Biofilm disruption of *Pseudomonas fluorescens* and *Chromobacterium violaceum* using fungal tinctures

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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 bacterium found in tropical and subtropical areas (Choo et al., 2006).

•*Pseudomonas fluorescens* is a Gram-negative bacteria often found in water and agricultural soils (Rainey, 1999).

• *C. violaceum* has been used as a bioindicator and also as a model organism to study quorum sensing, which are enabled by the production of violacein, a purple pigment (Poli et al., 2018).

•Medicinal mushrooms are known to produce a wide range of bioactive compounds, including antibiotics, but there are few studies into the potential of fungal metabolites to inhibit biofilms in bacteria.

•Biofilm inhibition can be achieved through quorum sensing inhibitors, which can include lactone compounds, which numerous fungi are known to produce.

• The objective was to quantify QS inhibition in *Chromobacterium violaceum* and *Psuedomonas fluorescens* (Figure 1) in response to medicinal mushroom tinctures from and assortment of mushrooms (**Table 1**).

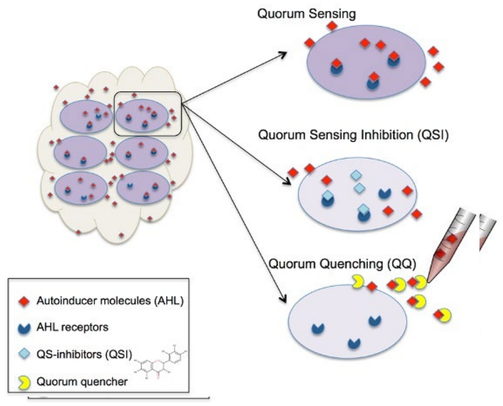

Figure 1. Quorum sensing inhibition (Skogman et al., 2016)

Fungal Tinctures
Reishi
Chaga
Turkey Tail
Maitake
Lions Mane
Cordyceps
Coprinus comatus

Table 1. Fungal tinctures used in this study. All tinctures except *Coprinus comatus* were obtained from the company Fungi Perfecti and were composed of up to 30% ethanol.

Materials and Methods

• Effects of DMSO and ethanol was tested using serial dilutions, to determine the baseline effect of these solvents on growth and cell death.

•Effects of tinctures on *C violaceum* and *P. fluorescens* were tested using spot plate assays.

•Effects of tinctures on cell growth of *C.violaceum* and *P .fluorescens* was analyzed using spectrophotometry and bacterial spread plates to determine colony forming units (CFUs).

•A biofilm assay was carried out to determine is disruption of the bacterial biofilm is possible using fungal tinctures.

Results

- No significant differences in CFU count were determined between treated and untreated samples or *P. fluorescens.*
- No significant reduction in biofilm formation was found in *P. fluorescens* after rapid biofilm assay with the tested fungal mushroom tinctures (**Table 2**).
- Spot plate assay did not reveal a reduction in biofilm for *P. fluorescens*.

	Absorbance at 570 nm				-
	Rep. 1	Rep. 2	Rep. 3	Mean	P-value
Control	0.572	0.554	0.598	0.575	N/A
Reishi	0.502	0.634	0.890	0.675	0.510
Chaga	0.620	0.604	0.614	0.613	0.721
Turkey Tail	0.925	0.572	0.680	0.726	0.782
Maitake	0.666	0.552	0.744	0.654	0.167
Lions Mane	0.568	0.396	0.534	0.499	0.652
Cordyceps	0.468	0.440	0.698	0.535	0.184
Coprinus comatus	0.528	0.675	1.08	0.761	0.398

Table 2. Spectrophotometry of crystal violet staining after rapid biofilm assay of *Pseudomonas fluorescens*. Cells of *P. fluorescens* were first grown in tryptic soy broth with fungal tinctures added to give a final concentration of ~5%. Cells were grown at room temperature for 72 hours. Cells adhering to culture tubes were stained with crystal violet and ethanol was added to dissolve crystal violet for spectrophotometry at 570 nm. Unpaired, two-tailed, Student's t-tests compared each tincture to the unamended control. Significant differences (p= <.05) are indicated by "*".

Results (continued)

• No significant results were found in spot plate assay with *C. violaceum* (**Figure 2**).

•No significant results were found in spectrophotometry measurement of biofilm formation in tubes after biofilm assay (**Figure 3**).

•Some experiments were unable to be performed with both bacteria due to time constraints caused by the pandemic (**Figure 4)**

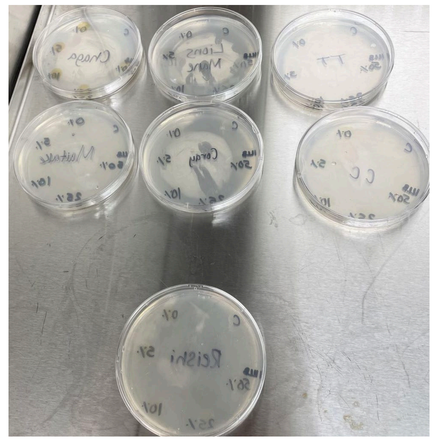

Figure 2. Spot plate assay with *Chromobacterium violaceum* and diluted concentrations of fungal tinctures

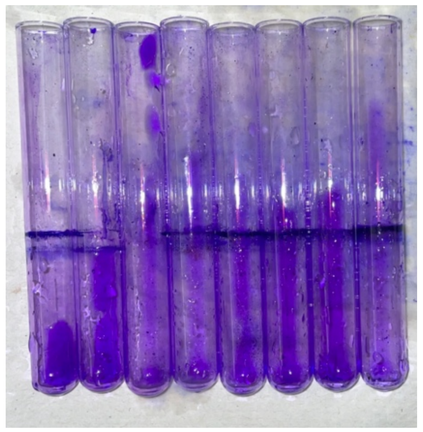

Figure 3. *Pseudomonas fluorescens* culture tubes from biofilm assay. Biofilms are composed of cells adhering to the glass culture tubes, which can be visualized with crystal violet staining. Tubes follow the order of Table 6 from left to right.

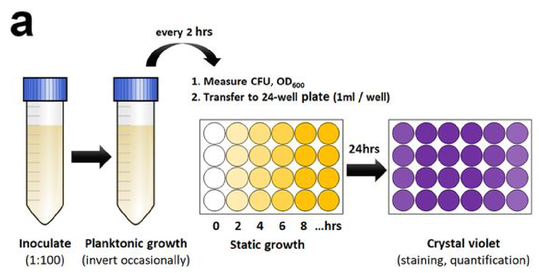

Figure 4. Methodology for future biofilm assays using microtiter plates (Matysik & Kline, 2019)

Discussion

• *Ganoderma luciderm* (Reishi medicinal mushroom) and *Inonotus obliquus* (Chaga mushroom) has been found to inhibit bacteria in combination with silver nanoparticles (Karwa et al. 2011; Nagajyothi et al. 2013). Silver nanoparticles are known to exhibit antibiotic and inhibitive properties in both Gram-negative and Gram-positive bacteria. Also, methanol-soluble particles that have been extracted from *G. luciderm* have been shown to inhibit quorum-sensing in *Chromobacterium violaceum* specifically (Zhu et al. 2011).

•*Coprinus comatus* and Cordyceps have been known to exhibit concentration-dependent inhibition against Gram-positive bacteria and Gram-negative bacteria (Hleba et al.2016; de Carvalho et al. 2016). The Maitake D-fraction has been found to boost the immune system and protect against cancer forming agents (Namba, 1997).

•In the future, the biofilm assay will need to be refined, but this study is a first attempt to develop this assay to screen potential inhibitors of biofilms. This sort of research could uncover molecules that could be useful in medicine and industry, to inhibit bacterial pathogens, and possibly help to prevent the spread of antibiotic resistant bacteria.

Literature Cited

1. Poli, J. P., Guinoiseau, E., De Rocca Serra, D., Sutour, S., Paoli, M., Tomi, F., ... & Lorenzi, V. (2018). Anti-Quorum Sensing Activity of 12 Essential Oils on chromobacterium violaceum and Specific Action of cis-cis-p-Menthenolide from Corsican Mentha suaveolens ssp. Insularis. Molecules, 23(9), 2125.
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