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Recent Studies on the Human Pathogen, *Vibrio vulnificus*

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Vibrio vulnificus is an estuarine bacterium which is the causative agent of food-borne disease and wound infection, both of which are potentially fatal. Fatality rates are typically over 50%, with incubation times as short as 7 hours. *V. vulnificus* is part of the normal bacterial flora of estuarine waters and occurs in high numbers in molluscan shellfish; disease most often results from ingestion of oysters harboring this organism. This pathogen is present in coastal waters and seafood around the world including Korea, Japan, China, Hong Kong, and Taiwan. In the United States, 95% of all deaths resulting from seafood consumption (30-40 cases per year) are caused by this bacterium, and 40-50 cases occur in Korea each year. Interestingly, over 90% of people developing a *V. vulnificus* primary septicemia are males. This is due to the hormone, estrogen, which we have found to be protective in females against the endotoxin produced by this bacterium. Further, most (~95%) cases occur in persons who are immunocompromised, have diabetes, or who have underlying diseases which result in elevated serum iron levels (primarily liver cirrhosis secondary to alcohol abuse/alcoholism or hepatitis). Despite this, a recent study revealed that of 235 emergency room physicians in Japan, less than 16% had a basic knowledge of V. vulnificus infection. In contrast to food-borne cases, wound infections typically occur in healthy persons, but often require surgical debridement or amputation of affected limbs. Wound infections in persons who do have liver disease or are immunocompromised carry a ~25% fatality rate.

Although *V. vulnificus* occurs in molluscan shellfish in high numbers, the incidence of disease is relatively low, leading to the hypothesis that not all strains of *V. vulnificus* are equally virulent. Unfortunately, no easy test to identify virulent strains of this species has been found. We have recently determined that all strains of *V. vulnificus* can be divided into two groups which correlate with clinical ("C-type") or environmental ("E-type") origin. Employing a simple and rapid PCR technique, we examined 55 randomly selected strains and found that 90% of isolates that had the C-type genome were from clinical sources, while 93% of environmental isolates had an E-type genome. Thus, being a "C-type *V. vulnificus*" is a strong indicator of potential virulence. Studies on the physiology of these two genomic types are currently underway; whether differences exist in their distribution and ecology

is not yet known, but the finding that only a small fraction of the *V. vulnificus* strains isolated from natural environments (including shellfish) are "C-type" suggests that only certain strains are capable of causing human infection.

In what we believe to be the first studies of their kind, we have conducted *in situ* gene expression studies on *V. vulnificus* by placing log phase cells into membrane diffusion chambers and suspending them into natural estuarine environments. Using RT-PCR, we have found that, as *V. vulnificus* enters the starvation-survival state in warm estuarine waters, the cells express *rpoS* (an alternate stress sigma factor) and *tufA* (an elongation factor) for at least 108h, and *katG* (catalase) for over 24h, suggesting a need for the continued expression of these genes during this state. *In situ* expression of the *V. vulnificus* hemolysin gene, *vvhA*, was observed to be constitutive in one clinical (C-type) and one environmental (E-type) strain, but no expression was observed in a second C-type strain in these warm waters.

An interesting aspect of the biology of *V. vulnificus* is that human infections show a distinct seasonality, with >90% of the 154 cases reported during 2000-2004 in the U.S. occurring between April and October. The decrease in infections observed during cold months parallels the general inability to isolate this pathogen from the environment when temperatures are below ~13°C. These decreases are due to entry of the cells into the "viable but nonculturable" state. In this state, the cells lose the ability to be cultured on routine media, but retain viability. In many species (including *V. vulnificus*), the cells can be resuscitated to the actively metabolizing and culturable state by a simple reversal of the stresses that induced the VBNC state. The VBNC state has now been described for nearly 100 species of bacteria contained in over 30 genera. A recent study from our lab (Kong *et al.*, 2004) indicated that entry into and resuscitation from the VBNC state by *V. vulnificus* is controlled to a great extent by the reduced activity of catalase when the cells are incubated at low temperature. Catalase activity is required for the cells to detoxify the H_2O_2 naturally present in routine laboratory media, and cells which lack this activity are not able to form colonies on such media. We have now confirmed this finding by RT-PCR studies; while *katG* (catalase) expression continues at a high rate in cells incubated at 20°C, it is greatly down-regulated in both C and E-type cells as they enter and persist in the VBNC state. On resuscitation (exposure to 20°C), *katG* expression is up-regulated, and the cells regain culturability. This indicates that the loss of catalase activity in VBNC cells is a result of decreased transcription of *katG,* and not of decreased activity of the enzyme after its synthesis. While *katG* expression is down-regulated, genes for the alternate stress sigma factor, *rpoS*, and an elongation factor (*tufA*) were constitutively expressed in cold estuarine waters (*in situ*) by both "E" and "C" type strains as these cells entered the VBNC state. Interestingly, whereas one C-type strain exhibited constitutive expression of the hemolysin gene (*vvhA*) in cold water, a second C-type did not express this gene *in vitro* at 5 or 20°C, but did express this gene transiently following initial exposure to cold waters.

V. vulnificus exhibits both encapsulated and non-encapsulated colony morphotypes, and these are easily discernable on agar plates. Cells that produce capsule produce "opaque" colonies and are virulent, whereas acapsular strains that produce "translucent" colonies are avirulent. Two genes, *wza* and *wzb*, are known to be necessary for capsule production. Our *in situ* studies have revealed that encapsulated cells continue to express *wza* and *wzb* for at least 24h after immersion in natural estuarine waters, providing the first evidence that cells which produce capsules *in vitro* also express capsule genes in the environment. During these studies, however, we noted that along with encapsulated (*wzb+*) and non-encapsulated (*wzb-*) morphotypes, a third "intermediate" capsular morphotype (*wzb+*) type exists. These cells produce colonies with less polysaccharide than the opaque morphotype. RT-PCR studies indicate that such cells exhibit greatly reduced expression of *wzb,* but whether such cells are virulent is not yet known.

This talk will describe *V. vulnificus* and the diseases it causes, emphasizing the newer studies from our laboratory on this pathogen.