

SOAR Proposal - Summer 2016

Establishing Preliminary Relationships Between Peptide Structure and Quorum Sensing Activity in *Bacillus cereus*

Faculty Mentor: Michael A. Bertucci, Assistant Professor of Chemistry

Student Researcher: Jessica Lynch

Start Date: May 31st, 2016

Length of Project: 10 weeks

Project Summary

This project focuses on bacterial quorum sensing and the role it plays in biofilm growth and bacterial virulence. Bacteria communicate with each other using a chemical “language”, resulting in synchronized group behavior. A pathogenic bacterial cell, by itself, will not make you sick. But, as the bacterial cell grows and divides, they release these chemical signals called “autoinducers” that allow them to communicate with each other. Once there are enough autoinducers (and therefore a lot of bacterial cells present), they bind to a receptor in the cells and spark a synchronized behavior. Specifically, the multicellular behaviors our project is interested in are biofilm production and the release virulence factors, both of which encourage bacterial infection of a host. If this communication pathway could be interrupted, it would prevent this synchronized behavior from occurring and attenuate the bacteria’s ability to cause harm.^{1,2}

Our project’s goal is to modify the autoinducer in *Bacillus cereus*, a mild bacterial pathogen, to interfere with the communication circuit so that biofilm production cannot occur. A technique called “Alanine scanning” will be used to accomplish this. The autoinducer responsible for quorum sensing in *B.cereus* is made up of seven amino acids (called a “peptide”).³ Alanine scanning involves replacing each of these amino acids, one at a time, with a different, simpler amino acid (alanine). Each peptide synthesized through this method will basically be missing one of its seven amino acids, with an alanine in its place. The purpose of this is to determine which amino acid “swap” would hypothetically keep the autoinducer from working properly, providing insight into which amino acids are most critical for initiating quorum sensing. Ultimately, we would like to test the peptides we synthesize in a bacterial plate to see if they successfully inhibit biofilm production. This has practical applications in creating new therapeutics that are an alternative to antibiotics and can hopefully help in solving some of the problems pertaining to the resistance of bacteria to current antibiotics.

Roles & Responsibilities

I, Dr. Bertucci, will serve as a mentor for all 10 weeks of the proposed project providing one-on-one instruction to the student, Jessica, in peptide synthesis and purification as well as bacterial culture techniques. I will also be responsible for supporting Jessica in her search and analysis of peer-reviewed literature related to our project and providing feedback on Jessica’s progress and results in daily individual meetings. Further, I will be in charge of procuring the supplies Jessica needs to complete her proposed project and arrange registration and travel for her to present her data.

Jessica will be the lead researcher on the project, responsible for carrying out all experiments towards our research goals. She will document her experiments in an approved laboratory notebook. At the end of her SOAR experience, Jessica will compile all of her

¹Bassler, Bonnie. “Bonnie Bassler: How Bacteria “Talk”.” TED. Feb 2009. Lecture.

<https://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate?language=en>

²Steven Rutherford, and Bonnie Bassler. *Cold Spring Harbor Perspectives in Medicine* (2012): n. pag. Cold Spring Harbor Laboratory Press. Web. 25 Feb. 2016.

³Grenha, R., Slamti, L., Nicaise, M., Refes, Y., Lereclus, D., Nessler, S., *PNAS.*, **2013**, 110, 1047 – 1052.

chemical products, analytical data, and relevant procedural documentation for reference by future students and for publication. Jessica will participate in daily individual research meetings with her mentor and formally present her progress to her labmates and mentor in the form of a powerpoint presentation at least 3 times throughout the summer. As a summative activity, Jessica will present her work at the National Meeting of the American Chemical Society and Moravian College's Undergraduate Student Scholarship and Creative Arts Day.

Timetable of Expected Milestones

WEEK 1: Orientation to literature, project goals, and experimental procedures; peptide design

WEEK 2 - 4: Synthesis of peptides derivatives

WEEK 5 - 7: Purification and identification of peptide derivatives

WEEK 8 - 10: Bacterial assay preparation and screening; poster preparation for ACS National Conference

Engagement in Discipline-Specific Research

This project directly relates to biochemistry, organic chemistry, and molecular genetics. Jessica is currently majoring in biochemistry and has taken organic chemistry with me this semester. She will be exposed to these subject areas more deeply through the described research in the context of a significant global health issue. She will be able to employ techniques and strategies she has learned in class and her laboratory courses, which will be beneficial in reinforcing the related chemical concepts.

Contributions to the Discipline & Community

Jessica's work will contribute to the discipline by gaining new information on specific molecular structures that are involved in quorum sensing in *Bacillus cereus* and how these molecules can be modified through synthetic organic chemistry in order to prevent cell communication. There is currently a deficiency in the literature with respect to chemical approaches to address this problem. *I consider this project to be foundational in creating a multi-year research program that will be a significant contribution to the greater quorum sensing community and produce publishable, novel data.* This project can be expanded upon with future research mentee due to the numerous ways the target peptide signaling molecule can be structurally altered to enhance inhibition of the bacteria's pathogenicity.

Jessica will have the opportunity to present the project at the National Meeting of the American Chemical Society in August in Philadelphia, PA. She will also present her findings to the college community at the Undergraduate Student Scholarship and Creative Arts Day in the Spring of 2017.

Student Purpose Statement

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Student name: Jessica Lynch

Major: Biochemistry

Graduation date: May 2017

Faculty Mentor: Dr. Michael Bertucci

On-Campus Housing: Yes

Of the two colleges I transferred from, none of them gave students an opportunity to participate in undergraduate research, as is offered at Moravian. The ability to contribute to a field of study and perform original research is something I am excited to be a part of. I had immediate interest in quorum sensing because it encompasses both biological and chemical processes. Since I am a biochemistry major, this project aligns well with my enthusiasm for both biology and chemistry. It gives a hands-on approach to chemical synthesis with a direct application to a biological system. This project will give me a head start for when I take biochemistry next semester.

I also like that it addresses a well-known problem that society faces today: bacterial resistance to antibiotics. Quorum sensing looks to develop an alternative to antibiotics, and this application in medicine also heightened my interest. Working in a veterinary hospital, I see the direct application of science through medicine. We dispense broad-spectrum antibiotics to our patients without hesitation, so I see the problems with antibiotics first hand on a weekly basis. I am excited to be a part of research that plays a role in cutting edge medicine and to learn more about the processes involved.

From this project, I hope to gain insight as to what I would like to pursue after graduation. While doing so, I will gain valuable experience and confidence in the lab. The opportunity to collaborate on an original experiment is something that goes beyond what we learn in a standard 3-hour lab period. Immersing myself in a summer long research project will allow me to develop advanced problem solving and troubleshooting skills that will help me in whatever I pursue after graduation.

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Expense Proposal

Towards the completion of the research and experiences discussed above, we are requesting funding for the following expenses:

ACS Membership Registration:	\$27.00
ACS National Meeting Registration:	\$105.00
(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) [HBTU] (500g)	\$100.00
Trifluoroacetic acid (100 mL)	\$77.20
Fmoc-Ala-OH (500 g)	\$205.00
Total	\$514.20

The ACS Membership Registration Fee will allow Jessica to become a student member of the American Chemical Society and receive a reduced registration fee for the National Meeting at which we plan to present her research. The ACS National Meeting Registration is that fee.

HBTU, Trifluoroacetic acid, and Fmoc-Ala-OH are all chemicals that are required for the completion of Jessica's proposed peptide synthesis and alanine scan. We do not have these chemicals present in the chemistry department at Moravian and they will be used exclusively for research purposes.