Biofilm Screening Assay with Plant and Fungal Extracts

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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).
- Biofilms form when planktonic microorganisms attach irreversibly to a surface (Figure 2). The polysaccharide layer provides protection from antibiotics (Figure 3).
- · Chromobacterium violaceum is a soil-borne gramnegative bacteria 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: Choo et al., 2006).
- The objective of this was to quantify QS inhibition in Chromobacterium violaceum and Pseudomonas fluorescens (Figure 1) by various medicinal mushroom tinctures concentrations including: Ganoderma lucidum, Hericium erinaceus, Ophiocordyceps sinensis, Grifola frondosa, Trametes versicolor & Inonotus obliquus

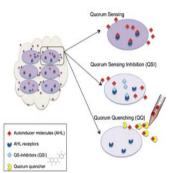


Figure 1. Quorum sensing inhibition (Skogman et al., 2016)

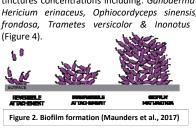


Figure 3. Antibiotic resistance in biofilms (Davies, 2003)

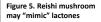
undiluted tinctures

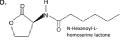
Chemical structure of (Pinya et al., 2019)

Figure 4. Ganoderma lucidum

Materials and Methods

- •A rapid biofilm formation assay (6 hours) was used to observe the effect of Reishi, Ganoderma lucidum and various essential plant oils on biofilm inhibition in an overnight TSB culture Chromobacterium violaceum (Cartagena et al., 2006).
- •A spot plate assay was used to observe the biofilm inhibition (Chan et al., 2014).
- •Spectrophotometric measurements were employed to measure the biofilm formation. Results were reported as a Abs_T/Abs_C ratio.







- · Colony forming unit (CFU counts) of undiluted tinctures suggest a lack of biocidal activity
- •Exposure to Inonotus obliquus, Trametes versicolor and Grifola frondosa showed potential anti-QS activity in Pseudomonas fluorescens represented by a decrease in crystal violet absorbance (Figure 7).

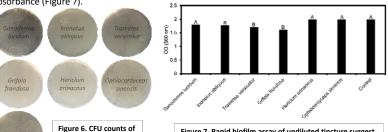


Figure 7. Rapid biofilm assay of undiluted tincture suggest anti-QS activity. "B" represents significantly different cell absorbances compared to the control

Results (continued)

- Exposure to 20% Ganoderma lucidum suggests anti-QS activity in CV1, represented by cellular growth with a loss of pigmentation, a comparison of loss of pigmentation is shown (Figure 8).
- Exposure to increasing EtOH concentrations in liquid, shows that <2% ethanol is non-biocidal to cells (Figure 9).
- · A one-tailed t-test comparing increasing concentrations of plant and fungal extracts to the control resulted in significant differences (Table 1).
- ·Absorbance values of the biofilm assay decreased as G. lucidum and peppermint concentrations increased (Table 1).
- •Ganoderma treatments between 5% and 10% exposure produced similar CFUs (Figure 10).
- Tincture treatments at 20% Ganoderma concentration showed biocidal activity on CV1 with a large decrease in bacterial plate count numbers (Figure 10).
- Decreased violacein production compared to the correlating colonyforming unit counts (CFU) suggest potential anti-QS activity (Figure 10).
- More replicates of these experiments are necessary.

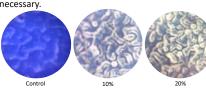


Figure 8. Microscopy of Ganoderma lucidum on CV1 indicates potential anti-QS activity (400x)

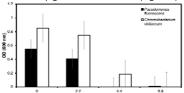


Figure 9. P. fluorescens and C. violaceum absorbances indicate that <2% EtOH is non-biocidal

Table 1. Potential biofilm inhibition focusing on Ganoderma lucidum				
and peppermint extracts				
Concentration	<u>Reishi</u>		Peppermint	
	Abs _T /Abs _C	P-value	Abs _T /Abs _C	P-value
5%	0.708	0.055	0.604	0.005
10%	0.462	0.001	0.673	0.004
20%	0.508	0.002	0.531	0.006
30%	0.169	0.000	0.408	0.003

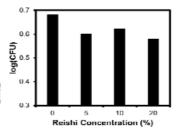


Figure 10. Bacterial plate counts suggesting anti-QS activity (Reishi)

- •Previous studies using a flask assay to assess biofilm inhibition in C. violaceum have been published and serve as a source for ideas on future experiments (Figure 11; Figure 11).
- Changes in cell growth morphology suggest that biofilms are being inhibited when treated with G. lucidum tincture. 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.
- Exposure to Ganoderma lucidum, Inonotus obliquus, Trametes versicolor and Grifola frondosa suggests evidence of quorum sensing inhibition in C. violaceum, however MQSIC/MIC ratios need to be calculated to confirm.
- In future experiments, the tinctures will be concentrated further to determine which concentrations are biocidal versus those inhibiting quorum sensing.
- The rapid biofilm assay method will also be adapted used a microtiter plate to obtain more precise results between trials (Figure 12).

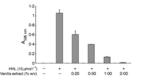


Figure 11. Inhibition of violacein production by vanilla extract (Choo et al., 2006)

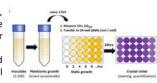


Figure 12. Methodology for future biofilm assays using microtiter plates (Matysik & Kline, 2019)

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