Investigating the antimicrobial and antibiofilm properties of coprinuslactone, a secondary metabolite from *Coprinus comatus*

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Introduction

- *Coprinus comatus*, the shaggy mane mushroom, is a common edible mushroom usually found on disturbed ground, grassy places, and roadsides[1] (Fig. 1).
- Members of the genus *Coprinus* are collectively known as the "inky caps" because they self-digest their gills to release their basidiospores[1] (Fig. 1).
- Coprinuslactone isolated from *Coprinus comatus* was found to disrupt quorum-sensing and to dissolve existing biofilms of *Pseudomonas aeruginosa*[2](Fig. 2).
- Biofilms are collections of microorganisms such as bacteria and fungi that grow on various surfaces and are often associated with many pathogenic forms of human diseases[3] (Fig. 3).

Figure 1. *Coprinus comatus* (Shaggy mane) mushroom
Picture credit: Alison Northrup
https://www.inaturalist.org/photos/45349949
https://botit.botany.wisc.edu/toms_fungi/may2004.html

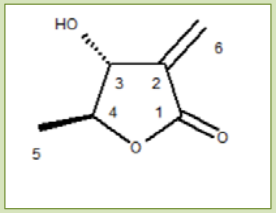

Figure 2. Coprinuslactone[2]
(3R,4S)-2-methylene-3,4-dihydroxypentanoic acid 1,4-lactone

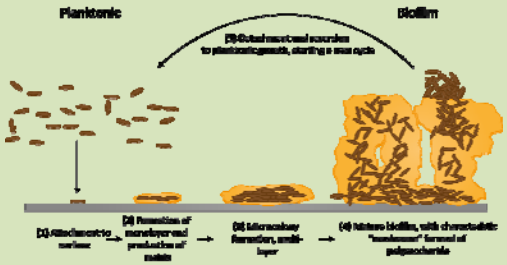

Figure 3. Biofilm formation schematic.
https://www.immunology.org/public-information/bitesized-immunology/pathogens-and-disease/biofilms-and-their-role-in

Materials and Methods

- A spot plate assay will be used to measure the effect of *Coprinus comatus* and *Coprinopsis cinerea* metabolites on bacterial growth of *Pseudomonas fluorescens* and *Chromobacterium violaceum.* A range of dilutions of *C. comatus* tincture and *C. cinerea* culture will be used to determine a minimal inhibitory concentration (MIC)[4] (Fig.4).
- A biofilm assay will be performed using the MIC determined of *C. comatus* tincture and *C. cinerea* culture to determine if there is antibiofilm activity at this concentration[5] (Fig. 4).

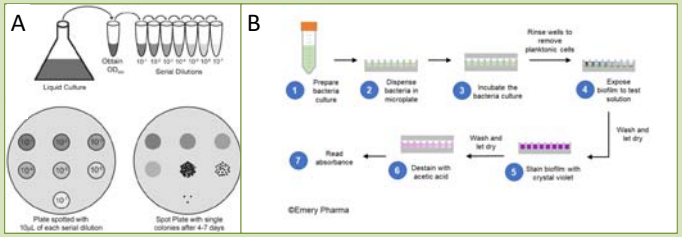

Figure 4. (A) Spot Plate Assay Procedure (B) Biofilm Assay Procedure

Anticipated Results

- If coprinuslactone is present in the *C. comatus* tincture and *C. cinerea* culture, bacterial growth of *P. aeruginosa* and *C. violaceum* should be inhibited.
- The estimated MIC of coprinuslactone should be approximately 150 µg ml^{-1} since this concentration has been previously found to have a bacteriostatic effect on *P. aeuroginosa*[2] (Fig. 5).
- The biofilms of *P. aeuroginosa* and *C. violaceum* should be disrupted using concentrations of 75 µg ml^{-1} and 150 µg ml^{-1} of the coprinuslactone. A previous study found that these concentrations were effective at dissolving existing biofilms of *P.aeruginosa in vitro*[2] (Fig. 6).

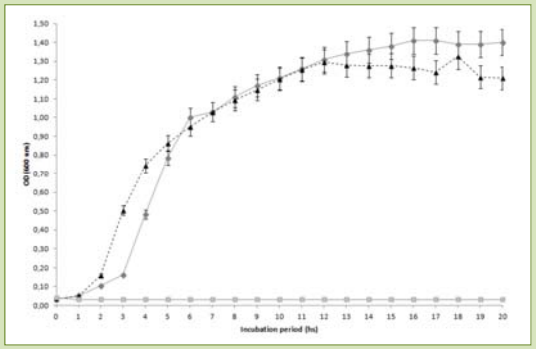

Figure 5. Growth curve of Pseudomonas aeruginosa PA14 under different concentrations of coprinuslactone. Triangles: untreated culture, Diamonds: 75 µg ml^{-1}, Squares: 150 µg ml^{-1}.

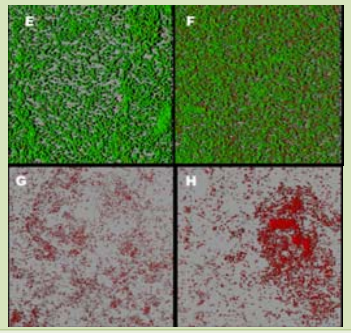

Figure 6. Effects of different concentrations of coprinuslactone on P. aeruginosa *in vitro* biofilms. Biofilms were stained using Live/Dead staining kit and visualized under a confocal laser scanning microscope. (E) P. aeruginosa biofilm treated with MeOH (positive control); (F) treatment with 37.5 ug/mL^{-1} of coprinuslactone; (G) treatment with 75 ug/mL^{-1} and (H) treatment with 150 ug/mL^{-1}.

Future Directions

- A potential obstacle we may encounter in this research is the possibility that other microbicidal metabolites in the C. comatus and C. cinerea extracts may obscure our observations of biofilm inhibition. This could potentially be avoided by isolating the coprinuslactone compound from the extracts to prevent confounding results.
- This work will be carried out concurrently with establishing a pipeline to test other fungal metabolite extractions from fruiting bodies we can obtain in Connecticut, such as, *Coprinopsis cinerea*.

Literature Cited
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