**The Effect of Temperature and Salinity on Motility in *Vibrio vulnificus***

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*Vibrio vulnificus* is a marine pathogen that is responsible for as much as 95% of all seafood-related deaths in the United States, typically following consumption of raw or undercooked oysters (Jones and Oliver, 2009; Oliver, 2005). With a fatality rate of 50-60%, it is particularly dangerous for those who are immunocompromised or have liver disorders, but can also cause wound infections in persons without underlying disease (Jones and Oliver, 2009; Oliver, 2005). The ability to infect a host requires capsular polysaccharide and a variety of putative virulence factors including proteases, lipopolysaccharide, and motility (Jones and Oliver, 2009). Motility of this marine bacterium involves a single, polar flagellum powered by a sodium ion pump, with non-motile strains exhibiting decreased lethality in mice as well as decreased attachment to human intestinal epithelial cells (Kim and Rhee, 2003; Kim et al., 2003). In this study, the effects of two environmental factors, temperature and salinity, on motility were examined. In addition, the role of the AI-2 quorum sensing system on motility was studied at three salt concentrations. These studies employed a *luxS* mutant, which is unable to produce LuxS, and its genetic complement. McDougald et al. (2000) reported that strains that cannot respond to AI-2 have been shown to have higher motility rates than those that can produce and respond to this molecule. In addition, *V. vulnificus* can be separated into two genotypes and the effect of genotype and isolate source on motility were examined.

*V. vulnificus* strains C7184K2, JDO1, and JDO2 were examined on 0.5%, 1%, and 3% NaCl motility agar. These strains represent the parent strain, *luxS* mutant, and the genetic complement of the *luxS* mutant, respectively. Motility was determined as the growth diameter in millimeters following inoculation onto the motility agar plates and incubation for 24 hours at room temperature and at 37°C.

There was a significant increase in motility (p<0.0001) in C7184K2 from 0.5% to 1% salt and a significant decrease in motility from 1% to 3% salt (Fig. 1a). There was also a significant difference in motility (Fig. 1b) between the strains examined (p<0.05) and at the different salt concentrations with the *luxS* mutant consistently more motile than the parent strain regardless of salinity. This phenotype was not seen in the complemented mutant. These results indicate that salinity affects motility in *V. vulnificus*. It also suggests that the inability to produce AI-2 results in increased motility, confirming previous work by McDougald *et al.* (2000)
*V. vulnificus* is genetically quite diverse which leads to great variation in the phenotype of this organism (Hilton *et al.*, 2006). *V. vulnificus* can be separated into two genotypes based on genetic polymorphisms in the *vcg* (virulence correlated gene) regions (Rosche *et al.*, 2005). Though these two genotypes are found in almost equal proportions in estuarine water, "C" and "E" genotypes are most commonly isolated from clinical and oyster/seawater sources, respectively (Warner and Oliver, 1998). It is important to note, however, that this is not definitive as there are clinically isolated E-genotypes and environmentally isolated C-genotypes.

Strains (n=21) of both C- and E-genotypes were incubated either at room temperature (~20°C) or 37°C for 24 hours, and the extent of motility determined using 0.5%, 1%, and 3% NaCl motility agar. There was a significant difference in motility (Fig 2a) demonstrated at the two temperatures (p<0.0001) and between the isolate sources when incubated at 37°C (Fig. 2b; p<0.05). There was no significant difference in motility between C- and E-genotypes at either temperature (data not shown).

Figure 1a: Effect of salinity on *V. vulnificus* strain C7184K2. A and B represent a statistically significant difference in motility of C7184K2 from 0.5% to 1% and 1% to 3%, respectively. Figure 1b: Effect of salinity on motility in C7184K2, the *luxS* mutant (JDO1), and *luxS* complemented strain (JDO2). A and B represent a statistically significant difference in motility with JDO1 compared to JDO2 and C7184K2 respectively. Each experiment was repeated four times with three pseudoreplicates each. Error bars represent the standard error of the means.

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In conclusion, salinity appears to have an influence on motility levels in *V. vulnificus*. It is noteworthy, however, that the luxS mutant was more motile than the parent and complemented strains. This observation agrees with a previous study by McDougald et al. (2000) in which an AI-2 mutant exhibited higher motility levels than strains that could respond to and produce AI-2. Temperature also had an effect on motility levels in our study in both C- and E-genotypes. In addition, there was a difference in motility between the clinical and environmental isolates, but not genotype, at 37°C. Both clinical and environmental isolates showed higher motility levels at 37°C than at room temperature, however, clinical isolates showed significantly higher motility levels than environmental isolates at 37°C. This raises the possibility that there may be an increase in motility upon entering the human body for those strains that are genetically more capable of attachment to human intestinal epithelial cells (Kim and Rhee, 2003) and, as a result, human infection (i.e. C-genotype strains).

References


