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# Isolation of *Vibrio vulnificus* from Seawater and Emerging *Vibrio vulnificus* Septicemia on Jeju Island

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*Vibrio vulnificus* is an opportunistic human pathogen, transmitted from seawater, raw oyster, and shellfish and responsible for severe septicemia. We studied *V. vulnificus* from surface seawater around Jeju Island between 2010 and 2011. In 2010, *V. vulnificus* was isolated and *V. vulnificus* septicemia was reported. Surface seawater temperature is an important factor for growth of *V. vulnificus*, and here we showed that high surface seawater temperature may influence growth of *V. vulnificus* and occurrence of emerging *V. vulnificus* septicemia on Jeju Island. This is the first report of isolation of *V. vulnificus* and emerging *V. vulnificus* septicemia on Jeju Island.

**Key Words:** *Vibrio vulnificus;* Sepsis; Seawater; Temperature

*Vibrio vulnificus* is a Gram-negative bacterium found commonly in estuarine and marine water and in association with shellfish and fish. It is responsible for rapidly progressive cellulitis and septicemia in the USA, Japan, and Southeast Asia [1, 2]. The growth of *Vibrio* spp. is dependent on the temperature of coastal water. A large outbreak of seafood-associated gastroenteritis, caused by the consumption of contaminated oysters containing *V. parahaemolyticus*, was linked to atypically high temperature in Alaskan coastal waters [3]. Jeju Island is located at the southern end of the Korea, where it receives direct influx from the warm Kuroshio Current. Therefore, surface seawater temperature of this island is warmer than other region of Korea.

In this study, we reported survey result of this bacterium from surface seawater around Jeju Island between 2010 and 2011 and emerging case of *V. vulnificus* septicemia on Jeju Island in August 2010. We conducted a research studying for the relationship between emerging vibrio sepsis in human and climate change around Jeju Island.

A total 192 specimens per year were collected from four samples obtained monthly from each port of four directions. DNA of *V. vulnificus* was extracted using bead beater extraction method and was used as the template for PCR [4]. A 492-bp segment of the 16S rDNA gene regions of *V. vulnificus* was amplified by PCR using the primers UFUL and URUL [5]. After sequencing of the PCR products, the 16S rDNA sequences

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**Figure 1.** Characteristic swelling accompanied by ecchymosis and necrotic cutaneous lesions in the left upper arm (A) and left calf area (B).

were compared with *V. vulnificus* 27562 (American Type Culture Collection 27562), X76333 (A) and X76334 (B) in GenBank using the multiple-alignment algorithm in the MegAlign program (Windows version 3.12e; DNASTAR, Madison, WI, USA) and the ClustalX program respectively [6]. Based on the aligned sequences, phylogenetic analyses were conducted in MEGA4 and phylogenetic trees were constructed by the neighbor-joining method [7]. The bootstrap consensus tree inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed [7]. *V. vulnificus* was divided A (X76333) and B (X76334) according to 16S rDNA sequences. Surface seawater temperature around Jeju Island were measured *in situ* with a CTD (Conductivity, Temperature, and Depth recorder, Sea-Birds Co., SBE-19) around 09:00 to 10:00 AM.

A 73-year-old woman was admitted in August 2010 with progressively worsening abdominal distention and edema in her extremities that had begun 3 months ago. She had a history of Child–Pugh class B cirrhosis caused by chronic hepatitis B. She is a diver. She denied drinking. At the emergency department, she displayed spiking fever, excessive thirst, and painful paresthesia in both arms and legs. The physical examination revealed a temperature of 38.3°C, blood pressure of 135/79 mmHg, heart rate of 128 beats/min, and respiratory rate of 26 breaths/min. A complete blood count showed hematocrit of 31.8%, white blood cell count of 6,600 cells/mm$^3$, polymorphonuclear leukocytes of 88.5%, and platelet count of 28,000 cells/mm$^3$. The results of liver function tests were: total bilirubin, 4.2 mg/dL; direct bilirubin, 2.7 mg/dL; alkaline phosphatase, 448 U/dL; aspartate transaminase, 91 U/dL; alanine transaminase, 40 U/dL; albumin, 1.9 g/dL; prothrombin time 23.7 seconds, and international normalized ratio 2.06. She had icteric sclerae, dry mucous membranes, distended abdomen, and abdominal tenderness. Both arms and legs were swollen and exhibited progressive hemorrhagic bullae, which had started as red and necrotic skin color changes on the day of admission (Fig. 1). She recalled having dived on Seongsan coast and eaten raw oysters the day before admission. Treatment was started with intravenous ceftazidime (4 g/day), and oral doxycycline (300 mg/day). The two blood cultures taken at the time of admission yielded *V. vulnificus*. The patient improved gradually and recovered fully after 2 weeks of medical treatment without needing an operation.

Ten out of 192 specimens were identified *V. vulnificus* from surface seawater of ports around Jeju Island in 2010 but not isolated in 2011 (Table 1), and *V. vulnificus* septicemia patient was first reported on Jeju Island in 2010. *V. vulnificus* was isolated from surface seawater of western (Hallim), northern (Jeju), and eastern (Seongsan) region in 2010, and *V. vulnificus* septicemia was reported in August 2010 at Seongsan. However, *V. vulnificus* was not isolated in human in 2011. Our phylogenetic tree showed that isolates of Jeju Island were closely related to B (X76334) and *V. vulnificus* 27562, and isolates from septicemia patient (08-SS-isolate from patient) was B (Fig. 2). The average sea surface temperature was 24.75 ± 1.47°C in 2010 and 23.71 ± 0.90°C in 2011.

*V. vulnificus* sepsis is one of the most serious infections during summer and fall season, especially in the southern and western part of the Korean Peninsula. Although, Jeju Island is located far south in Korea, the island is not an endemic region. In this study, we tried to isolate *V. vulnificus* from surface seawater of Jeju Island between 2010 and 2011. We isolated this bacterium, and the island had *V. vulnificus* septicemia patient in 2010. Surface seawater temperature is important factor for

**Table 1.** Strains isolated in this study

| Strain | Isolate location | Month isolated (month/year) | Isolate origin |
|---|---|---|---|
| [a]07-JJ-2 | Jeju station (Northern side) | 07/2010 | Surface seawater |
| [a]07-JJ-3 | Jeju station (Northern side) | 07/2010 | Surface seawater |
| [a]07-JJ-4 | Jeju station (Northern side) | 07/2010 | Surface seawater |
| [a]08-JJ-2 | Jeju station (Northern side) | 08/2010 | Surface seawater |
| [a]08-JJ-3 | Jeju station (Northern side) | 08/2010 | Surface seawater |
| [a]08-JJ-4 | Jeju station (Northern side) | 08/2010 | Surface seawater |
| [a]09-JJ-3 | Jeju station (Northern side) | 09/2010 | Surface seawater |
| [a]09-HL-4 | Hallim station (Western side) | 09/2010 | Surface seawater |
| [a]03-SS-1 | Seongsan station (Eastern side) | 03/2010 | Surface seawater |
| [a]07-SS-1 | Seongsan station (Eastern side) | 07/2010 | Surface seawater |
| [a]08-SS-Patient | [b]Seongsan (Eastern Area) | 08/2010 | Blood from patient |

JJ, Jeju; HL, Hallim; SS, Seongsan.
[a]Isolated month-site: 1, inner port; 2, entrance to port; 3, 500 m from port; 4, 1,000 m from port.
[b]This is the area where the patient lives.

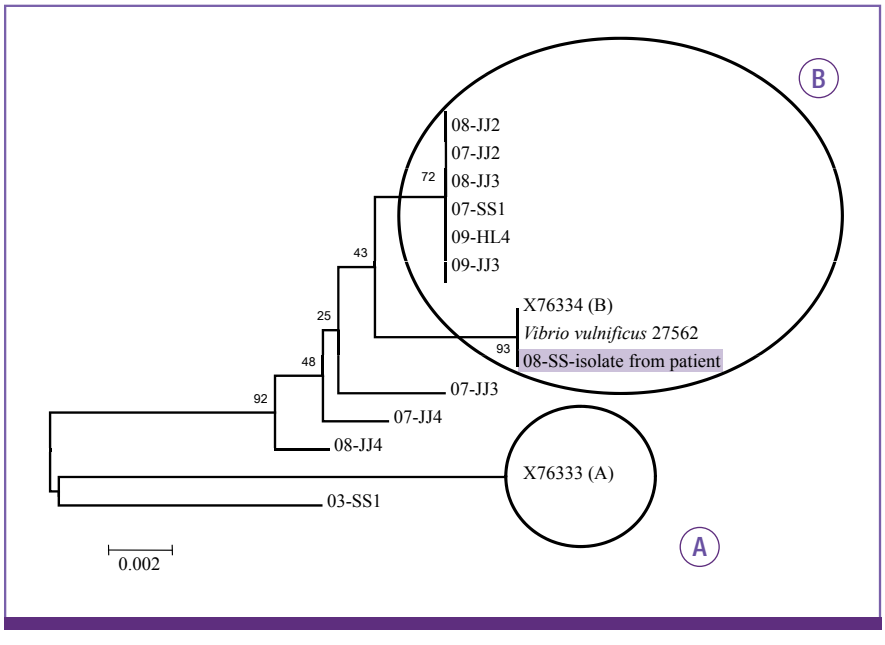


**Figure 2. 16S rDNA sequences phylogenetic tree. The tree was constructed using the neighbor-joining method in MEGA4 [7].** The bootstrap values presented at the corresponding branches were evaluated from 1,000. The data were displayed according the location where the samples were obtained: SG, *V. vulnificus* from Seogwipo (southern side); HL, *V. vulnificus* from Hallim (western side); JJ, *V. vulnificus* from Jeju (northern side); SS, *V. vulnificus* from Seongsan (eastern side). 1, inner port; 2, entrance to port; 3, 500 m from entrance to port; 4, 1,000 m from entrance to port. Isolates compared with *V. vulnificus* 27562 (American Type Culture Collection 27562), X76333 (A, which includes most strains isolated in the Gulf of Mexico, US Atlantic coast, and Asia) and X76334 (B, which included the strains isolated from clinical isolates and in surface seawater Korea) in GenBank.

the growth of *V. vulnificus*. The optimum temperature of *V. vulnificus* is 37°C, and it cannot grow below 20°C [8, 9]. However, we did not find this bacterium in 2011. We measured and compared surface seawater temperature between 2010 and 2011. Increasing surface seawater temperature is strongly associated with outbreaks of *Vibrio* disease [3, 10]. Surface seawater temperature of 2010 was higher than that of 2011. Therefore, we assume that increasing surface seawater temperature of Jeju Island in 2010 may contribute to growth of *V. vulnificus* and to occurrence of emerging *V. vulnificus* septicemia on Jeju Island. Isolates from the Gulf of Mexico and the US Atlantic coast are type A, and clinical and environmental isolates of Korea are type B [5]. 16S rDNA phylogenetic tree showed that isolates from surface seawater of Jeju Island were closely related to B than A, and clinical isolate from septicemia patient was B (Fig. 2). This result shows that the predominant type around surface seawater of Jeju Island is type B rather than A and suggests that *V. vulnificus* in surface seawater of Jeju Island is a more virulent strain because B type can infect humans [5]. This is the first report which sought to know

the relationship between emerging *V. vulnificus* septicemia and changing of temperature of sea surface on Jeju Island. If the surface seawater temperature around Jeju Island increases due to climate change, *V. vulnificus* septicemia may occur more frequently on Jeju Island in near feature.

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