Isolation of plant-growth promoting rhizobacteria from mixed-conifer forest in Sierra Nevada, California

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Introduction

Climate change enhances the occurrence of extreme weather: wildfires, drought, and rising summer temperatures.[1] Heat stress, in particular, is extremely threatening to the successful recruitment and establishment of conifer seedlings.[2] Since 2010, 147 million trees in California have died from extreme weather events, a majority of which have been conifer species from the Sierra Nevada Mountain Range.[3] As we attempt to restore and reforest the conifers of Sierra Nevada, how can we facilitate their successful establishment in the face of increasing summer temperatures? Potentially with the use of **plant-growth promoting rhizobacteria (PGPR).**

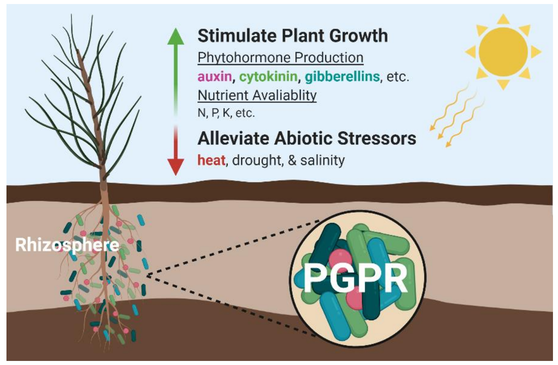


Figure 1. Plant-growth promoting rhizobacteria (PGPR), produce plant hormones (phytohormones) that can enhance overall growth and tolerance to environmental stressors.[4]

Objective

Isolate and characterize novel phytohormone-producing PGPR native to the rhizosphere of mixed- conifer forests in Sierra Nevada, California that can potentially promote overall growth and alleviate heat stress in two ecologically and economically significant conifer seedlings *Pinus ponderosa* (Ponderosa pine) and *Pseudotsuga menziesii* (Douglas fir).

Hypothesis

Potential PGPR will produce the following phytohormones: **auxin/indole-3-acetic acid (IAA)**, **gibberellic acid (GA)**, and **cytokinin (CK)**.

Methods

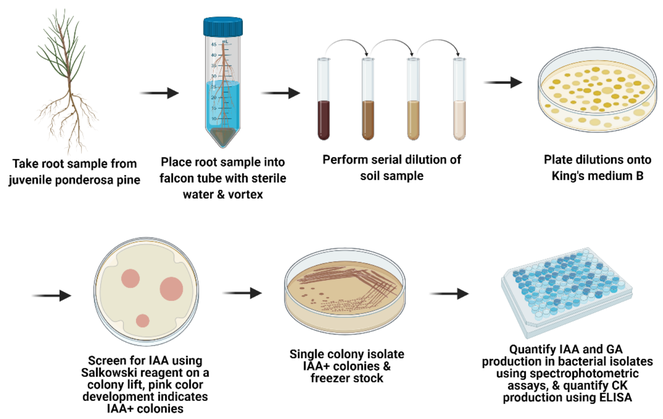


Figure 2. Schematic overview of methods used to isolate bacteria from root soil, screen colonies for (IAA) production, and quantify IAA, GA, and CK production.[4]

Results: Soil Dilutions, Phytohormone Production, & Bacterial Characterization

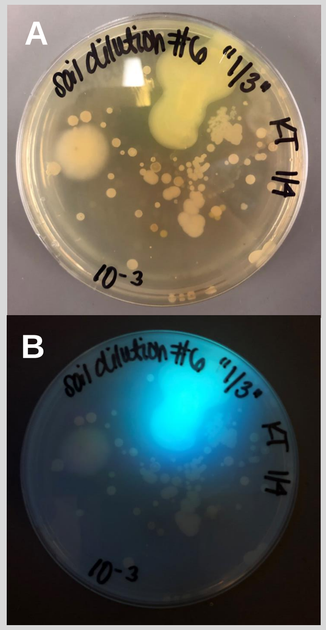
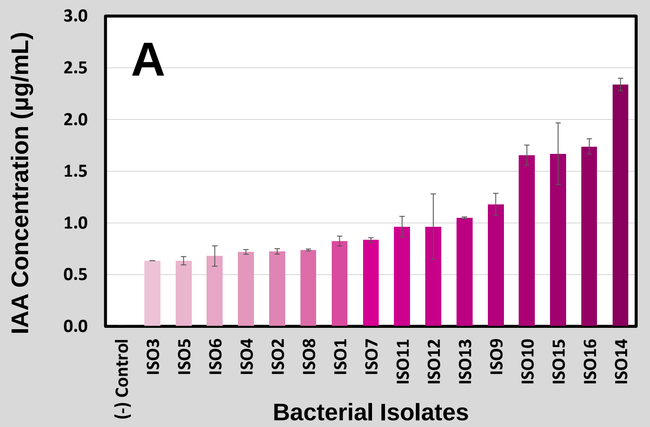
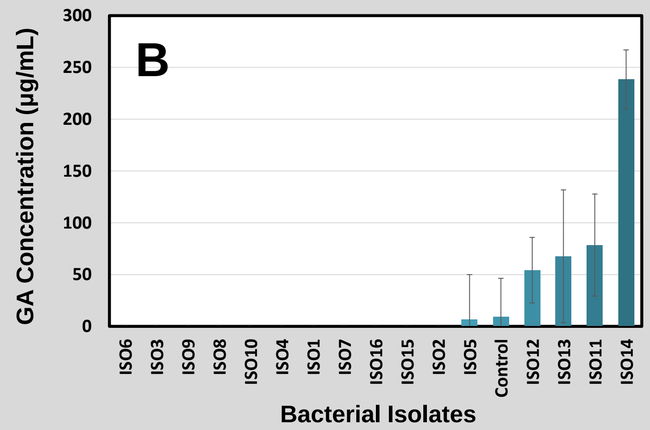


Figure 3. Soil dilution plated onto King's medium B under normal light (A) and UV light (B). King's medium B is diagnostic for fluorescent Pseudomonas; a genus that has been found in many studies to promote plant-growth and alleviate abiotic stressors.[5]





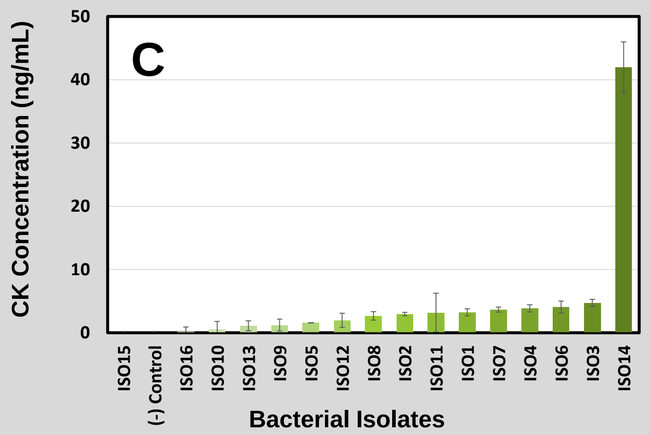


Figure 4. ISO14 produces that highest concentrations of indole-3-acetic acid (IAA), gibberellic acid (GA), and cytokinin (CK). Each isolate (ISO1-ISO16) was incubated in Nutrient Broth for 72 hours at 28°C along with a negative control (sterile media) **(A-C)**. Spectrophotometric assays were performed using culture supernatant to quantify IAA and GA production, and an ELISA was used to quantify CK production.

ISO14 appears to be a Gram-negative bacilli that produces a yellow fluorescent pigment when plated on King's medium B; key characteristics of the Pseudomonas genus.

→ Sanger sequencing will be used to molecularly confirm: genus, specie, & strain

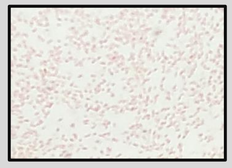


Figure 5. Gram stain of ISO14 at 1000X magnification. Pink coloration indicates that ISO14 is Gram-negative.

Future Research

- To test if the presence of ISO14 can promote overall growth and mitigate the effects of heat stress in *Pinus ponderosa* (Ponderosa pine) and *Pseudotsuga menziesii* (Douglas fir), a plant growth experiment will be conducted **(Fig. 6)**.
- It is hypothesized that the inoculation of ISO14 in *P. ponderosa* and *P. menziesii* seedlings will increase root elongation, dry weight, and stomatal conductance after 21 days under both normal and heat stressed conditions.

Upon completion of this research, potential PGPR may by identified in hopes to support the growth and transplantation of conifer seedlings in Sierra Nevada, California as summer temperatures continue to rise due to the effects of climate change.

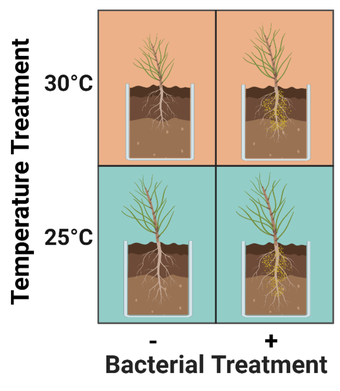


Figure 6. Experimental design for plant growth treatments.

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