Quantifying Anti-Quorum Sensing Activity of *Ganoderma lucidum* Tincture on *Chromobacterium violaceum*

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Introduction

• Quorum sensing (QS) is a bacterial cell communication pathway relying on the synthesis, release, and uptake of autoinducers, correlated to population concentration (Poli et al., 2018).

• *Chromobacterium violaceum* is a soil-borne gram-negative bacteria residing in tropical and subtropical areas (Choo et al., 2006).

• *C. bacterium* has been used as a biological bioindicator and also to screen potential quorum sensing inhibitors, which is enabled by the production of violacein (Poli et al., 2018).

• The objective of this was to quantify QS inhibition in *Chromobacterium violaceum* (Figure 1) by a medicinal mushroom tincture from the reishi mushroom (*Ganoderma lucidum*) tincture concentrations.

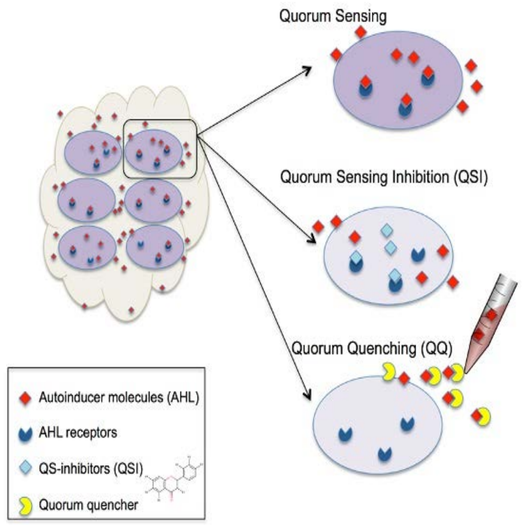

Figure 1. Quorum sensing inhibition (Skogman et al., 2016)

Figure 2. The Reishi mushroom has been used medicinally for hundreds of years in Chinese herbal medicine.

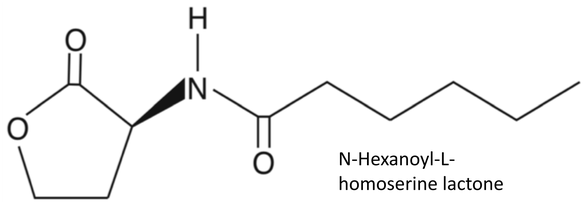

Figure 3. Autoinducer molecule for Gram-negative bacteria. Reishi metabolites may "mimic" lactones.

Materials and Methods

• A spot plate assay was used to observe the effect of Reishi, *Ganoderma lucidum,* on biofilm inhibition in our *Chromobacterium violaceum* isolate (CV1) (Chan et al., 2014). Tinctures were dissolved in ethanol to give a range of concentrations for the pilot experiment (Poli et al., 2018).

• Spectrophotometry and plate counts to determine colony forming units (CFUs) of *C. violaceum* were employed to measure the violacein production and determine biocidal activity of the dilutions of Reishi tincture.

Results

• Exposure to *Ganoderma lucidum* showed potential anti-QS activity in CV1, represented by cellular growth with a loss of pigmentation (Figure 2).

• CV1 showed an evident loss of pigment when exposed to 10% *G. lucidum* concentration. (Figure 3; Figure 4) A comparison of loss of pigmentation is shown.

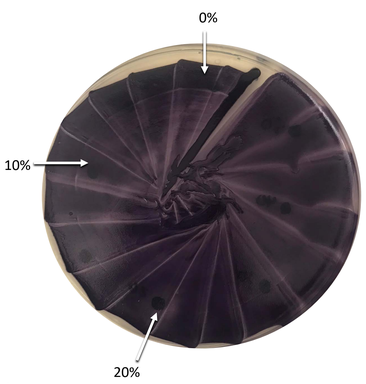

Figure 5. Reishi effects on CV1 spread plate shows potential anti-QS activity.

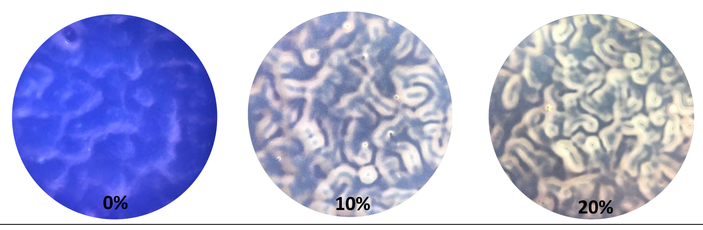

Figure 6. Loss of pigment in biofilms at increasing concentrations of Reishi exposure (400x magnification)

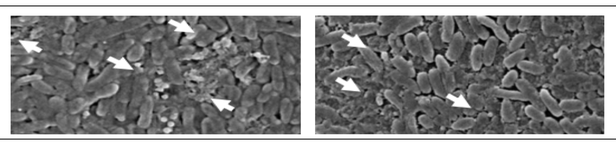

Figure 7. Biofilm Degradation in Chromobacterium Violaceum (Poli et al., 2018)

Results (continued)

• In each trial, there was only one replicate. The second method shown in Trial 2 was also adapted and improved based on biocidal activity observed in Trial 1 (Figure 8).

• Similarly, more replicates of the colony plate counts need to be recorded. If the decrease in cell count is shown to be nonsignificant, this would suggest that quorum sensing was inhibited without killing the cells (Poli et al

• The absorbance at 585 nm decreased as *G. lucidum* tincture concentration increased (Figure 8).

• Decreased violacein production (Figure 8) and consistent CFU counts suggest potential anti-QS activity (Figure 11).

• Tincture treatments at 20% showed biocidal activity on CV1 with a large decrease in bacterial plate count numbers (Figure 10; Figure 11).

• Replication is needed to test if the decrease in CFU values are significantly different.

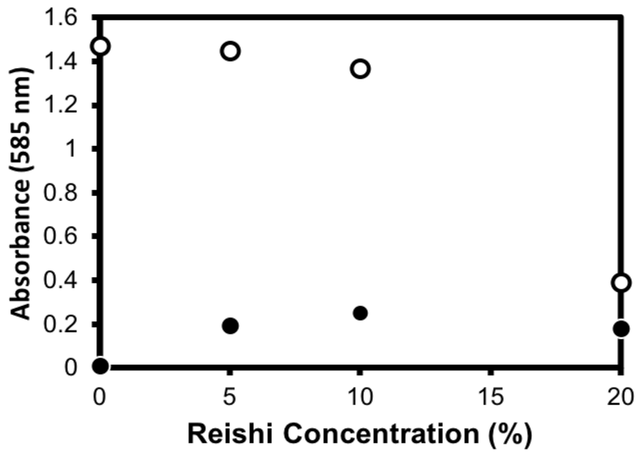

Figure 8. Absorbance values of varying Reishi concentrations. Trial 1 is indicated by (●) . Trial 2 is indicated by (○)

Figure 10. CFU plate counts suggesting anti-QS activity

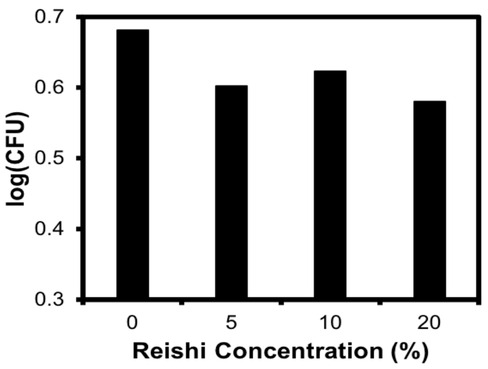

Figure 11. Bacterial plate counts suggesting anti-QS activity

Discussion

• Here we present evidence of anti-QS activity of Reis however MQSIC/MIC ratios would need to be determined confirm.

• Efficiency of inhibiting biofilms using this methodology in *violaceum* is recorded (Figure 12; Figure 13).

• Changes in cell growth morphology suggest that biofilms are being inhibited when exposed to *G. lucidum.* These results are supported by the observed spectrophotometer measurements, exhibiting little change in absorbance values between 5-10% Reishi concentration and the bacterial plate counts.

• The 20% Reishi tincture shows biocidal effects

• More replicates in each experiment are needed, along with the refined methodology to ensure more valid results.

•Future work will focus on calculating MQSIC/MIC to quantify the suggested anti-QS activity.

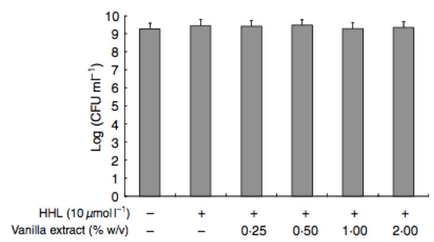

Figure 12. Bacterial cell count of vanilla extract exposure flask assays (Choo et al., 2006)

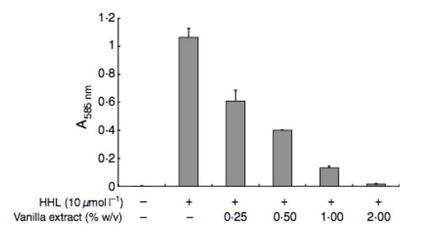

Figure 13. Inhibition of violacein production by vanilla extract (Choo et al., 2006)

Literature Cited

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