

Mortality and Infection Following *in vivo* dsRNA Knockdown of Crustins in the Pacific Whiteleg Shrimp, *Litopenaeus vannamei*

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As invertebrates, penaeid shrimp rely solely upon an innate, non-adaptive immune response to survive in marine environments under a variety of stresses, including bacterial, fungal, and viral infection. The invertebrate immune response includes both cellular responses, such as phagocytosis, and humoral responses, such as the secretion of antimicrobial peptides. The crustin family of putative antimicrobial peptides was identified via shrimp EST libraries. Sequence homology to an 11.5kD antimicrobial peptide from the shore crab, as well as the discovery of a putative serine protease inhibitor domain (whey acidic protein) made crustins excellent candidates for further analysis. In previous experiments these peptides have been shown to play a role in the shrimp immune response. In this study, the activity of crustins is being assessed following immune challenge with *Vibrio penaeicida* and *Fusarium oxysporum*, both are known shrimp pathogens. In order to focus on the function of crustins *in vivo*, the knockdown of crustin by injection of dsRNA was undertaken in our bioassay facilities. Successful knockdown was demonstrated (lasting approximately 7 days post injection). In further experiments, crustins were knocked down followed by subsequent challenge with either bacteria or fungi, and changes in mortality were determined. Other shrimp control genes (both immune and non-immune) have been knocked down, and do not cause any change in mortality compared to negative controls. This study shows a different response to infection by bacteria and fungi. It appears that crustins are providing some immune protection within the shrimp.

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