INTRODUCTION & HYPOTHESIS

Secondary metabolites are compounds that are made by organisms but are not required for the primary phase of growth, normal development, or reproduction. Secondary metabolites can have diverse functions and include a number of antimicrobial compounds. The B. thailandensis genome is rich in genes that code for predicted and characterized secondary metabolites.

Our goal in this project is to investigate the antimicrobial activities of B. thailandensis to potentially uncover novel antimicrobial factors. Recent work showed that a type of cell-to-cell signaling system called quorum sensing is a global regulator of secondary metabolite production and antimicrobial activity in B. thailandensis. B. thailandensis quorum sensing controls genes in 11 putative and characterized secondary metabolites, including the previously described antibiotic bactobolin. When we started our experiments, we sought to target the quorum sensing-controlled genes for toxic activities towards other bacterial species.

METHODS & RESULTS

B. thailandensis produces the antimicrobial bactobolin as well as another product with activity against B. subtilis

- **B. thailandensis** produces antimicrobial compounds.
- **B. subtilis** is used for testing antimicrobial activity.
- **Figure 1.** Antimicrobial activities of wild-type (WT) and a bactobolin mutant of B. thailandensis were tested by the disc diffusion assay and by the outgrowth diffusion assay. A) B. thailandensis stationary phase culture fluids were filter sterilized and tested for antimicrobial activity on a lawn of B. subtilis on LB agar. The zone of inhibition around only the WT B. thailandensis culture fluid after B. subtilis outgrowth can be attributed to the previously described antimicrobial, Bactobolin. Next, unfiltered cultures were directly spotted on LB agar or C) LB agar containing 5% NaCl. Zones of inhibition were present for the WT and bacterial-negative B. thailandensis strains indicating the presence of an additional antimicrobial, which was eliminated in the sterilization process used for the disc diffusion assay.

**Figure 2.** Coculture compositions with B. thailandensis and B. subtilis were inoculated in liquid LB broth in test tubes at a starting ratio of 10:1. Wild type B. subtilis was competed against the following B. thailandensis strains: wild-type (squares), a bactobolin-negative mutant (triangles), or a double mutant in bactobolin production and the AHL regulator BtaR1 (circles). Colony forming units (CFU) per mL of culture are shown for B. subtilis in each competition.

**Figure 3.** BtaR1 is a quorum sensing regulator in B. thailandensis. BtaR1 controls many genes, including those for four secondary metabolites. Two have been previously characterized (tertphenyl and malleolactone) and two are uncharacterized.

**Figure 4.** Individual mutants in BtaR1-controlled putative and characterized secondary metabolites were tested for antimicrobial activity against a bactobolin-resistant isolate of B. subtilis in the outgrowth diffusion assay described in Figure 1. All strains show equivalent zones of inhibition indicating a diffusible factor that is not quorum sensing controlled has antimicrobial activity.

CONCLUSIONS

- B. thailandensis produces a previously uncharacterized diffusible antimicrobial that can be removed by the filter sterilization process (Figure 1).
- The regulator BtaR1, which is involved in cell-to-cell signaling called quorum sensing, promotes antimicrobial activity (Figure 2 and 3).
- Initial studies show that none of the known BtaR1-controlled putative and characterized secondary metabolites are responsible for the antimicrobial activity seen in the outgrowth diffusion assay (Figure 3 and 4).

FUTURE DIRECTIONS

- There are many exciting discoveries yet to be made working with the antimicrobial factors of B. thailandensis.
- We will continue to investigate bactobolin-independent antimicrobial activity in B. thailandensis by generating mutations in secondary metabolite genes in a bactobolin-negative background. We will use the assays described in Figure 1 as our readout.
- We will pursue factors that are QS-controlled as well as those which are not.
- We hope to isolate and characterize the antimicrobial compounds. The results of the filter sterilization experiment caused the compound to be removed or inactivated and suggest that we will have to carefully approach this.
- We will test the bactobolin-independent antimicrobial activity of B. thailandensis against other bacterial species (including relevant pathogens and other soil-dwelling organisms).

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