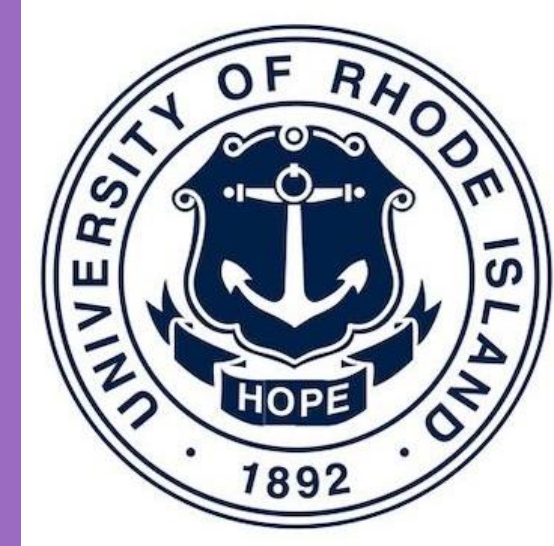


Investigating the Antimicrobial Activity of the Sesquiterpene Lactone Laurenobiolide



Oli Horyn¹, Hannah Trautmann³, Kathryn M. Ramsey^{2,3}, Matthew Bertin^{1,3}

¹Department of Pharmacy Practice, University of Rhode Island, Kingston, RI 02881

²Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02881

³Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881

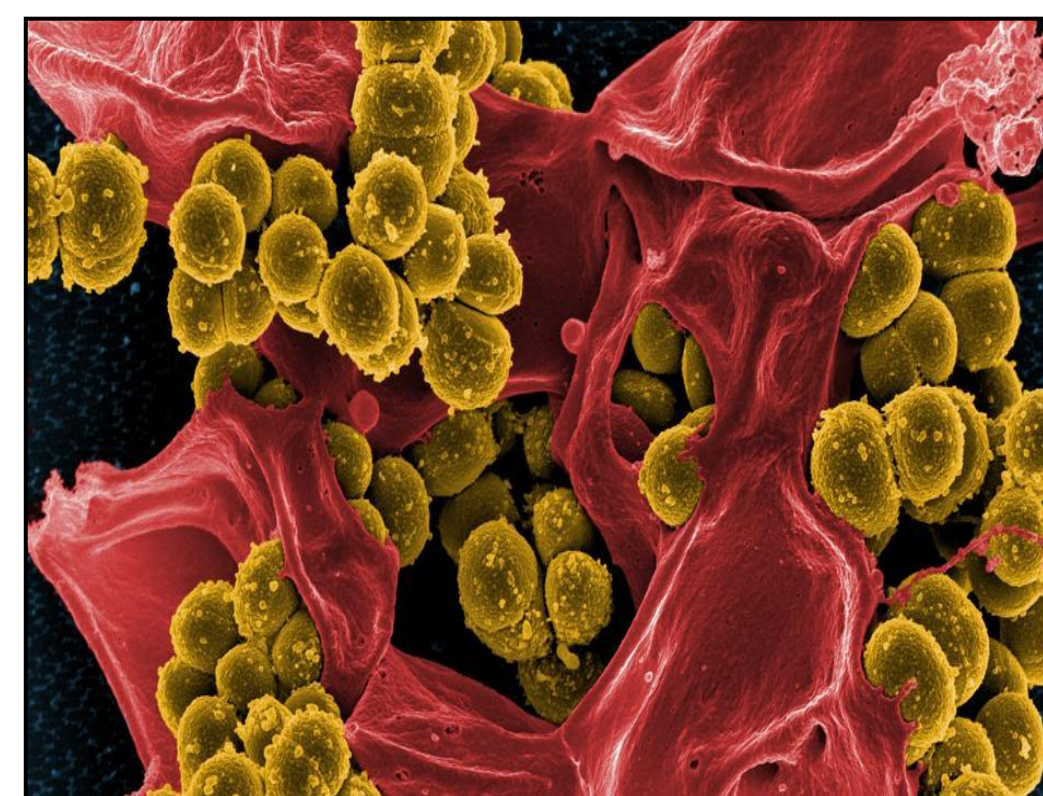
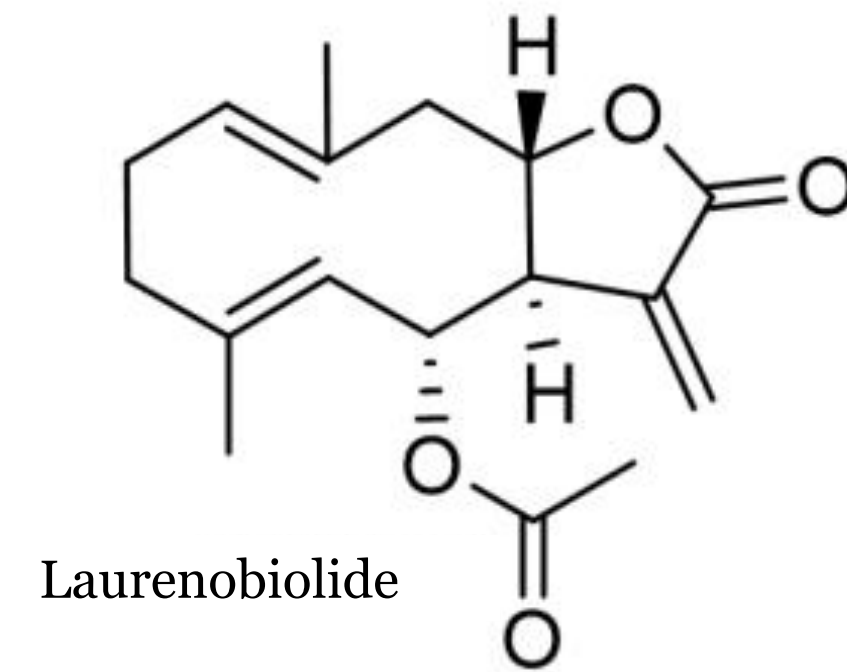


INTRODUCTION

With increasing antibiotic resistance, looking for novel antimicrobials is essential for public health. A novel antimicrobial Laurenobiolide was isolated from the URI PRISM library by the Bertin Laboratory and tested its antimicrobial activity against a variety of bacterial organisms by the Ramsey Laboratory.

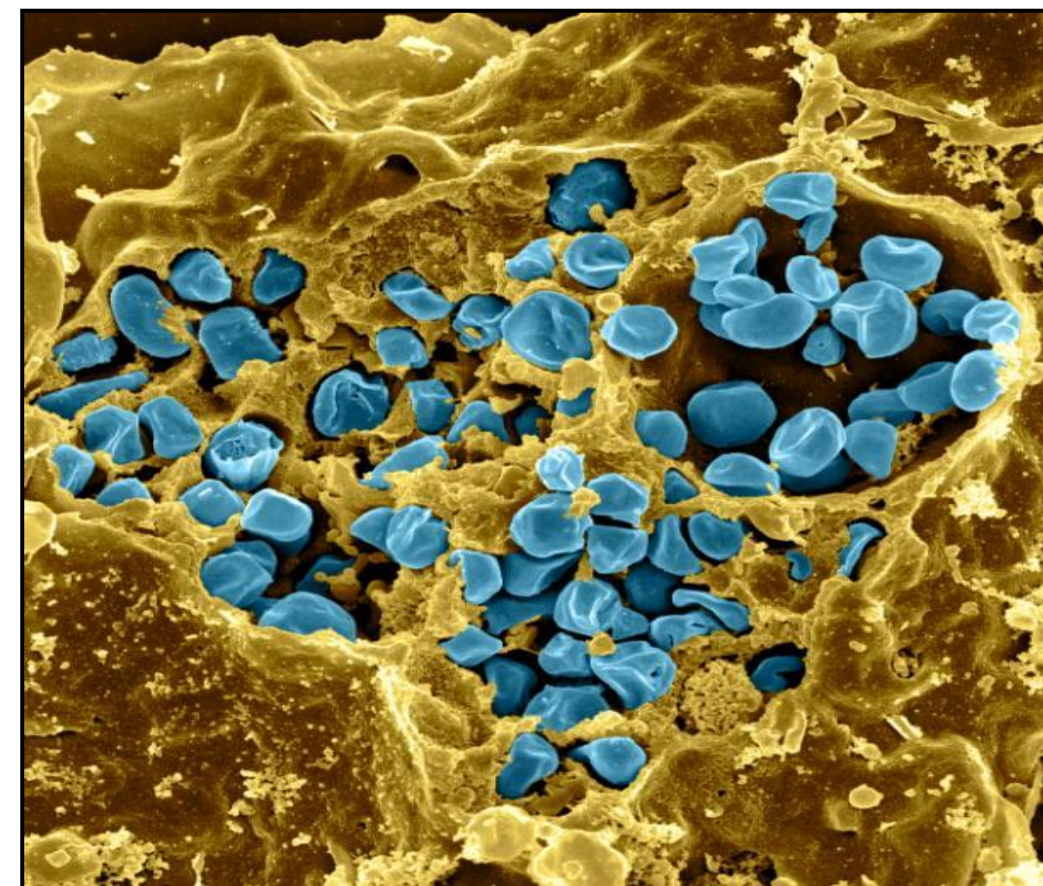
Laurenobiolide

- Known sesquiterpene lactone
- Isolated from North American tulip tree *Liriodendron tulipifera*
 - Found to be most abundant in twigs
- Used by indigenous tribes as a treatment for malaria
- A former graduate student identified laurenobiolide as effective against MRSA



Staphylococcus aureus

- Gram-positive bacterium
- Causes skin infections
- Methicillin-resistant staphylococcus aureus (MRSA)



Francisella tularensis

- Gram-negative bacterium
- Causes tularemia, a potentially lethal illness
- Potential bioweapon due to its highly infectious nature



Escherichia coli

- Gram-negative bacterium
- Causes foodborne illness

METHODS AND MATERIALS

Organisms Tested

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

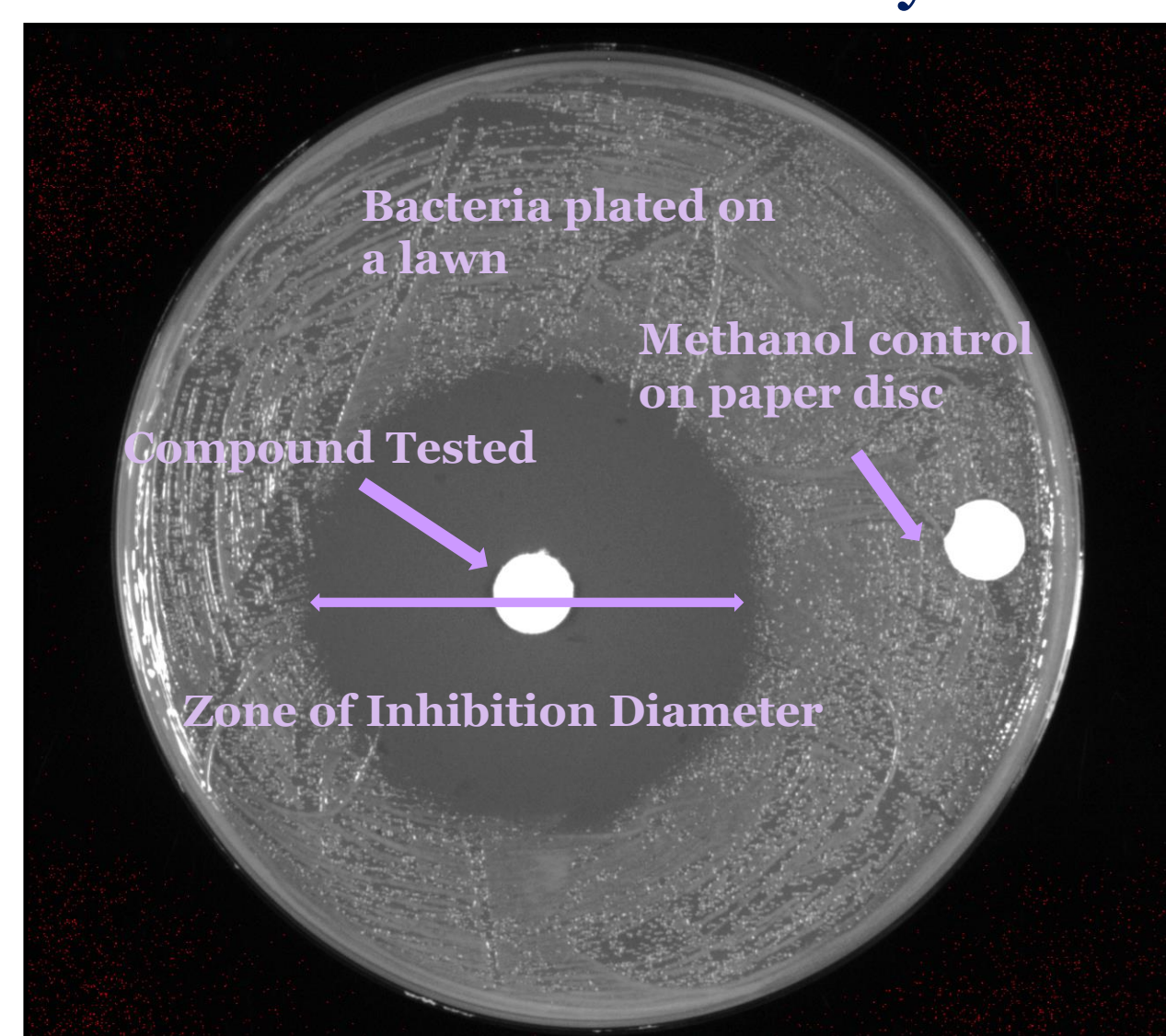
Lawn Optimization

All strains were grown at different concentrations to optimize cell growth and coverage

Compounds Tested

Laurenobiolide
 Crude *L. tulipifera* extract
 Crude *L. chinense* extracts
 Crude hybrid of both *L. chinense* and *L. tulipifera*

Disc Diffusion Assay



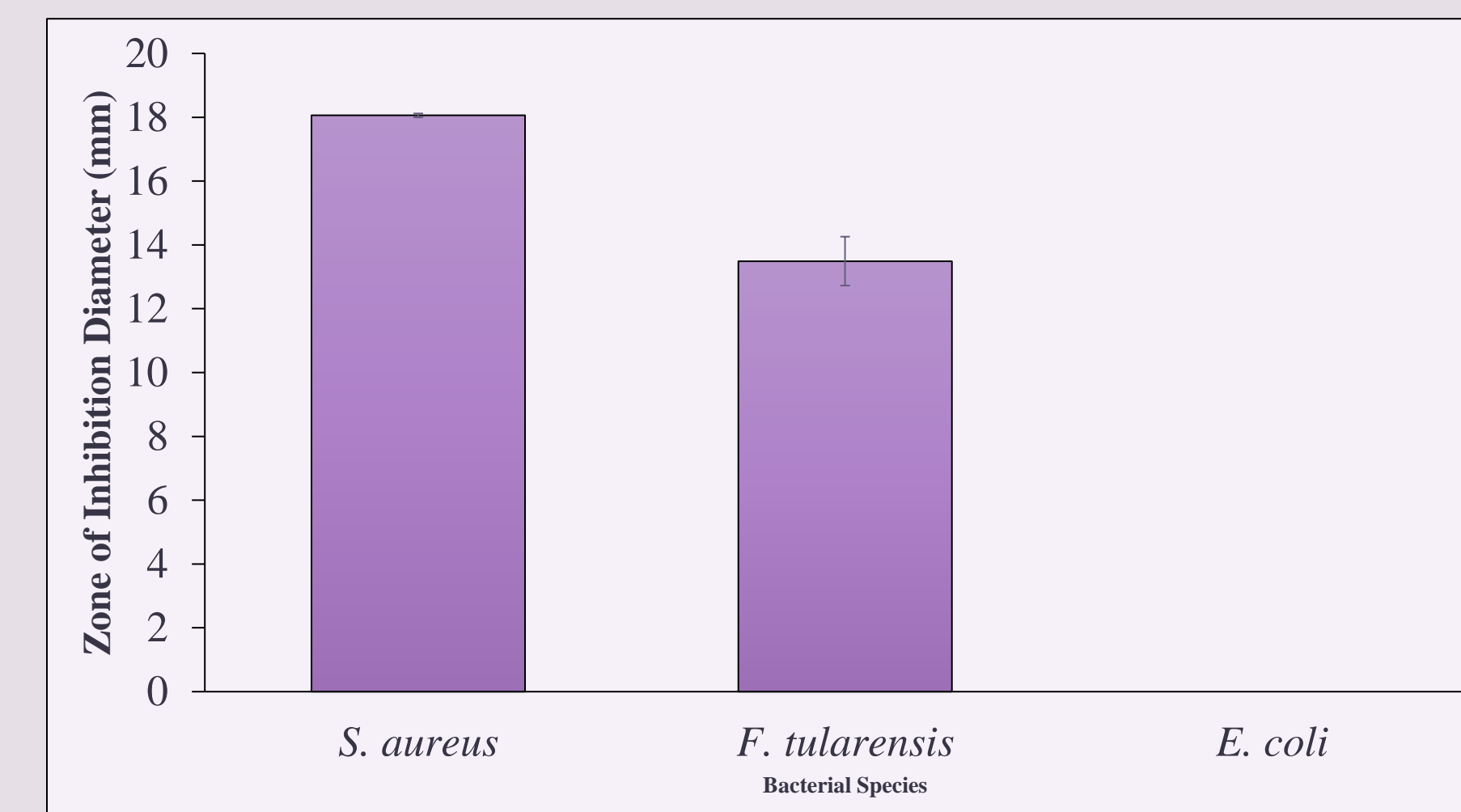
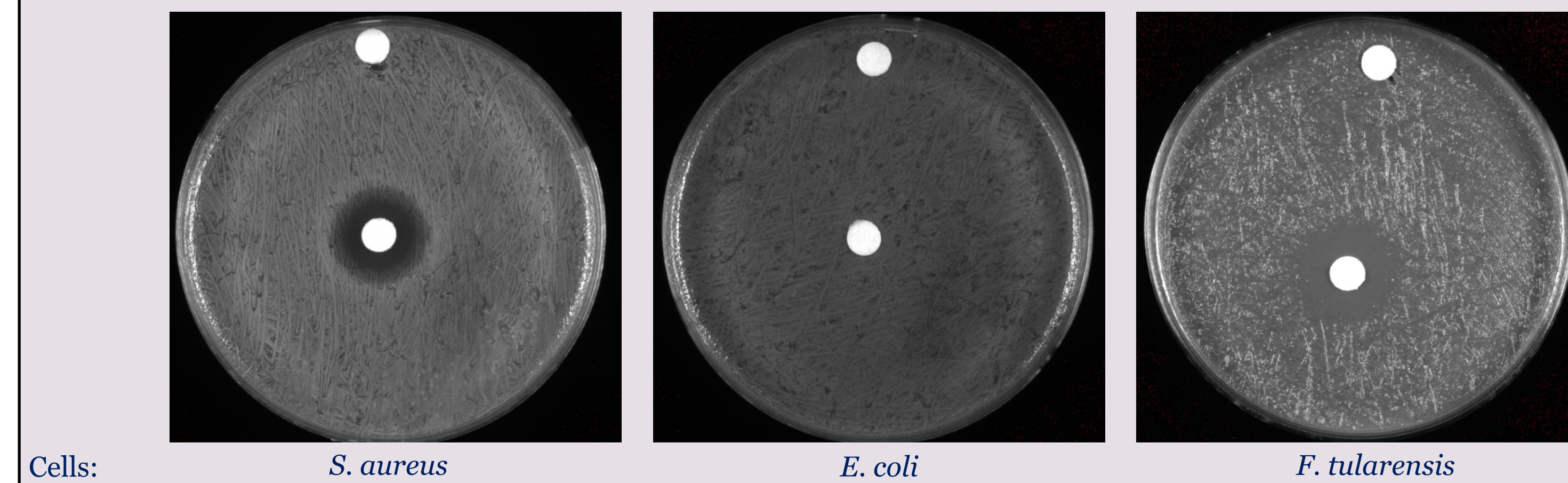
Bacteria plated on agar at specific concentration

Two discs per plate: control (methanol/CH₃OH) and disc impregnated with compound and concentration (see indicated)

Incubated for 24 hours (*E. coli* and *S. aureus*) or 48 (*F. tularensis*)

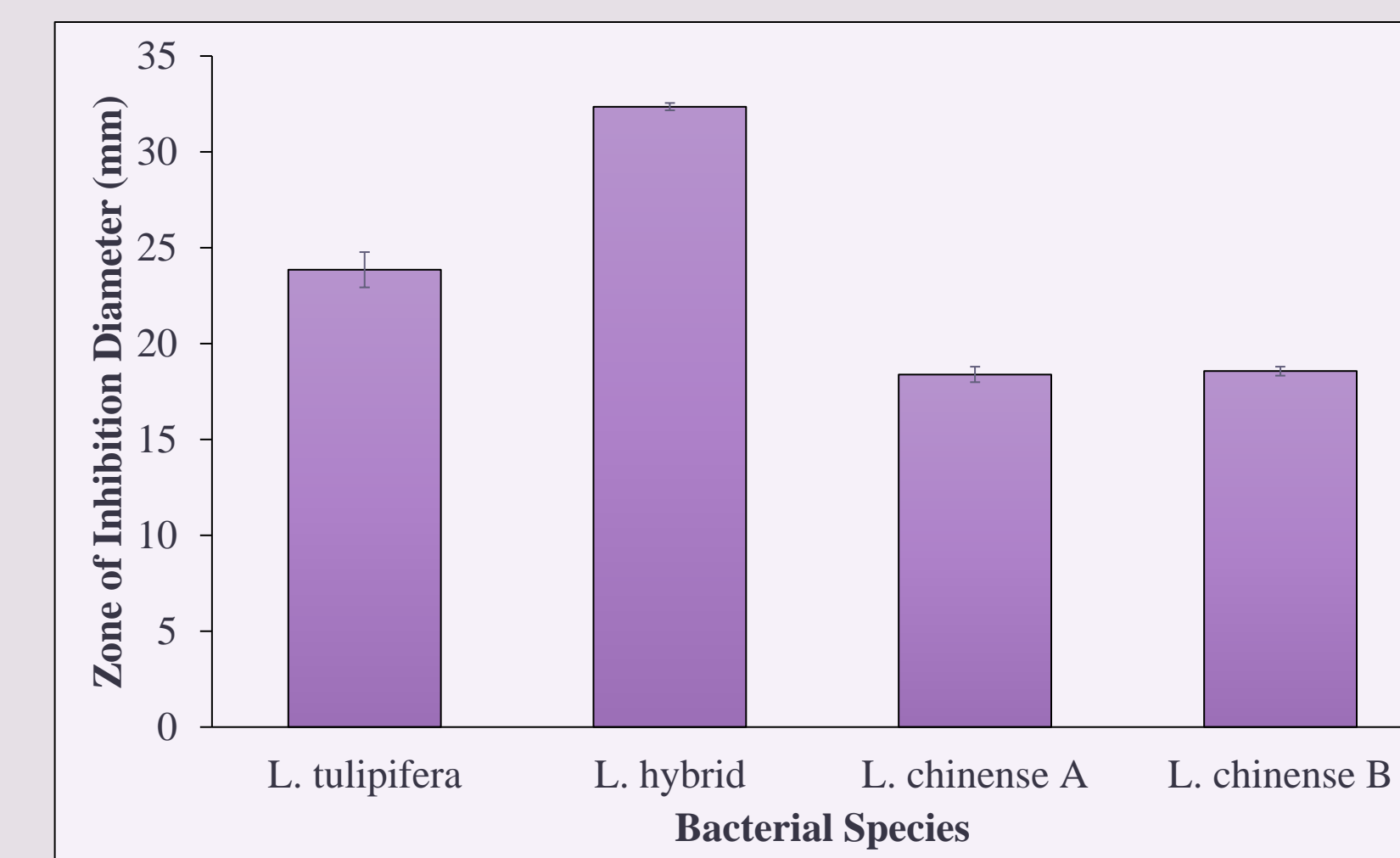
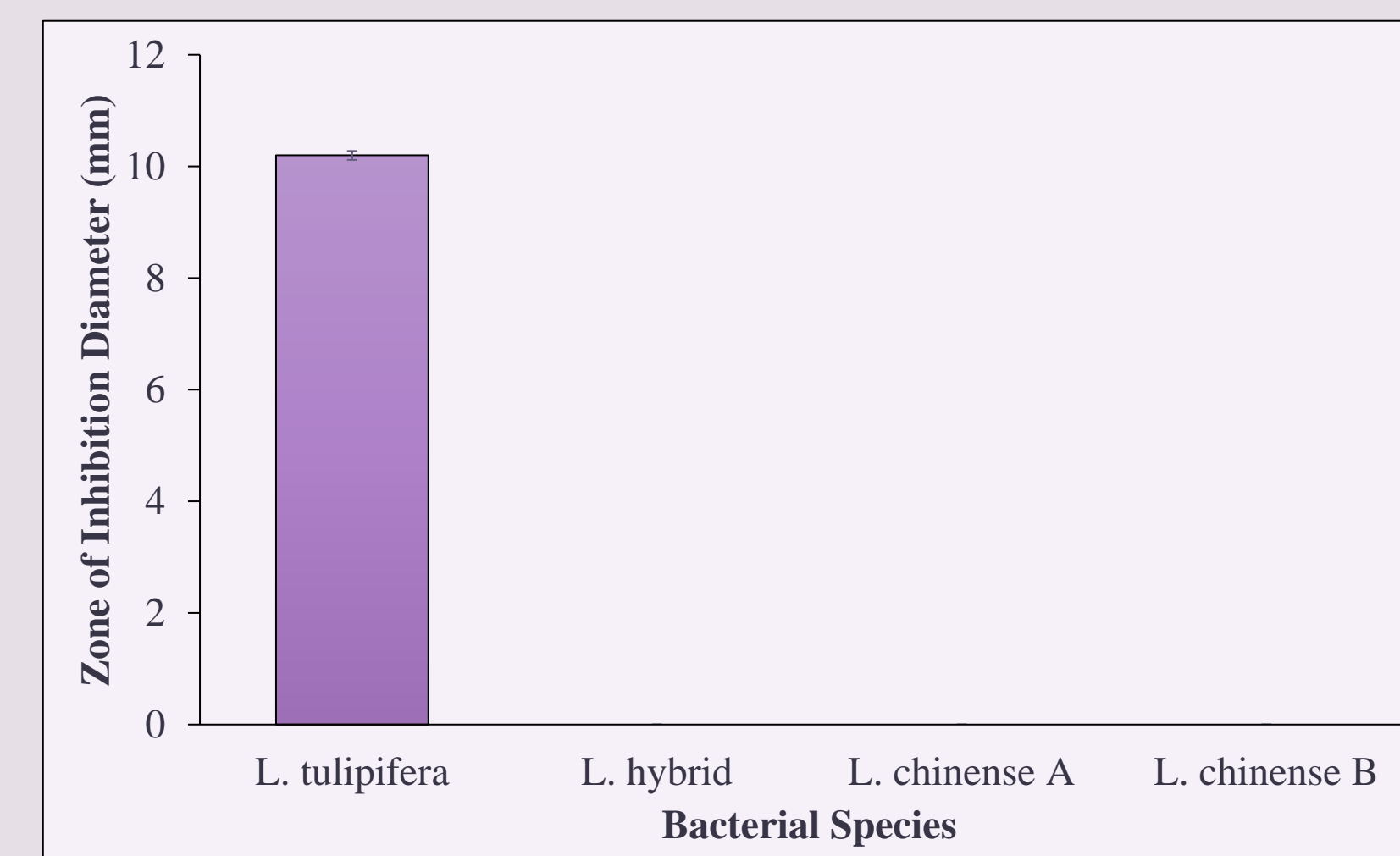
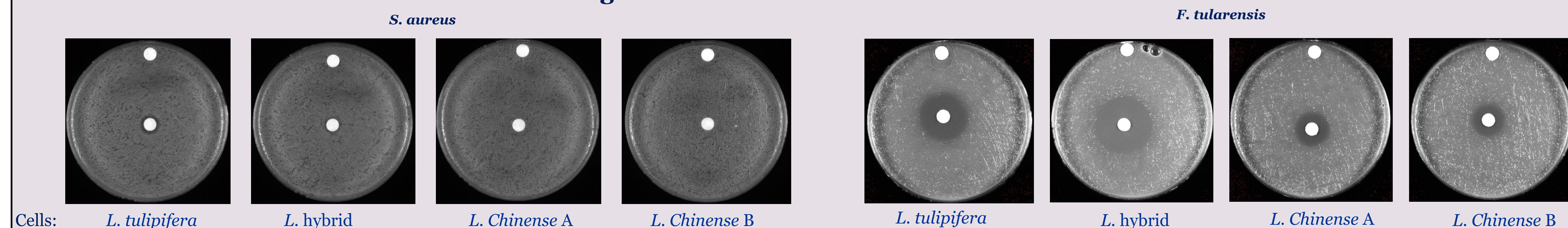
RESULTS

Laurenobiolide tested on *S. aureus*, *F. tularensis*, and *E. coli*



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.

Four Extract Testing on *S. aureus* and *F. tularensis*



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.

Identification of Laurenobiolide Resistant Mutants

Images of preliminary screening (waiting for data)

Bar Graph here vs WT

Preliminary screening was performed with *S. aureus* with one biological replicate using discs impregnated with 8 mg/ml sample extract. Plates were incubated for 24 hours, and zones of inhibition were measured in mm. Replicates were compared against Wild Type to determine mutants of note to send for whole genome sequencing. Error bars represent standard deviation.

CONCLUSIONS AND FURTHER STEPS

Laurenobiolide Sensitivity

- S. aureus* and *F. tularensis* confirmed sensitive
- E. coli* did not exhibit sensitivity

Investigating Four Extracts

- Verified action of laurenobiolide into *S. aureus* and *F. tularensis*
- Potential other sesquiterpene lactone action in *F. tularensis*

Isolated Laurenobiolide Resistant Mutants

- Next step into determining mode of action

References

A new antibiotic selectively kills Gram-negative pathogens

Imai Y, Meyer KJ, Iinishi A, Favre-Godal Q, Green R, Manuse S, Caboni M, Mori M, Niles S, Ghiglieri M, Honrao C, Ma X, Guo JJ, Makriyanis A, Linares-Otaya L, Böhringer N, Wuisan ZG, Kaur H, Wu R, Mateus A, Typas A, Savelkoul HF, Koozebakhsh A, Nashed KZ, Pankov G, Giamberini M, Nishimura T, Nishimura T, Nofrio A, Lewis K. Nature. 2019 Dec;576(7787):459-464. doi: 10.1038/s41586-019-1791-1. Epub 2019 Nov 20. Erratum in: Nature. 2020 Apr;580(7802):E3. PMID: 31747680; PMCID: PMC7188312.

Acknowledgments

Research reported in this presentation was supported by the Rhode Island Institutional Development Award (IDeA) Network for Biomedical Research Excellence from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103430. We would like to thank Dr. Bertin and lab and Dr. Gregory and lab for gifts of antibiotics and productive joint lab meetings.