T6SS Expression of *Vibrio parahaemolyticus* in Intraspecific Competition

I. Abstract

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a type of gram negative bacteria found in marine environments that may express the type VI secretion system (T6SS) defense mechanism. This mechanism is used by some bacteria to defend themselves against what they perceive as "enemies" which ranges from eukaryotic cells to other bacteria. Studies of T6SS have found that different species of bacteria have evolved different ways to regulate and use the mechanism, often depending on their environment. Some bacteria even use the T6SS mechanism against different strains of their own species. Finding out how different strains of *V. parahaemolyticus* interact with each other could potentially help us reveal more about the T6SS mechanism. If more information is known about the T6SS mechanism, new methods of creating antibiotics could potentially be discovered. This project will employ assays to determine the presence of Dienes lines, which is associated with T6SS expression. Additionally, I will conduct RT-qPCR to determine the expression of genes involved in T6SS. Further, I will compare the gene expression of T6SS between virulent and avirulent strains in order to discover more about how the T6SS mechanism functions in *V. parahaemolyticus*.

II. Introduction

Type VI secretion system (T6SS) is a defense mechanism used by some gram negative bacteria in both interspecific and intraspecific competition (Miyata et al., 2011, Unterweger et al., 2014). The T6SS mechanism occurs when a bacterium senses an "enemy" and injects the enemy cell with virulence factors that kill it (Macintyre et al., 2010). Previous studies testing a variety of gram negative bacteria that possess the T6SS mechanism have shown that different bacteria have unique ways to activate and regulate this process (Salomon et al., 2013). For example, T6SS is initiated in *V. cholerae* when another cell engulfs it whereas *P. atrosepticum* must be in acidic conditions with potato tuber extracts to activate the mechanism (Salomon et al., 2013).

In the bacterial species *Vibrio parahaemolyticus* (*V. parahaemolyticus*), Salomon et al. (2013) revealed that some strains of *V. parahaemolyticus* initiates the T6SS mechanism when they are in "warm, marine-like conditions". Since *V. parahaemolyticus* is usually found in marine conditions - and is known to cause gastroenteritis when consumed by humans in shellfish - it makes sense that the T6SS mechanism would be active in environments that match their habitat (Salomon et al., 2013).

In *V. cholerae, T6SS* has been observed as a mode of intraspecific competition *(Unterweger et al., 2014).* While some strains of V. cholerae can coexist, other strains use T6SS to compete with one another *(Unterweger et al., 2014).* When two competing strains of the same bacteria are placed on opposing sides of a trypic soy agar (TSA) plate and meet one another, a line where neither strain of bacteria can occupy appears on the plate. The line is called "Dienes line" (Budding et al., 2009).

Since bacteria use the T6SS mechanism against other types of bacteria, further studies of how the T6SS mechanism functions may help reveal new methods of

creating antibiotics for medical purposes (Macintyre et al., 2010). Innovations in antibiotics is becoming increasingly important since antibiotic resistant microbes are becoming more prevalent (WHO 2015). If more is known about the T6SS mechanism, it could potentially be harnessed as a defense against antibiotic resistant bacteria.

This project intends to determine if the expression of T6SS is correlated with virulence of *V. parahaemolyticus*. To determine whether or not certain strains express T6SS, strains of *V. parahaemolyticus* will be plated on opposite ends of agar plates to observe Dienes line formation (or lack thereof) which suggest T6SS expression. After this screening step, strains will be subjected to RT-qPCR to determine expression of virulence factors and T6SS. The comparison of gene expression between the strains will be used to discover more about the T6SS mechanism in *V. parahaemolyticus.*

Bibliography

"Antimicrobial Resistance." *World Health Organization*. World Health Organization, (2015). Web. 31 Jan. 2016.

Budding, A. E. et al. "The Dienes Phenomenon: Competition and Territoriality in Swarming *Proteus Mirabilis* ." *Journal of Bacteriology* 191.12 (2009): 3892–3900. *PMC*. Web. 31 Jan. 2016.

Macintyre, D. L., S. T. Miyata, M. Kitaoka, and S. Pukatzki. "The Vibrio Cholerae Type VI Secretion System Displays Antimicrobial Properties."*Proceedings of the National Academy of Sciences* 107.45 (2010): 19520-9524. Web. 31 Jan. 2016.

Miyata, S. T., M. Kitaoka, T. M. Brooks, S. B. Mcauley, and S. Pukatzki. "Vibrio Cholerae Requires the Type VI Secretion System Virulence Factor VasX To Kill Dictyostelium Discoideum." *Infection and Immunity*79.7 (2011): 2941-949. Web. 31 Jan. 2016.

Salomon, Dor, Herman Gonzalez, Barrett L. Updegraff, and Kim Orth. "Vibrio Parahaemolyticus Type VI Secretion System 1 Is Activated in Marine Conditions to Target Bacteria, and Is Differentially Regulated from System 2." *PLoS ONE* 8.4 (2013): n. pag. Web.

Unterweger, Daniel, Sarah T. Miyata, Verena Bachmann, Teresa M. Brooks, Travis Mullins, Benjamin Kostiuk, Daniele Provenzano, and Stefan Pukatzki. "The Vibrio Cholerae Type VI Secretion System Employs Diverse Effector Modules for Intraspecific Competition." *Nature Communications Nat Comms* 5 (2014): n. pag. Web. 31 Jan. 2016.

III. Objective(s)

1. Determine which combination of strains of *V. parahaemolyticus* result in Dienes lines when inoculated on the opposite end of agar plate.
2. Use RT-qPCR to compare gene expression in strains of *V. parahaemolyticus* that use the T6SS mechanism against other strains of *V. parahaemolyticus* to the gene expression of strains that do not use the T6SS mechanism.

IV. Methodology

Dienes Lines: To achieve the first objective, bacterial cultures of different

strains of *V. parahaemolyticus* will be obtained from Dr. Yeung. TSA supplemented with 2% NaCl (i.e., TSAS) plates will be inoculated with varying combinations of two different strains of *V. parahaemolyticus* placed on opposite sides of the plate. The plates will be placed in conditions ideal for the bacterial growth and will be left until the bacteria have grown enough to interact in the center of the plate. At this point, Dienes lines (or the lack thereof) will be observed and recorded.

RT-qPCR: To achieve the second objective, RNA will be extracted from different strains of *V. parahaemolyticus* expressing T6SS (such as in contact with competing strains), as well as strains that do not form Dienes line. Primers will be designed for genes involved in T6SS and other virulence mechanism. RNA will be reverse transcribed, followed by quantitative PCR to determine the relative expression of these genes.

V. Timeline

Spring Quarter 2016-

1. Obtain 30 strains of *V. parahaemolyticus* from Dr. Yeung
2. Prepare agar, maintain viability of all strains.
3. Test many combinations of strains of *V. parahaemolyticus* for Dienes lines. Begin designing primers for Objective #2, include housekeeping genes determination. Then, order primer sets after verification.

Fall Quarter 2016-

1. Run traditional PCR to test accuracy of primer sets
2. Identify conditions when T6SS is expressed in some strains
3. Extract and then quantify RNA
4. Conduct reverse transcription
5. Quantify cDNA and then learn qPCR

Winter Quarter 2017-

1. Run many rounds of qPCR with the cDNA prepared in previous quarter. Then, calculate relative gene expression.
2. Write reports and prepare presentation

VI. Final Products and Dissemination

- Present at 2017 CSU Biotechnology Symposium
- Present at 2017 CSM Student Research Conference

VII. Budget Justification

Operating Expenses	Amount
Media (to grow many *V. parahaemolyticus* strains)	$500
Primer sets (genes involved in T6SS, other virulence mechanism, and housekeeping genes)	$100
RNA extraction kits	$500
RT reagents (to covert RNA to cDNA)	$200
qPCR kits	$400
Disposable (e.g., petri plates, pipet tips, Eppendorf tubes)	$100
Total:	$1800

PROPOSAL BUDGET

Student Applicant(s): Alex Campbell		
Faculty Advisor: Dr. Marie Yeung		
Project Title: **T6SS Expression of *Vibrio parahaemolyticus* in Intraspecific Competition**		**Requested Endowment Funding**
Travel	*subtotal*	**$0**
Travel: In-state		**$0**
Travel: Out-of-state		**$0**
Travel: International		**$0**
Operating Expenses	*subtotal*	**$1800**
Non-computer Supplies & Materials		**$1800**
Computer Supplies & Materials		**$0**
Software/Software Licenses		**$0**
Printing/Duplication		**$0**
Postage/Shipping		**$0**
Registration		**$0**
Membership Dues & Subscriptions		**$0**
Multimedia Services		**$0**
Advertising		**$0**
Journal Publication Costs		**$0**
Contractual Services	*subtotal*	**$0**
Contracted Services		**$0**
Equipment Rental/Lease Agreements		**$0**
Service/Maintenance Agreements		**$0**
	TOTAL	**$1800**