

Antimicrobial Production in *Burkholderia thailandensis*

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ABSTRACT

Burkholderia thailandensis is a soil-dwelling bacterium that has been proven to produce antimicrobial compounds. We are interested in understanding how *B. thailandensis* regulates production of these compounds and also hope to identify and characterize novel bacterial factors that possess antimicrobial activity. In our research, we use a variety of methods including disc diffusion assays, competition experiments, and mutational analysis to investigate the genes responsible for the antimicrobial activity of *B. thailandensis*.

The current research reports the presence of a previously undescribed diffusible antimicrobial factor produced by *B. thailandensis*. We have yet to identify the chemical nature of this factor or the genes responsible for its production. However, this factor is removed or inactivated by filter sterilization.

We tested if cell-to-cell communication and a selection of candidate secondary metabolites are associated with this antimicrobial factor. The BtaR1 regulator for bacterial communication may control antimicrobial activity, but we do not believe it to be a different factor. Additionally, mutations in known BtaR1-controlled secondary metabolites do not influence antimicrobial activity against *B. subtilis* in our assays.

Moving forward with this project we will continue to investigate the genes responsible for producing antimicrobial compounds by testing mutant strains of *B. thailandensis*. We hope to isolate and further characterize the antimicrobial factor(s).

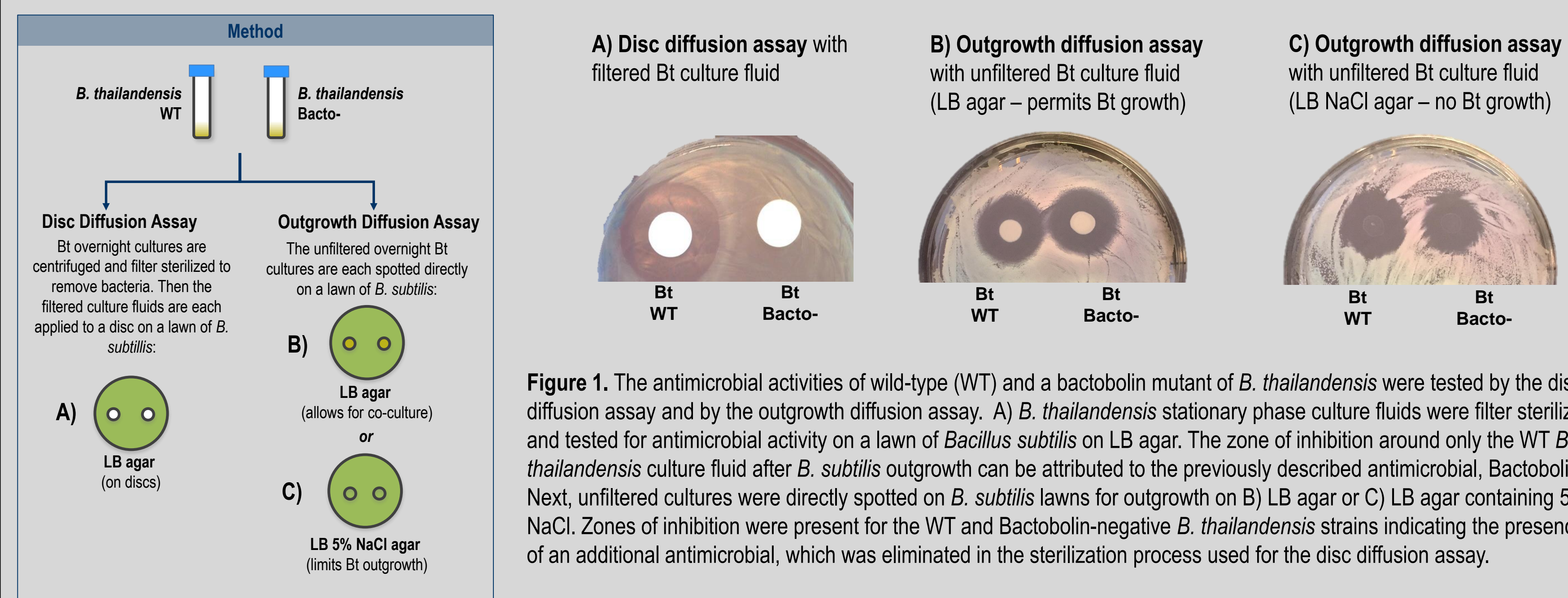
INTRODUCTION & HYPOTHESIS

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*



The quorum sensing regulator BtaR1 controls antimicrobial activity against *B. subtilis*

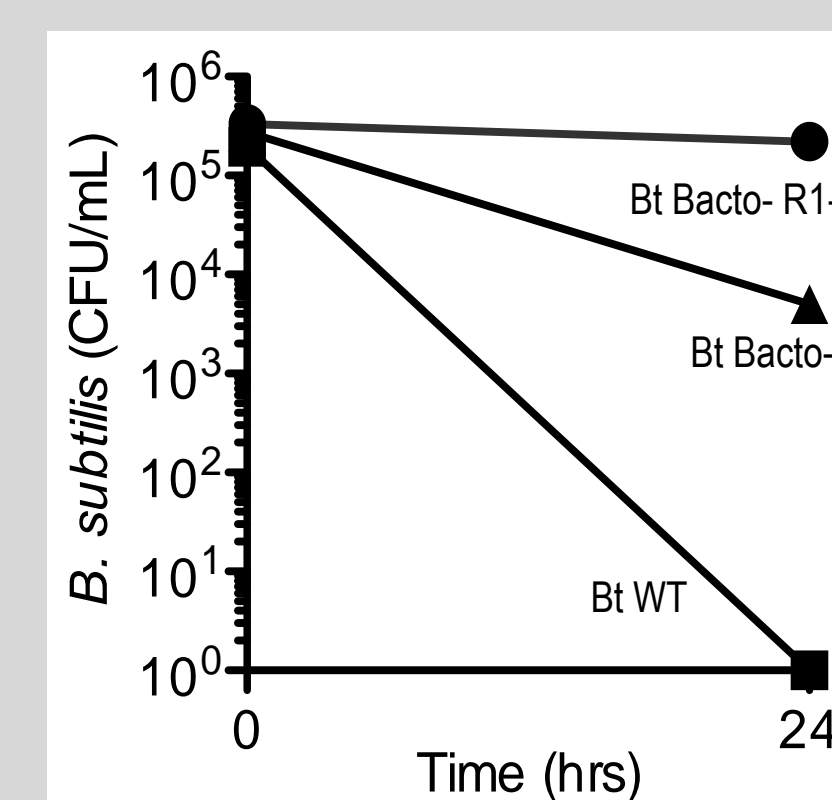


Figure 2. Coculture competitions with *B. thailandensis* and *B. subtilis* were inoculated in liquid LB broth in test tubes at a starting ratio of 10:1. Wild type *B. subtilis* was competed against the following *B. thailandensis* stains: wild-type (squares), a bactobolin-negative mutant (triangles), 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.

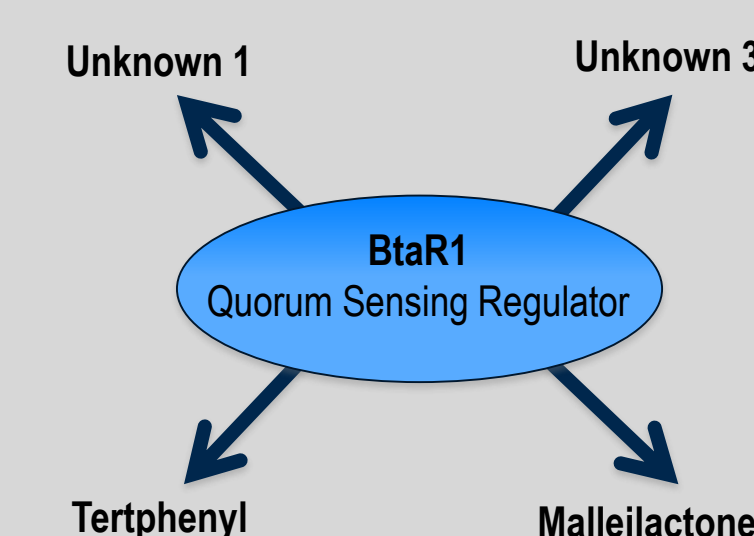


Figure 3. BtaR1 is a quorum sensing regulator in *B. thailandensis*. BtaR1 controls many genes, including those for four secondary metabolites. Two have been previously characterized (tertphenyl and malleilactone) and two are uncharacterized.

Another *B. thailandensis* factor may also have antimicrobial activity against *B. subtilis*

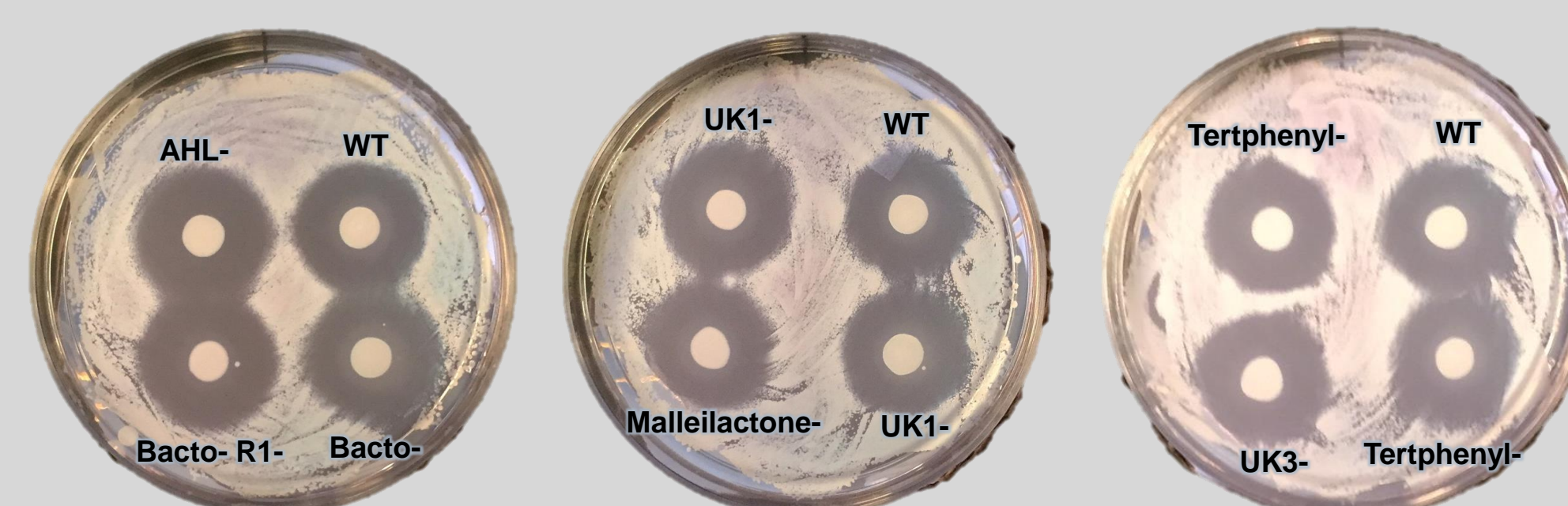


Figure 4. Individual mutants in BtaR1-controlled putative and characterized secondary metabolites were tested for antimicrobial activity against a bactobolin resistant isolate of *B. subtilis* in the Outgrowth Diffusion Assay described in Figure 1. All strains show equivalent zones of inhibition indicating 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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