between 5 and 25 ppt contained the highest numbers of CFU. V. vulnificus could not be recovered from deionized water (0 ppt), probably because of lysis.

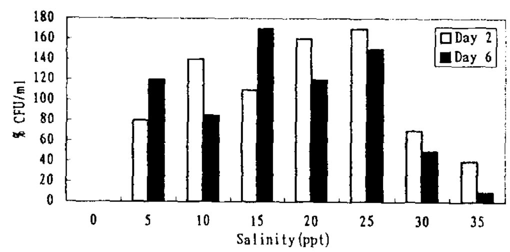

Fig. 3. Effect of salinity on the survival of V. vulnificus in sterile seawater. Mean percentages for triplicate tubes are shown. Percentages were calculated by using the number of CFU/ml following inoculation as 100%.

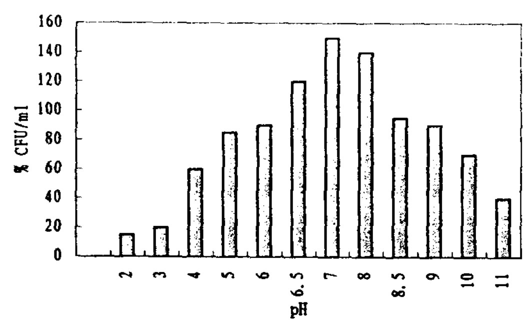

Fig. 4. Effect of pH on the survival of V. vulnificus. Mean percentages for triplicate tubes are shown. Percentages were calculated by using the number of CFU/ml following inoculation as 100%.

3.3 Effect of pH on survival

Numbers of V. vulnificus increased in the pH range of 6.5 to 8.0 (mild acidic to mild basic condition), but pH outside this range (i.e., pH 2 to 6 and pH 9 to 11), numbers of V. vulnificus decreased (Table 4, Fig. 4).

3.4 Effect of UV light on survival

At 15 and 25℃, UV radiation was bactericidal for pure cultures of V. vulnificus suspended in aquarium seawater (Table 5, Fig. 5). V. vulnificus counts were reduced approximately 10,000-fold to undetectable levels after 2 h of UV light treatment at both temperatures; no significant reduction in V. vulnificus counts was observed in untreated water.

The unusually high temperature optima of V. vulnificus and other human pathogenic marine bacteria may be important in the ability of these organisms to produce human infections. However, these characteristics also suggest that many marine environments of normally high salinity and low temperature may be hostile to the survival of V. vulnificus. This organism may grow in select, localized environments where optimal conditions exist and then be disseminated to other environments by tidal flow or fresh water runoff. Studies are currently under way to test this hypothesis.

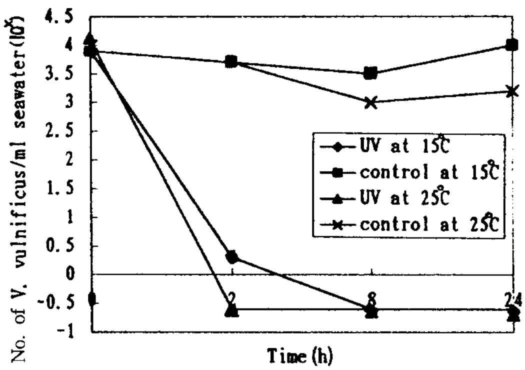

Fig. 5. Effect of UV light on the survival of V. vulnificus at 15 and 25℃.

The salinity and temperature effects on the occurrence of V. vulnificus are also interesting in relation to the incidence of human infections due to this organism. Eight-five percentage of V. vulnificus infections occur in the warm months of the year and this organism overwinters in bottom sediments and enters the water column again when warm temperatures return. The possibility that a similar mechanism exists for V. vulnificus is currently under investigation by analysis of sediment cultures. In conclusion, V. vulnificus is a human pathogen capable of producing highly lethal bloodstream infections and destructive wound infections. All evidence suggests that infections due to this organism are acquired from seawater or seafood (Kelly et al., 1980). Our results suggest that the occurrence of this organism is especially favored by warm water environments of low salinity. Such environments

Table 5. Effect of UV light on the survival of V. vulnificus at 15 and 25℃

Time (h)	No. in UV at 15℃	No. of control at 15℃	No. of UV at 25℃	No. of control at 25℃
0	8×10^{3}	$8\times^{3}$	10^{4}	8×10^{3}
2	$2\times^{0}$	$5\times^{3}$	2×10^{-1}	5×10^{3}
8	2×10^{-1}	3×10^{3}	2×10^{-1}	10^{3}
24	2×10^{-1}	10^{4}	2×10^{-1}	2×10^{3}

may pose a significant hazard for acquisition of V. vulnificus infections.

Detailed studies over broad ranges of environmental factors demonstrated that temperatures outside the range of 15 to 25℃, salinities greater than 25 ppt, pH outside the range of 6.5 to 8.0, and UV radiation for 2h reduced the survival of V. vulnificus in seawater. In addition, unidentified environmental agents also contribute to the demise of V. vulnificus in unsterilized seawater.

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해양 Vibrio vulnificus의 생존에 미치는 환경적 요인의 영향

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본 연구는 생명을 위협하는 Vibrio vulnificus의 생존에 온도, 염분, pH 및 자외선과 같은 환경적 요인이 어떠한 영향을 미치는 가를 알아보기 위하여 수행하였다. 6일 동안 15에서 25℃의 온도 범위에서 V. vulnificus의 수는 증가하였지만 이 범위외에서 V. vulnificus의 생존수는 감소하였다. 1 에서 10℃ 사이에서의 실험 결과, V. vulnificus가 10℃ 이하에서는 생존수가 적었다. 한편 6일 동안 5에서 25 ppt 정도의 염분 조건에서는 V. vulnificus의 수는 증가하거나 거의 일정한 수를 유지하였다. 이 범위 이상의 염분에서는 V. vulnificus수는 감소하였다. 최적의 pH 영역은 6.5에서 8.0이었고 이 영역밖에서는 V. vulnificus의 생존율은 낮았다. 15와 25℃에서 자외선은 V. vulnificus에 대해 살균 효과를 나타내어서 2시간 동안 자외선 처리후 V. vulnificus수가 약 10,000배 감소되었다. 이상과 같은 결과들은 환경적 요인이 V. vulnificus의 생존에 미치는 영향이 크다는 것을 보여주었다.