Research Outline

Project Name: Investigating the Antimicrobial Activity of the Sesquiterpene Lactone Laurenobiolide

Description – We will investigate the effects of laurenobiolide, a sesquiterpene lactone, on the growth of three bacterial species: *Staphylococcus aureus*, *Escherichia coli*, and *Francisella tularensis* subspecies *holarctica*.

Background –Laurenobiolide is a known sesquiterpene lactone isolated from the North American tulip tree *Liriodendron tulipifera*. It has known antimicrobial activity on Methicillin-resistant *Staphylococcus aureus* (MRSA) and is found to be most abundant in the twig bark. We are working with purified laurenobiolide that was isolated by Dr. Matthew Bertin's lab at URI from the twigs of the *L. tulipifera*. This molecule is also part of an extract found in the Principal Rhode Island Secondary Metabolite (PRISM) library, a collection of extracts from specimens from the URI Heber W. Youngken Jr. Medicinal Garden. We will also be testing the antimicrobial activity of three other related compounds: two samples from two *Liridendron chinense* trees, which has a similar genetic composition but unlike the *L. tulipifera*, does not contain laurenobiolide, and an extract from a hybrid of the *L. tulipifera* and the *L. chinense*.

S. aureus is a spherical shaped, Gram-positive bacterium and pathogen which typically causes infections that afflict the skin, presenting as sores, bumps, redness, among other symptoms. MRSA is a nosocomial pathogen that commonly afflicts patients and is difficult to treat. *E. coli* is a Gram-negative bacterium that typically lives in human intestines but will often cause foodborne illness in humans. *F. tularensis* is a Gram-negative, highly infectious pathogen that is classified as a potential bioweapon because it has the capability to cause lethal illness. Depending on the mode of entry, it can have varying presentations but typically causes a flu-like illness. In the Ramsey laboratory, we use *F. tularensis* subsp. *holartica* Live Vaccine Strain (LVS), which is closely related to pathogenic strains but is attenuated for virulence in humans.

Hypothesis – Laurenobiolide shows antimicrobial activity on *S. aureus* and potentially other bacterial species and functions by inhibiting an essential cellular process.

Aims

1. Verify antimicrobial action of laurenobiolide on *S. aureus*
2. Test the antimicrobial effects of laurenobiolide on other bacteria, including Gram-negative bacteria.
3. Identify specific cellular process inhibited by laurenobiolide

Experiment	Justification	Figure/Data	Expected Results
Aim 1: Verify antimicrobial action of laurenobiolide on *S. aureus*			
Confirm efficacy of antimicrobial compound on organism	Validating those previous results are replicable	Figure – Images of disc diffusion assays Table – Reporting measured zones of inhibition	The compound shows antimicrobial activity on the organism
Aim 2: Test the antimicrobial effects of laurenobiolide on other bacteria including Gram-negative bacteria			
Test activity against other species of bacteria	Allows us to see if there is potential broad spectrum antimicrobial activity	Figure: Images of disc diffusion assays Table – Reporting measured zones of inhibition	Either broad spectrum antimicrobial activity or antimicrobial activity restricted to either *S. aureus* or Gram-positive organisms.
Aim 3: Identify specific cellular process inhibited by laurenobiolide			
Isolation of laurenobiolide resistant mutants	These mutants may allow us to find a gene that could inform us about the mode of action	Figure: Images of disc diffusion assays Table – Reporting measured zones of inhibition	Growth of resistant mutants
Whole Genome Sequencing (Illumina) of laurenobiolide-resistant mutants	Identifying mutation in gene will identify cellular process that leads to resistance	Table containing information about mutations identified and what cellular processes might be affected	Identification of gene mutation is located on
Research mutation	Determine whether cellular processes or general drug resistance (via changes to cell permeability) are impacted		Determining mode of action