Applicant: Oli Horyn
Faculty Advisor: Dr. Kathryn Ramsey
ASP Faculty Advisor: Dr. Matthew Bertin

Investigating the Mechanism of Action of the Sesquiterpene Lactone Laurenobiolide

With constantly evolving bacteria threatening the efficacy of antibiotics, the discovery of novel antimicrobials is imperative. Laurenobiolide is a sesquiterpene lactone isolated from the North American Tulip Tree *Liriodendron tulipifera*. It has been shown that laurenobiolide has antimicrobial activity against methicillin-resistant *Staphylococcus aureus (*MRSA) *(*Kirk et al., 2022). I was able to confirm this activity against a methicillin-susceptible strain of *S. aureus* and I further determined it has antimicrobial activity against an attenuated strain of the Gram-negative human pathogen *Francisella tularensis*, *F. tularensis* susbsp. *holarctica* LVS (**Figure 1**). Due to the inhibition of growth on these two species, laurenobiolide may have broad-spectrum activity; however, the compound necessitates further investigation of susceptible strains. The goal of this project is to determine how laurenobiolide exerts its effects to prevent bacterial cell growth.

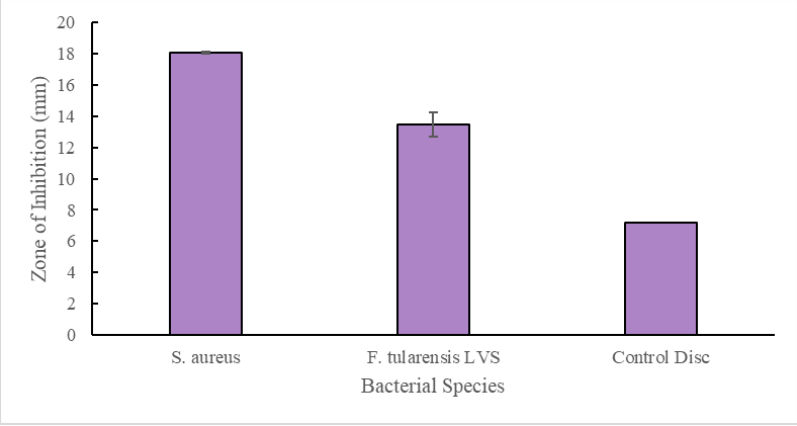

Figure 1. Laurenobiolide inhibits the growth of *S. aureus* and *F. tularensis* LVS. Representative data from a disc diffusion assay. Assays are performed in biological triplicate and have been repeated at least twice for validation.

Although we know laurenobiolide inhibits cell growth of certain bacteria, the specific mechanism is unknown. I am interested in identifying the mechanism of action of laurenobiolide within species *S. aureus* and *F. tularensis*. To accomplish such tasks, I will identify spontaneously arising mutant bacteria that grow on media treated with laurenobiolide, as would indicate the cells are no longer affected by the antimicrobial. Once we've obtained such mutants, we will isolate and sequence their DNA to determine how the mutation has altered the genome allowing for cell proliferation in the presence of laurenobiolide.

Approach

Wild-type (WT) cells are exposed to laurenobiolide using a disc diffusion assay. By exposing bacteria to a range of concentrations of laurenobiolide present in the media as the drug diffuses away from the concentrated laurenobiolide on the disc, we expect to find spontaneously arising mutant colonies that grow within the zone of inhibition that phenotypically appear resistant. To confirm resistance, we will isolate the potentially resistant bacteria and test them using a disc diffusion assay in comparison to WT cells. Once a laurenobiolide-resistant mutant is identified and confirmed, the DNA of each bacterial strain will be isolated and sent for whole genome sequencing.

Methodology

A disc diffusion assay is initiated by growing *S. aureus* and *F. tularensis* on either LB agar and CHAH (cystine heart agar with hemoglobin), respectively, at 37°C overnight, resuspending the cells into liquid media (LB media and Mueller Hinton Broth, respectively) and then diluting the cells to a low concentration (optical density at 600 nm [OD_{600}] of 0.05 for *S. aureus* and an OD_{600} of 0.01 for *F. tularensis*). A specific volume (100 µl) of the bacteria in media is spread onto

the corresponding 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 8mg/mL laurenobiolide (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 where the bacteria are absent in response to laurenobiolide. The WT strain and the potential laurenobiolide-resistant mutants are tested in biological triplicate and the zones of inhibition are averaged for comparison. 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 with the addition of Lysostaphin for the isolation of *S. aureus* gDNA. The concentration and quality of the extracted genomic DNA will be assessed using a Nanodrop spectrophotometer. The DNA will then be sent for whole genome resequencing at SeqCenter.

Once results are obtained from SeqCenter, the analysis of mutations will be reviewed to compare the mutant strains to WT to identify which genes contain potential causative mutations of resistance. Then we can begin to assess the potential mechanisms of action of laurenobiolide by inference of relevant pathways. This is a common and effective method to identify drug targets (Imai et al., 2019). If time permits, we will start to validate the resistance-causing mutations by creating clean deletions of the mutated genes and testing for resistance to laurenobiolide; however, completion of these studies and final validation of the drug target may be beyond the scope of this project.

In summary, the identification of the mechanism of action for laurenobiolide may be an advancement toward the development of a novel broad-spectrum antimicrobial. The amount awarded would contribute to the materials required to facilitate the isolation of laurenobiolide-resistant mutants, genomic DNA extraction, and bacterial whole genome resequencing.

References

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