Vibrio vulnificus uptake by the larvae of the Eastern oyster, *Crassostrea virginica*

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Vibrio vulnificus is a gram negative, halophilic bacterium which is typically isolated from marine and coastal waters around the world. This bacterium is responsible for nearly 95% of all seafood related deaths in the United States, primarily through the ingestion of raw or undercooked oysters (Oliver and Kaper, 2001). There are two genotypes associated with *V. vulnificus*. Based on variations in the virulence correlated gene (*vcg*), the C-genotype is strongly related to clinical origin whereas the E-genotype is closely linked to environmental sources (Rosche, et al., 2005). It has been shown that the distribution of the two *V. vulnificus* genotypes is nearly equal in the water column. However, there is a much greater proportion (ca. 84%) of E-genotype cells present in the Eastern oyster, *Crassostrea virginica*, which is a natural reservoir for *V. vulnificus* (Warner and Oliver, 2008). The spawning season for *C. virginica* occurs during the warmer months. After fertilization of free floating sperm and eggs, the larvae undergo various developmental stages. Cleavage of the embryo occurs between 4-6 hours after fertilization. This is followed by gastrulation when the first gut is formed, although the organism is still incapable of feeding at this point. From 12-18 hours the larvae transition into swimming trochophores and finally into veligers near 24 hours (Buroker, 1983). This final veliger stage has been previously reported to be the first feeding stage.

The purpose of this study was to determine if there is a differential uptake of the two genotypes of *V. vulnificus* by various *C. virginica* larval states. To this end, gametes were aseptically extracted from fertile adult oysters, observed for quality, and sexed. Sperm and eggs were combined in artificial seawater (ASW) to allow fertilization. Two strains of *V. vulnificus* (CMCP6 and JY1701, C- and E-genotype, respectively) were separately incubated with various stages (initial fertilization, first cleavage, gastrulation, trochophore, and veliger) of the larvae. After incubation, the larvae were collected by filtration through a sterile 22µ mesh screen. The collected embryos were gently rinsed with ASW to remove non-ingested bacteria then recollected. A subsample was used to quantify the embryos present while a second subset was homogenized and plated onto cellobiose-polymyxin B-colistin (CPC+) agar to determine and quantify the presence of *V. vulnificus*.

As shown in Figure 1, uptake of *V. vulnificus* was first observed during the trochophore larval stage of *C. virginica* and continued to be detected through the veliger stage. However, there appeared to be no difference between the two *V. vulnificus* genotypes regarding their larval uptake.

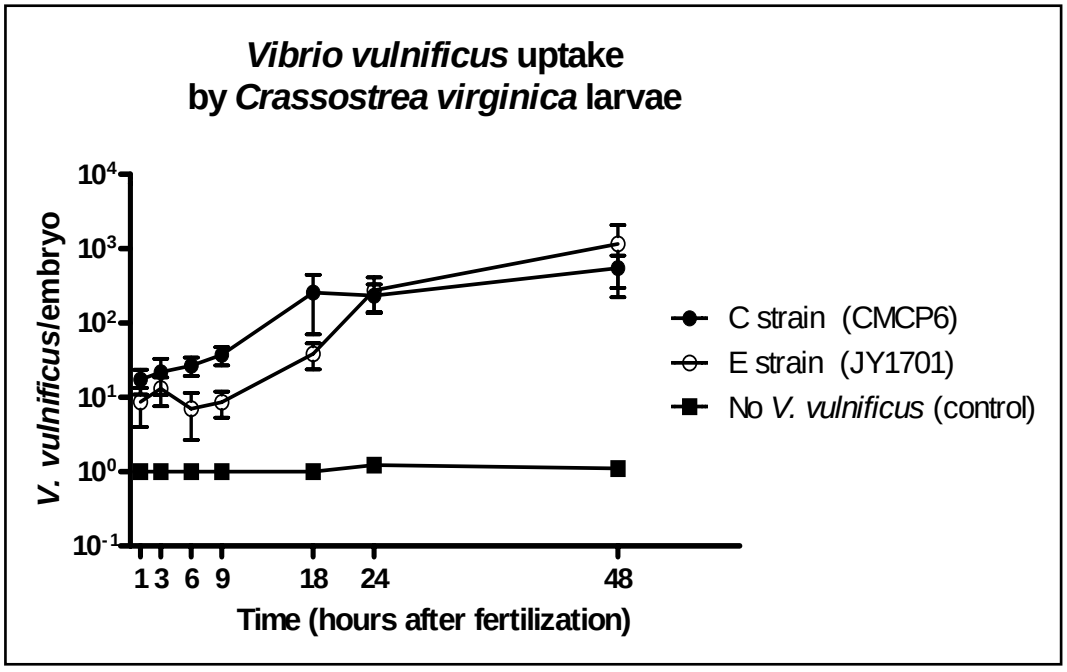

Figure 1: Comparison of the uptake of the two genotypes of *V. vulnificus* by *C. virginica* oyster larvae over a 48 hour time period. The control is shown to indicate that uptake was a result of added bacteria and not previously established bacteria within the embryos.

This study demonstrates that there is an association that develops between *V. vulnificus* and *C. virginica* prior to reaching the adult stage. However, our study did not explain the observed difference between the levels of *V. vulnificus* genotypes observed within adult oysters and their surrounding waters.

References

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