CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

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Project Number

36158

Project Title

New Antibiotics: Conjugative Transfer of Cytotoxic Genes for Targeted Cell Elimination

Abstract

Objectives/Goals

With the increasing problem of antibiotic resistance in bacteria, a different approach is needed to combat bacterial infections beyond the continual overuse of antibiotics. This experiment demonstrates the viability of an alternative strategy: repurposing bacterial horizontal gene exchange, or conjugation, to transmit cytotoxic genes within a bacterial population for rapid and sustainable toxin delivery.

Methods/Materials

To emulate the population dynamics of the toxin delivery system, a MatLab-based predictive simulator was first written using conditional probabilities to determine necessary conditions for transfer of a toxin-encoding plasmid into recipient populations. Results were then lab verified using custom plasmid constructs of the ccdB genetic toxin regulated by the araBAD promoter: dubbed pT-BAD, these plasmids were synthesized from gene fragments isolated from different bacterial systems. Cytotoxic ccdB experimental and YFP control plasmids were transformed into donor K12 Escherichia coli and incubated with recipient K12 cells to conjugate the ccdB toxin or control YFP genes. Following arabinose induction, surviving populations were finally quantified using spectrophotometry.

Results

Eighty iterations of 5 minute conjugation intervals in the simulation yielded 240 minutes to be sufficient for complete transfer of the toxin-encoding plasmid into all potential recipient cells. In vitro data demonstrated that within 30 minutes of post-conjugation arabinose induction, populations receiving the ccdB toxin experienced significant population decline and remained at an unrecoverable flatline for the four hour duration, while control populations receiving nonlethal YFP continued to proliferate normally.

Conclusions/Discussion

Based on paired T-test analysis of the lab experimental results, the toxin experimental group showed statistically significant variances from the YFP control group, indicating that the toxin transmission system successfully targeted and inhibited cell growth in bacterial populations.

Here, reprogramming conjugation as an efficient drug delivery tool is shown to effectively transmit lethal cytotoxic genes within bacterial populations for inducible cell death. Future research could expand upon this genetic system to combat antibiotic resistant bacterial infections, as well as induce genetic cell death in other pathogens and illnesses using tissue-specific promoters and cytotoxic genes.

Summary Statement

My project addresses the problem of antibiotic resistance in bacterial infections using an alternative to antibiotics that employs bacterial gene transfer mechanisms for efficient drug delivery of lethal genes to kill bacterial populations.

Help Received

I carried out and designed my experiments independently, using lab facilities and equipment at the J. Craig Venter Institute, under the supervision of Dr. Philip Weyman.

Preliminary