Second part
Modeling Phage-induced Infection Dynamics of *Escherichia coli*

Abstract

As previously discussed in the main page, we are interested in engineering a system (the killer strain) that is able to kill a desired *E. coli* strain (the prey strain). We have developed a model which is able to describe the dynamics of the phage-killing system when the killer cells have already swum to the prey cells by chemotaxis and are equally distributed among the prey cell population. After introducing the model, we will analyze the stability and efficiency of the system. Numerical values for the parameters were obtained partly in the lab and partly from the literature. After analyzing one simplified model and doing simulations for the full model we have found out that the system we want to design in the lab should be quiet stable and efficient. It is shown that ten killer cells can kill more than 10^9 prey cells in about five hours.

Contents

1 Introduction

The details of the system we want to model are documented in the main page of the killing group. Here we want to summarize the system in a few words: When the killer cells have reached the prey cell population conjugation is initiated. After the F-pili, which are produced by helper-plasmid pUB307 in the killer cells, are attached to the prey cells, the copy of the lambda plasmid with the oriT will be transported across the pili into the prey cells, transforming an uninfected prey cell into an infected prey cell. The killer cell then leaves the infected prey cell and is ready for the next conjugation. For conjugation a binary vector system is used. It consists of a helper plasmid (pUB307) and the mobilizable lambda genome. The helper plasmid contains all the genes necessary to carry out conjugation, which need not to be included in the mobilizable plasmid. These elements are trans acting ones. Our helper plasmid is derived from a broad host range conjugative plasmid of *E. coli*, which is called RP1. The only elements necessary for conjugation that are cis acting ones and therefore have to be on the plasmid to transfer are the oriT and some mob genes, which are included in the lambda genome. If the helper plasmid does not have a functional oriT, it cannot be transported into the prey cells by conjugation. Consequently, the infected prey cells are not able to perform a conjugation with uninfected prey cells. The phages should then be produced after a certain time, when the replication of the Lambda DNA, the expression of the Lambda proteins and the packaging are finished. The Lambda phages in turn can also attach to the uninfected prey cells and infect them by transferring their DNA, causing the prey cells to begin to produce Lambda phages again, which initiates a snowball effect. In the literature you can find several models on phage-bacteria and conjugation dynamics. A very early consideration for phage-bacteria dynamics is presented by M. Delbrück in 1940 [4]. The more detailed models for phage-bacteria dynamics are presented by R. J. H. Payne et. al [9], E. Beretta et. al [2], Ing-Nang Wang [10], P. Mudgal et. al [7] and E. Chapman-McQuiston et. al. [3]. A very simple, but good model for kinetics of conjugative transfer is presented by B. R. Levin et. al [5].

Since in our system the kinetics of conjugation and the phage-bacteria dynamics run in parallel, we needed to construct a model, which combines these two interactions in one single model. In this modeling approach we assume that the diffusion of the cells and the phages has already reached equilibrium, allowing us to neglect the spatially uneven distribution of the cell densities. The model hereby naturally complements the PDE approach of our other model by using ODEs and delay differential equations (DDEs) to be able to better resolve the time dynamics of the actual killing part, which takes place after the sensing.

For the mathematical analysis we simplified the model, because, as a DDE sys-

tem, our extended model is rather complex and we have to fall back to numerical methods. By performing the stability analysis for the simplified model analytically, we gained further insights on the solution of our highly non-linear system.

For the numerical analysis we used Matlab 7.1, especially the functions ode45 and dde23. For the most of the parameters in our model, we used the numerical values provided in the dissertation of R. G. Moldovan [6] and the published values of Ing-Nang Wang [10]. For the conjugation rate we used the experimental value of the characterization of the oriT+pUB307 in our lab, which is presented in our main page.

2 Model Description and Assumptions

The model presented here consists of the following five components:

- x: density of uninfected prey cells,
- y: density of infected prey cells,
- u: density of killer cells,
- z: density of conjugation complexes consisting of one uninfected prey cell and one killer cell,
- v: free phages.

The model scheme is presented in figure 1. The cell densities are given in $\frac{\text{number of cells}}{\text{ml}}$ and the phage density is given in $\frac{\text{number of phages}}{\text{ml}}$

For this model, we have the following assumptions:

A1 The conjugative transport consists of two steps. In the first step, the killer cell meets the uninfected prey cell, and a conjugation complex z is formed. In the second step, after the Lambda plasmid with the oriT is transported, the conjugation complex z disintegrates into one infected prey cell y and one killer cell u. We assume that this disintegration can be described with a simple first order rate k_1, which means that the conjugation complex will exist the time $1/k_1$ in average. During this time the transport of the copy of the Lambda plasmid takes place. The state z is introduces an effective time delay of $\approx 1/k_1$ for the overall process $x + u \to z \xrightarrow{k_1} y + u$.

A2 Because modeling all the sub states involved in the production of phages would not be feasible, we simplify this process by assuming that phage maturation occurs during a time Δ. This means that a new infected cell produces free phages by lysis with a time delay Δ.

A3 We assume free phages can only bind to uninfected prey cells. We neglect the possibility of a new phage infecting a previously infected prey cell.

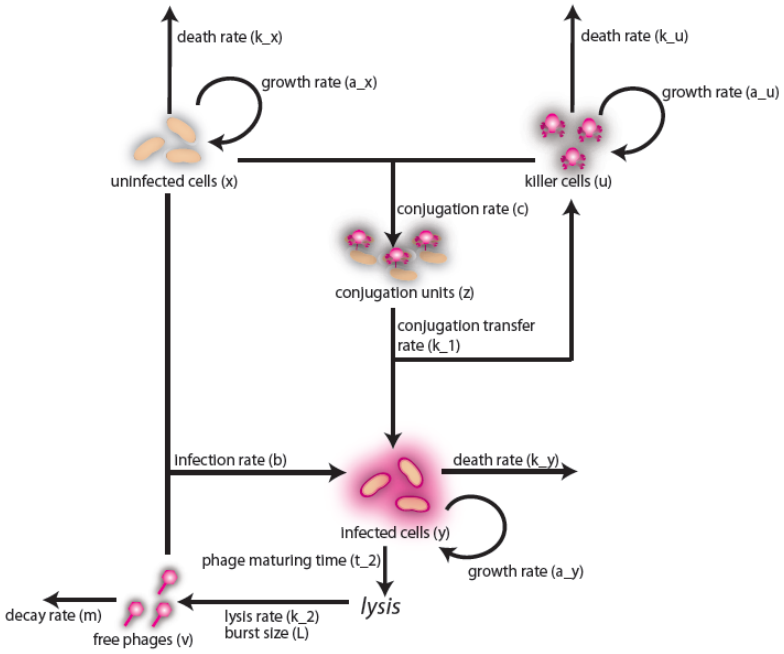

Figure 1: Model Scheme.

A4 We assume a new infected cell exists as soon as one free phage binds to the uninfected prey cell.

A5 As discussed above, we assume all concentrations to be spatially independent and that our biological system can be described well using mass action kinetics.

Based on this five assumptions we can describe the change in concentration over time by a system of five delay differential equations, which we will now discuss in detail.

Considering the uninfected prey cells, we have an input caused by cell growth and an output caused by infection by killer cells and free phages. We assume that in the absence of phages and killer cells, the uninfected prey cell population grows according to a logistic equation with carrying capacity κ and an intrinsic growth rate constant a_x, resulting in

$$\frac{\mathrm{d}x(t)}{\mathrm{d}t} = a_x \left(1 - \frac{x(t)+y(t)+u(t)+z(t)}{\kappa}\right) x(t) - b\,v(t)\,x(t) - c\,u(t)\,x(t)\,, \qquad (1)$$

where the term $x(t)+y(t)+u(t)+z(t)$ describes the total density of all the cells in the environment competing for the same nutrients. In this equation, b is the adsorption

rate of the phages binding to uninfected prey cells and c is the rate at which a killer cell binds to an uninfected prey cell to form what we called the conjugation complex z. Because the goal of our system is to kill the prey cells very quickly, we here neglected the normal cell death, which would result in an additional term $-k_x\, x(t)$.

The input terms in the killer cell population u consist of the logistic growth with rate a_u and of the killer cells which result from the dissociation of the conjugation complex z with rate k_1 (as mentioned above, this step takes place after the Lambda plasmid was transported into the prey cell). As output we only have the killer cells which bind to the uninfected prey cells forming a conjugation complex z. We again neglect normal cell death, since the survival time of the killer cells is assumed to be much greater than the time scale of the killing process. We thus arrive at

$$\frac{\mathrm{d}u(t)}{\mathrm{d}t} = a_u \left(1 - \frac{x(t)+y(t)+u(t)+z(t)}{\kappa}\right) u(t) - c\, u(t)\, x(t) + k_1\, z(t)\,. \tag{2}$$

For the conjugation complex we have:

$$\frac{\mathrm{d}z(t)}{\mathrm{d}t} = c\, u(t)\, x(t) - k_1\, z(t)\,. \tag{3}$$

We now consider the population of infected cells y. In order to implement the delay Δ in a consistent way, we will first write down the integral equation for y, which we shortly want to motivate by the following arguments. Let us first look at the differential equation

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = \mathrm{in}(t) - k_{\mathrm{death}} N(t)\,. \tag{4}$$

Using the initial conditions at $t = 0$ we can equivalently rewrite the system as the integral equation

$$N(t) = \int_0^t \mathrm{d}\theta\, \mathrm{in}(\theta)\, e^{-k_{\mathrm{death}}(t-\theta)} + N(t=0)\,. \tag{5}$$

This equivalence will help us modeling the following situation for the infected cells y: Right after the infection, taking place with the rate $b\, v(t)\, x(t) + k_1\, z(t)$ (infection by phages and conjugation), the cells rest in the infected state for the time Δ, producing the phages, or die at a rate k_y. After the delay Δ, we want to allow the infected cells to lyse at rate k_2.

We will denote the state of infected cells that are *not* ready for lysis by y_1. Cells in this state at time t must have been infected between $t - \Delta$ and t, so, analogous to eqn. 5,

$$y_1(t) = \int_{t-\Delta}^t \mathrm{d}\theta\, \left[b\, v(\theta)\, x(\theta) + k_1\, z(\theta)\right] e^{-k_y(t-\theta)}. \tag{6}$$

Let now y_2 be the cells which *are* ready for lysis. These cells got infected at a time θ between 0 and $t-\Delta$ and thus had the time $t-(\theta+\Delta)$ to lyse and the time $t-\theta$ to die:

$$y_2(t) = \int_0^{t-\Delta} \mathrm{d}\theta \; [b\, v(\theta)\, x(\theta) + k_1\, z(\theta)]\, e^{-k_y(t-\theta)} e^{-k_2(t-(\theta+\Delta))}. \tag{7}$$

Accordingly, all infected cells correspond to the sum

$$y(t) = y_1(t) + y_2(t)\,. \tag{8}$$

We can now rewrite the eqns. 6 and 7 into a system of two differential equations

$$\frac{\mathrm{d}y_1(t)}{\mathrm{d}t} = b\, v(t)\, x(t) + k_1\, z(t) - (b\, v(t-\Delta)\, x(t-\Delta) + k_1\, z(t-\Delta))\, e^{-k_y\,\Delta} - k_y\, y_1(t) \tag{9}$$

$$\frac{\mathrm{d}y_2(t)}{\mathrm{d}t} = (b\, v(t-\Delta)\, x(t-\Delta) + k_1\, z(t-\Delta))\, e^{-k_y\,\Delta} - (k_y + k_2)\; y_1(t)\,. \tag{10}$$

Note that we cannot reduce the system to a single equation in y.

Lastly, we have to specify the dynamics of the free phages. The influx of phages comes from the lysing of the infected cells and can be read off from eqn. 10. In each lysis, L (burst size) new free phages are produced. Furthermore, the phages can perform conjugations and can be degraded at a rate m. Thus,

$$\frac{\mathrm{d}v(t)}{\mathrm{d}t} = L\, k_2\, y_2 - m\, v(t) - b\, v(t)\, x(t)\,. \tag{11}$$

From the published experimental values in [10] (fig. 2) we found out that the infected cells are stable enough to live much longer than the phage maturing time Δ, because the cell density does not decrease after inducing the replication and expression of the Lambda plasmid and proteins. This means we can assume $k_y = 0$.

As indicated in fig. 2, we can use this measurement to estimate both Δ and $1/k_2$. But, in contrast to Δ, $1/k_2$ is measured with a big uncertainty since the temporal uncertainty of the time of induction is so big that $1/k_2$ could also be close to zero, meaning k_2 would be very big. We will for this reason simplify our system by assuming $k_2 \to \infty$.

In this limit we get

$$y(t) = y_1(t) = \int_{t-\Delta}^{t} \mathrm{d}\theta \; [b\, v(\theta)\, x(\theta) + k_1\, z(\theta)]\,. \tag{12}$$

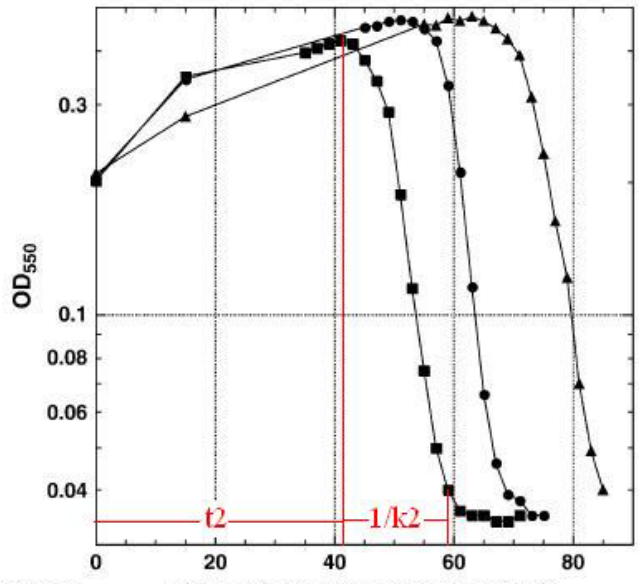

Figure 2: Lysis time determination: A standardized method similar to the previously described procedure [10] was used to determine the lysis time for each λ Sallele. Briefly, a 25 ml culture of $\lambda-$lysogen was grown to $A_{550} \approx 0.2$ with constant aeration in a 30° water bath. The culture was then aerated at 42° for 15 min for thermal induction of the prophage, followed by aeration at 37° until lysis. Beginning $\approx$ 5-6 min before the onset of lysis, the A_{550} of the induced culture was recorded every 2 min using a spectrophometer equipped with a fast-acting sipper device. The figure has been modified by Yin Cai and Maria Münch.

We can now follow the same steps as above and arrive at the following final set of equations:

$$
\begin{aligned}
\frac{dx}{dt} &= a_x \left(1 - \frac{x(t) + y(t) + u(t) + z(t)}{\kappa}\right) x(t) - bv(t)x(t) - cu(t)x(t) \\
\frac{dy}{dt} &= bv(t)x(t) + k_1 z(t) - bv(t-\Delta)x(t-\Delta) - k_1 z(t-\Delta) \\
\frac{dv}{dt} &= L(bv(t-\Delta)x(t-\Delta) + k_1 z(t-\Delta)) - mv(t) - bv(t)x(t) \\
\frac{du}{dt} &= a_u \left(1 - \frac{x(t) + y(t) + u(t) + z(t)}{\kappa}\right) u(t) - cu(t)x(t) + k_1 z(t) \\
\frac{dz}{dt} &= cu(t)x(t) - k_1 z(t)
\end{aligned}
$$

a_x – replication coefficient of prey cells
a_u – replication coefficient of killer cells
b – infection rate
c – conjugation adsorption rate
κ – growth capacity
L – burst size
m – decay rate of free phage
k_1 – reaction rate of $z \rightarrow u + y$
Δ – time delay for phage maturing time

The equations $\frac{dy}{dt}$ and $\frac{dv}{dt}$ are delay differential equations, because the time delay Δ is directly included. For DDEs additional information to initial values is required to specify the system. In the section simulation results we will specify which additional information we used.

3 Mathematical Analysis

3.1 Simplifying the Full Model

The full model is very difficult for non-numerical mathematical analysis, so we have to simplify our model. We do this by making the following additional assumptions:

A1' Instead of logistic growth, all cells grow with the same exponential rate a. Cell death is neglected, except for the killer cells which die with a rate e_1. Because the goal of our system is to kill the prey cells very quickly, we here neglected the cell death of prey cells.

A2' Conjugation involves only one step: The transport of the Lambda plasmid during conjugation does not need time and thus no conjugation complex state is necessary. Cells become infected as soon as the killer cells touch them.

A3' Infected cells are ready for lysis right after infection, we do not consider the phage maturation time Δ.

Based on these assumptions we get the following model, which is illustrated in fig. 3:

$$\begin{aligned}
\frac{dx}{dt} &= a\ x - b\ v\ x - c\ u\ x \\
\frac{dy}{dt} &= a\ y + b\ v\ x + c\ u\ x - k\ y \\
\frac{dv}{dt} &= k\ L\ y - b\ v\ x - m\ v \\
\frac{du}{dt} &= ((a\ - e_1)\ - e_2\ u)\ u
\end{aligned}$$

a	–	replication rate
b	–	infection rate
c	–	conjugation rate
k	–	lysis rate
L	–	burst size
m	–	decay rate of free phages
e_1	–	normalized death rate
e_2	–	inner stress rate

The nomenclature of the states is the same as in the full model.

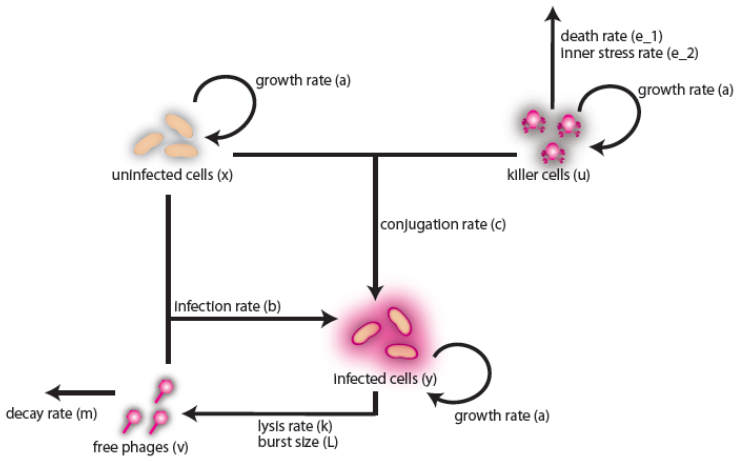

Figure 3: Scheme of the simplified model.

3.2 Linear Stability Analysis

A good place to start analyzing the non-linear system is to determine the equilibrium points and describe the behavior of $\frac{dx}{dt} = f(x)$ near its equilibrium points.

Definition 1:
A point x^* is called an ***equilibrium point*** of $\frac{dx}{dt} = f(x)$ if $f(x^*) = 0$.

Four equilibium points can be found:
(1)

$$\begin{aligned} x &= 0 \\ y &= 0 \\ v &= 0 \\ u &= 0 \end{aligned}$$

(2)

$$\begin{aligned} x &= \frac{(k-a)\,m}{b\,(a+k\,(L-1))} \\ y &= \frac{am}{b\,(a+k\,(L-1))} \\ v &= \frac{a}{b} \\ u &= 0 \end{aligned}$$

(3)

$$\begin{aligned} x &= -\frac{(ce_1 + a\,(e_2-c))\,(a-k)\,m}{b\,(ce_1 + a\,(e_2-c))\,(a-k) + abe_2kL} \\ y &= -\frac{a\,(-ce_1 + a\,(c-e_2))\,m}{b\,(ce_1 + a\,(e_2-c))\,(a-k) + abe_2kL} \\ v &= \frac{ce_1 + a\,(e_2-c)}{be_2} \\ u &= \frac{a-e_1}{e_2} \end{aligned}$$

(4)

$$\begin{aligned} x &= 0 \\ y &= 0 \\ v &= 0 \\ u &= \frac{a-e_1}{e_2} \end{aligned}$$

Definition 2:
Let $f : U \to \mathbb{R}^n$ be a differentiable function in $t_0 \in U, U \subset \mathbb{R}^r$, U open. Then the matrix

$$(f_j^{(i)}(t_0))_{1\leq i\leq n, 1\leq j\leq r}$$

is called the ***Jacobian matrix*** J of f.

The Jacobian matrix of our system is given by

$$J = \begin{pmatrix} a - bv - cu & 0 & -bx & -cx \\ bv + cu & a - k & bx & cx \\ -bv & kL & -m - bx & 0 \\ 0 & 0 & 0 & a - e_1 - 2e_2u \end{pmatrix}$$

Definition 3:
An equilibrium point x^* is said to be ***stable*** if for every neighborhood U of x^* there exists a neighborhood $U' \subset U$ of x^* such that every solution of $\frac{dx}{dt} = f(x)$ with initial condition in U' (i. e. $x^* \in U'$), satisfies $x(t) \in U$ for all $t \geq 0$. Consequently an equilibrium point x^* is said to be ***unstable*** if it is not stable.

Eigenvalues corresponding to the four equilibrium points:

(1) $\underline{\lambda_1 = a}, \underline{\lambda_2 = a - e_1}, \lambda_3 = a - k, \lambda_4 = -m \Rightarrow$ unstable state

(2) $\lambda_1 = a - e_1, \lambda_2 = ..., \lambda_3 = ..., \lambda_4 = ... \Rightarrow$ unstable state

(3) Cumbersome considerations $\Rightarrow$ no state of interest. With the Hurwitz criterion we tried to find out if it can be stable.

Theorem (Hurwitz criterion) [8]:
Let $a_4\lambda^4 + a_3\lambda^3 + a_2\lambda^2 + a_1\lambda + a_0$ be the characteristic polynomial of our Jacobian matrix. Then the following conditions have to be fulfilled to get $\mathrm{Re}(\lambda_i) < 0 \; \forall i = 1, ..., 4$:

$$H_1 := a_3 > 0$$

$$H_2 := \det \begin{pmatrix} a_3 & a_1 \\ a_4 & a_2 \end{pmatrix} > 0$$

$$H_3 := \det \begin{pmatrix} a_3 & a_1 & 0 \\ a_4 & a_2 & 0 \\ 0 & a_3 & a_1 \end{pmatrix} > 0$$

$$H_4 := \det \begin{pmatrix} a_3 & a_1 & 0 & 0 \\ a_4 & a_2 & a_0 & 0 \\ 0 & a_3 & a_1 & 0 \\ 0 & a_4 & a_2 & a_0 \end{pmatrix} > 0$$

In our case we have:

$$a_0 = \frac{(a-e_1)(-ce_1 + a(c-e_2))(a-k)m}{e_2}$$

$$a_1 = -\frac{(a-k)((ce_1 + a(c-e_2))^2(e_1-k) + e_2(-ace_1 + ce_1^2 + a^2e_2)kL)m}{e_2((ce_1 + a(-c+e_2))(a-k) + ae_2kL)}$$

$$a_2 = \frac{(ce_1 + a(-c+e_2))^2(a-k)m}{e_2((ce_1 + a(-c+e_2))(a-k) + ae_2kL} - \frac{ae_2(a-k)kLm}{(ce_1 + a(-c+e_2))(a-k) + ae_2kL}$$

$$+\frac{(ce_1 + a(-c+e_2))(a-k)kLm}{(ce_1 + a(-c+e_2))(a-k) + ae_2kL} + (-a+e_1)(a-k-\frac{ae_2kLm}{(ce_1 + a(-c+e_2))(a-k) + ae_2kL})$$

$$a_3 = -e_1 + k + \frac{ae_2kLm}{(ce_1 + a(-c+e_2))(a-k) + ae_2kL}$$

$$a_4 = 1$$

We have calculated that $H_1 > 0$ if we assume $m, b, a, k, L > 0$ and $k > e_1$, but it hard to find general rules to determine whether $H_i > 0$, $i = 2, 3, 4$. Whether or not our system reaches this state will dependent on the chosen parameter values and also on the initial conditions. That is why in the next section we did an analysis of initial value conditions.
(4) $\lambda_1 = -a + e_1, \lambda_2 = a + \frac{c(e_1-a)}{e_2}, \lambda_3 = a - k, \lambda_4 = -m \Rightarrow$ stable, if $c > \frac{a}{u}$

To illustrate the dependence of the system on condition (4), we picked the following two sets of parameters and ran simulations. Later on we will give more details on the estimation of realistic parameters.

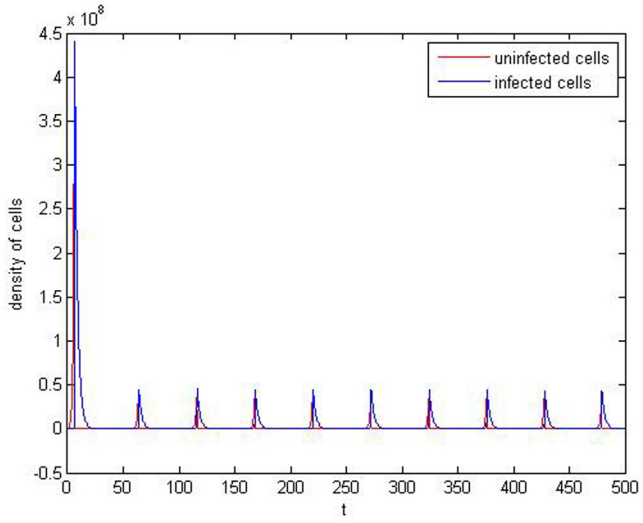

Figure 4: $a = 1.1, b = 5e-8, c = 2e-20, k = 1.5, L = 30, m = 0.5, e1 = 0, e2 = 1.1e-9, x(0) = 500000, y(0) = 0, v(0) = 0$ and $u(0) = 50$

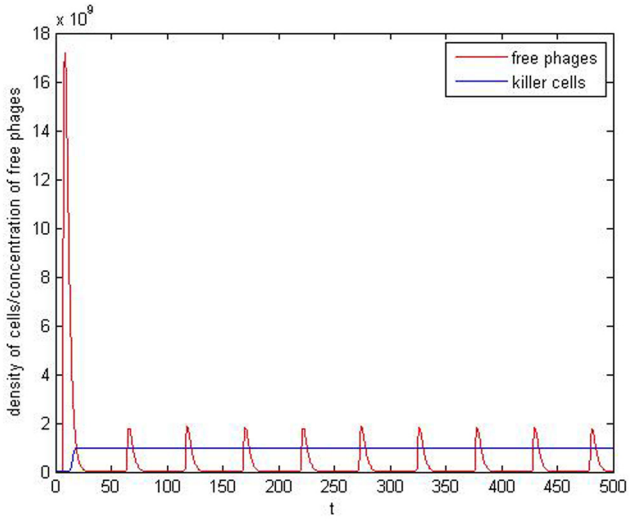

Figure 5: $a = 1.1, b = 5e-8, c = 2e-20, k = 1.5, L = 30, m = 0.5, e1 = 0, e2 = 1.1e-9, x(0) = 500000, y(0) = 0, v(0) = 0$ and $u(0) = 50$

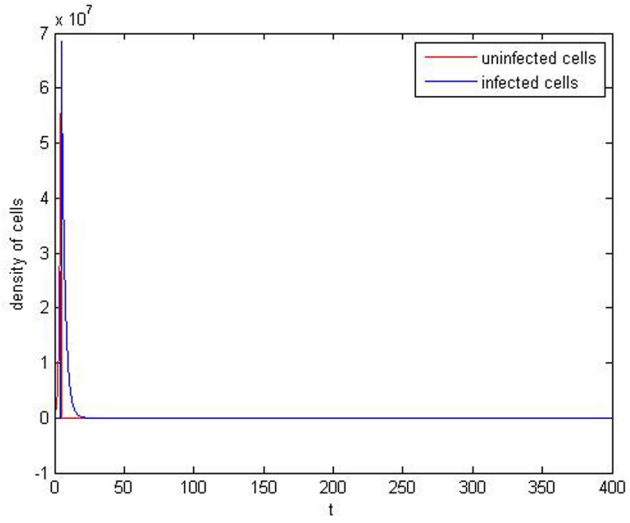

Figure 6: $a = 1.1, b = 5e-8, c = 2e-9, k = 1.5, L = 30, m = 0.5, e1 = 0, e2 = 1.1e-9, x(0) = 500000, y(0) = 0, v(0) = 0$ and $u(0) = 50$

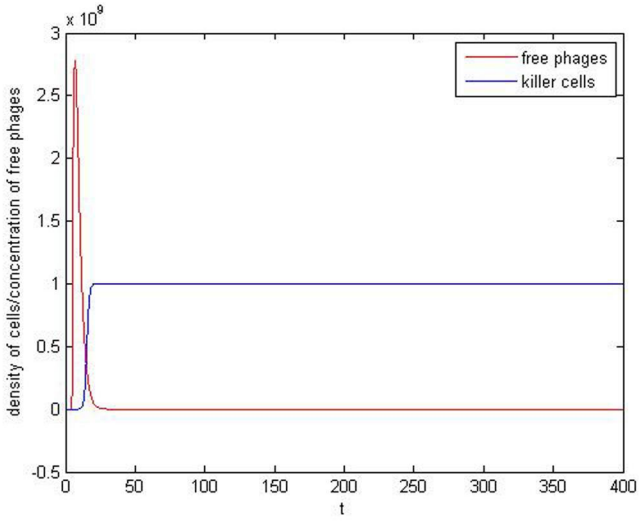

Figure 7: $a = 1.1, b = 5e-8, c = 2e-9, k = 1.5, L = 30, m = 0.5, e1 = 0, e2 = 1.1e-9, x(0) = 500000, y(0) = 0, v(0) = 0$ and $u(0) = 50$

In agreement with the analytic results above, we observe oscillations in the case $c < \frac{a}{u}$ and the steady state (4) otherwise.

3.3 Initial Value Analysis

In the previous section we have calculated four steady states, but actually we do not know which of these states will be achieved by our system. Finally we want to know more about the global properties of our non-linear system, which is analytically difficult. Since numerically we can vary the initial values for the prey and killer cells and check the effects on our steady states, we have written a matlab program, which solves our ODE system in dependance of different initial values of prey and killer cells.

To check whether our system achieves a steady state or not, we have solved the ODE system in the time interval from 851 minutes to 1000 minutes in one minute steps. We chose this time interval, because if the model system reaches a biologically meaningful stable state, it should already have reached it before this time range. We then plotted the sum of all calculated values of $x(t)$ in two dimensions: if $\sum_{t=851}^{1000} x(t) < 1$, which corresponds to no prey cells being present, we plot a red point, else we plot a green point (meaning that there are still prey cells). Oscillations starting much later than after one day are not interesting from the biological point of view, since at that time scale environmental factors, which the model cannot describe anyway, will become important.

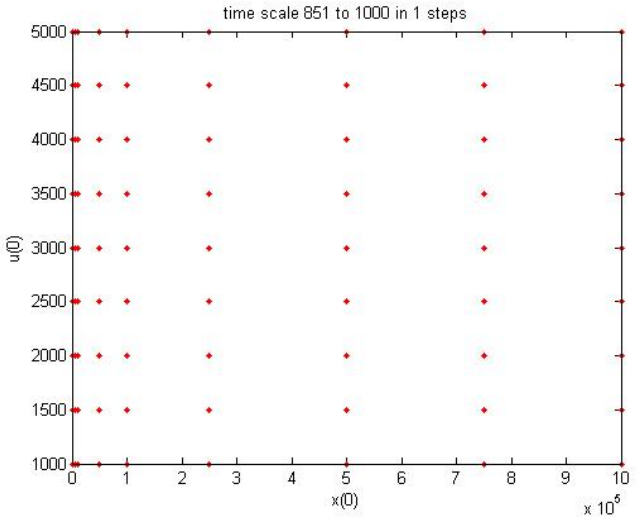

Figure 8: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.322, L = 50, m = 0.00272, e1 = 0, e2 = 1.83e-12, v(0) = 0$ and $y(0) = 0$

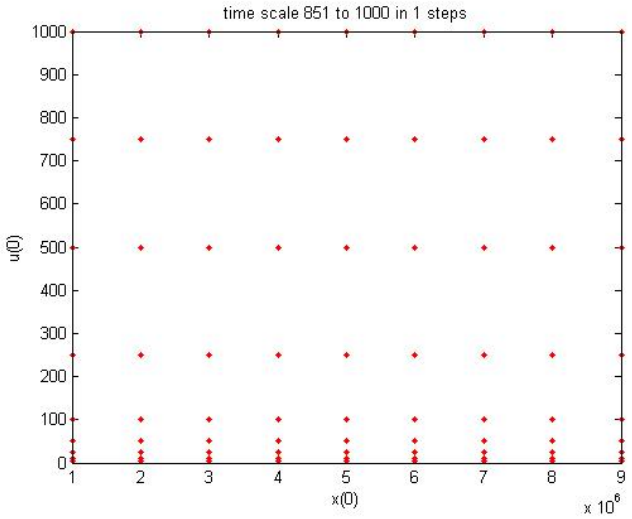

Figure 9: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.322, L = 50, m = 0.00272, e1 = 0, e2 = 1.83e-12, v(0) = 0$ and $y(0) = 0$

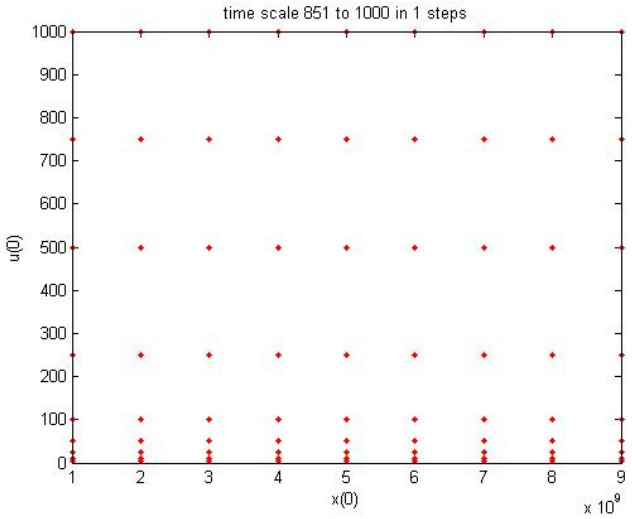

Figure 10: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.322, L = 50, m = 0.00272, e1 = 0, e2 = 1.83e-12, v(0) = 0$ and $y(0) = 0$

The parameters which will be explained in the next section are appropiate for our system. After some tests with different initial values of prey cell and killer cell densities we have found out that the initial concentration of killer cells is not as important as the initial concentration of prey cells. The tests also show that the

system will end up in the desired steady state with zero prey cells after about one day very easily. We did not find any initial value which lead to an oscillation or high values of prey cells.

⇒ If the experimental system could indeed be described approximately with this model and this set of parameters, this killing module would be a very efficient module for a broad range of initial conditions.

3.4 Critical Densities, Critical Concentrations and Critical Times

In this section we want to provide critical thresholds for concentrations of free phages, densities of killer cells and proliferation-onset time. Our aim is to categorize our system with conditions that lead to different outcomes. At first we will only consider conjugation to simplify the analysis and then test whether the conjugation processes alone can lead to total clearance of our prey cells. After that we take a look at the phage dynamics and check whether phage-induced infections alone are able to provide the desired effect. At the end we combine both analyzes.

Conjugation Processes

First we only regard conjugation terms. The system we analyze is therefor given by

$$\begin{aligned} x_t &= a\ x - c\ u\ x \\ y_t &= a\ y + c\ u\ x - k\ y \\ u_t &= a'\ u - a''\ u^2 \end{aligned}$$

with $a' = a - e_1, a'' = e_2$.

We assume that the concentration of uninfected bacteria may be reduced if the density of killer cells, $u\,(t)$, exceeds a critical threshold U_I, given by $U_I = \frac{a}{c}$. This threshold only expresses whether the killer cells can to some degree reduce the number of bacteria throughout conjugation. Whether or not the killer cells will actually succeed in clearing the prey cells is a different matter. Under inundation therapy the prey cells will be cleared if the number of killer cells is greater than a certain threshold, U_C, which will be calculated below. Furthermore we assume that the behaviour of the uninfected cells is dominated by the conjugation term, so that $\mathrm{d}x/\mathrm{d}t \approx -cux$. We now consider the expression

$$\frac{du}{dx} = \frac{dv}{dt} \Big/ \frac{dx}{dt} = \frac{a''u - a'}{cx}.$$

The explicit solution obtainable from integration by separation of variables provides a relationship between x and u:

$$\left(\frac{a''u\left(t\right)-a'}{a''u_{\Phi}-a'}\right)^{c}=\left(\frac{x\left(t\right)}{x_{\Phi}}\right)^{a''}$$

with $x_{\Phi}=x_{0}e^{at_{\Phi}}$. To calculate the clearance threshold U_C, we apply the condition that clearance continues right up to the time when the uninfected bacteria disappear, that is $u\left(\tau\right)>U_I$ where τ is such that $x\left(\tau\right)=\delta$, and δ is the concentration equivalent to only one bacterial cell remaining. Substituting into the above equation and rearranging yields the condition for full clearance under passive process to be $u_{\Phi}>U_C$, where

$$U_C\approx\frac{a'}{a''}\left(1-x_0^{\frac{a''}{c}}\right)+\frac{a}{c}x_0^{\frac{a''}{c}}.$$

If $u_0>U_C$, the prey cells will rapidly be killed by the killer cells throughout conjugation. From this we can conclude that the conjugation processes are negligible, because for full clearance of, for example, 500000 prey cells you would need about 2 x 10^8 killer cells (even if the conjugation rate is about 5 x 10^{-9}, which would be pretty high).

Phage Dynamics

There is a threshold density of bacteria that must be present in order for the virus numbers to increase, which we shall refer to as the proliferation threshold X_P. To calculate X_P we consider the condition that the basic reproductive number of the phages is greater than one. The basic reproductive number R_0 is defined as the number of infections of uninfected bacteria caused per infected cell. Each infected prey cell can divide and will thus give rise to a cell line which, on average, will exist for a time $\frac{1}{k-a}$, during which this lineage will produce $\frac{Lk}{k-a}$ virus particles. Each of these will cause on average $\frac{bx}{bx+m}$ new infections. The total number of secondary infections per infection is therefore

$$R_0=\frac{Lk}{k-a}\frac{bx}{bx+m}.$$

Because the basic reproductive number of the phage depends on the density of bacteria, the appropriate condition for $R_0>1$ can be expressed in a convenient form: the phage and the lytic bacteria increase in number only when $x\left(t\right)>X_P$, where

$$X_P=\frac{m\left(k-a\right)}{b\left(k\left(L-1\right)+a\right)}\approx\frac{m\left(k-a\right)}{bkL}.$$

Passive Process
Here we focus on phage production throughout a conjugation, which has taken place before. So at the beginning we assume there will be a lot of phages which are going to kill our prey cells, meaning we have a passive process. Our system is simplified in the following way:

$$\begin{aligned} x_t &= a\ x - b\ v\ x \\ y_t &= a\ y + b\ v\ x - k\ y \\ v_t &= -b\ v\ x - m\ v \end{aligned}$$

We assume $cux \to 0$, because we focus on phage dynamics, so this term does not appear in the equations anymore. Our aim is to derive a clearance threshold V_C of phages, which indicates how many phages have to be present to kill all prey cells (passive process), so secondary infections are negligible. As before, we neglect cell growth, because infection dynamics outbalance $\Rightarrow \frac{dx}{dt} \approx -bvx$. We consider the expression

$$\frac{dv}{dx} = 1 + \frac{m}{bx},$$

which has an explicit solution obtainable from integration by separation of variables

$$v\left(t\right) - v_\Phi = x\left(t\right) - x_\Phi + \frac{m}{b}\ln\left(\frac{x\left(t\right)}{x_\Phi}\right)$$

with $x_\Phi = x_0 e^{at_\Phi}$. Because the concentration of bacteria has to be reduced, which means $\frac{dx}{dt} < 0$, $v\left(t\right)$ has to exceed a critical inundation threshold V_I given by

$$V_I = \frac{a}{b}.$$

This term only expresses whether the phages reduce the number of bacteria to some degree.

As before we consider the time when the prey cells disappear, that is $v\left(\tau\right) > V_I$, where τ is such that $x\left(\tau\right) = \delta$, and δ is the concentration equivalent to only one bacterial cell remaining. Substituting into the above equation and rearranging yields the condition for full clearance under passive process to be $v_\Phi > V_C$, where

$$V_C = V_I + x_0 e^{at_\Phi} + \frac{m}{b}\ln\left(x_0 e^{at_\Phi}\right).$$

So, if $v_\Phi > V_C$ and $x_\Phi < X_P$, we obtain full clearance of prey cells. In our case $v_\Phi = L$ and $t_\Phi = t_1 + \Delta$ with the conjugation running time t_1 and the phage maturing time Δ.

Active Process
In this situation we assume that at the beginning there are few phages and that they are mainly produced by the lysis of infected cells, so we obtain an active process which is very fast.

$$\begin{aligned} x_t &= a\ x - b\ v\ x \\ y_t &= a\ y + b\ v\ x - k\ y \\ v_t &= -b\ v\ x - m\ v + k\ L\ y \end{aligned}$$

If X_P is not exceeded at the time of phage inoculation, it is still possible that, with the exponentially increasing bacterial numbers, the threshold will be surpassed at a later time. There is thus an intrinsically temporal context to the problem, imposed by the critical moment at which the bacterial density passes the proliferation threshold. We call this the proliferation-onset time T_P, so $x\left(T_P\right) = X_P$. Whether or not active phage replication is achieved depends on whether there is still any phage present when the proliferation-onset time is reached. We obtain the expression

$$T_P \approx \frac{1}{a}\ln\left(\frac{X_P}{x_0}\right) = \frac{1}{a}\ln\left(\frac{(k-a)\,m}{b\,(k\,(L-1))}\right).$$

The final threshold definition relates to the requirement to prevent the phage of becoming fully purged from the system prior to T_P. A certain timing and size of phage inoculation is needed for some phages to be still present at the time when X_P is surpassed, and thus is required for active phage replication to become possible. This threshold can be thought of either in terms of a critical inoculation size V_F for a given inoculation time, or a critical inoculation time T_F for a given inoculation size. Prior to T_P the behaviour of the uninfected bacteria is dominated by the growth term, so that $\mathrm{d}x/\mathrm{dt} \approx ax$. At the same time the numbers of infected bacteria will always be very small, which means that a quasi-steady-state hypothesis can be imposed on the equation for y_t, so that we approximate

$$y\left(t\right) = \frac{bv\left(t\right)x\left(t\right)}{k-a}.$$

Substituting for $y\left(t\right)$ into $\mathrm{d}v/\mathrm{d}t$ and using the formula for X_P we arrive at

$$\frac{\mathrm{d}v}{\mathrm{d}x} = mv\left(\frac{1}{aX_P} - \frac{1}{ax}\right).$$

Integration by separation of variables yields the relationship

$$\frac{a}{m}\ln\left(\frac{v\left(t\right)}{v_\Phi}\right) = \frac{\left(x\left(t\right) - x_\Phi\right)}{X_P} + \ln\left(\frac{x\left(t\right)}{x_\Phi}\right),$$

with $x(T_P) = X_P, x_\Phi = x_0 e^{at_\Phi}$ and $v(T_P) \to 0$. To calculate the threshold V_F, we apply the condition that some phages are still present when the proliferation onset time is reached, that is $v(T_P) > \epsilon$, where ϵ is the concentration equivalent to only one phage being present. Substituting into the above equation gives the condition $v_\Phi > V_F$, where

$$V_F \approx \epsilon \exp\left(m(T_P - t_\Phi) + \frac{m}{a}\left(e^{-a(T_P - t_\Phi)} - 1\right)\right).$$

If $t_\Phi << T_P$, the condition is that $t_\Phi > T_F$, where

$$T_F \approx T_P - \frac{1}{m}\ln\left(\frac{v_\Phi}{\epsilon}\right) - \frac{1}{a}.$$

For a given initial killer cells population size, attempts at active therapy will fail outright if treatment is made prior to the failure threshold time T_F.

Conditions for Full Clearance

- $x_\Phi < X_P, v_\Phi > V_I$ and $v_\Phi > V_C \Rightarrow$ passive removal

- $x_\Phi < X_P, v_\Phi > V_I$ and $v_\Phi < V_C \Rightarrow$ bacteria decline only until $v(t) < V_I$ but given sufficient time this case might progress to the active phase and so to resolution

- $x_\Phi < X_P$ and $v_\Phi < V_I \Rightarrow$ number of phages initially falls, uninfected bacteria increase and eventually $x(t) > X_P$ at time $T_P \Rightarrow$ active phase

- $x_\Phi > X_P$ and $v_\Phi < V_I \Rightarrow$ rapid increase of phages with positive feedback, soon $v(t) > V_I \Rightarrow$ resolution

- $x_\Phi > X_P$ and $v_\Phi > V_I \Rightarrow$ resolution

- $v_\Phi > V_C \Rightarrow$ bacteria are cleared by primary infection alone

Critical Values for Our System

When we assume the parameters given in section four, we obtain the following critical values:

$$\begin{aligned} X_P &= 64135.4 \\ V_I &= 22875000 \\ V_C &> 22875000 \\ T_P &= 605.95 \\ T_F &< 0 \\ v_\Phi &= L = 50 \\ t_\Phi &= 42 \\ U_C &= 1.30089e16 \ (x(0) = 1000) \text{ or } 1.40467e18 \ (x(0) = 10000) \end{aligned}$$

Phase 1: $x_\Phi < X_P$, $v_\Phi < V_I \rightarrow$ number of free phages stays low (it is nearly zero), number of prey cells increases and eventually $x(t) > X_P$ at time T_P, so that the system goes to the active phase (see below)

Phase 2: $x_\Phi > X_P$, $v_\Phi < V_I \rightarrow$ number of free phages increases with positive feedback, soon $v(t) > V_I$ and the system goes to resolution (see below)

Phase 3: $x_\Phi > X_P$, $v_\Phi > V_I \rightarrow$ resolution: number of prey cells decreases very fast

In our system, v_Φ means the first production of free phages caused by the lysis of the first infected cell by conjugation, so v_Φ should be the burst size of phages in the model. It means v_Φ is definitely much smaller than $V_I = 22875000$. On the other side the initial number of prey cells is unknown. It could be higher than X_P or smaller. This analysis tells us that when we have a small density of prey cells at the beginning, the model will start with phase one. Then the prey cells grow until the model reaches phase two. In the second phase the prey cells and the free phages increase together until the model comes into phase three. Afterwards, the prey cell population will be killed in phase three. When we have a high density of prey cells at the beginning, the model starts in the second phase, then comes later into the third phase, which leads to resolution. That means this module should be efficient!

Other phases: cannot be achieved, because

- resolution caused by conjugation: $u(0) > U_C$ (U_C is too hight for $u(0)$)

- passive removal: $x_\Phi < X_P, v_\Phi > V_I$ and $v_\Phi > V_C$ (V_I is too high for v_Φ, for $v_\Phi > V_I$, number of killer cells multiplied with number of prey cells = 1e16)

- bacteria decline only until $v(t) < V_I$, but given sufficient time this case might progress to the active phase and so to resolution: $x_\Phi < X_P, v_\Phi > V_I$ and $v_\Phi < V_C$ (V_I is too high for v_Φ)

- killing of the prey population only by conjugation: $u(0) > U_C$ (U_C is too high for $u(0)$)

- absolutely inefficient: $T_\Phi < T_F < 0$ (not possible)

- primary infection resolution: $v_\Phi > V_C$ (V_C is too high for v_Φ)

4 Parameter Estimation and Measurement

Our test environment for the system should be in M9 soft agar with ideal temperature (37°C).

a
Value: 0.0183
Meaning: growth rate of cells in M9, only for the simplified model
Unit: 1/min
Source: Dissertation [6]

e_1
Value: 0
Meaning: death rate of killer cells, only for the simplified model
Unit: 1/min
Remark: We could not find a value for this parameter in the literature. We just assume that the killer cells have the same feasibility as prey cells to die which corresponds to $e_1 = 0$.

e_2
Value: 1.83e-12
Meaning: inner stress rate (logistic growth of killer cells), only for the simplified model

Unit: ml/(min number of cells)
Remark: We assume that 10^{10} cells/ml is the highest possible density of killer cells. When $e_1 = 0 \Rightarrow \mathrm{d}u(t)/\mathrm{d}t = u(t)(a - e_2 u(t)) = 0$ which corresponds to $e_2 = 1.83e{-}12$ ml/[cells min]

a_x
Value: 0.0183
Meaning: growth rate of prey cells in M9, only for full model
Unit: 1/min
Source: Dissertation [6]

a_u
Value: 0.0183
Meaning: growth rate of killer cells in M9, only for full model
Unit: 1/min
Source: Dissertation [6]

κ
Value: 1e10
Meaning: growth capacity in M9, only for the full model
Unit: number of cells/ml
Source: Dissertation [6]
Remark: In the literature κ is much smaller normally (4e9 cells/ml). But there it has always been measured in the liquid. We want test our system in soft agar and that is why κ may be higher.

b
Value: 8e-10
Meaning: infection rate
Unit: ml/(min number of phages) or ml/(min number of cells)
Source: Dissertation [6]

c
Value: 9e-13
Meaning: conjugation rate
Unit: ml/(min number of cells)
Remark: We have measured this rate for the simplified model. The conjugation rate in the full model does not have the same meaning as in the simplified model (in the full model, c is an absorbtion rate), but because it is quiet difficult to measure these rates in the lab, we neglect the difference. The measurement protocol for c can be found in the mean page - parts characterization.

k_1
Value: 0.5
Meaning: reaction rate of $z \rightarrow y + u$, only for the full model (assuming that the transport of the plasmid needs two minutes)
Unit: 1/min
Remark: We have to determine c for the full model. We know that the transport of a plasmid via conjugation depends on the size of the plasmid, but it is a fast process and the difference of k_1 between different plasmids is not very high. We find k_1 for a plasmid with 10 kb by measuring the time which is needed for transporting the plasmid with 10 kb and this is about two minutes, so k_1 for a plasmid with 10 kb is 0.5/min. Larger plasmids need more time for the transport: RP4 with 60 kb need about 4 min [1]. We neglect the difference.

k
Value: 0.322
Meaning: lysis rate, only for the simplified model
Unit: 1/min
Source: caculated from figure three of [10]

L
Value: 50
Meaning: burst size in M9
Unit: number of phages/cell
Source: Dissertation [6] and [10]
Remark: In the literature $L \approx 30$ to 170 phages/cell and the value depends on the medium and the experiment. We choose 50 phages/cell, because M9 is not a rich medium and may be the cells are not very fit for producing of phages.

m
Value: 0.00272
Meaning: decay rate of free phages in LB
Unit: 1/min
Source: [10]
Remark: We could not find out the decay rate of free phages in M9.

Δ
Value: 40
Meaning: time delay for phage maturing, only for the full model
Unit: min
Source: [10]

5 Simulation Results

5.1 Simplified Model

As initial values we choose a cell density of one billion prey cells per ml and a cell density of ten killer cells per ml. The initial values of free phages and infected cells are zero. We simulated the model for 1440 minutes which corresponds to one day.

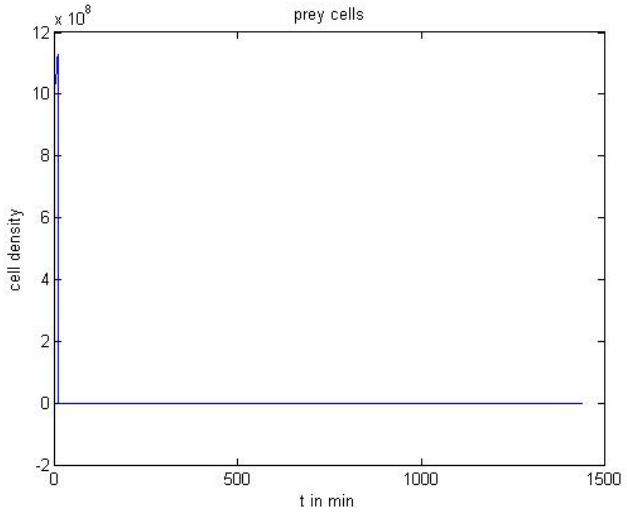

Figure 11: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.33, L = 50, m = 0.00272, e1 = 0$ and $e2 = 1.83e-12$

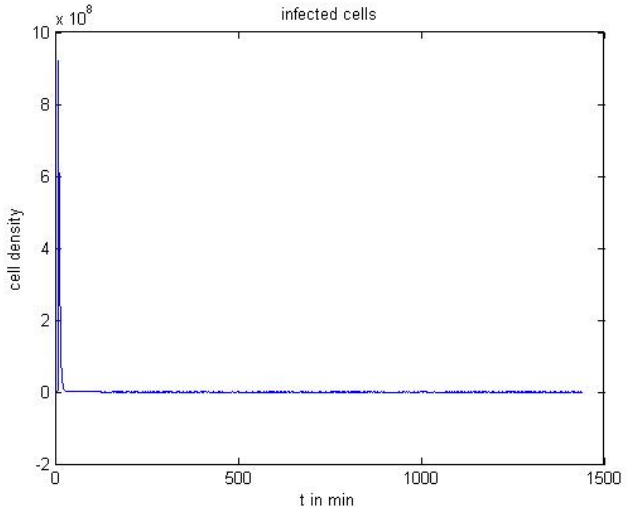

Figure 12: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.33, L = 50, m = 0.00272, e1 = 0$ and $e2 = 1.83e-12$

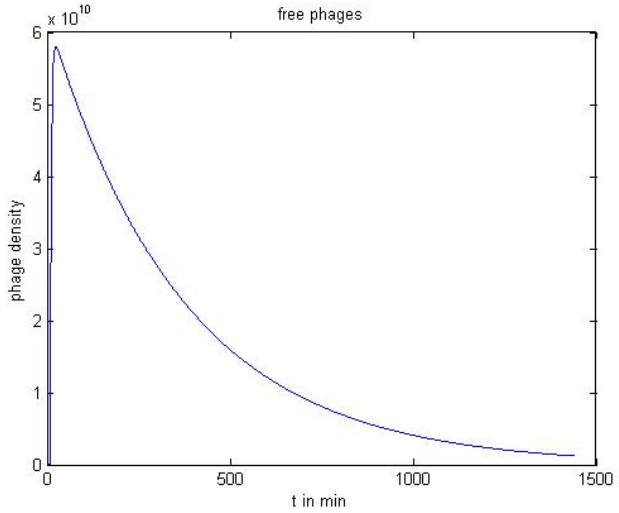

Figure 13: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.33, L = 50, m = 0.00272, e1 = 0$ and $e2 = 1.83e-12$

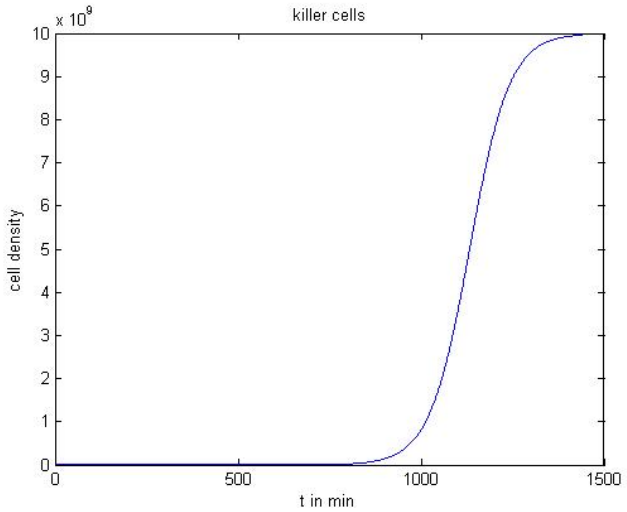

Figure 14: $a = 0.0183, b = 8e-10, c = 9e-13, k = 0.33, L = 50, m = 0.00272, e1 = 0$ and $e2 = 1.83e-12$

Analysis

- No delay visible.
- At the beginning the prey cells grow and then they decrease very fast.
- The free phages increase very fast.
- All prey cells are killed. The small oscillation of infected cells is caused by numeric effects.
- Although we have a very small conjugation rate, all prey cells will be killed $(x(t) \to 0) \Rightarrow$ The module is efficient.

5.2 Full Model

As initial conditions we chose the same as for the simplified model. As mentioned before, additional information is required to specify our DDE system. Because the derivatives of infected cells and free phages depend on the solution at the previous

time t - Δ, it is necessary to provide an initial history function to specify the value of the solution before time $t = 0$. As in many common models we have set the history a constant vector, zero in our case. Because we have the time delay for phage maturing Δ, there should not be any influx of free phages caused by lysed infected cells before time Δ, which corresponds to history = constant = zero if $t < 0$.

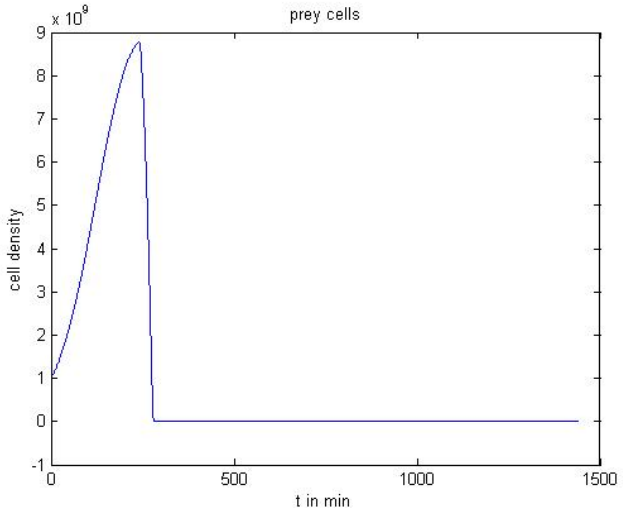

Figure 15: $a_x = 0.0183, a_u = 0.0183, b = 8e-10, c = 9e-13, \kappa = 1e10, L = 50, m = 0.00272, k_1 = 0.5$ and $\Delta = 40$

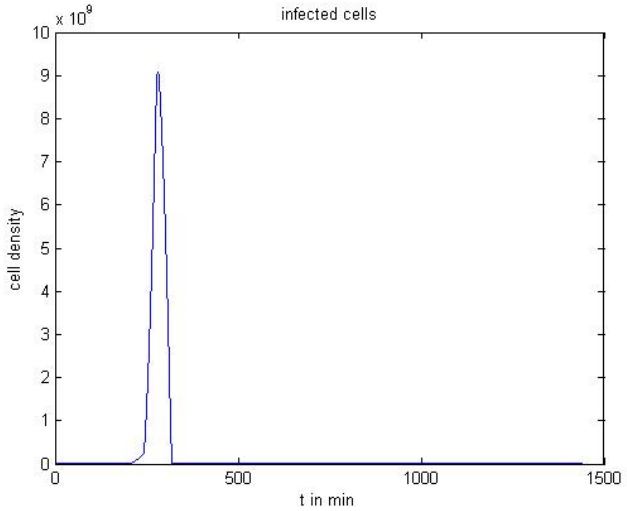

Figure 16: $a_x = 0.0183, a_u = 0.0183, b = 8e-10, c = 9e-13, \kappa = 1e10, L = 50, m = 0.00272, k_1 = 0.5$ and $\Delta = 40$

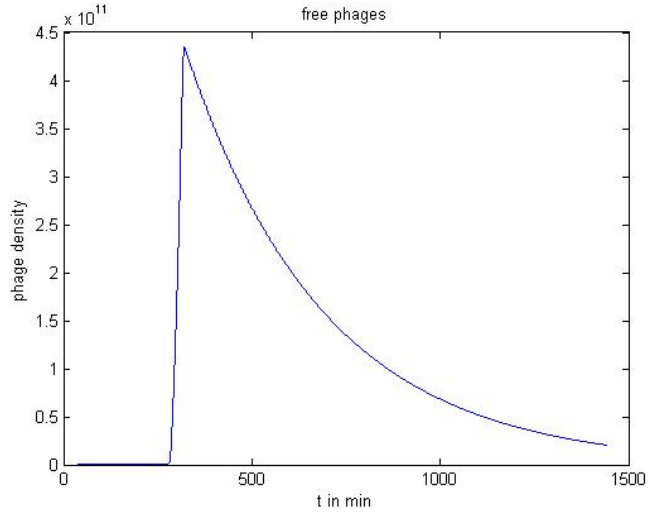

Figure 17: $a_x = 0.0183, a_u = 0.0183, b = 8e-10, c = 9e-13, \kappa = 1e10, L = 50, m = 0.00272, k_1 = 0.5$ and $\Delta = 40$

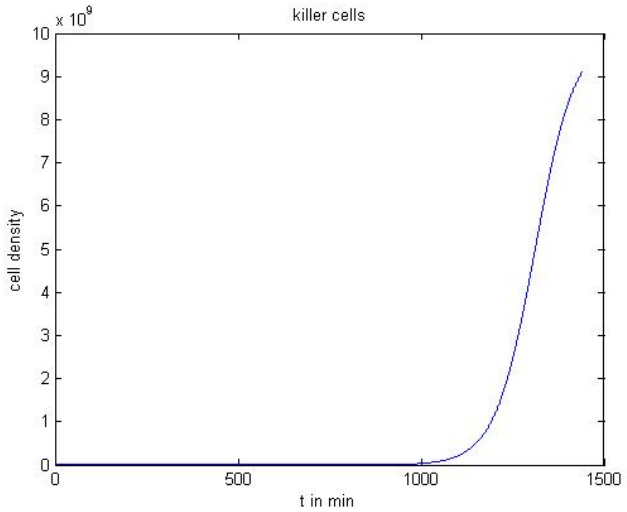

Figure 18: $a_x = 0.0183, a_u = 0.0183, b = 8e-10, c = 9e-13, \kappa = 1e10, L = 50, m = 0.00272, k_1 = 0.5$ and $\Delta = 40$

Analysis

- The delay is visible.
- At the beginning the prey cells grow and then they decrease very fast.
- In comparison to the basic model the prey cells grow slower at the beginning, but they can reach a much higher concentration. Full clearance is reached much later, but a at a more realistic time scale.
- The free phages increase very fast, but much later in comparison to the simplified model.
- Again all prey cells will be killed even with a small conjugation rate.
- $\Rightarrow$ The killing is very efficient.

6 Conclusions

Comparing the simulations of the simplified and full models, we can see the differences which were expected. But these differences do not change the stability and the general shape of the curves. This means the simplified model can reflect the general properties of the full model very well. Accordingly, the results of the mathematical analysis performed for the simplified model will also reflect the general properties of the full model.

By linear stability analysis we showed that our model has two different stable steady states of which only one corresponds to an effective and fast killing of all prey cells. The initial value analysis and the critical value analysis however showed that with realistic parameter values full clearance will be obtained for a wide range of initial values.

Furthermore, for the killing mechanism to be effective, it is not necessary to have a very high density of killer cells initially. Because of the snowball effect in the infection process, ten killer cells per ml are able to kill 10^9 prey cells per ml efficiently.

The parameter values we used were obtained partly from our own lab and partly from the literature. We also believe all model assumptions to be biologically reasonable, making us confident that our model is indeed realistic.

We can predict that it is possible for the prey cells to grow to a very high density initially to be though eventually killed. The duration of this process can be predicted to be four to six hours.

In the future, it might be interesting to try to combine the modeling approach described here with the PDE approach of the colicin-induced killing model described in the first part of the documentation. Unfortunately, this could not be done for the full model as the explicit delay could not be incorporated in a PDE system.

Concluding, we have developed a model, which is able to simulate the killing procedure in a realistic way. All our theoretical considerations have reinforced our expectation that the system is very efficient. For us, this is also a motivation for our future work in the lab.

References

[1] L. Andrup, L. Smidt, K. Andersen, and L. Boe. Kinetics of conjugative transfer: a study of the plasmid pXO16 from Bacillus thuringiensis subsp. israelensis. *Plasmid*, 40:30–43, Jul 1998.

[2] E. Beretta and Y. Kuang. Modeling and analysis of a marine bacteriophage infection with latency period. *Nonlinear Analysis: Real World Applications*, 2:35–74, 2001.

[3] E. Chapman-McQuiston and X.L. Wu. Stochastic receptor expression allows sensitive bacteria to evade phage attack. Part II: theoretical analyses. *Biophys. J.*, 94:4537–4548, Jun 2008.

[4] M. Delbruck. The growth of bacteriophage and lysis of the host. *J. Gen. Physiol.*, 23(5):643–660, 1940.

[5] B.R. Levin, F.M. Stewart, and V.A. Rice. The kinetics of conjugative plasmid transmission: fit of a simple mass action model. *Plasmid*, 2:247–260, Apr 1979.

[6] Radu Gheorghe Moldovan. The interaction between lambda phage and its bacterial host. *Dissertation*, 2006.

[7] P. Mudgal, F. Breidt, S.R. Lubkin, and K.P. Sandeep. Quantifying the significance of phage attack on starter cultures: a mechanistic model for population dynamics of phage and their hosts isolated from fermenting sauerkraut. *Appl. Environ. Microbiol.*, 72:3908–3915, Jun 2006.

[8] J. D. Murray. *Mathematical Biology*, volume 18 of *Interdisciplinary Applied Mathematics*. Springer, New York, 2003.

[9] R.J. Payne and V.A. Jansen. Understanding bacteriophage therapy as a density-dependent kinetic process. *J. Theor. Biol.*, 208:37–48, Jan 2001.

[10] I.N. Wang. Lysis timing and bacteriophage fitness. *Genetics*, 172:17–26, Jan 2006.