Antibiotic resistance

Antibiotic resistant bacterial strains were identified even before antibiotics entered widespread use. Subsequent overuse of these drugs has resulted in a steady increase in the number of resistant strains. Many have gained resistance to multiple classes of antibiotics, and we are in danger of entering a post-antibiotic era in which strains exist for which no known drugs are effective. Several pan-drug-resistant strains have been identified, and others will doubtless arise in the future as the rate of introduction of novel drug continues to decline.

Phage therapy

Our project aims to address antibiotic resistance by furthering the feasibility of phage therapy, the use of bacteriophages (viruses that infect bacteria) to treat bacterial infections. Phage therapy was first explored in 1919 but never became widespread due to both a lack of conclusive evidence of efficacy and the development, in the 1940s, of the ability to produce large quantities of antibiotics. Subsequent phage therapy research has been limited. A major advantage of phage therapy is host specificity: each kind of bacteriophage infects only a specific bacterial host species. Thus, phage therapy can be directed towards only the pathogen that is causing an infection. By contrast, most antibiotic drugs are broad-spectrum and may disrupt the body’s microbiome by killing beneficial bacteria.

Current implementations of phage therapy involve lytic infection. In the lytic cycle, a phage particle injects its genome into the host cell whereupon new virions are immediately assembled and released by lysis of the host. However, about half of phages are temperate, meaning they may enter a dormant state called lysogeny in which the viral genome is integrated into the host chromosome. Since hosts containing a dormant phage may grow normally, temperate phages can not currently be used for phage therapy.

KillerRed and Superoxide

The goal of our project is to design a method of making temperate phages a viable option for phage therapy. This is accomplished by constructing a λ g11 phage containing the gene for KillerRed. KillerRed is an engineered dimeric red fluorescent protein that is photosensitive (A). Upon absorption of light energy, KillerRed can react with water to form superoxide (O2•), the radical anion of molecular oxygen (B,C,D). In large quantities, superoxide is toxic to the cell because it reacts with protein side chains and with lipid chains, causing cross-linking of both. It also indirectly causes higher levels of hydroxyl radicals resulting in nucleic acid damage. The addition of KillerRed to the λ genome provides a deadly lysogenic state; when the λ genome is integrated into the host chromosome, the host will produce KillerRed and then superoxide production is controlled by exposure to light.

Results

Our photobleaching experiments demonstrate the killing ability of KillerRed in a plasmid, and KillerRed was constructed as a β-galactosidase fusion protein into λ g11. We used a nonphotosensitive mRFP as a control.

Conclusions and Future Directions

- We have demonstrated fluorescent protein expression in the λ lysogen. We believe that other temperate phage could be engineered in a similar way in order to fight other bacterial species.
- Our construct’s production of KillerRed was limited by the presence of several rare codons in the KillerRed gene. Codon optimizing the gene for E. coli would likely result in a higher amount of killing.
- SuperNova, a monomeric version of KillerRed, has recently been developed. It may result in more efficient killing.

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3D Printing

Tiny bacteriophages to raise awareness for antibiotic resistance and promote phage therapy!

Human practices

Presentations

Presentations to high school students about synthetic biology, GEM, our project and its implications.

3D Printing

Tiny bacteriophages to raise awareness for antibiotic resistance and promote phage therapy!

Modeling

Host-phage population dynamics: The purpose of this agent-based model is to illustrate the impact that lambda phages have on a population of E. coli cells. The factors that can be altered are shown in the interface in the blue boxes. With this model we can run simulations to gain insight about the host-phage environment. Graphs constructed with data from this model can be found in the “Results” section.

KillerRed intracellular model: This model uses differential equations to determine the levels of mRNA transcript, immature and mature KillerRed, and superoxide. The model parallels a typical experimental setup by incorporating three stages: (1) induction at 37°C by IPTG, (2) incubation at 4°C to allow the KillerRed chromophore to mature, and (3) photobleaching. Superoxide production is modeled using the various photochemical states of the KillerRed chromophore, and the cell’s defense against superoxide is incorporated using a Michaels-Menten model of superoxide dissimutation.

4b) Light Activated Production of Superoxide

5b) Host Death

4a) Rolling Circle Replication

2a) Rolling Circle Replication

3a) Lysis

3b) Expression of KillerRed

1) Infection