Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

FINAL REPORT

Final reports will be published on the Cal Poly Digital Commons website (http://digitalcommons.calpoly.edu).

I. Project Title

T6SS Expression of *Vibrio parahaemolyticus* in Intraspecific Competition

II. Project Completion Date

3/24/17

III. Student(s), Department(s), and Major(s)

(1) Alex Campbell, Biological Sciences Dept., Biological Sciences Major

IV. Faculty Advisor and Department

Dr. Marie Yeung, Biological Sciences Dept.

V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)

Cal Poly Biological Sciences Department, Cal Poly College of Science and Mathematics

VI. Executive Summary

The rise of antibiotic-resistant bacteria has reached an alarming level. The recently discovered type VI secretion system (T6SS) appears to be a bacterial defense mechanism that could potentially become involved with addressing this concern. Previous studies suggest *Vibrio* spp. may express T6SS. In this study, *Vibrio parahaemolyticus*, a common foodborne pathogen, is used as a model to study T6SS. The objectives of this study were to evaluate culturing conditions that would stimulate swarming motility in strains of *V. parahaemolyticus* and to detect for the presence of T6SS based on phenotypic criteria. Different media (BHI, TSAS), agar concentration (0.7%, 1%, 2%) and incubation temperature (25°C and 37°C) were tested to determine the optimal environment that induces swarming in strains of *V. parahaemolyticus*. The diameter of growth from the center of a 150-mm agar plate was measured in centimeters to quantify swarming in varying conditions. The greatest extent of swarming was observed when *V. parahaemolyticus* strains (n=15) were incubated in BHI medium containing 1% agar at 25°C. In these conditions, the strains swarm the surface of an entire 150-mm agar plate within 3 days of incubation. When two T6SS-positive strains of the same species swarm towards each

other, instead of forming a continuous mass, a Dienes line forms at the junction indicating competition between the two strains. Thus, the next step was to detect Dienes line formation. Pairwise testing was performed from the 15 strains, with each pair inoculated 0.5 cm apart on a 150-mm agar plate. Pairwise testing of identical strains served as the negative control while strains of Proteus mirabilis served as the positive control. Eight strains (53%) of *V. parahaemolyticus* were positive for Dienes line formation under the test conditions. PCR was conducted to confirm the presence/absence of T6SS genes in the sample genome. RT-qPCR method was optimized.

VII. Major Accomplishments

(1) Identified strains of *Vibrio parahaemolyticus* that potentially express T6SS through the observation of Dienes Lines.

(2) Presented at 2017 CSU Biotechnology Symposium as the first author (and the only student) of the poster.

(3) Developed molecular biology skills and acquired information from literature review that will further assist investigation of T6SS in *Vibrio parahaemolyticus*.

(4) Designed primers to continue the project for the future.

(5) Acted as a team leader while working with three other undergraduate students.

VIII. Expenditure of Funds

Expense	Amount ($)
Media and culturing supplies	600
Disposable	300
Extraction Kits	360
PCR reagents including primers	350
CSU Biotechnology Symposium Student Fee	90
CSU Biotechnology Symposium Poster	100
Total:	1800

IX. Impact on Student Learning

When I began working on "T6SS Expression of *Vibrio parahaemolyticus* in Intraspecific Competition", I had limited knowledge of microbiology/molecular biology research techniques. Additionally, I was uncertain of my career aspirations. Overall, this project has helped me grow in 3 primary ways: developing laboratory skills, guiding my learning at Cal Poly, and by contributing to me figuring out what my career interests are.

The laboratory skills that this project helped me develop have been both technical and interpersonal. I learned a variety of laboratory techniques in both microbiology and molecular biology such as cultivating different types of microbes, PCR, gel electrophoresis, and quantitative PCR. Additionally, throughout the course of the project, 3 other Cal Poly students assisted with the lab work and learned about the same techniques. Working with others on the project was also essential to learning about working in a laboratory. Since I was the lead for the project, I had to refine my understanding of the project as a whole and the lab techniques so that I could bring new team members up to date with the project when they joined. Leading other team members to collect data also helped me practice delegating. Most importantly, throughout the process, I realized how it is important to balance independently solving problems with asking for help. At first I was striving to figure most things out on my own, however I learned that in some situations it is necessary to get help from my PI or other more experienced individuals to avoid common or critical mistakes.

Another area where this project helped me was by guiding and complementing my learning in classes at Cal Poly. The quarter I started the project was also the quarter when I took an introductory microbiology course. Working on the microbiology based research outside of class helped me understand what was going on in class. Additionally, the material that I learned in class helped me understand what I was doing in research. Later on when I began working on PCR and gel electrophoresis for the project, which are molecular biology techniques, I discovered that I needed to learn a lot more about the techniques that I was using in research. This encouraged me to take a molecular biology laboratory class earlier than I would have if I was not involved in research. In a similar way as the microbiology class, the molecular biology class and research complemented each other and I believe that the knowledge I gained from each experience helped me with the other.

Finally, the project played an important role in helping me figure out what I am aiming to do after I graduate from Cal Poly. At the time when the project started, I thought that I may want to be a scientist working in a laboratory in industry post-graduation. However, throughout the course of this project I became torn between pursuing a PhD. and working in industry directly after graduation in a non-laboratory role. Having the opportunity to attend the CSUPERB Biotechnology Symposium because of this project helped me to make my decision. This opportunity allowed me to learn about the variety of academic research being done in biotechnology. I was introduced to many incredible areas of research at the symposium, however, I never saw a type of research that I could imagine myself doing for years (or decades) after Cal Poly. Furthermore, I met many students who were passionate about their research and have a desire to continue on to get their PhDs. This helped me determine that although I enjoy research, I am not passionate about it enough to pursue a doctorate degree. If it was not for this project allowing me to experience research and to attend the symposium, I think I would still be debating about whether or not I should pursue a PhD.

I am grateful that the Baker and Koob Endowments funded the“T6SS Expression of *Vibrio parahaemolyticus* in Intraspecific Competition” project. This project provided me with unique challenges and opportunities that have enriched my Cal Poly experience. I am also appreciative that through my experiences with this project I was able to understand my career interests more clearly. Regardless of where my career path leads me in the future, I know that there are many important skills and experiences that this research has brought that will continue to help me in years to come.