Antimicrobial peptides (AMPs) from Lobsters: Comparing Approaches to Testing for Antimicrobial Activity

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My project focuses on determining the antibacterial properties of antimicrobial peptides (AMPs) found in lobster hemolymph and shells. Focusing on the big picture, antibiotic resistance has been widely acknowledged as a public health threat due to the misuse of antibiotics. Researchers are working to find a solution, one of which could come from lobsters. Lobsters, and crustaceans in general, are remarkable when it comes to the peptides and proteins they contain because of how they protect the crustacean. Lobsters use AMPs, which are released from cells (hemocytes) in their circulatory fluid (hemolymph), to fight off pathogens as part of their innate immune system. In addition to helping the lobsters, AMPs could potentially be used to fight bacteria in other organisms. While bacteria are the main focus of my project, AMPs likely have other bioactive properties in addition to their antibacterial properties[1]. This is particularly useful due to the prevalence of antibiotic resistant bacteria, since these AMPs could provide a solution as a new pipeline of antibiotics.

Previously, the Stemmler lab has worked to isolate and analyze these AMPs using liquid chromatography and mass spectroscopy, successfully establishing the identity of the peptide Hoa-D1[2]. While identifying this AMPs is a critical step, it is still not known if this peptide is biologically active. This summer, I tested the bioactivity of Hoa-D1 towards *E. coli* using the broth dilution and disk diffusion methods of testing. Due to the difficulties in collecting enough material, I started the summer by testing the bioactivity of lobster shells, as a previous paper indicated that they may have bioactivity[3]. I hoped to use the shells, which I had no shortage of, to find the best method of testing before moving on to Hoa-D1. However, four rounds of testing indicated no bioactivity associated with shell extracts, so I moved on to collecting and testing cell extracts from the lobster hemolymph containing Hoa-D1.

Past research by Rube Ahaiwe[4] in the Stemmler lab tried the disk diffusion method with Hoa-D1 and found no activity, leading to the hypothesis that AMPs do not diffuse due to being positive charged. My data supported her hypothesis, as I also found no activity with the disk diffusion method. However, I did see that Hoa-D1 exhibited bioactivity against *E. coli* when testing using the broth dilution method (see Fig. 1) which involves inoculating bacteria in a liquid medium with the AMP rather than relying on the AMP to diffuse off of a paper disk onto agar. This is the first evidence of bioactivity that we have seen from Hoa-D1, and it brings us one step closer to confirming Hoa-D1 and other AMPs as new antibiotics, hopefully bringing an end to the antibiotic resistance epidemic.

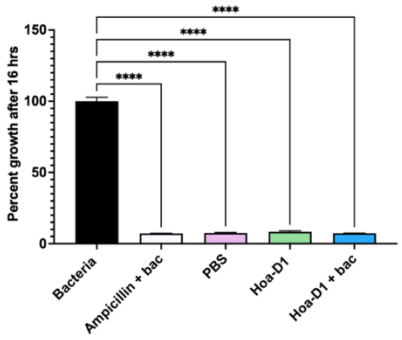

Figure 1. Percent growth of *E. coli* in the presence of ampicillin and Hoa-D1 using the broth dilution method, where 100% represents the positive control. Two additional negative controls not containing bacteria also shown. Hoa-D1 inhibits bacterial growth at 16 hours. Error bars represent standard error mean, **** p<0.0001 (ANOVA)

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References:

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4. Ahaiwe, R. (2021). Antimicrobial peptides (Amps) in the lobster, *Homarus americanus*: Isolation and activity. *Honors Projects*.