

# ***Vibrio vulnificus* biofilm formation is related to salinity and AI-2 production**

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*Vibrio vulnificus* is an estuarine bacterium that causes both food-borne illnesses and wound infections. It is the leading cause of seafood related deaths in the United States, most commonly resulting from the ingestion of raw or undercooked oysters. Because *V. vulnificus* is routinely subjected to varying salinities in estuarine environments, we performed a series of experiments to

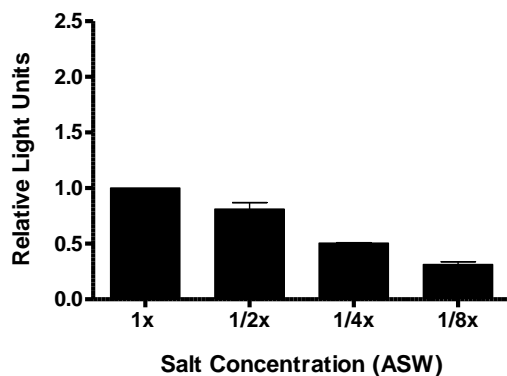


Figure 1: AI-2 production in varying salinities after 4 h. All data normalized to 1x ASW.

observe some of the effects salinity has on *V. vulnificus* physiological processes. In the first set of experiments, it was observed that the amount of the quorum sensing molecule AI-2 produced by *V. vulnificus* decreased proportionally as salinity decreased from 1x to 1/8x artificial salt water (ASW) after 4 h (Fig 1).

Kaneko *et al.* (1975) observed *Vibrio parahaemolyticus* had decreased attachment to chitin in increasing salt concentrations. From that observation, our next step was to observe biofilm formation in the same ASW concentrations used for the AI-2 production assay. Overnight cells of *V. vulnificus* strain,

C7184-K2, were grown to stationary phase and then inoculated into 96 well polystyrene plates at a 1:100 dilution into 1/8x, 1/4x, 1/2x, and 1x ASW and incubated at 30°C for 4 h. Subsequently, the plate was removed and the degree of biofilm formation determined as described by Stepanovic *et al.* (2000).

McDougald *et al.* (2006) showed that over a short (2 h) time period, *V. vulnificus* showed no difference in biofilm formation between low and high salinities. However, our observations found an increase in biofilm formation from high to low salinities in ASW after 4 h (Fig 2). AI-2 has been linked to biofilm formation and other processes related to biofilm formation, such as motility (Kim *et al.*, 2003). In addition, McDougald *et al.* (2006) showed that motility and attachment increased in a *V. vulnificus smcR* mutant, which is unable to respond to AI-2. Their data suggested that a decrease in AI-2 may lead to increased attachment in *V. vulnificus*. Our study suggests an inverse relationship between the amount of AI-2 produced and the amount of biofilm formed in varying salinities of ASW. Salt is a predominant abiotic factor in estuaries and can fluctuate dramatically over short periods of time.

These results suggest that salinity may have a much larger effect on the physiological functions of *V. vulnificus* than what was previously thought.

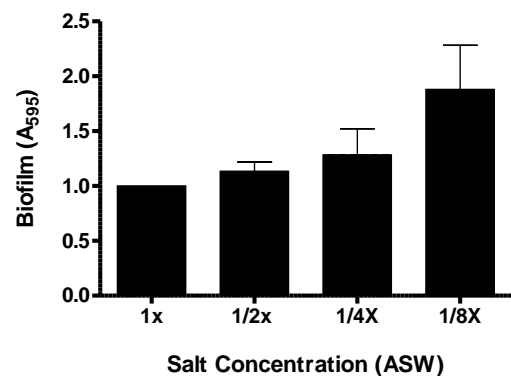


Figure 2: Biofilm formation in varying salinities after 4 h. All data normalized to 1x ASW.