SOAR Proposal - Summer 2017

Optimizing cyclization of LamD derivatives in preparation for bioassays of *Lactobacillus plantarum*

Faculty Mentor: Michael A. Bertucci, Assistant Professor

Student Researcher: Jonathan Nadraws

Start Date: May 30th, 2017
Length of Project: 10 weeks

Project Summary

Quorum sensing is the scientific term for the ability of bacteria to communicate with one another on a cell to cell basis by chemical means. Bacteria produce molecules that serve as a means of communication with other bacteria called autoinducers. All species of bacteria have a unique chemical autoinducer and can detect the type and concentration of other autoinducers present in their environment. Autoinducers bind to receptors on the cell walls of bacteria, allowing them to relay information about what types of bacteria are present around them (including their own kind), as well as how many are present. When the concentration of autoinducers from their own species is sufficiently high, this triggers a synchronized behavior by a colony of bacteria, allowing them to effectively coordinate their activities. These behaviors are diverse, including biofilm formation, pathogenic attack, and even some behaviors that are beneficial to humans. The bacteria in the human gut, for example, can utilize quorum sensing to coordinate their efforts, allowing them to gain critical nutrients and resources, as opposed to foreign, potentially harmful bacterial species.[1,2]

Our project is focused on the effects of *Lactobacillus plantarum*. Due to its beneficial effects, it has been labelled, along with thousands of other bacterial species, as a probiotic. It has been shown to treat the symptoms of Irritable Bowel Syndrome (IBS) and reduce LDL-cholesterol ("bad" cholesterol). Other benefits of probiotics include their antimicrobial properties, as well as their ability to exclude harmful bacteria (pathogens) in the gut by not giving these pathogens sufficient surface area to develop their colony.[2]

The goal of this research is to study the autoinducer unique to *Lactobacillus plantarum* called LamD, a molecule referred to as a peptide, composed of 5 structural units known as amino-acids. This is being done by a method known as an alanine scan, and a library of 5 different LamD derivatives have been designed to test how integral each individual amino acid is to the function of LamD. This library has yet to be completed, and the goal of this project is to create an efficient, high yielding chemical procedure for the cyclization of the peptide derivatives, a crucial step in their synthesis. Past efforts at doing this have given low yields, and

---

[1] Bassler, Bonnie. "Bonnie Bassler: How Bacteria "Talk"." TED. Feb 2009. Lecture. Available from: http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate

[2] Ouwehand, Arthur C., Seppo Salminen, and Erika Isolauri. "Probiotics: An Overview of Beneficial Effects." Antonie Van Leeuwenhoek 82 (2002): 279-89. Print.

made the assembling of this library inefficient. Once this protocol has been developed, future researchers will be able to quickly and easily create the molecules necessary to begin bioassays.[3]

From this research we will potentially be able to construct molecules which can up-regulate, or enhance the abilities of *Lactobacillus plantarum*, which could prove useful in aiding digestive health and decreasing the chance of bacterial and viral infection in humans.

Roles and Responsibilities

I, Dr. Bertucci, will serve as Jonathan's mentor during all 10 weeks of his proposed project. I will be responsible for monitoring Jon's progress, working with him one-on-one as he explores academic literature and formulates his experiments, and providing consultation on learning new techniques and interpreting data. In addition, I will gather the supplies and chemicals Jonathan needs to carry out his proposed research and arrange registration and travel to the conferences at which Jonathan will present his results.

Jonathan will be the individual conducting all the research and experiments to forward his project goals. As common in our discipline, Jonathan will be responsible for maintaining a laboratory notebook to document his results and progress. At the end of the summer, Jonathan will compile all of the peptides that he has synthesized, the data he has acquired, and other relevant documentation in an organized fashion for reference by future students and publication. Jonathan will participate in daily research meetings with Dr. Bertucci and any other students being advised by Dr. Bertucci to set a daily research agenda and discuss results from the previous day's work. As summative evaluations of his progress and opportunities for academic and professional development, Jonathan will present his work at the Moravian College Undergraduate Student Scholarship and Creative Arts Day, Landmark Undergraduate Research Conference, and the National Meeting of the American Chemistry Society in Washington, D.C.

Timetable of Expected Milestones

WEEK 1: Investigation of peer-reviewed literature, introduction to the organic chemistry of peptide synthesis, establishment of project goals

WEEK 2 & 3: Synthesis and purification of LamD linear peptides; cyclization reaction design

WEEK 4: Initial cyclization testing and evaluation

WEEK 5 & 6: Screening of alternative reagents for cyclization optimization; purification of initial cyclized peptides

WEEK 7 & 8: Resynthesis of Lam D linear peptides (if necessary); recyclization of linear peptides with optimized protocol; cyclic peptide purification

WEEK 9 & 10: Purification of recyclized peptides; establishment of bioassay protocol & conditions; preparation of poster for ACS national conference

---

[3]Sturme, Mark H. J., Jiro Nakayama, Douwe Molenaar, Yoshiko Murakami, Ryoko Kunugi, Toshio Fuji, Elaine E. Vaughan, Michiel Kleerebezem, and Willem M. De Vos. "An Agr-Like Two-Component Regulatory System in Lactobacillus Plantarum Is Involved in Production of a Novel Cyclic Peptide and Regulation of Adherence." Journal of Bacteriology (2005): 5224-235. Print.

Qualifications of the Student: Jonathan has completed organic chemistry I, in which he earned an A, and is currently enrolled in organic chemistry II this spring. Thus, he has the necessary background to understand and command his project with my guidance. I have been very impressed with Jonathan's performance in class (and diligence in completing this application) that give me tremendous confidence in his ability to succeed as an independent researcher. In addition, he has gained extra laboratory skills working as a laboratory assistant throughout the fall and spring for our nursing chemistry course. He is very enthusiastic about this project and opportunity. As a chemistry major who plans to continue onto a career in industry and/or graduate school, I know this would be a valuable experience in helping him achieve his future goals.

Engagement in Discipline-Specific Research

As a chemistry major, Jonathan will engage in advanced organic synthesis, utilizing cutting-edge technology to synthesize and purify complex molecules. He will be able to use his knowledge of chemistry to create an efficient and streamlined chemical procedure that future researchers can make use of to efficiently synthesize Lam D derivatives for use in future *Lactobacillus* quorum sensing projects. He will also gain valuable insights into related fields such as medicine and biochemistry, and how these fields can be connected through interdisciplinary scientific research. Working in an independent laboratory setting will allow Jonathan to reinforce learned concepts and learn new laboratory techniques, as well as develop scientific critical thinking and problem solving skills. Collecting and interpreting scientific data will allow him to hone his analytical skills, all while making significant contributions to a new area of scientific research.

Contributions to the Discipline and Community

Jonathan's work will serve as a significant contribution to a new and expanding area of scientific research in my laboratory here at Moravian. His development of an efficient cyclization procedure will be foundational for my future undergraduate mentees to synthesize novel LamD derivatives through their own independent research. From there, meaningful data can be collected regarding the function of LamD and ways to modify the bacterial communication in *Lactobacillus*. More broadly, this project is posed to fill a void in the chemical literature regarding the impact quorum sensing molecules have on non-pathogenic (commensal) bacteria and the benefits they have on human health. Consequently, Jonathan will be producing novel, publishable data as he completes his SOAR project.

Jonathan will have the opportunity to present his research at the Moravian College Undergraduate Student Scholarship and Creative Arts Day, Landmark Undergraduate Research Conference, and the National Meeting of the American Chemical Society in Washington, D.C.

Student Statement of Purpose

Optimizing cyclization of LamD derivatives in preparation for Bioassays of *Lactobacillus plantarum*

Student: Jonathan Nadraws

Major: Chemistry

Expected date of graduation: May 2019

Faculty Mentor: Michael Bertucci

Request for on-campus housing: Yes

My passion for science has led me to the opportunity to engage in discipline-specific research in a field I am very passionate about. I have taken courses in chemistry that have sparked my interest for organic synthesis and this research provides a unique opportunity to make use of my skills as a scientist to contribute to scientific literature, as well as the study of quorum sensing, and the larger biochemical community at large. As a chemistry major, this research will allow me to further hone my critical thinking and analytical skills as I collect scientific data, which I will use to draw conclusions and answer questions, while also creating new demand for further research into a brand new and promising area of study.

This research is immensely valuable for the medical community, and has major implications for the study of bacteria. This project deals specifically with bacterial species that are beneficial to human health. Gaining insight into this field may allow us to modify the behavior of this bacteria so as to increase their functionality. Using this bacteria in products consumed by humans will allow us to strengthen the human immune defenses and increase our quality of life. It also has importance in studying bacterial infection, and how scientists can combat it, as well as broader significance in the study of the evolutionary development of cell to cell communication.

From this amazing opportunity to engage in a new and developing area of research I will gain valuable insights into disciplines closely related to my own, and to see how tightly interwoven they are. From a chemical point of view I would be engaging in chemical synthesis of complex molecules involved in biochemical processes. This would give me valuable insight into many different subjects of biochemistry, preparing me for my future studies. Additionally, it will provide me insight into my potential interests for graduate studies. Working closely with Doctor Bertucci I will get to see firsthand the methodology and techniques experienced scientists use in research, giving me valuable experience in the laboratory and developing my skills as an independent scientist. Devoting my time to a single project will allow me to immerse myself in my research and develop the ability to problem shoot and experiment, skills which are difficult to fully develop in weekly 3-hour labs, as is normal for many science courses. I will carry this valuable experience over into my graduate studies, where I will continue to engage in amazing research.

Optimizing cyclization of LamD derivatives in preparation for bioassays of *Lactobacillus plantarum*

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

Student Researcher: Jonathan Nadraws

Expense Proposal

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

PyBOP (25 g):	$88.50 + shipping
Dawson Dbz AM resin (5 g):	$292.00 + shipping
Fmoc-Gly-OH (250 g):	$108.00 + shipping
Total:	**$488.50 + shipping**

PyBOP and Dawson's resin are chemicals that have been documented in the literature as cyclization reagents. Fmoc-Gly-OH is an amino acid found in LamD, Jonathan's target peptide. These reagents will be integral for screening in Jonathan's experiments to discover an optimal cyclization protocol. We do not have these chemicals present in the chemistry department at Moravian and they will be used exclusively for research purposes.