Purdue University

Bacterial Warfare
The Rock Paper Scissors Approach
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- Purdue Department of Chemistry
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The Concept

Brainstorming Ideas

- Microbe board game?
- Chess?
- Risk?
- Some kind of microbe warfare?

[Diagram showing a rock-paper-scissors cycle and a triangular KILLS interaction network between A, B, and C.]
How is diversity maintained in nature?

Environmental factors and intrinsic dynamics prevent equilibrium of a system.

Two Species – one will displace the other.

Many Interacting Species = Very complex network of competitive relations.

Three Species – can provide interesting results.

Rock–Paper–Scissors model developed.
Bacterial Growth 101

- Exponential Model
  \[ X = X_0 e^{ut} \]
  - \( X \) is concentration of the cell population

- Logistic Growth Model
  \[ \frac{dX}{dt} = X(u - bX) \]
  ![Logistic Growth Model](http://www.math.duke.edu/education/postcalc/predprey/logistic.gif)
Killing Mechanism

- A kills B
- B kills C
- C kills A
- Kill Term Must be Accounted for in Model
- Therefore:

  \[
  \frac{dX_a}{dt} = X_a \left( u_1 - bX_a - M_{ca} X_c \right)
  \]

  \( M_{ca} \) is a term for c kills a

  \[
  \frac{dX_b}{dt} = X_b \left( u_2 - bX_b - M_{ab} X_a \right)
  \]

  \[
  \frac{dX_c}{dt} = X_c \left( u_3 - bX_c - M_{bc} X_b \right)
  \]
Assumptions

- Growth Terms will be the same for all three types of cells.
- Natural Death coefficient will be the same for all three types of cells.
- Clarification: Population values are normalized by setting initial population equal to 1 which corresponds to 100% cell survival.
Simulations

Murder Rates
$M_{ab} = 0.2$, $M_{bc} = 0.2$, $M_{ca} = 0.2$

Initial Population
$A_0 = B_0 = C_0 = 1$
C is the weakest but dominates?

Murder Rates
A=0.3, B=0.2, C=0.1

Initial Population
A₀=B₀=C₀=1
Oscillations

Murder Rates
A=0.15, B=0.118, C=0.15

Initial Population
\( A_0 = B_0 = C_0 = 1 \)
IN THE LAB

- Original concept involved 3 e. coli strains
- In iGEM parts registry, found 2 regulators with corresponding producer genes
- Simplified problem to a 2-strain model
The Mechanism

- Indirect Killing:
  - Inducible regulators are normally “OFF”
  - Inducible regulator + Death gene = controlled death
  - Each strain’s “weapon” is a gene producing the chemical corresponding to the opposite strain’s regulator

**STRAIN A:**
- Makes chemical X
- Regulator controlled by chemical Y

**STRAIN B:**
- Makes chemical Y
- Regulator controlled by chemical X
The Parts

- Death Gene: ccdB gene
  - Usually used to regulate part-making
- Chemical Producer genes: AraC (arabinose), cl
- Inducible Regulators: cl, AraC
- GFP/mRFP (colors): Red and Green
  - For distinguishing strains and measuring their growth

**STRAIN A:**
- RED
- AraC
- cl Reg.
- ccdB

**STRAIN B:**
- GREEN
- cl
- AraC Reg.
- ccdB
• In order to make Bacteria kill each other in a meaningful way, the death gene used had to be regulated.

• AraC and cl regulators were used to control the activity of the death gene.

• Upon contact with AraC (or Arabinose) or cl the death gene is activated and the cell “commits suicide.”
The genes used had to be ordered such that AraC or cl were constitutively produced, the regulator would be induced by opposite molecule, and after induction, the ccdB gene would initiate death.

The use of regulators for the molecule that is not produced is the key because otherwise the cell would effectively kill itself upon constitutive production of its main molecule instead of causing the other strain to kill itself.
Transform plasmids into bacteria and grow on agar medium to amplify the desired DNA
Extract and purify plasmid DNA
Digest DNA with necessary restriction enzymes
Gel Electrophoresis and blotting to separate desired gene from excess DNA
Mix and ligate genes together in proper order
Transform new plasmids with desired arrangement of genes into E. Coli
BIOLOGICAL PROBLEMS

How fast will the signaling protein diffuse?

- Will the protein toxin diffuse out of the dead cell and kill the nearby bacteria?

- Can we watch the bacteria warfare (red vs. green)?
Modeling The Lab

- Does genetic engineering effect?
  - Growth rate
  - Death rate
- What are the murder rates?
  - A → B
  - B → C
  - C → A
Recruit iGEM members
- Flyers and posters
- Go to related lectures and give short speeches
- Let academic advisers send emails to related majors

Generate plans & ideas in spring. Start experiments & modeling in summer.

IBE- Institute of Biological Engineers
iGEM PLANS

- Keep the project student run

- Continue this project if the next generation members are interested

- Encourage members with little experience to join