Modelling the growth and evolution of interacting microbial communities

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Abstract

The evolution and adaptation of microbial populations play an essential role in the development of bacterial antibiotics resistances. Here we present a combined theoretical-experimental study to understand the mechanisms governing the evolution of microbial populations and the role of different factors involved in bacterial interactions. We have experimentally studied dynamics of interactions between the strain of S. aureus (SH1000) with different inhibitor producing strains of S. epidermidis (B180, B155 and TU3298) differing in their level of toxicity. We have performed a set of experiments to analyse the growth rate, toxicity level and evolution of each strain separately. Also, a set of experiments involving mutual invasions of toxin-producing populations of S. epidermidis and susceptible populations of S. aureus with varied initial frequencies were conducted. In order to explain our experimental findings, we have developed a few mathematical models which we used for simulation of the bacterial dynamics in the above experiments. Our simulations have confirmed that the toxicity level can drastically affect the evolutionary outcomes. Furthermore, our models reproduce and explain the way how competitive interactions between toxin-producing bacteria with their susceptible/adapted counterparts influence the two-lineage community response to toxins exposure.

Introduction

The emergence of antibiotics helped to eliminate many of the epidemics and infections. However, the spread of antibiotic resistance can bring us to the post-antibiotic era which may appear to be similar to the pre-antibiotic one when minor infections have caused people to die.

Types of community interactions

Coexisting populations of microorganisms form a community. There are different types of interactions within and between these communities. Competition for resources and space, symbiosis and predations are the most common types of such interactions.

Competition models

As an example of how two or more species compete for the limited resources or inhibit each other's growth, we consider the basic 2-species *Lotka-Volterra* competition model:

$$\begin{cases} \frac{du}{dt} = D_u \, \Delta \, u + f(u,v) \\ \frac{dv}{dt} = D_v \, \Delta \, v + g(u,v) \end{cases}$$

where species u and v have logistic growth in the absence of the other. First terms in the RHS describe motility of bacteria, while $f(u,v)$ and $g(u,v)$ describe local dynamics of interacting bacteria. This model can be extended in many ways. For instance, we can include another variable to account for the emergence of adapted populations, that can survive the competition with their counterparts. Furthermore, when modelling the interactions between inhibitory and susceptible populations, we extend the model further with another variable representing the toxin concentration.

Experimental methods

Growth rate and generation time of bacteria

Eight replicates for each strain were incubated at 37°C for 24 h, and OD_{600} readings were taken at 30-min intervals.

Inhibition assays

- ✓ Preparing the Bacterial Overnight Culture(s).
- ✓ Spot Culture of the Inhibitor-producing Isolate.
- ✓ Preparing the spray inoculum of the test competitor strain(s).
- ✓ Replicates

Competitions

- ✓ All bacterial strains were cultured at *37°C* in *10 mL BHI* broth shaken at *200 rpm* and on agar solidified *BHI* medium. The *cfu/mL* in each tube was equalized
- ✓ Both species were then mixed together in a final volume of *10 mL PBS*, $(invader : resident = 0.01 : 1 = 10\ \mu l : 1\ ml)$.
- ✓ The controls and mixtures were vortexed thoroughly before $50\ \mu l$ was plated onto *25 mL BHI* agar and incubated at 37°C.
- ✓ Viable counts for each isolate were calculated everyday .

Theoretical methods

Competitions for resource

The toxins in B180 produced inhibition zones against the competing S. aureus. However, these zones were considered to be small when comparing to the other inhibition zones produced by other strains. Hence, we decided to neglect the effect of the toxins when modelling the interaction dynamics between B180 and SH1000.

Inhibition + competition for resources

Four- variables model was constructed to model the interaction dynamics between toxin producing epidermidis strains and S. aureus.

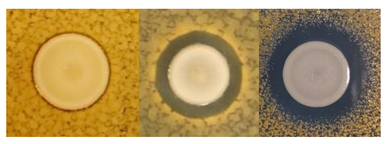
Experimental results

Growth rate and generation time of bacteria

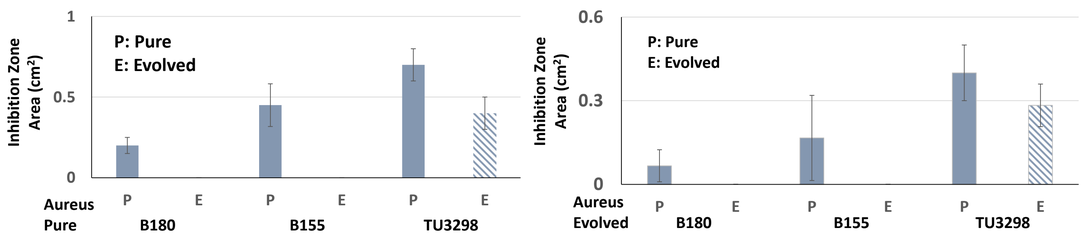
For measurements of doubling times we used the growing population of SH1000 (S.aureus) as a control. The doubling time were determined per minute as follow: SH1000= 36.48 , B180= 32.75 , B155= 42.88 and TU3298= 33.54 , in addition, we were able to determine the growth rate for the used strains.

Inhibition assays

Inhibition of aureus growth by epi… was indicated by the shadow along perimeter…. of SH1000 before and after invasion was .



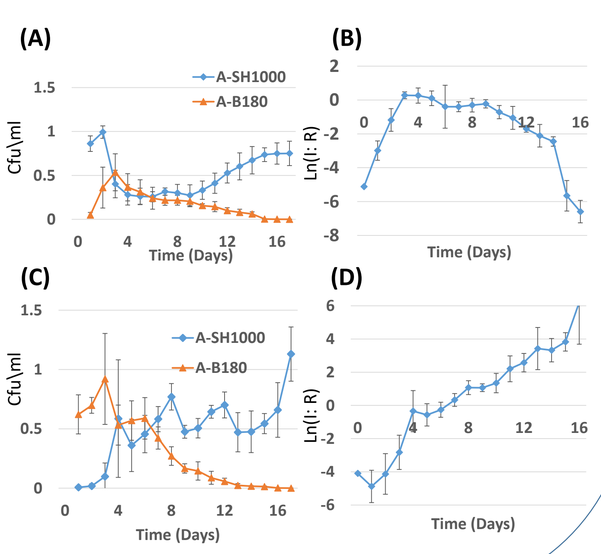
B180 B155 TU3298



Right panel shows inhibition zones produced by pure S. epidermidis strains, and left - by the evolved S. epidermidis strains. Both show the inhibition zone area (cm^2) produced by the inhibitory S. epidermidis strains against the pure SH1000 (P) and the evolved SH1000 (E).

Competitions

A and B, Isolates of S. epidermidis (B180), (Orange) invading populations of S. aureus (SH1000), (Blue) at frequency of (0.01:1). C and D, Isolates of S. aureus (SH1000), (Blue) invading populations of S. epidermidis (B180), (Orange) at frequency of (0.01:1). The x-axis is the time in days in all figures, in (figure A and C), y-axis is the colony-forming units (cfu) per plate. In (figure B and D), the y-axis is the natural log of the invader to resident ratio. Error bars represent the standard error of the mean (n=5).



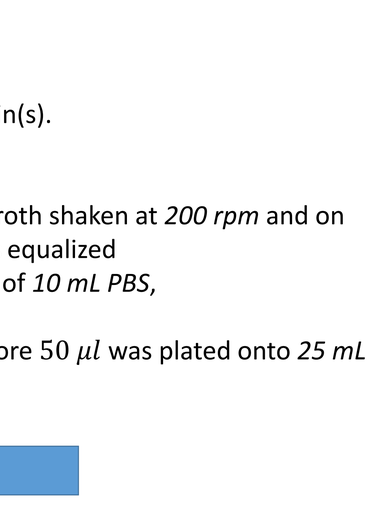
Theoretical results

Growth rate and generation time of bacteria

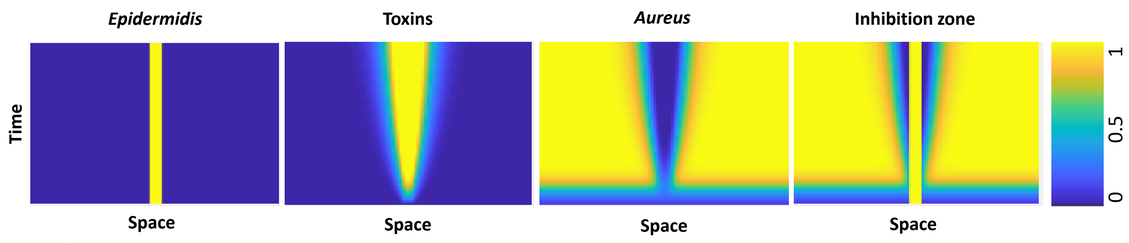
This was modelled using the simplest model where a single strain (N) using a single resource (R) was considered. The strain N was assumed to have a logistic growth while the resource R to be not renewable:

$$\begin{cases} \frac{dN}{dt} = r\, N\, (1 - \frac{N}{R}) \\ \frac{dR}{dt} = -c\, N \end{cases}$$

Panels to the right show experimental measurements (dots) and modelled dynamics (solid lines).

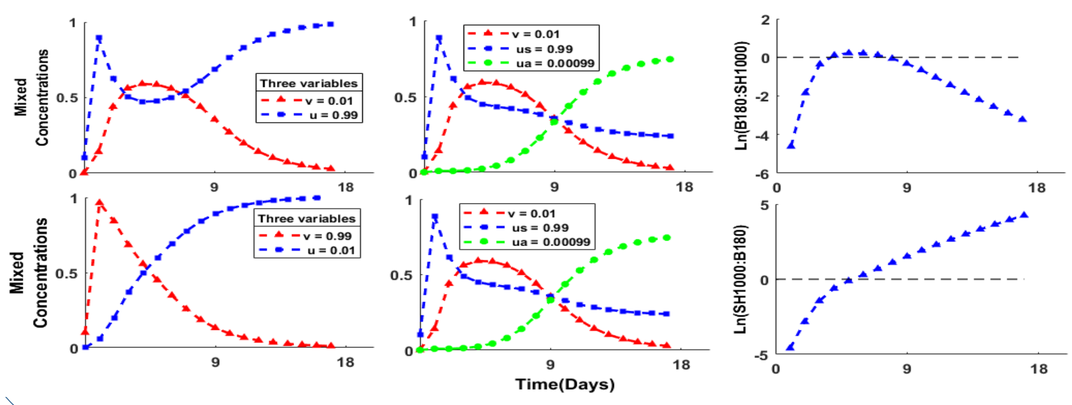


Inhibition assays



- ✓ Spot Culture of the Inhibitor-producing Isolate. We assume that $D_{epidermidis} = 0$.
- ✓ The toxin substance diffuses after overnight incubation, $D_{toxins} \neq 0$.
- ✓ The spray inoculum of the test competitor strain (SH1000).
- ✓ The inhibition zone.

Competitions



Conclusion

Our main findings include experimental and theoretical evidence for:

- *Positive association between the level of toxicity expressed by S. epidermidis and the time consumed by S. aureus populations to adapt to this toxicity.*
- *Negative association between the size of S. aureus populations and the time required for its adaptation and appearance of the resistance to toxin.*
- *A positive relationship between the level of S. epidermidis toxicity and its ability to survive and avoid extinction while interacting with S. aureus.*