Identifying the effectiveness of plant extracts for treating biofilms of *Escherichia coli* and *Myxococcus xanthus*

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Research Goals

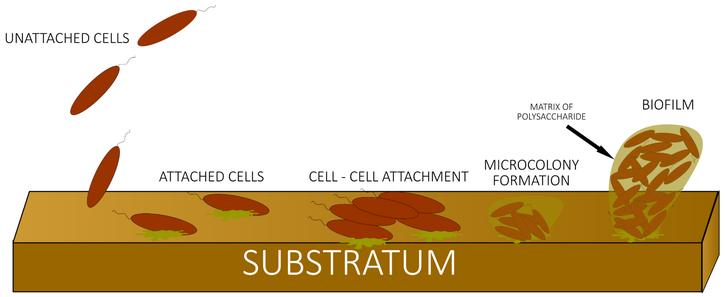
To identify a possible broad-spectrum treatment for biofilm related infections using garlic and ginseng plant extracts.

Introduction

Antibiotic resistance is a topic that is of growing concern in the field of medicine. Antibiotics act as growth blockers or act as toxins, killing the organism they affect. These biological weapons generate selective pressure leading to the current antibiotic crisis. This selective pressure causes resistant organisms to begin to emerge and over generations antibiotic resistance can develop and be spread among a bacterial population.

One of the key factors contributing to antibiotic resistance in bacteria is the ability for some bacteria to form and shield themselves within biofilms. Biofilms are masses of bacteria protected by a polysaccharide/ extracellular DNA matrix, can help prevent the penetration of antibiotics to the cells within and can unknowingly lead to lingering, viable cells that can cause further infections. Biofilms can adhere to surfaces in hospitals and can become resistant to even the harshest disinfectants. In hospitals nosocomial infections can lead to chronic illnesses and can be pointed towards surfaces contaminated with biofilms that resist antibiotics as well as disinfectants.

Figure 1: Mechanism of Biofilm Formation



Significance.

Sufferers of the genetic disease known as cystic fibrosis are vulnerable to bacterial infections in the airways of the lungs. The thickened mucus caused by this disease allows for the bacterium *Pseudomonas aeruginosa* to form biofilms more easily. This allows *P. aeruginosa* to linger in the airways of the lungs of these patients causing chronic lung infections, inflammation and possible lung failure. The magnitude of this rising problem elicits a need for novel treatments regarding biofilm-associated infections. There has been a growing interest in the use of plant extracts as a means other than antibiotics in combating bacteria. Two such extracts include garlic and ginseng which have been shown to clear *P. aeruginosa* biofilms *in vivo* and *in vitro*.

Escherichia coli serves as a model organism in the biological sciences, being well characterized and reliable for study. *Myxococcus xanthus, a soil bacterium* possesses a similar mechanism for motility in the form of type IV pili like *P. aeruginosa* which allow better adherence to surfaces, an essential step in the manifestation of biofilms. Studies on these two organisms can introduce the use of garlic and ginseng plant extracts as possible treatments against biofilm formation across bacterial species.

Methods

Bacterial motility, growth and biofilm formation were monitored using motility assays, spectrophotometry and live imaging techniques.

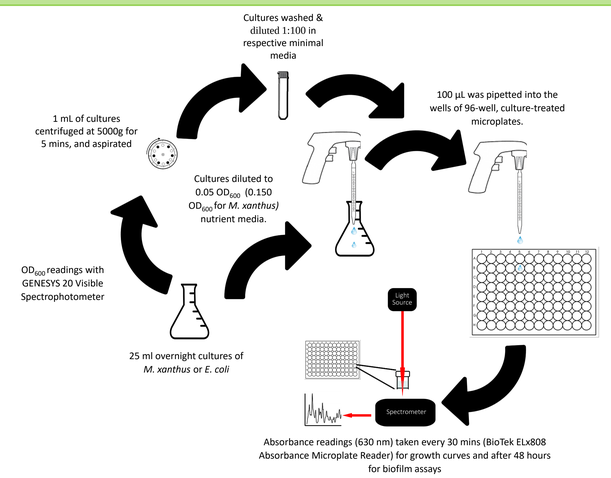
Bacterial Strains, Media, and Cultivation

Wild-type *M. xanthus* strain DZ2 and wild-type *E. coli* strain DH5α were used in this study. *M. xanthus* was cultured for everyday use in 25 mL CYE (peptone 1%, yeast extract 0.5%, 10 mM morpholinepropanesulfonic acid (MOPS) and 8 mM magnesium sulfate ($MgSO_4$) at pH 7.6) broth or agar plates (CYE + 1.5% Bacto agar). *E. coli* was cultured in 25 mL Miller's LB (Fisher Scientific) broth or agar plates (LB + 1.5% Bacto agar). CF (10 mM MOPS, 1 mM potassium phosphate, 8 mM $MgSO_4$, 0.02% ammonium sulfate, 0.2% sodium citrate, 0.2% glycerol, and 150 mg/L peptone at pH 7.6) media for *M. xanthus* and M9 salts (22 mM potassium phosphate, 22 mM sodium phosphate, 85mM sodium chloride, 0.1% ammonium chloride, 0.2 mM $MgSO_4$, 0.1mM calcium chloride and 0.4% glucose at pH 7.2) media for *E. coli* were used as minimal media for biofilm production. Garlic extract (*Allium sativum*) suspended in sugar alcohol (1000 mg/mL garlic extract) and Ginseng extract (*Panax quinquefolius)* were filter sterilized through 0.22 µm cellulose acetate membrane filters.

Analysis of Biofilm Inhibition

To quantify biofilm inhibition of garlic and ginseng extracts on *M. xanthus* and *E. coli*,1.4 % ethanol was used to monitor the effect of alcohol on growth as the garlic extract was suspended in alcohol. Cultures were grown in 2.5, 5, 10 or 20 mg/mL garlic extract per 100 µL or 0.25, 0.5, 1 or 2% ginseng extract per 100 µL. Cultures were then incubated at 32°C (*M. xanthus)* or 37 °C (*E. coli)* for 48h.

Figure 2: Bacterial Growth and Inhibition Analysis



For inhibition studies, after incubation, the plate was dumped of liquid culture and then washed with deionized water. After washing, 125 µL of 0.1% crystal violet was added to each well and allowed to incubate for 15 minutes following which, the tray was dumped and then washed twice with deionized water. The plate was then air dried and 200 µL of 30% acetic acid was added to the wells and allowed to incubate for 15 minutes. Each well was mixed via pipetting and 125 µL of the liquid mixtures were transferred to a new plate for optical readings at 630 nm using a plate reader.

Treatment Effects on Growth

Qualitative studies of organism growth on treated media were conducted by incubating cultures for 48 hours and examining colonies at 20x magnification using an Olympus EX51 light microscope.

Results

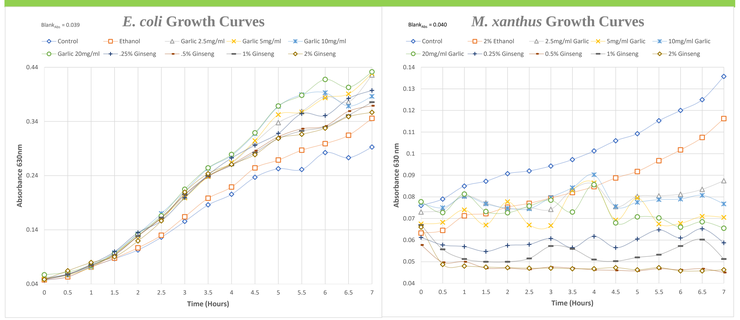


Figure 3: *E. coli* and *M. xanthus* growth over 7 hours. Displayed values were taken from the mean of three replicates per treatment.

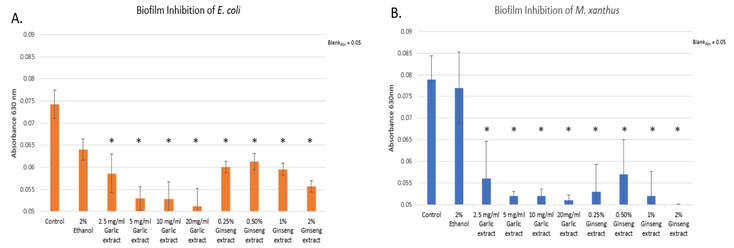


Figure 4: Biofilm inhibition assay results. Values are taken from the mean of seven replicates per treatment. Error bars denote one standard deviation. Stars signify $p < 0.05$.

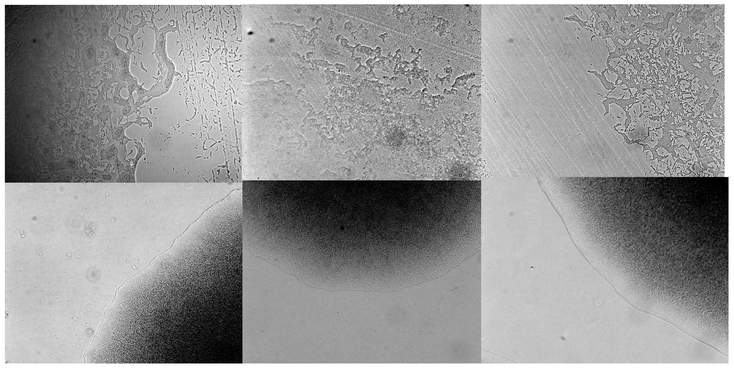


Figure 5: Images of *M. xanthus* (top) and *E. coli* (bottom) at 20x magnification. Left to right image panels are; control, 20 mg/ml garlic extract and 0.5% ginseng extract nutrient agar plates.

Discussion and Future Studies

The growth curves of *E. coli* depict an apparent increase in growth rate during logarithmic phase with additions of 2.5, 5, 10 or 20 mg/mL garlic extract per 100 µL or 0.25, 0.5, 1 or 2% ginseng extract per 100 µL (Figure 3). Results from the biofilm assay conducted on *E. coli* show a significant decrease in biofilm production in comparison to untreated control *E. coli* cultures when treated with either garlic or ginseng extracts. 5 mg/mL garlic extract nearly eliminated biofilm development, with 10 and 20 mg/mL showing similar results (Figure 4). Ginseng was not as effective of a biofilm inhibitor compared to garlic, taking at least 2% to yield similar inhibition as compared to 2.5 mg/mL garlic extract (Figure 4). Together, the two assays display support for garlic and ginseng as inhibitors of biofilms in *E. coli* as inhibition of cell growth was not observed, but biofilm inhibition was.

M. xanthus growth curves were not as easy to obtain due to the slow growth of the microbe. However, qualitative testing by growing the bacterium on treated and not treated plates showed that garlic at a concentration of 20mg/mL caused cell lysis in the culture (Figure 5). This could mean that at high concentrations the biofilm inhibition observed by garlic was most likely due to cell death. 0.5% ginseng extract did not inhibit growth in agar plate culture (Figure 5) and thus ginseng extract may act as a biofilm inhibitor in *M. xanthus* as well. 2% ginseng was observed to completely inhibit biofilm growth in *M. xanthus.*

Further tests will need to be conducted on higher concentrations of ginseng in *E. coli*, and *M. xanthus* growth on 2% ginseng will need to be assessed along with another set of growth curves. Predation assays will also be conducted to observe if ginseng interferes with the motility of *M. xanthus* during predation.