Investigating the Molecular Mechanism of Action of the Sesquiterpene Lactone Laurenobiolide

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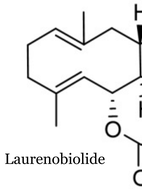
INTRODUCTION

- Antibiotic resistance is a significant threat to public health
- Novel antimicrobials may be developed based on molecules derived from plants

Laurenobiolide

- Sesquiterpene lactone
- Isolated from North American tulip tree *Liriodendron tulipifera*
- Used by indigenous tribes as a treatment for malaria
- Bioassay-guided approach identified laurenobiolide as effective against MRSA[1]



Laurenobiolide

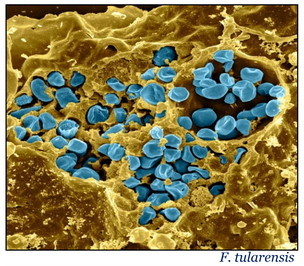
Staphylococcus aureus

- Gram-positive
- Causes skin infections
- Can be multi-drug resistant

S. aureus

Francisella tularensis

- Gram-negative
- Causes tularemia
- Potential bioweapon due to its highly infectious nature



F. tularensis

Escherichia coli

- Gram-negative
- Causes foodborne illness

E. coli

METHODS AND MATERIALS

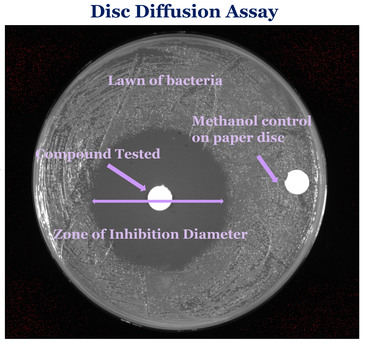
Disc Diffusion Assay Workflow

- Plated a lawn of bacteria in triplicate
- Two discs per plate: control (methanol) and disc impregnated with compound
- Incubated at 37°C
  - 24 hours for *E. coli* and *S. aureus*
  - 48 hours for *F. tularensis*
- Performed with indicated organisms in biological triplicate using discs impregnated with 8 mg/mL laurenobiolide.
- Plates were incubated for 24 or 48 hours (organism dependent), and zones of inhibition were measured in mm.

Organisms Tested

Staphylococcus aureus - SA113
Escherichia coli - ATCC25922
Francisella tularensis – Live Vaccine Strain (LVS)

Disc Diffusion Assay



RESULTS

Antimicrobial Activity of Laurenobiolide

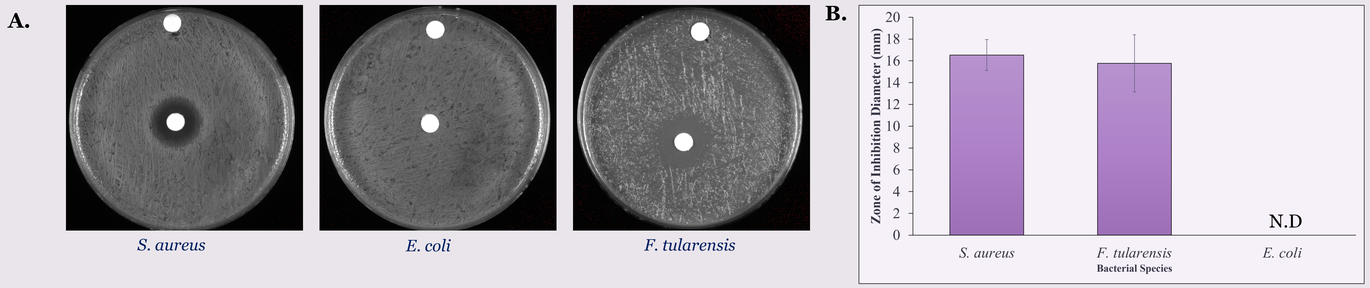


Figure 1. Multiple bacterial species are sensitive to laurenobiolide. A. Representative image of disc diffusion assay results, using indicated bacteria. **B.** Quantification of disc diffusion results. Error bars represent standard deviation. ND indicates no zone of inhibition detected.

Isolation of Laurenobiolide-Resistant Mutants

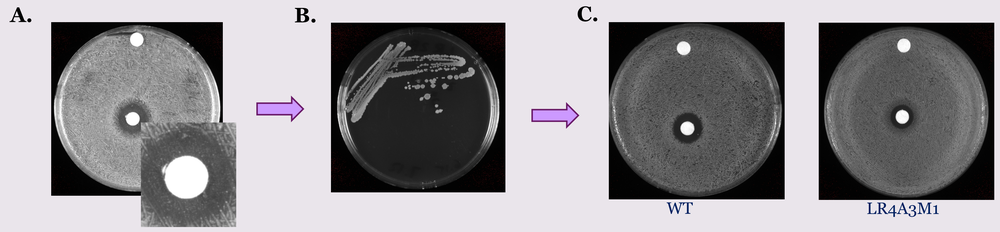


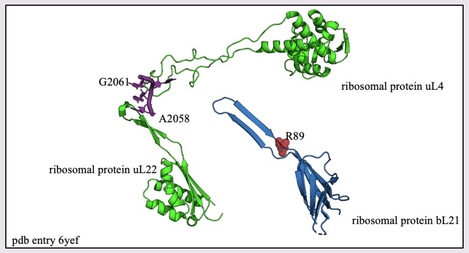
Figure 3. Testing colonies that grew within the laurenobiolide zone of inhibition for resistance to laurenobiolide. A. Selected potential mutants from laurenobiolide disc diffusion plates. **B.** Isolated single colonies of potential mutants. **C.** Disc diffusion assay assessed sensitivity of potential resistant mutants to laurenobiolide compared to wild-type *S. aureus*. **D.** 3 isolates tested (LR4AM1, LR4AM3, and LR4A3M4) have decreased levels of laurenobiolide sensitivity compared to the wild type.

Identification of Mutations

Position	Mutation	Genetic Variant	Annotation	Gene	Description
1,528,329	A→T	Synonymous	I189I (ATT→ATA)	*SAOUHSC_01602*	maltose operon transcriptional repressor
1,659,236	C→G	Missense	R89P (CGT→CCT)	*rplU*	50S ribosomal protein bL21
2,079,882	$(T)_{8\rightarrow7}$	Frameshift Deletion	coding (780/846 nt)	*SAOUHSC_02245*	SAM-dependent methyltransferase

Figure 4. Mutations associated with LB resistance identified across all three mutant strains that were not present in the wild type SA113. These mutations were identified by whole genome sequencing, and all were found in each mutant genome.

A.



B.

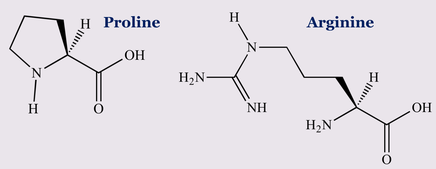


Figure 5 Impact on R89P mutation on bL21. A. R89 is the 89th amino acid on the bL21 protein and is far from the macrolide binding site. Figure generated by Dr. Steven Gregory. **B**. Structures of proline and arginine. Protein 89 in bL21 altered from an arginine into a proline. The difference in structure may alter binding affinity and conformation.

SAOUHSC_02245
MKQLVGIPESMLIPLIARAKEYENEKPIIKDALSKKIFDGLDDMYKNVTCDDMSQIG
ISIRTVIIDCVTKRLIKDNKDLIVVNIGCGLDTRFQRFNKEKISWIDLDVPESIEIRKT
FFKESNSYKMISKSMLDYSWIDDVKNYKFFNSKSDILFIIEGVLMYFDESVMTQLLD
TIIKKMGDHNLTFAIEFCSKTIANNTKRHQSVSKLSSQPVFKYGYNDLKKLNEILPN
TIRVIHEYNYFDYYKNRWGLFGYCRFIPYLKK**RLNNKIVLMKYKVPKRQRH**

Figure 6. Impact on frameshift mutation on SAM-dependent methyltransferase
The wild-type SAM-dependent methyltransferase protein sequence is shown; the domain is highlighted in purple. A single nucleotide deletion results in frameshift, causing the mutant protein to lose 18 amino acids, potentially altering the protein structure and folding.

CONCLUSIONS AND FURTHER STEPS

Laurenobiolide Antimicrobial Activity

- *S. aureus*, a Gram-positive bacterium, confirmed sensitive
- Active against *F. tularensis*, a Gram-negative bacterium
  - Suggests possibility for broad spectrum antibiotic
- *E. coli* did not exhibit sensitivity at concentration of 8mg/mL

Isolation of Laurenobiolide-Resistant Mutants

- May aid us in identifying the mechanism of action

Identification of Mutations

- Two genes with potential resistance-causing mutations were identified: rplU and SAOUHSC_02245

Ongoing work: Complementation

- For each gene of interest, reintroduce the wild-type gene into the resistant mutant.
- We expect that the wild-type version of one of the two genes will restore sensitivity. This would suggest that in the laurenobiolide-resistant mutant, the mutation is the complemented gene is the cause of laurenobiolide resistance.

References

1. Kirk, Riley D., "Evaluating natural product libraries with emphasis on in vitro permeability workflows" (2021). *Open Access Dissertations*. Paper 1294. https://digitalcommons.uri.edu/oa_diss/1294

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