

Oli Horyn, Pharm.D. major

Investigating the Mechanism of Action of the Sesquiterpene Lactone Laurenobiolide

Goal

Antibiotic resistance is a major threat to public health which makes the discovery of new antibiotics imperative. Laurenobiolide is a naturally occurring compound found in the North American Tulip Tree that has antimicrobial activity on two species of bacteria. Initial work with laurenobiolide revealed that it has antimicrobial activity on methicillin-resistant *Staphylococcus aureus* (MRSA) (Kirk et al., 2022). I have subsequently validated the antimicrobial activity on a methicillin-susceptible strain of *S. aureus* and identified antimicrobial activity against the Gram-negative human pathogen *Francisella tularensis*. The goal of this project is to determine, at the molecular level, how laurenobiolide prevents bacterial cell growth.

Project Objectives

Although we know laurenobiolide inhibits cell growth of certain bacteria, the specific molecular interactions that allows it to exert its effects are unknown. **I am looking to identify the mechanism of action of laurenobiolide on *S. aureus* and *F. tularensis*,** or in other words, what interactions allow laurenobiolide to inhibit growth of these cells at the molecular level. To do this, I will identify spontaneously arising mutant bacteria that will grow on media treated with laurenobiolide, as would indicate the cells are no longer affected, or have become resistant, to the compound. Once we have these mutants, we will isolate their DNA and sequence it to determine how the mutation has altered the cells so they can grow in the presence of laurenobiolide.

Project Plan

Laurenobiolide is a naturally occurring sesquiterpene lactone found in the North American Tulip Tree (*Liriodendron tulipifera*) and inhibits growth of methicillin-resistant *Staphylococcus aureus* MRSA. I have confirmed this inhibitory activity on *S. aureus* and explored its ability to inhibit growth of *Escherichia coli* and *Francisella tularensis*. This revealed that laurenobiolide can inhibit growth of *F. tularensis* but not *E. coli*.

To find the mechanism of action of laurenobiolide, we will look for mutants that are not affected by laurenobiolide. Non-mutant (wild type) cells are exposed to the laurenobiolide using a disc diffusion assay. Disc diffusion assays are described further below but, in short, the presence of laurenobiolide leads to a zone of inhibition, an area in which the compound is present, and the bacteria do not grow. By exposing bacteria to media containing laurenobiolide, we expect to find spontaneous mutant colonies that grow within the zone of inhibition, which may be resistant to laurenobiolide. To confirm resistance, we will isolate the bacteria and test them using a disc diffusion assay, comparing to non-mutant (wild type). Once a laurenobiolide-resistant mutant is found, the DNA of the bacteria will be isolated and sent for whole genome sequencing.

A disc diffusion assay is done by growing bacteria overnight, resuspending the cells into liquid media, and then diluting the cells to a low concentration (optical density at 600 nm [OD₆₀₀] of .05 for *S. aureus* and an OD₆₀₀ of .01 for *F. tularensis*). A specific volume (100 µl) of the bacteria in media is spread onto solid growth media and allowed to dry. Then, two filter paper discs are pressed onto the plate: a control disc impregnated with methanol placed near the edge of the plate, and a disc with laurenobiolide (which is dissolved in methanol) in the center of the plate. The plates are incubated at 37°C for a time period dependent on the species (24 hours and 48 hours for *S. aureus* and *F. tularensis* respectively). The plates are then evaluated by measuring the diameter of the zone of inhibition, a ring where the bacteria did not grow in response to laurenobiolide. The wild-type (WT) strain and the potential laurenobiolide-resistant mutants are tested in biological triplicate and the zone of inhibitions are averaged. The diameters of each are then compared. If we have isolated a laurenobiolide-resistant mutant, we expect that the zone of inhibition is significantly smaller compared to WT or absent. This assay typically takes 3-4 days to complete, depending on the species of bacteria.

Once a mutant is identified, the next step will be to isolate the genomic DNA from the bacteria by using the Lucigen Masterpure Complete DNA & RNA Purification Kit, and then to measure the concentration of the extracted genomic DNA using a Nanodrop spectrophotometer. The DNA would then be sent for whole genome resequencing at SeqCenter. I am experienced in this process, as I have previously confirmed resistance of *F. tularensis* to the antibiotic kasugamycin resulting from inactivating mutations in the *ksgA* gene.

Your Contribution to the Project Concept

This project is based on work I completed over the summer to evaluate the impacts of laurenobiolide on different bacterial species: it was initially tested on *Staphylococcus aureus*, but our goals were to validate the results of *S. aureus* and additionally test it on *Francisella tularensis* and *Escherichia coli*. After the conclusion of the project, I wanted to continue this project and look deeper into function of laurenobiolide, specifically by identifying the mechanism. I proposed an experiment outline and schedule of experiments that I plan to do throughout the rest of the semester to Dr. Ramsey.

Plans for Results

I plan to present my research at the 2023 American Society of Pharmacognosy Annual Meeting. I also would hope to present my research at a more local symposium or conference if the opportunity arises. I am also hoping to contribute to a paper that Dr. Bertin and his lab are working on by providing details about the microbiological work I have done with laurenobiolide.

Value of the Project to You

I plan to work in the pharmaceutical industry, specifically research and development of drugs, and am passionate about the incorporation of natural products into medicine, especially as being the basis for new drugs. I have done research with antibiotics in Dr. K. Ramsey's lab previously and with the addition of working with this compound from Dr. Bertin's lab, it been an amazing opportunity for me to get an insight into what I plan on doing in the future. The hands-

on experience and mentorship have allowed me to further my critical thinking and has challenged me to work more independently. I have also discovered that communicating research is a skill that I enjoy and wish to further develop. Continuing working on this project, especially if I am to present my research in the future, will also help me become a competitive candidate when applying for jobs or to a PGY1 residency after graduation.

Reference List

Kirk RD, Rosario ME, Oblie N, Jouaneh TMM, Carro MA, Wu C, Kim AM, Leibovitz E, Hunter ES, Literman R, Handy SM, Rowley DC, Bertin MJ. Screening the PRISM Library against *Staphylococcus aureus* Reveals a Sesquiterpene Lactone from *Liriodendron tulipifera* with Inhibitory Activity. ACS Omega. 2022 Sep 30;7(40):35677-35685. doi: 10.1021/acsomega.2c03539. PMID: 36249352; PMCID: PMC9558601.

November 14, 2022

Undergraduate Research Initiative Committee
University of Rhode Island
Kingston, RI 02881

Dear Committee Members,

I am extremely pleased to offer my support to Oli Horyn for her undergraduate research project, "Investigating the Mechanism of Action of the Sesquiterpene Lactone Laurenobiolide." This project is a joint effort with the laboratory of Dr. Matthew Bertin. It is worth noting that while my laboratory studies the human pathogen *Francisella tularensis*, we exclusively use the model organism *F. tularensis* subsp. *holarctica* LVS (Live Vaccine Strain), which does not infect or cause disease in humans. Additionally, all of Oli's work using *Staphylococcus aureus* is performed using a methicillin-sensitive (non-MRSA) strain.

Oli is an academically outstanding upper-level student and has been a member of my laboratory since the spring of 2021. During the summer of 2021, when she held a RI-INBRE Summer Undergraduate Research Fellowship (SURF), she became proficient at using a particular technique (disc diffusion assays), validating the susceptibility of several *F. tularensis* mutant strains to various antibiotics. We then focused on a particular antibiotic, kasugamycin, and Oli identified a number of spontaneous kasugamycin-resistant mutants. After isolating these mutants and extracting their DNA, Oli was able to validate that they all had mutations in the *ksgA* gene, which is known to lead to kasugamycin-resistance. While we did not identify any novel mutations, this work provided the ideal training for her current proposed research project.

Over this past summer, Oli was awarded a RI-INBRE Summer Undergraduate Research Fellowship + (SURF+), having already participated in the RI-INBRE SURF program. During this time, she started working collaboratively with the laboratory of Dr. Matthew Bertin to investigate a novel antimicrobial, laurenobiolide. Work performed in Dr. Bertin's lab revealed that this compound inhibits growth of methicillin-resistant *Staphylococcus aureus* (MRSA). Oli's goal was to determine if this antimicrobial activity extended to organisms other than *S. aureus*. She was able to validate sensitivity of methicillin-sensitive *S. aureus* and *F. tularensis*, but not *E. coli*, to laurenobiolide. In our discussions of her results, this raised an important question: how does laurenobiolide exert its antimicrobial effects at the molecular level? Given her experience, she hypothesized that identifying a resistant mutant would provide insight into how laurenobiolide functions to inhibit bacterial growth. I enthusiastically support the continued development and undertaking of her independent research project.

Together with my graduate students, I will supervise all the proposed work in this project to ensure safety, rigor, and reproducibility. The success of this project will be based on Oli's ability to (i) learn and implement research protocols, (ii) critically analyze the resulting data, (iii) make connections between the resulting data and the current scientific literature, and (iv) effectively communicate research findings, both within and outside our research group.

Oli is an outstanding student with great enthusiasm for laboratory research. Her undergraduate research experience in my laboratory, including the exciting work proposed here, will benefit her as she works towards her goal of working in the pharmaceutical industry. She is

extremely well-prepared to carry out the proposed experiments and I support her application for an undergraduate grant for original student research without reservation and with enthusiasm.

Sincerely,

A handwritten signature in black ink, appearing to be 'KR' followed by a horizontal line.

Kathryn M. Ramsey, PhD
University of Rhode Island
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