Virginia Tech iGEM 2007

VT iGEM team
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Around the world in 80 days? Not anymore…

- **A public health risk:** people travel faster than symptoms develop
  - SARS – 2003 – Four hospitalized in San Jose in SARS airplane probe
  - Drug Resistant TB – 2007 – 31 yr old male travels between US, Europe, Canada

- **How much can an infection grow before there is a risk of an epidemic developing?**
Engineering an Epidemic

➢ ...to build a model and observe epidemic development within and between populations.
  ▶ Use *E. coli* and phage λ as a model population
  ▶ Understand events from the beginning of the epidemic
    ■ Engineer *E. coli* to fluoresce upon infection
    ■ Use an engineered fluorescent phage
  ▶ Design a network for the spread of infection
    ■ Examples: simple diffusion, air traffic
  ▶ Verify models experimentally
Brainstorming: Epidemics and “Air Travel”

Planning for Wet Lab
- Bacterial and Phage Cultures
  - Observe Infection
- Construct Reporter Plasmid

Multi-scale Modeling
- Bacterial Growth Model
  - Single Population Infection Model
- Inter-Population Model: “Air Travel”

KEY: Spring Summer Fall
Testing Models Biologically

- In order to build a mathematical model, we had to engineer a biological model.
- Experiments must be simple and easy to reproduce
- Utilize well known species and molecular techniques
  - Bacteriophage λ
  - *E. coli*
  - Reporter Plasmid
λ Phage: Lytic or Lysogenic?

- Primarily controlled by Cro and CI protein production
  - If enough Cro produced first, cell goes lytic
  - If enough CI produced first, cell goes lysogenic
How Do We Test?

- **Reporter Plasmid**
  - Lambda promoter
  - Fluorescent protein genes

[Diagram showing Lysogeny and Lysis processes with RFP and GFP]
Microscopy

- Images constructed from overlay of multiple filters
  - Show our working plasmid
  - Lytic cell expresses GFP, lysogenic cell expresses dsRED
Observing Lysis
A Biological Model System and a Computer Simulation

<table>
<thead>
<tr>
<th>Other Models in Epidemiology</th>
<th>Our Approach with <em>E. coli</em>…</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use data from past epidemics</td>
<td>Full time course experimental data</td>
</tr>
<tr>
<td>Exclude data from infections that did not become epidemics</td>
<td>Observe infection before epidemic</td>
</tr>
<tr>
<td>Deterministic</td>
<td>Inherent random variations</td>
</tr>
<tr>
<td>Cannot test</td>
<td>Test and repeat</td>
</tr>
</tbody>
</table>

➢ **Hybrid Modeling**
  ▶ Developed for simulation of stiff gene-network models
  ▶ Useful for evaluation of epidemiological models?

➢ **The model has some limitations...**
Mechanisms modeled as reaction equations

- Bacteria + Food → 2 Bacteria
- Bacteria + Phage → Infected Bacteria

Why use reaction equations?
- Allows stochastic modeling
- Can be approximated by differential equations
- Hybrid simulator allows for both
Epidemic Spread among Populations
Gene Network
Bacterial Growth
Model
Single Population Infections
The multi-scale model
Initial Attempt at Matching Bacterial Growth

- Exponential Growth
- Unrealistic
  - The bacteria grows unhindered
- The match could be improved with a sigmoid curve

Modeling with Exponential Bacterial Growth

- Simulated Growth
- Actual Growth

Graph showing bacterial growth over time with concentration on the y-axis and time on the x-axis.
Building the Bacterial Growth Model

- Monad's bacterial growth model and Modified Michaelis Menten's enzyme kinetics model

\[
\begin{align*}
B(\text{bacteria}) + S(\text{substrate}) & \xrightleftharpoons[k_1]{k_2} X(\text{intermediate}) \\
X(\text{intermediate}) & \xrightarrow{k_2} 2B(\text{bacteria})
\end{align*}
\]

- Lag phase
  - Caused by stresses from new environment

- Exponential Growth
  - Growth occurs at the rate assigned by \( k_2 \)

- Saturation
  - Growth levels off
  - Saturation point is controlled by

\[
k_m = \frac{k_{-1} + k_2}{k_1}
\]
Matching Bacterial Growth

- Modified Monad’s bacterial growth model and Michaelis Menten's enzyme kinetics model creates a sigmoid curve
- Stress into new environment causes growth to lag initially
- Growth begins to rise exponentially
  - no constraints
- Growth levels off at saturation point
  - not enough resources for it to continue
Building the Infection Model

- **Add Phage to Growth Model**
  
  \[(\text{uninfected bacteria}) + (\text{phage}) \longrightarrow (\text{infected bacteria})\]

- **If cell becomes lytic**
  
  \[(\text{infected bacteria}) \longrightarrow (\text{lytic})\]
  \[(\text{lytic}) \longrightarrow 100 (\text{phage}) + (\text{space})\]

- **If cell becomes lysogenic**
  
  \[(\text{infected bacteria}) \longrightarrow (\text{lysogenic})\]
  \[(\text{lysogenic}) + (\text{space}) \longleftrightarrow (\text{lysogenicS})\]
  \[(\text{lysogenic S}) \longrightarrow 2 (\text{lysogenic})\]
Matching Infection

- Bacterial growth causes population to increase.
- First decline - bursting of lytic cells
- Cells begin bursting at 40 minutes
  - takes longer for lysis to overtake bacterial growth
  - cause decline in concentration
- Population increases at 550 minutes - lysogenic cells continue to grow

Debris from the dead cells are distorting the OD measurements.
Population Interaction model

- Predicts the spread of infection between populations
- Grown in 96 well plates
- Can implement a variety of patterns
  - Simple diffusion
  - Air Traffic Data

[Map of the United States with air traffic data points: LAX, JFK, ATL]
Toolkit
Discussion

- **Current Projects**
  - Post-doc working on lysis publication
  - Sequencing the entire registry
  - Modeling publications

- **Used hybrid simulator, but not necessary**

- **Refine biological model for more randomness**
  - Smaller concentrations of *E. coli* and phage λ
  - Smaller volume for infections
  - Engineer phage to be less stable
Contributing Back

- Defined in the Registry
  - Modified Lambda Promoter
  - Reporter Plasmid
  - Reversed Fluorescent Protein gene: dsRED

- Contributed to Registry
  - Fluorescent Phage
  - Sequencing: with so many parts, it’s possible to run into contamination...

- Other Contributions
  - Models
  - Toolkit
  - Publications
Acknowledgements

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**Hybrid Simulator:** PERFORM Laboratory, directed by William Sanders

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Questions?