Repurposing Microbes as Living Biologic for Antibiotic Resistance

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Introduction/Background

Antibiotic resistant diseases are a huge problem across the world as traditional antibiotics are becoming ineffective on these diseases.

One antibiotic resistant strain common worldwide is methicillin-resistant Staphylococcus aureus (MRSA).

According to the CDC, there were nearly 120,000 cases of bloodstream S. aureus infections in the United States in 2017, of which more than 20,000 resulted in the death of the patient (CDC, 2019 March 5).

Our goal is to create a wound dressing that will fight against MRSA infections by:

- Using Escherichia coli (E. coli) that has been genetically engineered to combat MRSA.
- Fabricating a hydrogel-based wound dressing that can be easily applied to promote wound healing.
- Designing a model to predict the efficiency of the genetic circuit.

Using this strategy, it can be possible to apply this bacteria-killing technique to many other types of antibiotic resistant diseases, not just fighting MRSA.

Genetic Engineering

- Bacterial Quorum Sensing
- Tapping into Inherent Bacterial Communication System
  - Quorum sensing occurs when the cells have a population over a specific threshold which synchronize group behavior.
  - Using S. aureus's natural communication system and splitting it between two plasmids, we can use it to sense when MRSA is present and genetically engineer a release of a kill mechanism.

Design of Genetic Switch

- Plasmids that sense different levels of AIP
  - agr/agrC – P2
  - agr/agrC – P3
  - The two sensing plasmids will allow us to determine sensibility of promoters and work with the one that is the most selective to AIP.
  - RFP will fluoresce to prove circuit works, it is a substitute for luxophastin, which would kill MRSA.

Plasmid that produces AIP

- agr/agrD
- AIP is expensive to buy and if synthesized by E. coli, we could have the ability to not only produce AIP but also perform several co-culturing experiments.

Hydrogel

- Hydrogel Precursor Solution
  - Distilled water 32.9% w/v
  - Poly(ethylene glycol) diacrylate (PEG-DA) 8.9% w/v
  - 2-Hydroxy-2-methylpropiophenone (HOMPP) 0.3% w/v
  - Sodium alginate 7.5% gl

- Exposed precursor solution to a broad-spectrum ultraviolet (UV) light, causing the molecules to crosslink.

- The mechanical properties were optimized:
  - Completed 10 iterations over the semester, varying UV exposure times and the composition of the precursor solution.

- To be sure that the UV light would not kill the cells inside the hydrogel, we exposed E. coli cells to UV light for various periods of time and plated the resulting cells.

- To ensure that no E. coli could escape the hydrogel, and possibly get on the wound, E. coli immobilization tests were performed.

Modeling

- Expanded ODE system translated from gene regulatory networks
- Multi-Compartment Analysis

- Production (Hill Function)
  - Complete construction of proof of concept P2 and P3 plasmids.
  - Sequence confirm P2 and P3 plasmids.
  - Move confirmed parts to Staphylococcus carnosus (S. carnosus).

- Based on Mass Action Kinetics
- Stochastic system that tracks molecule counts over long periods of time

Conclusions

- Genetic Engineering
  - Proof of concept circuits could be created using our designed plasmids and synthetic biology techniques.
  - Hydrogel
    - Hydrogel is flexible and shows promising results for immobilizing E. coli.
    - Tests show E. coli cells able to survive under UV exposure for up to 1 minute.

- Modeling
  - Simple stochastic models can give rise to intuitive understanding of the underlying system.

Future Directions

- Genetic Engineering
  - Expand proof of concept P2 and P3 plasmids.
  - Sequence confirm P2 and P3 plasmids.
  - Move confirmed parts to Staphylococcus carnosus (S. carnosus).
  - Begin co-culturing experiments with and without hydrogel.

- Hydrogel
  - Complete with E. coli immobilization tests.
  - Perform AIP and luxophastin diffusion tests.
  - Consider ways to secure hydrogel onto wound.
  - Consider storage and transportation of hydrogel.

- Modeling
  - Expand Gro model to represent entire system.
  - Gather quantitative experimental data from hydrogel and genetic engineering experiments.
  - Parameter fit model to experimental data.

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