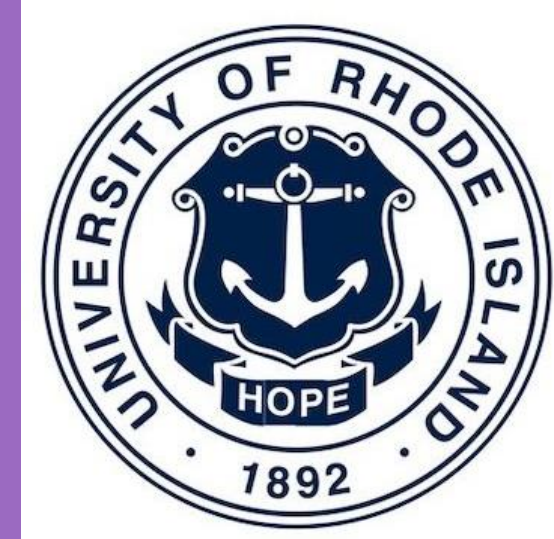


Investigating the Antimicrobial Activity of the Sesquiterpene Lactone Laurenobiolide

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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 MRSA¹

Staphylococcus aureus

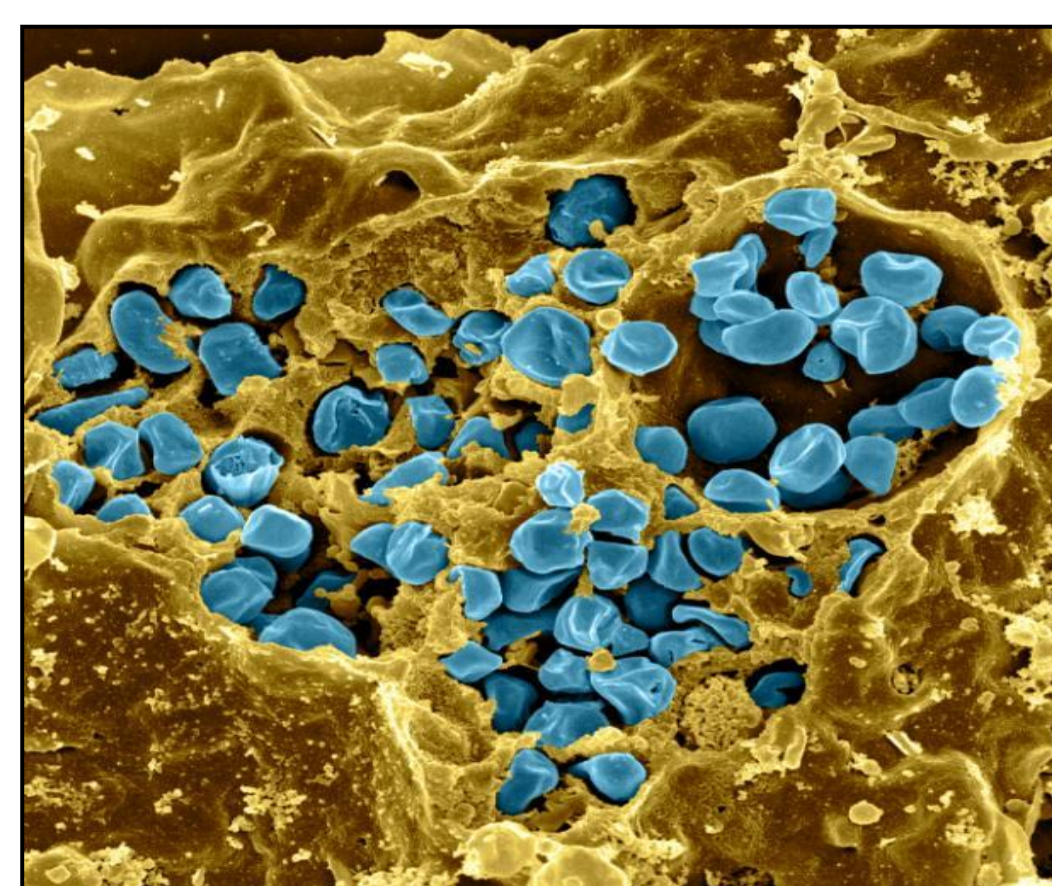
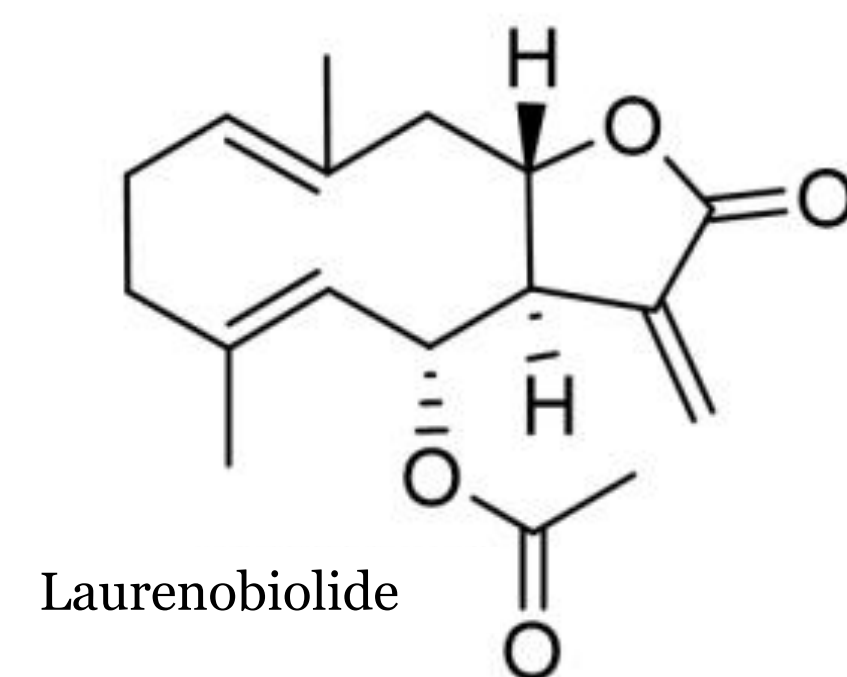
- Gram-positive
- Causes skin infections
- Can be multi-drug resistant

Francisella tularensis

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

Escherichia coli

- Gram-negative
- Causes foodborne illness



METHODS AND MATERIALS

Disc Diffusion Assay Workflow

- Plated bacteria at optimized concentration 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*

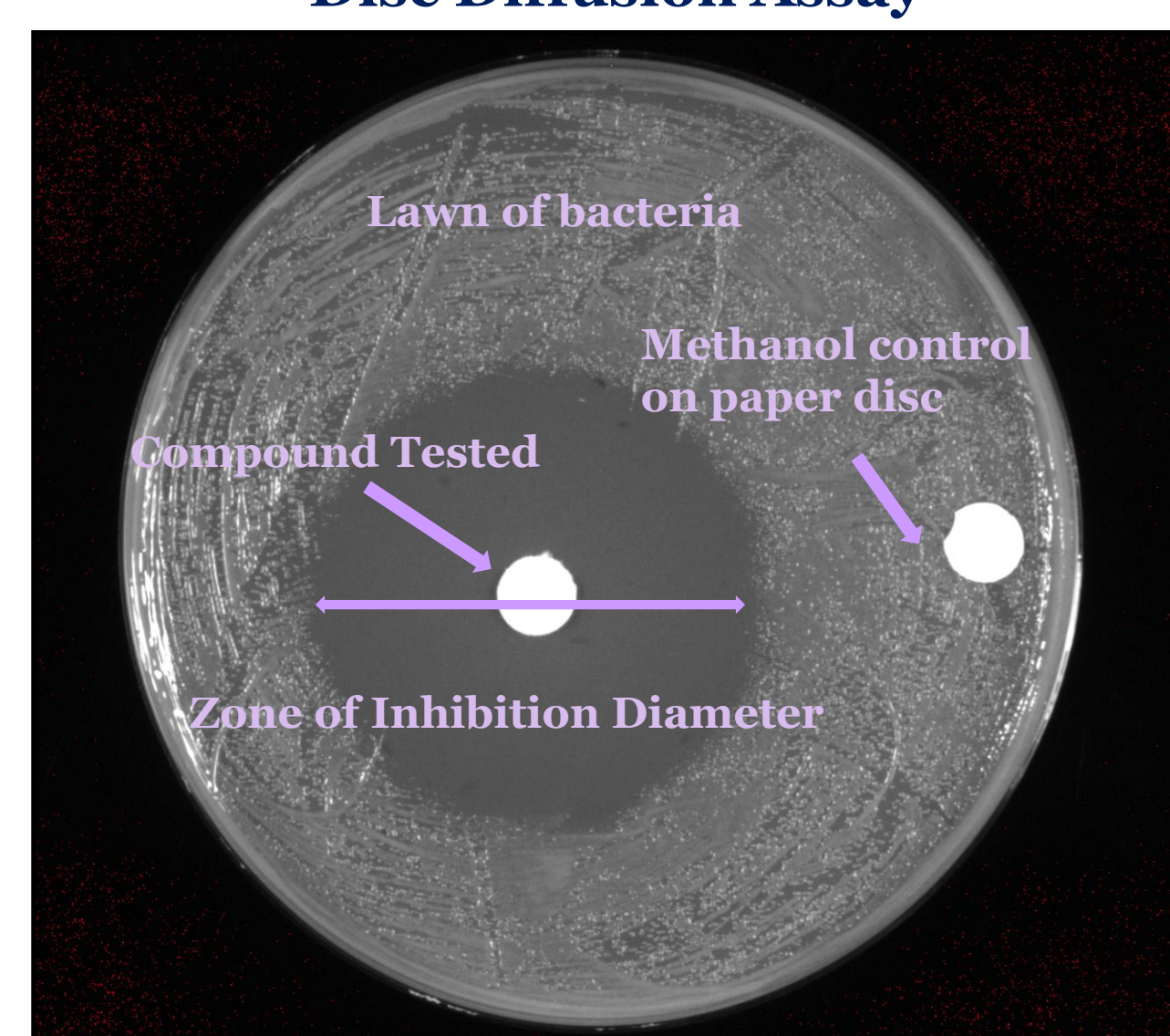
Compounds Tested

- Laurenobiolide
- Extracts from
 - *L. tulipifera*
 - *L. chinense*
 - *L. tulipifera* – *L. chinense* hybrid

Organisms Tested

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

Disc Diffusion Assay



RESULTS

Antimicrobial Activity of Laurenobiolide

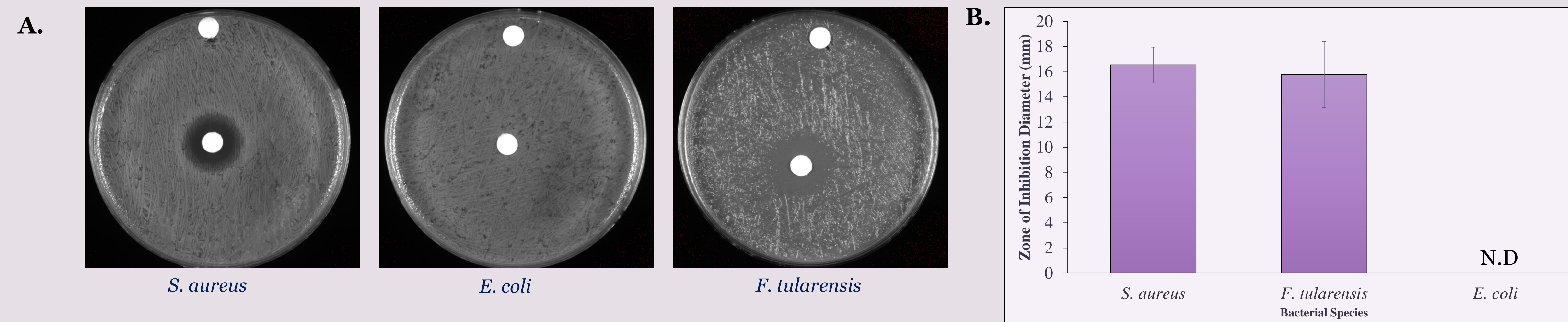


Figure 1. Multiple bacterial species are sensitive to laurenobiolide. A. Representative image of disc diffusion assay results, using indicated bacteria B. Quantification of disc diffusion results. Disc diffusion assays were performed with the indicated strains in biological triplicate using discs impregnated with 8 mg/mL laurenobiolide. Plates were incubated for 24 or 48 hours (strain dependent) and zones of inhibition were measured in mm. Error bars represent standard deviation. ND indicates no zone of inhibition detected.

Antimicrobial Activity of Tree Extracts

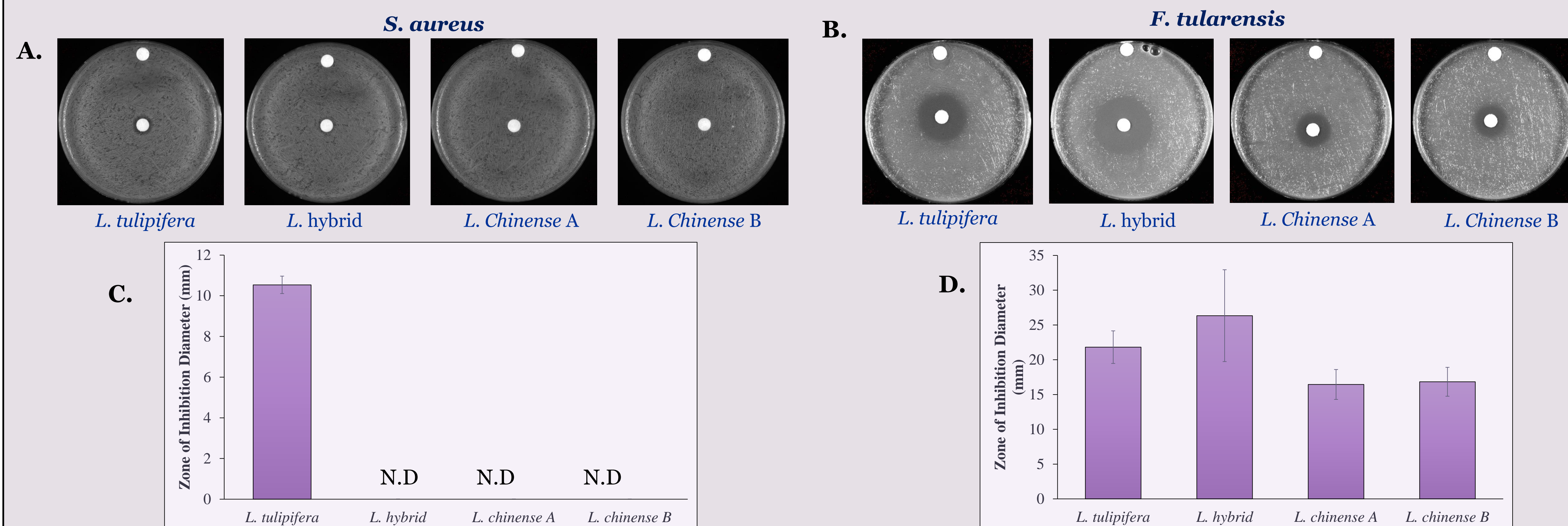


Figure 2. Testing of tree extracts performed to verify sensitivity consistent with laurenobiolide assay results. A and B. Representative image of disc diffusion assay results, using indicated bacteria. C and D. Quantification of disc diffusion results. Disc diffusion assays were performed with the indicated strains in biological triplicate using discs impregnated with 10 mg/mL sample extract. Plates were incubated for 24 or 48 hours depending on strain, and zones of inhibition were measured in mm. Error bars represent standard deviation. ND indicates no zone of inhibition detected.

CONCLUSIONS AND FURTHER STEPS

Laurenobiolide Antimicrobial Activity

- *S. aureus* confirmed sensitive
- Laurenobiolide 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

Antimicrobial Activity of Tree Extracts

- *S. aureus* is only sensitive to *L. tulipifera* extract, consistent with presence of laurenobiolide.
- *F. tularensis* is sensitive to all tested extracts, suggesting the presence of other inhibitory compounds

Looking for Laurenobiolide-Resistant Mutants

- May allow us to determine mode of action

References

- 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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Looking for Laurenobiolide-Resistant Mutants

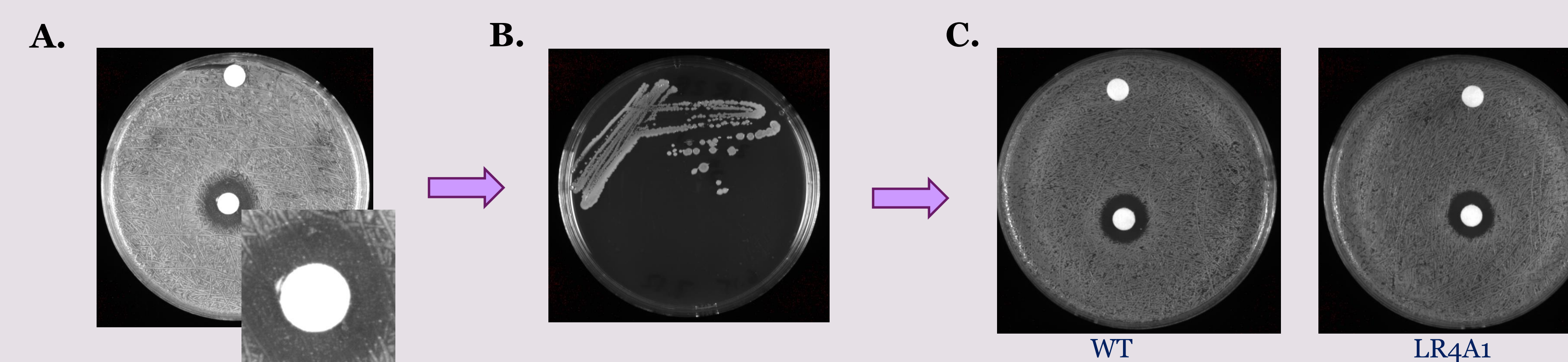


Figure 3. Testing colonies that grew within the laurenobiolide zone of inhibition for resistance to laurenobiolide. A. Selected potential mutants from laurenobiolide disc diffusion plates. B. Isolated single colonies of potential mutants. C. Used disc diffusion assay to assess sensitivity of potential laurenobiolide-resistant mutants to laurenobiolide compared to wild type *S. aureus*. Eight potential laurenobiolide-resistant colonies were purified. Three purified colonies were then picked per each original potential laurenobiolide-resistant colony for a total of 24 isolates. Nine isolates have been compared to wild type *S. aureus*. All isolates tested so far have wild type levels of laurenobiolide-resistant sensitivity.