

Title : Isolating High Efficient Killing Bacteriovorax (*Bx*) Strains that Prey on Virulent *Vibrio vulnificus*

*Vibrio vulnificus*, a Gram-negative halophilic bacterium, is the leading cause of reported seafood related deaths in the United States. *Bacteriovorax* (*Bx*), a group of *Bdellovibrio* and like organisms (BALOs), are halophilic, predatory prokaryotes that prey on susceptible Gram-negative bacteria including *V. vulnificus*. Since the host range and predation efficiency of *Bx* strains vary, selecting the most appropriate strain is important for possible therapeutic applications of the predator. The aim of this study was to isolate from environmental samples high efficient killing (HEK) *Bx* strains against *V. vulnificus*.

Water samples were collected from different aquatic systems and filtered through 0.8µm filters. To establish natural mesocosms, 500 ml of filtered sample was spiked with *V. vulnificus* FLA042 to reach an optical density (OD) of 0.7. Equal volume of prey was spiked into autoclaved water samples which served as control. The mesocosm flasks were incubated at room temperature on a shaker and monitored for 5 days. At each 24 h interval, OD values and plate counts for both *Bx* and prey were obtained. The predominant *Bx* plaques were selected and analyzed by 16S rRNA gene amplification and sequencing using specific primers. The dynamics of the bacterial community in the mesocosms were examined by denaturing gradient gel electrophoresis (DGGE). With this method, the highest efficient killing *Bx* isolates against *V. vulnificus* FLA042 were successfully identified from natural water samples. The results show that the inoculated prey bacteria decreased significantly (ANOVA,  $p < 0.05$ ) with a simultaneous thousand fold increase in *Bx* numbers within 24 to 48 h, indicating the predation of *V. vulnificus* by the predator. The results also revealed that *Bx* isolates of ribotype clusters IX and X were consistently the most efficient for killing *V. vulnificus*, although other *Bx* ribotypes were present. This is the first report demonstrating that different ribotypes of *Bx* exhibit different prey preferences. The results may carry long-term implications for the novel approach of using *Bx* to eradicate pathogenic bacteria.