Directed evolution of phage lysins: using mathematical models to explore feasibility/design of new antibacterial drugs

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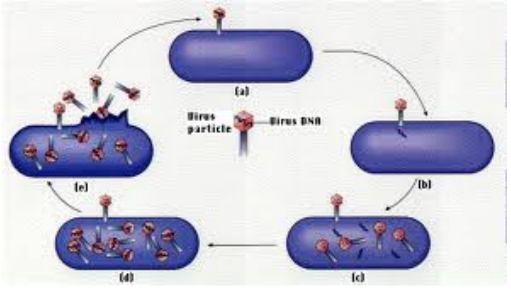
- Antibiotic resistance $\Longrightarrow$ search for novel methods to combat bacterial infections
- Directed evolution

Phage lysins

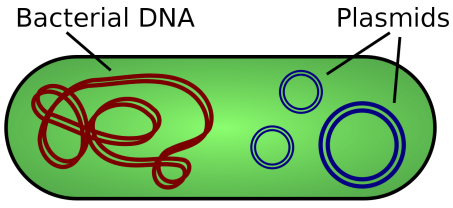


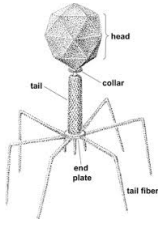
- Lysins have evolved to kill host cell from inside and not harm future hosts
- potential for using lysins to "kill from the outside" (Fischetti, Nelson)
- promising as antimicrobials: only harm bacterial cells; specific bacterial targets; no bacterial resistance
- obstacles to therapeutic use: short half life, etc.

Evolving a better lysin

- Mathematical models: explore feasibility of experimentally evolving better lysins (longer half life, toxicity, diffusivity, adsorption, ...)
- Goal: Improve killing of pathogen R (e.g., group A strep) by lysin/toxin
- *Experimental setup: rigged so that "producer" strain G benefits from enhanced killing of competitor R

Two possible delivery systems

- toxin (that kills R) carried on gene in
  - plasmid in G 

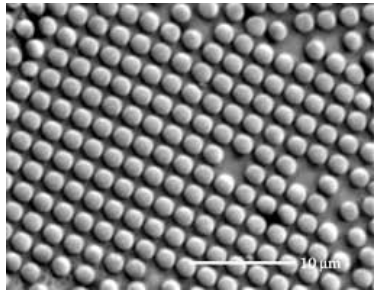
  - phage that infects G 

Experimental system

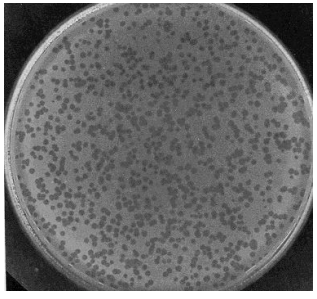
- co-culture R + WT G and/or mutant strain(s) G' (standing genetic variation)
  - select or screen for G'
  - 10 incubation cycles involving R + (G and/or G')
  - Selection: after each incubation, collect G-types (or phage) together and resample to start next round of competitions with R
  - Screening: only keep samples with "enough G" (fluorescence for sorting)

**** simulation

- Some type of spatial structure needed to localize the benefit; in liquid, G and G' would both benefit from improved toxin in G'
  - emulsion droplets (≤ 100 cells, G+R or G'+R) for mini competitions



  - agar plates

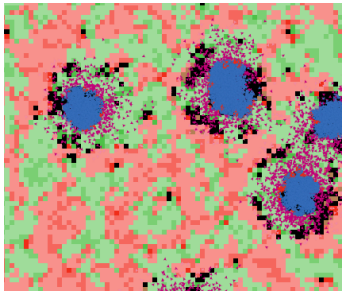


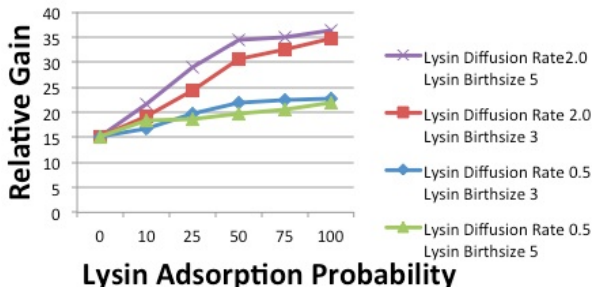
Mathematical Models

Emulsion droplets (endpoint analysis, ODEs): mean q
Poisson distribution for initializing G-types in droplets $\Longrightarrow$
ratio of G' to G after one incubation cycle is

$$\frac{G_1'}{G_1} = \frac{h'}{h} \cdot \frac{G_0'}{G_0} \cdot \frac{2+q}{2+q+qG_0'(\frac{h'}{h}-1)}$$

Plate growth (spatial agent-based models):





Emulsion droplets: Endpoint analysis

Ignore details of what goes on in droplets. Focus on outcome of each incubation:

- Droplets with only G (+R) produce h G during incubation
- Droplets with only G' (+R) produce h' G' during incubation
- Assume (for now) ... only these "pure" droplets

Let $p_0 =$ initial frequency of G' and $p_k =$ frequency at the end of cycle k of G' among all 'G-types' (G' + G) in the population. Evolutionary dynamics has a simple form:

$$\frac{p_1}{1-p_1} = \frac{h'}{h}\left(\frac{p_0}{1-p_0}\right) \tag{1}$$

for the first cycle, and

$$\frac{p_k}{1-p_k} = \left(\frac{h'}{h}\right)^k \left(\frac{p_0}{1-p_0}\right) \tag{2}$$

after k cycles. Relevant measure of mutant fitness (W) in this protocol is simply

$$W = \frac{h'}{h}. \tag{3}$$

accounting for mixed droplets

Mean q Poisson distribution for initializing G-types in droplets $\implies$ ratio of G' to G after one incubation cycle is

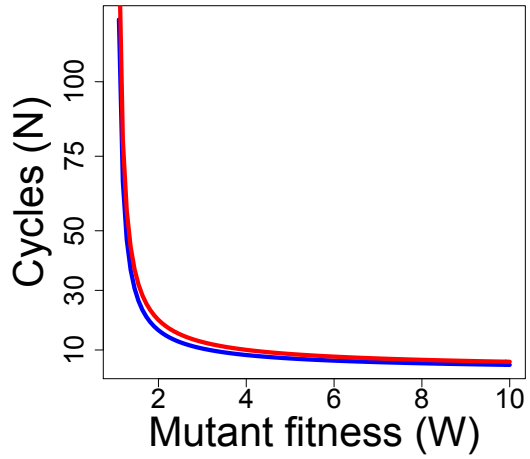
$$\frac{p_1}{1-p_1} = \frac{h'}{h} \cdot \frac{p_0}{1-p_0} \cdot \frac{2+q}{2+q+qp_0(\frac{h'}{h}-1)}$$

Not a problem when p_0 small. Previous result is a good approximation.

How many incubation cycles?

$$p_N = \frac{W^N p_0}{W^N p_0 + 1 - p_0}$$

$$N\,[\ln(\mathrm{W})] = \ln\left[\frac{p_N(1-p_0)}{p_0(1-p_N)}\right]$$



To go from initial frequency of 10^{-7} to detectable levels (0.01 blue; 0.1 red)

Dynamics within droplet for plasmid model

$$\dot{G} = \psi_{_G} G(1 - \frac{G+R}{K})$$

$$\dot{R} = \psi_{_R} R(1 - \frac{G+R}{K}) - \gamma RL$$

$$\dot{L} = \alpha G(1 - \frac{G+R}{K}) - \delta L$$

G outgrows R if

$$L \ > \ \frac{(\psi_{_R} - \psi_{_G})(1 - \frac{G+R}{K})}{\gamma} \ ,$$

but both may be increasing. R decreases if

$$L \ > \ \frac{\psi_{_R}(1 - \frac{G+R}{K})}{\gamma}.$$

Dynamics within droplet for phage model

$$\dot{P} = -a_1 PG \ + b a_1 P_\tau G_\tau$$

$$\dot{G} = -a_1 PG \ + \psi_{_G} G(1 - \frac{G+R}{K})$$

$$\dot{R} = \psi_{_R} R(1 - \frac{G+R}{K}) - a_2 LR$$

$$\dot{L} = Z a_1 P_\tau G_\tau \ - a_2 LR \ - \delta L$$

Dynamics within droplet for phage model

$$\dot{P} = -a_1 PG \; + b a_1 P_\tau G_\tau$$

$$\dot{G} = -a_1 PG \; + \psi_{_G} G(1 - \frac{G+R}{K})$$

$$\dot{R} = \psi_{_R} R(1 - \frac{G+R}{K}) - a_2 LR$$

$$\dot{L} = Z a_1 P_\tau G_\tau \; - a_2 LR \; - \delta L$$

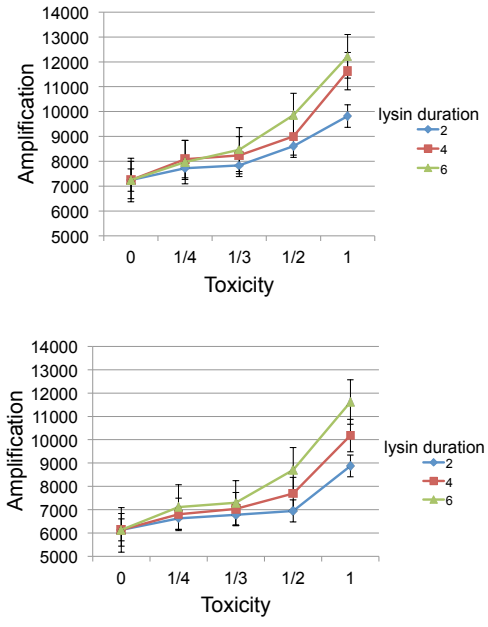
FAILURE: Phage reproduction much faster than bacterial growth. G can't take advantage of removed R.

For phage-delivered lysin to work, need real spatial structure (plates).

Individual-based models of plate growth

Phage model

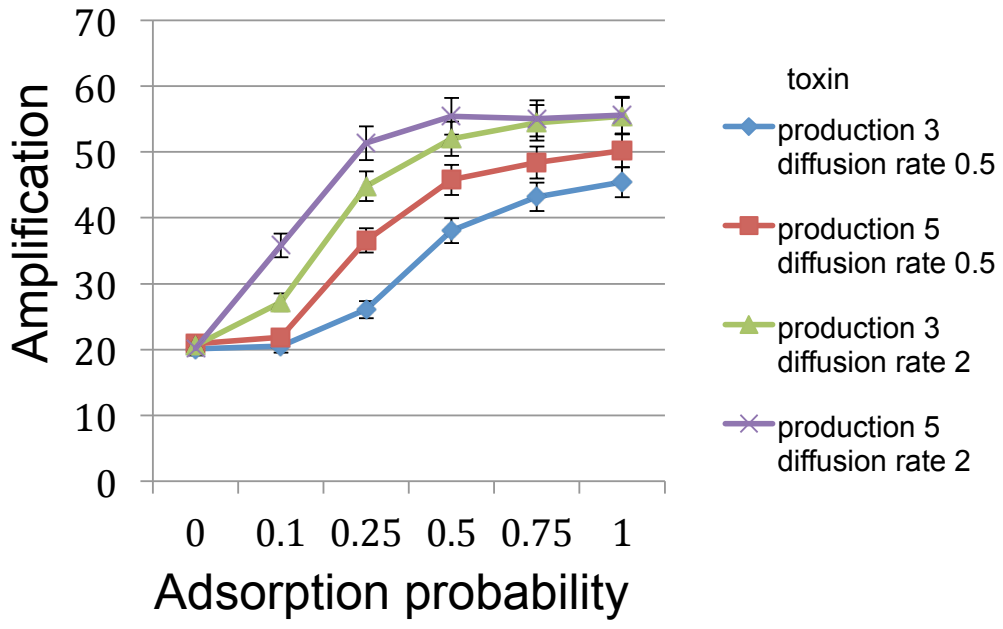
****simulation



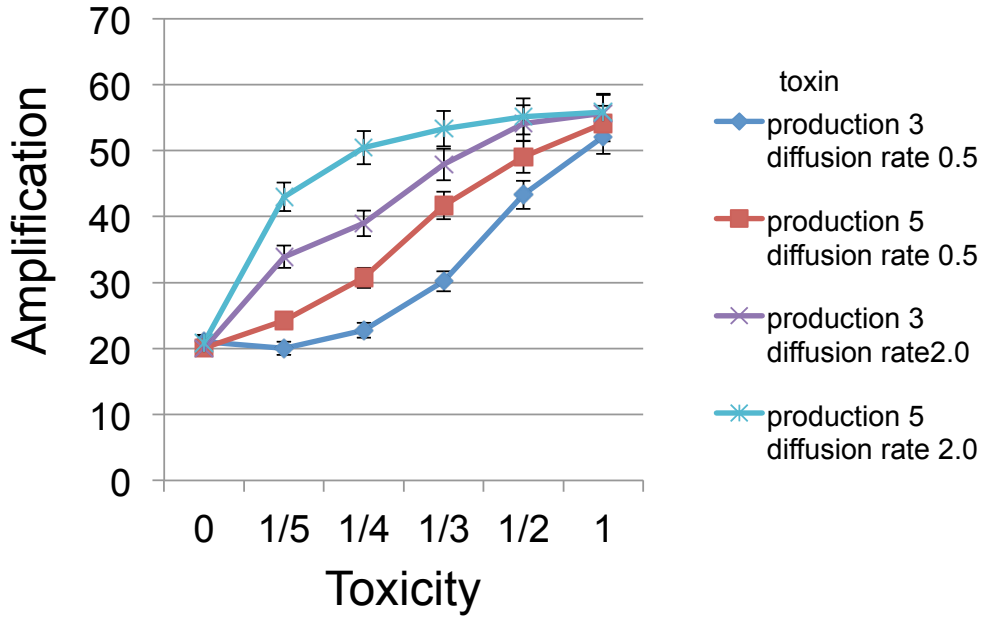
Amplification in number of phage as a function of lysin toxicity and duration for fast (top) and slow (bottom) phage reproduction; 1.7-1.9X increase. [0 point $\Longrightarrow$ no lysin]

Individual-based models of plate growth

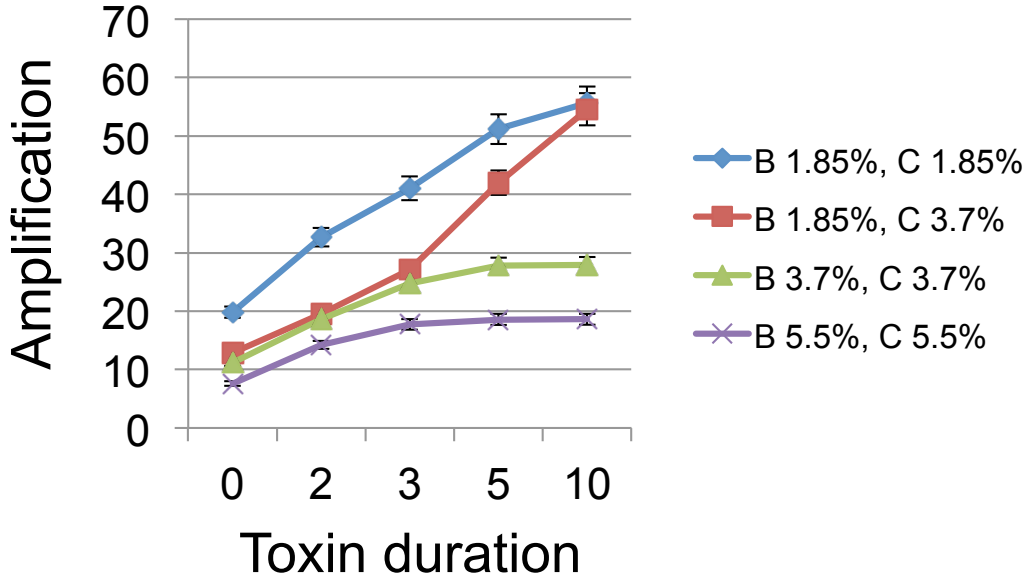
Plasmid model



Amplification in number of G. Select for faster toxin diffusion, enhanced production, higher adsorption rate.
Overall amplification 2.8X.



Amplification in number of G as function of toxicity. Overall amplification 2.7X.



Amplification in number of G as function of toxin half life. Larger dynamic range with lower initial cell densities and more R than G. Overall amplification 2.8-4.3X.

Bull et al. (2015), Models for the directed evolution of bacterial allelopathy: bacteriophage lysins. PeerJ 3:e879; DOI 10.7717/peerj.879

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