M1 Project: Bacteriophages - Host interaction using DDE models

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Abstract

The growing emergence of bacterial resistance to the currently available antibiotics, is a critical therapeutic problem. Bacteriophage Therapy is one among the fascinating alternative approaches applied in order to fight antibiotic-resistant pathogens. Bacteriophage Therapy is based on the interaction of bacteria and bacteriophages, viruses that infect and replicate within the bacterium. Mathematical models had began to provide a better conceptual understanding of this complex system. They consist of a set of differential equations each of which tracks a component population within the model. The majority of the models focus on one type of phage and bacteria. In my project I focus on studying two classes of phages with different life cycles using delay differential equations. I do that to explore a wider range of ecological concepts. First I examine the interaction of M13, T7 phages and their host in a chemostat. I investigate under which circumstances coinfection of the cell emerges and what kind of population dynamics the systems might have. I found that coinfection remains in a steady state when the proportion of T7 is higher than that of M13. Then I study the model of T7 phage-host interaction with multiple receptors in a chemostat. The model consists of three types of cells and three genetically engineered T7 phages. The aim is to evolve these phages through mutation into a phage that binds to a single type of receptors on the surface of the cell. I investigate which set of dilution rates could give the highest concentration of this phage and if the third type of cells is necessary in order to achieve that. I found the that the latter is essential for the scope of the model and that initial conditions do not affect the optimal set of dilution rates. These two models in companion with a future experimental work could open up new possibilities to the evolution of synthetic bacteriophage cocktails.

1 Introduction

1.1 Bacteriophage therapy

Bacteriophages were independently discovered by Frederick Twort (1915) and Felix D'Herelle (1917). The term "Bacteriophage" is a synthesis of the word "bacteria" and the Greek ancient word "phagein" which means "to devour". Bacteriophages, or simply phages, are parasites and for this reason they need a bacterial host in order to replicate themselves. Since their discovery they have contributed to the evolution of many other fields including molecular biology and bacterial genetics [**Daniel et al.**, **2010**], [**D'Herelle**, **2007**]. They have also been applied to treat bacterial infections, but due to the lack of knowledge of phage biology and the advent of antibiotics in the 1940s, the usage of therapy was abandoned in the West. However, the growing emergence of bacterial resistance to the currently available antibiotics, revitalized bacteriophage therapy and particularly new strategies towards engineering synthetic bacteriophages which includes modification of phages' genomes.

1.2 Structure

Bacteriophages vary in sizes and shapes but the majority of them have the same basic features: a head or capsid and a tail. They are composed of proteins that encapsulate a DNA or RNA genome (Figure 2). In order to infect the cell, they attach to receptors on the bacterial cell surface and once they become tightly bound to them, they penetrate and inject their genetic material into the host cell

1.3 Life cycles

Bacteriophages can be dinstinguished in two principle categories according to their life cycle: the lytic and the lusogenic. The main difference between these two types of cycle is that a lytic phage, after injecting its genetic material into the host, replicates and after a fixed priod of time (latent period) kills the cell by lysis in order to release new phage particles. On the other hand lysogenic phages are able to integrate their DNA or RNA into the cell's chromosome and replicate without bursting the host, allowing it to grow through division. In my project I studied two particular types of phages, M13 and T7 phage. M13 is a lysogenic phage (Figure 7 in Appendix) while T7 is a lytic phage (Figure 8 in Appendix).

1.4 Bacteriophage cocktails

Although Bacteriophage Therapy have many advantageous characteristics over antiobiotics, it has limitations to some applications. In particular, it has been observed that bacteria can also develop resistance to phages through various mechanisms

[**Nobrega et al.**, **2015**]. To overcome this limitation many strategies have been considered. A prominent approach is to use a cocktail of different types of phages but complementary characteristics [**Chan et al.**, **2013**],[**Goodridge**, **2010**]. Bacteriophage cocktails can impact a wider range of bacterial types while succeeding effectiveness under a greater diversity of conditions [**Chan et al.**, **2012**]. In addition [**Weitz et al.**, **2005**] suggests that multiple quasispecies of bacteria and phage can coexist in a homogeneous medium with a single resource. Therefore it would be interesting to explore the potential for coexistence of phages with different life cycles and this is what I examine in the first model of my project.

1.5 Evolution of lytic bacteriophages

One main property of phages is that they are target- specific, which means that they target a specific range of bacterial receptors on the surface of the cell. However, it would be desirable to use lytic phages that can only target and bind to single protein receptors on the surface of pathogenic bacteria. This enables phages to target and kill specific bacteria rather than other types of bacteria that coexist in the environment (microbiome). I investigate this idea in the second model of my project.

1.6 Mathematical models of phage-host interaction

Mathematical models play an important role on the conceptual understanding of bacteriophages-host interaction. Even though they do not capture the realities of *in vivo* dynamics they can capture qualitative properties that can be generalized [**Bull et al.**, **2014**]. A wide variety of them has been introduced, covering an area of interest, such ecological environment [**Levin et al.**, **1977**], food industry [**Cattoen**, **2003**], [**Mudgal et al.**, **2006**] and therapeutics [**Cairns et al.**, **2009**], [**Levin et al.**, **1996**], [**Payne et al.**, **2000**]. Most of them are based on the classical Lotka- Voltera equations known also as predator (lytic phage) - prey (host) equations and use differential equations to describe them.
More realistic models in terms of biological properties of the species are suggested by the following papers. In [**Payne et al.**, **2000**] the authors introduce a simple and generic kinetic model that describes the dynamics of a lytic phage and its host population. It is based on the assumption that the rate of reaction between phages and host is proportional to the product of their concentration (mass action kinetics). The model shows a variety of qualitative outcomes of phage treatment. However due to its simplicity it doesn't take into account the fact that the dynamics at each time t not only depend on the concentrations of phages and cells at that time , but also at time $t - k$, where k is the latent period of a lytic phage (Figure 8 in Appendix).
Another interesting phage-host interaction kinetic model, is in [**Mudgal et al.**, **2006**]. The model quantifies the growth of phage and host population for different initial conditions. The model uses delay differential equations to capture more accurately the population dynamics and introduces a step function for a better description of

the adsorption rate of phages (rate at which free phages become attached to bacteria) that changes with time. However the results show a systematic difference between the parameters obtained from model optimization and those from the experiments. The model described in [**Cairns et al.**, **2009**] is the most realistic version of all before mentioned models. It is a delay differential equations system, based on the mass action kinetics law which compares the model results with experimental work to estimate thresholds and rate constants.

1.7 Interaction environment

The environment in which the phage-host interaction occurs can vary from culture plates to chemostats. A chemostat is a well mixed culture in which inflow of susceptible bacteria enters and culture liquid outflows continuously [**Abdelhamid et al.**, **2011**] (Figure 9 in Appendix). The control parameters of phage evolution depend on environmental conditions which are neither constant nor controllable in culture plates [**Husimi**, **1989**]. For that reason it is preferable to use continuous cultures such as a chemostat. The first models of phage growth within a chemostat were developed by [**Levin et al.**, **1977**] and [**Levin et al.**, **1985**].

1.8 Overview of what follows

The remainder structure of the report is the following. First I briefly refer to the software I used for my project. Then I elaborate on the two models I studied. I start by presenting a set of delay differential equations I came up with in order to describe them mathematically, then I solve the system numerically and analyse the main results. In the end, I discuss some further work that can be done, and give a conclusion.

2 Methodology

To solve numerically the two systems I studied, and visualize the results I used the software Matlab.

3 Models and Results

3.1 Model 1: M13, T7 - Host interaction in a Chemostat

3.1.1 Description

The scope of this model is to create a system where coinfection of the cell by phages can emerge, investigate under which circumstances it occurs and what kind of population dynamics this system might have.
The bacteriophages I studied in the model were the lysogenic phage M13 (Figure 7 in

Appendix), and the lytic phage T7 (Figure 8 in Appendix). In the model the latter phage underwent a genome modification. More specifically the T7 DNA Polymerase which is an enzyme used during the replication of the T7 phage, was removed from the phage's genome and placed into the genome of M13 phage. As a result, T7 was able to replicate only when coexisted with M13 phage in a cell. The possible events that occur in this system are the following :

(i) M13 infects a susceptible cell. Both M13 and cell replicate (Figure 11, Event 1 in Appendix).

(ii) T7 infects a susceptible cell. The cell bursts (Figure 11, Event 2 in Appendix).

(iii) M13 infects a cell with T7 and vice versa. T7 replicates, The cell bursts, M13 dies (Figure 12, Event in Appendix).

The environment in which the interaction takes place is a Chemostat (Figure 10 in Appendix). The culture initially contains concentrations of M13 and T7 phage. When the experiment starts, concentration of susceptible cells S_{in} inflows continuously in the culture with a dilution rate D while culture liquid outflows continously from it with the same dilution rate.
The mathematical model I derived to describe this system consists of a set of delay differential equations. It is based on the existing model [**Cairns**, **2009**] in companion with [**igem**, **2011**].
DDE System

$$\frac{dS}{dt} = -b_T S T_7 - b_M S M_{13} + D S_{in} - D S, \qquad S \geq 0 \tag{1}$$

$$\frac{dI_M}{dt} = b_M S M_{13} + \beta I_M - b_T T_7 I_M - D I_M, \qquad I_M \geq 0 \tag{2}$$

$$\frac{dI_T}{dt} = b_T S T_7 - b_T T_7(t-k) S(t-k) - b_M I_T M_{13} - D I_T, \qquad I_T \geq 0 \tag{3}$$

$$\frac{dI_{TM}}{dt} = b_T T_7 I_M + b_M M_{13} I_T - b_T S(t-k) T_7(t-k) - D I_{TM}, \qquad I_{TM} \geq 0 \tag{4}$$

$$\frac{dT_7}{dt} = b_T h S(t-k) T_7(t-k) - b_T S T_7 - b_T T_7 I_M - m_M T_7 - D T_7, \qquad T_7 \geq 0 \tag{5}$$

$$\frac{dM_{13}}{dt} = \gamma M_{13} - b_M S M_{13} - b_M M_{13} I_T - m_T M_{13} - D M_{13}, \qquad M_{13} \geq 0 \tag{6}$$

Population types

(i) S : Concentration of susceptible cells to M_{13}, T_7 phage at time t, measured in CFU/ml (Colony forming units per ml)

(ii) I_M : Concentration of infected cells by M_{13} at time t, in CFU/ml.

(iii) I_T : Concentration of infected cells by T_7 at time t, in CFU/ml

(iv) I_{TM} : Concentration of infected cells by T_7, M_{13} at time t, in CFU/ml.

(v) M_{13} : Free M_{13} phage at time t in PFU/ml (plaque forming units per ml).

(vi) T_7 : Free T_7 phage at time t in PFU/ml.

The control parameters of the model are listed in Table 1 in Appendix.

3.1.2 Resutls

The table 1 shows the experimental values I used for the control parameters of the model. When I started working on the model we didn't know the values for the binding rates. The initial idea was to do the experiment of the model in the lab and infer most of the values through the experiment. However one of the collaborators of the lab did not provide the material needed for the experiment. To overcome this difficulty we followed a different approach.
First I chose a range of different initial concentrations of M13 and T7 phage such that M13, T7 $\in \{10, 10^2, 10^3, 10^4\}$ (16 pairs of M13,T7 in total). Second I selected the binding rates $b_M, b_T \in \{10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, ..10^{-12}\}$ (100 combinations of b_M, b_T in total). Then for each initial concentration of M13 and T7 I varied the binding rates and solved the system numerically.
Since we were interested to know under which circumstances the model can exhibit coinfection of the cell, for each pair of binding rates (b_M, b_T) I counted the number of initial concentrations that led to coinfection of the cell by the two phages. Figure 1 shows us that of binding rates $(b_T, b_M) = (10^{-4}, 10^{-8})$ corresponding to the white box, lead to coinfection for all initial concentrations. Given this optimal set of binding rates, I plotted the population dynamics for different initial concentrations of M13 and T7. The main results I found are the following:

- M13 $>$ T7: concentration of infected cell by both phages drops to zero, while concentration of infected cells by M13 reaches a steady state (Figure 2).
- M13$<$ T7: concentration of infected cells by M13 initially increases,then decays until it becomes zero. On the other hand concentration of infected cell by both phages reaches a steady state.(Figure 3).

3.2 Model 2: T7- Host with multiple receptors interaction in a Chemostat

3.2.1 Description

This model consists of three genetically engineered T7 phages (T^1, T^2, T^3) and three different types of bacteria (S_1, S_2, S_3) with the following characteristics:

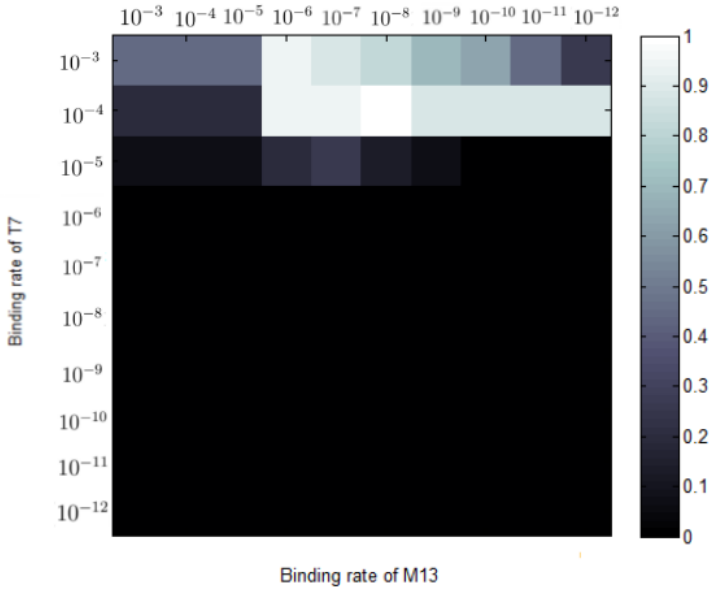

Figure 1: Heatmap of the binding rates b_M, b_T for different initial concentrations of M13, T7.

- T^1: T7 phage that can bind to multiple receptors on the surface of the cell.
- T^2: T7 phage that can bind only to one type of receptor, the one in red colour in Figure 14 in Appendix.
- T^3: T7 phage that can bind to multiple receptors, excluding the receptor in red in Figure 14 in Appendix.
- S_1: Susceptible cell to T^1, T^2, T^3. After being infected by one of them it bursts and new phage particles are released.
- S_2: Susceptible cell to T^1, T^2. After being infected by T^1 or T^2 it bursts and new phage particles are released.
- S_3: Susceptible cell to T^1, T^3. The cell after being infected does not burst.

In addition to that, in the model, bacteriophages undergo mutations. We considered two types of mutations: T^3 mutates to T^1 , T^1 mutates to T^2 with rate μ (Figure 15 in Appendix).
As in model 1, the interaction of phages and cells takes place in a chemostat. The culture initially contains concentrations of T^1, T^2, T^3 phages. When the experiment starts, constant concentration of susceptible cells $S_{1in}, S_{2in}, S_{3in}$ inflows continuously into the culture with dilution rates $D1, D2, D3$ respectively while culture liquid outflows continuously from it with dilution rate D ,where $D = D1 + D2 + D3$ (Figure 16) .

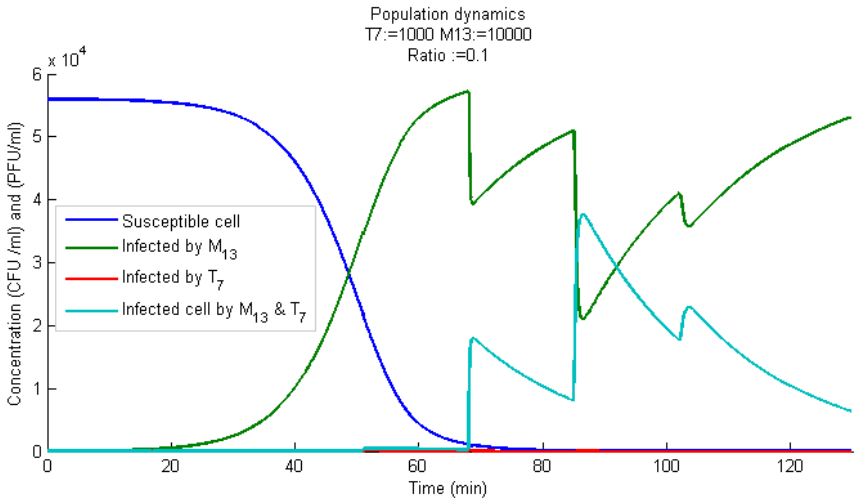

Figure 2: Dynamics of cells with initial concentrations: T7=10^3, M13=10^4

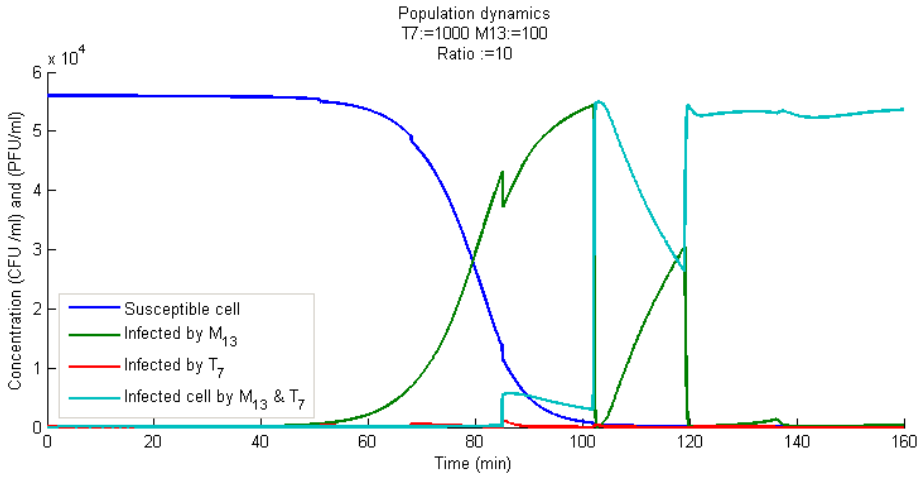

Figure 3: Dynamics of cells with initial concentrations: T7=10^3, M13=10^2

The mathematical model that I came up with to describe tthis system consists of a set of 13 dealy differential equations. It is based on [**Cairns et al.**, **2009**].

DDE model

Notation: I_i^j : Infected cell S_i by phage T^j

$$\frac{dS_1}{dt} = D_1 S_{1in} - DS_1 - bS_1(T^1 + T^2 + T^3)S_1, \qquad S_1 \geq 0 \quad (7)$$

$$\frac{dS_2}{dt} = D_2 S_{2in} - DS_2 - bS_2(T^1 + T^2) \qquad S_2 \geq 0 \quad (8)$$

$$\frac{dS_3}{dt} = D_3 S_{3in} - DS_3 - bS_3(T^1 + T^3) \qquad S_3 \geq 0 \quad (9)$$

$$\frac{dI_1^1}{dt} = -DI_1^1 - bS_1(t-k)T^1(t-k) + bS_1T^1 \qquad I_1^1 \geq 0 \quad (10)$$

$$\frac{dI_1^2}{dt} = -DI_1^2 - bS_1(t-k)T^2(t-k) + bS_1T^2, \qquad I_1^2 \geq 0 \quad (11)$$

$$\frac{dI_1^3}{dt} = -DI_1^3 - bS_1(t-k)T^3(t-k) + bS_1T^3, \qquad I_1^3 \geq 0 \quad (12)$$

$$\frac{dI_2^2}{dt} = -DI_2^2 - bS_2(t-k)T^2(t-k) + bS_2T^2, \qquad I_2^2 \geq 0 \quad (13)$$

$$\frac{dI_2^1}{dt} = -DI_2^1 - bS_2(t-k)T^1(t-k) + bS_2T^1, \qquad I_2^1 \geq 0 \quad (14)$$

$$\frac{dI_3^1}{dt} = -DI_3^1 + bS_3T^1, \qquad I_3^1 \geq 0 \quad (15)$$

$$\frac{dI_3^3}{dt} = -DI_3^3 + bS_3T^3, \qquad I_3^3 \geq 0 \quad (16)$$

$$\begin{aligned}\frac{dT^1}{dt} &= -DT^1 + (1-\mu)hbT^1(t-k)S_1(t-k) + \mu hbT^3(t-k)S_1(t-k) \\ &\quad + (1-\mu)hbT^1(t-k)S_2(t-k) - dT^1 - bT^1(S_1 + S_2 + S_3), \qquad T^1 \geq 0 \quad (17)\end{aligned}$$

$$\begin{aligned}\frac{dT^2}{dt} &= -DT^2 + \mu hbT^1(t-k)S_1(t-k) + hbT^2(t-k)(S_1(t-k) + S_2(t-k)) \\ &\quad + \mu hbT^1(t-k)S_2(t-k) - bT^2(S_1 + S_2) - dT^2, \qquad T^2 \geq 0 \quad (18)\end{aligned}$$

$$\frac{dT^3}{dt} = -DT^3 + h(1-\mu)bS_1(t-k)T^3(t-k) - dT^3 - bT^3(S1 + S3), \quad T^3 \geq 0 \quad (19)$$

3.2.2 Results

As mentioned in the introduction, one main property of phages is their ability to target a specific range of bacterial receptors on the surface of the cell. Consequently, we are interested in enabling lytic phages to bind only to a single rather than multiple types of receptors on the bacterial surface, in order to specifically kill the bacteria that we

are targeting and not some other types of bacteria in the environment.
Therefore the scope of this model is the evolution the phages (through mutation) so as to accomplish a high proportion of T^2 (the phage which targets and binds only to the desired receptor) . In order to do that the following questions arose:

- which proportion of dilution rates $D1, D2, D3$ could give the highest concentration of T2?
- Is S_3 (the cell that does not burst) necessary for this purpose?

First given that $D = 0.05$ min^{-1}, I varied the dilution rates $D1, D2$ (as $D3 = D - D1 - D2$) and solved the system numerically. At the end of the simulation, I computed the amount of T^2 percentage of the total phage concentration for different sets of dilution rates and initial concentrations of phages.

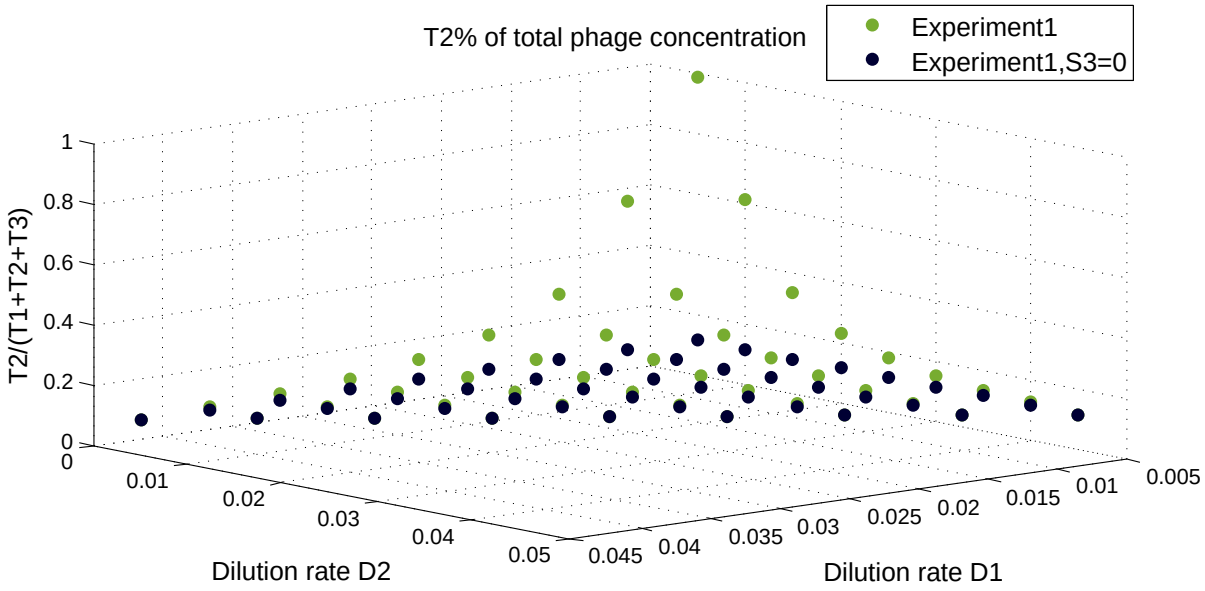

Figure 4: T^2 % of total phage concentration with Initial concentrations: $T^1 = 10^3, T^2 = 0, T^3 = 0$

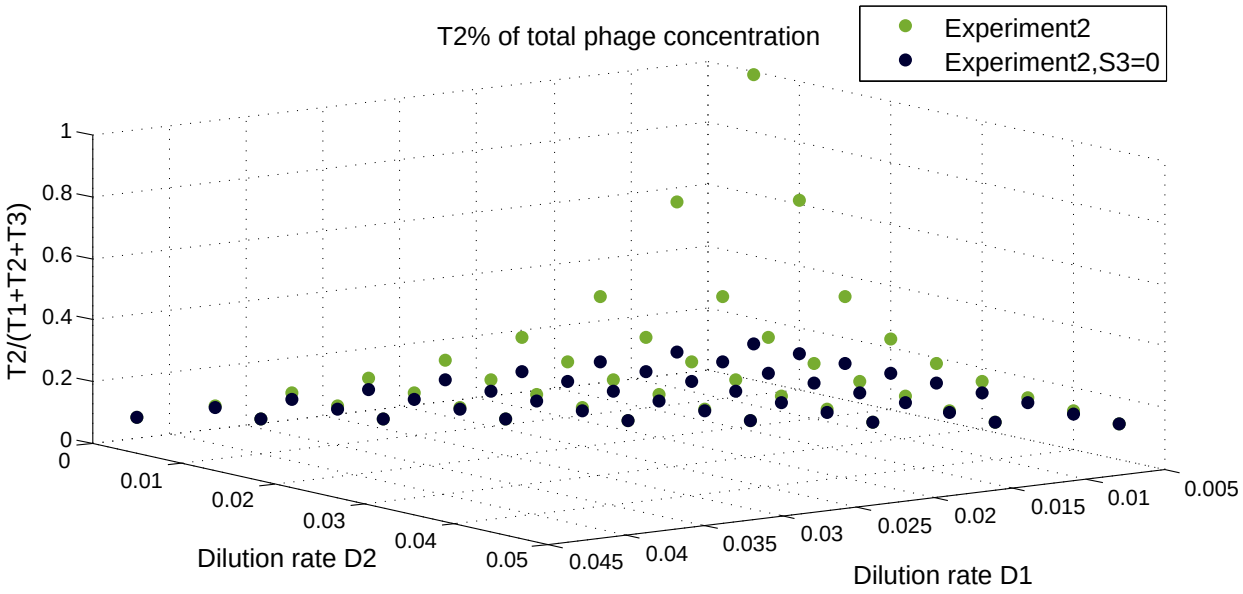

Figure 5: T^2 % of total phage concentration with Initial concentrations: $T^1 = 0, T^2 = 0, T^3 = 10^3$

I examined the concentration of T^2 phage in two experiments with different initial conditions. I found that in both the set of dilution rates which gives the highest concentration of T^2 is the same: $D1 = 0.005, D2 = 0.005, D3 = 0.04$ (data in green, Figure 16-17).

Then I investigated how the dynamics of the system would change in the absence of the third type of cell, S_3 (data in blue, Figure 16-17). I came to the conclusion that in both experiments the presence of S_3 is necessary to succeed a high T^2 % of total phage concentration (data in green compared to data in blue, Figure 4-5).

Given this optimal set of dilution rates, I plotted the dynamics of phages for the two experiments to examine which of them would give the highest proportion of T^2 phage. In Figure 6 we can see that Experiment 1 gives a higher T^2 concentration than Experiment 2. Therefore it would be more advisable to work on the model with the initial conditions of Experiment 2.

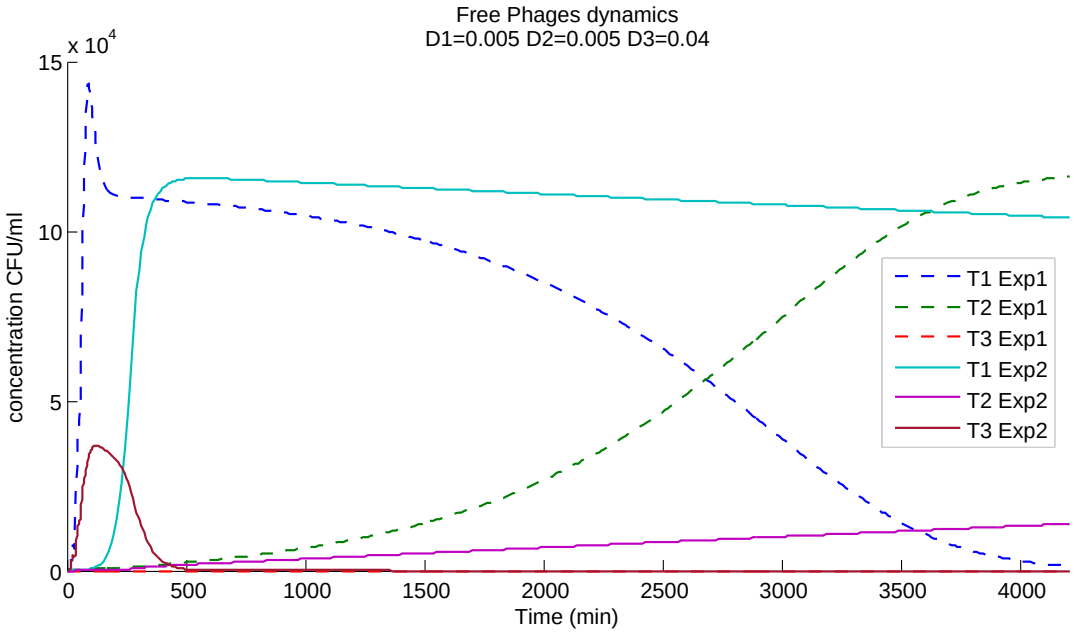

Figure 6: Phage dynamics for with initial concentrations
Experiment1: $T^1 = 10^3, T^2 = 0, T^3 = 0$
Experiment2: $T^1 = 0, T^2 = 0, T^3 = 10^3$

4 Further work

The research that has been undertaken in this project was to formulate mathematically the two models and explore the population dynamics using computer simulations for various initial conditions. The next step we need to take regarding the "M13 - T7 - Host interaction" model, is to compare the results with experimental lab work and apply inference techniques to accomplish more realistic parameters. Regarding the" T7 phages- Host interaction with multiple receptors" model , we could set up the experiment in the chemostat using the optimal set of dilution rates and initial conditions we found and compare the computational results with data. As a second step we could apply parameter inference to the model and possibly extend it in such a way that the values of dilution rates can vary throughout the experiment.This will allow us adopt a better strategy of increasing the T^2 phage concentration in time.

5 Conclusions

In this work I focus on studying two models of Bacteriophages - Host interaction in a Chemostat. In the first model I investigate under which circumstances coinfection of the cell by a cocktail of T7, M13 phages emerges and what kind of dynamics the system exhibit. I found that coinfection remains in a steady state when the proportion of T7 is higher than that of M13. In the second model I studied the intreaction between three genetically engineered T7 phages and three types of bacteria. I investigated under which set of ditution rates we could evolve the phages to target and bind only to single proteins receptors on the surface of the cell. I found that the optimal set of dilution rates does not depend on the initial conditions and that the third type of cell is an essential component of the model. These two models in companion with a future experimental work could open up new possibilities to overcome current limitations of synthetic Bacteriophage Therapy.

6 Acknowledgments

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7 Apprendix

This appendix contains plots for the two models and tables with values for all parameters used in the models.

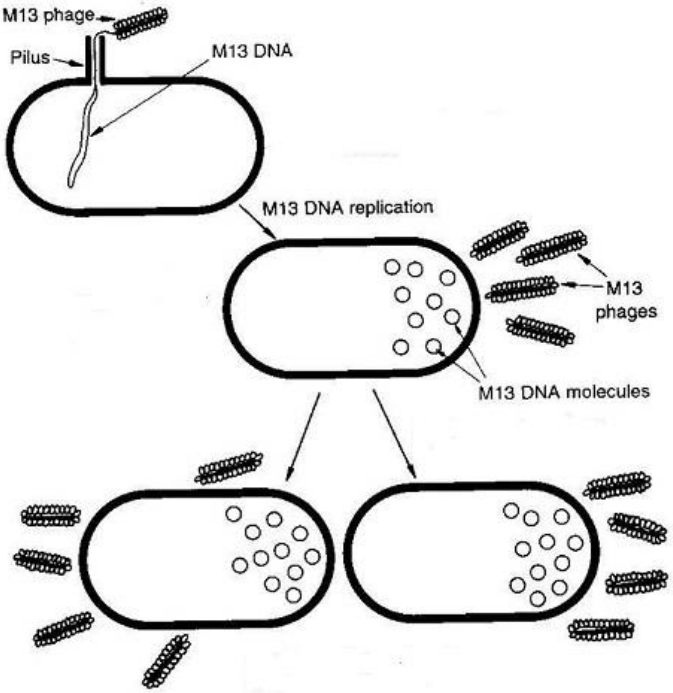

Figure 7: Life cycle of M13 phage.
https : *//learning.uonbi.ac.ke/courses/SZL*311*/scormPackages/path*$_2$*/leaky.JPG*

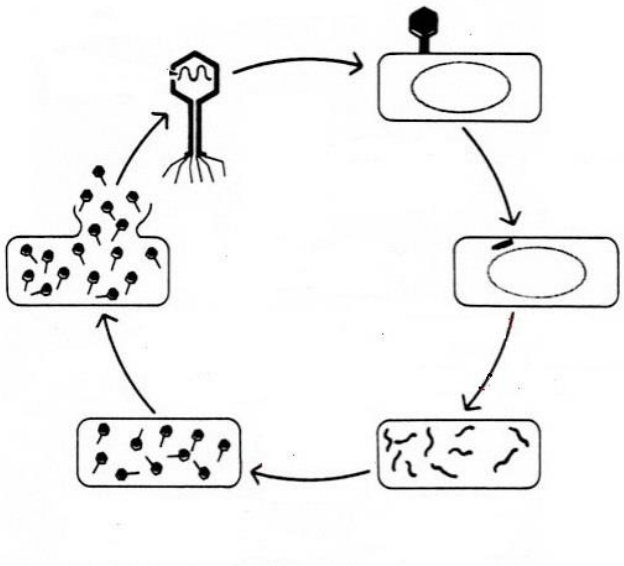

Figure 8: Life cycle of T7 phage.
http : *//textbookofbacteriology.net/phage.html*

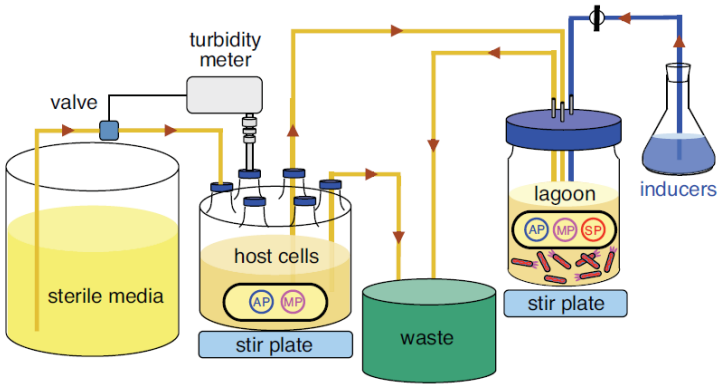

Figure 9: Schematic of phage-assisted continuous evolution (PACE). Host E. coli cells continuously flow through a lagoon vessel containing phage **[Esvelt et al., 2011]**

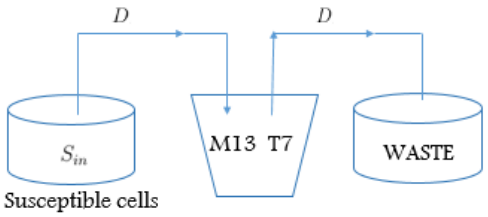

Figure 10: Schematic of the model 1 Chemostat.

Table 1: Control parameters meaning and values for model 1

Symbol	Meaning	Value
D	Dilution rate	0.05 min^{-1}
S_{in}	Input concentration of susceptible cells	$5.6 * 10^4$ CFU/ml
β	Growth rate of infected cell with M_{13}	0.0041 min^{-1}
b_T	Binding rate of T_7 to a cell	10^{-4}ml $\text{CFU}^{-1}\text{min}^{-1}$
b_M	Binding rate of M_{13} to a cell	10^{-8}ml $\text{CFU}^{-1}\text{min}^{-1}$
k	Latent period	17 min
h	Burst size of a cell	100 PFU
γ	Replication rate of M_{13} phage	0.2 min^{-1}
m_T	Decay rate of phage T_7	0.009 min^{-1}
m_M	Decay rate of phage M_{13}	0.009 min^{-1}

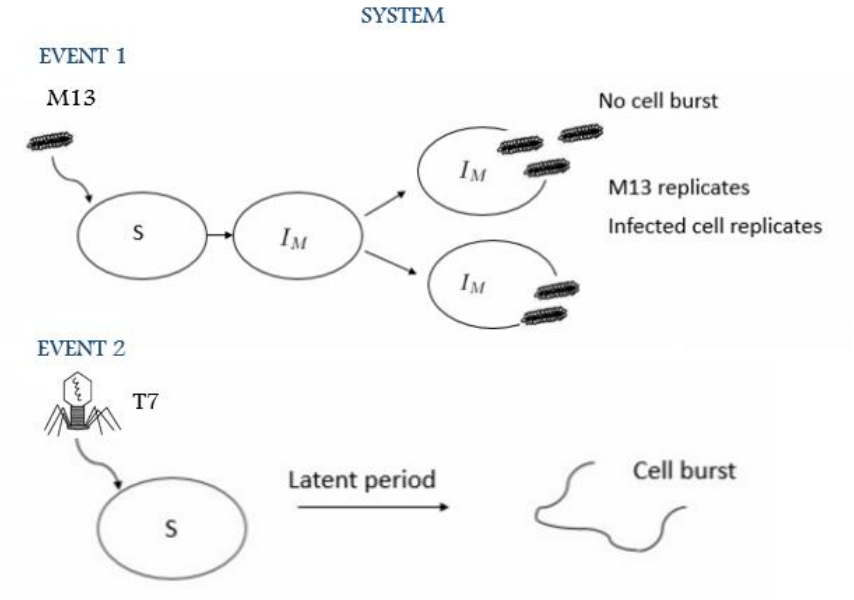

Figure 11: Event 1: M13 infects a susceptible cell, Event 2: T7 infects a susceptible cell.

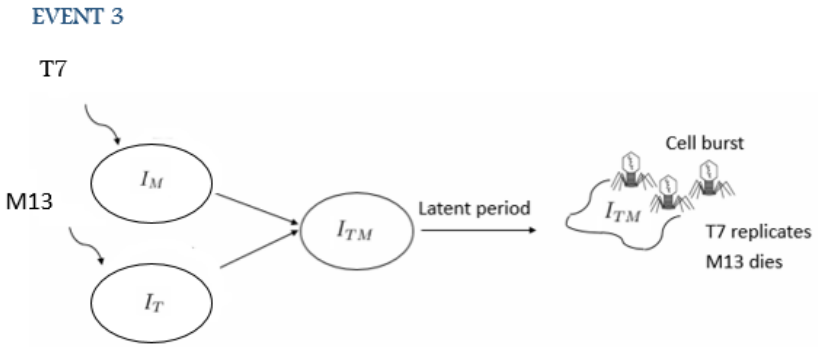

Figure 12: Event 3: M13 infects a cell with T7 and vice versa.

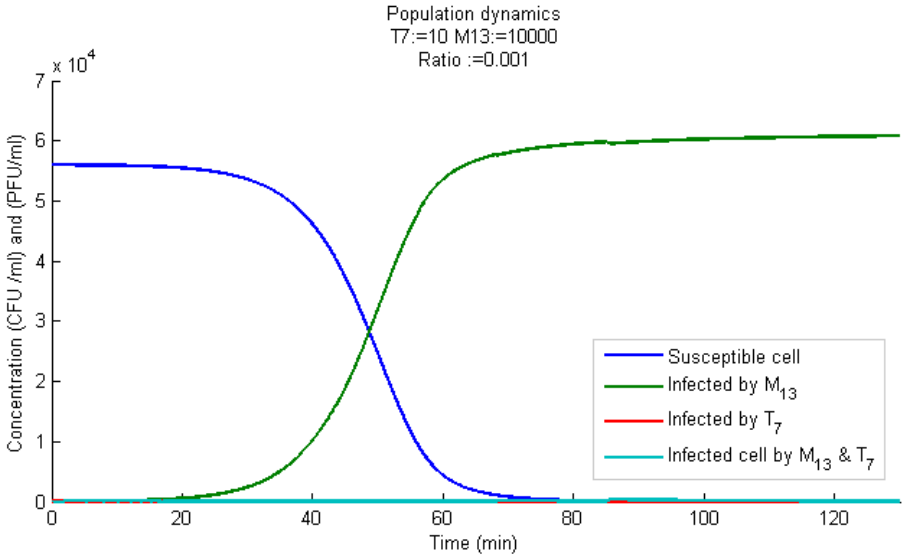

Figure 13: Dynamics of cells with initial concentrations: T7=10, M13=10^4

Table 2: Control parameters meaning and values for model 2

Symbol	Meaning	Value
D	Dilution rate	0.05 min^{-1}
S_{1in}	Input concentration of susceptible cells S_1	$5.6 * 10^4$ CFU/ml
S_{2in}	Input concentration of susceptible cells S_2	$5.6 * 10^4$ CFU/ml
S_{3in}	Input concentration of susceptible cells S_3	$5.6 * 10^4$ CFU/ml
b	Binding rate of T_7 to a cell	$10^{-6}\text{ml CFU}^{-1}\text{min}^{-1}$
k	Latent period	17 min
h	Burst size of a cell	100 PFU
d	Decay rate of phages	0.009 min^{-1}
μ	Mutation rate	10^{-3}PFU^{-1}

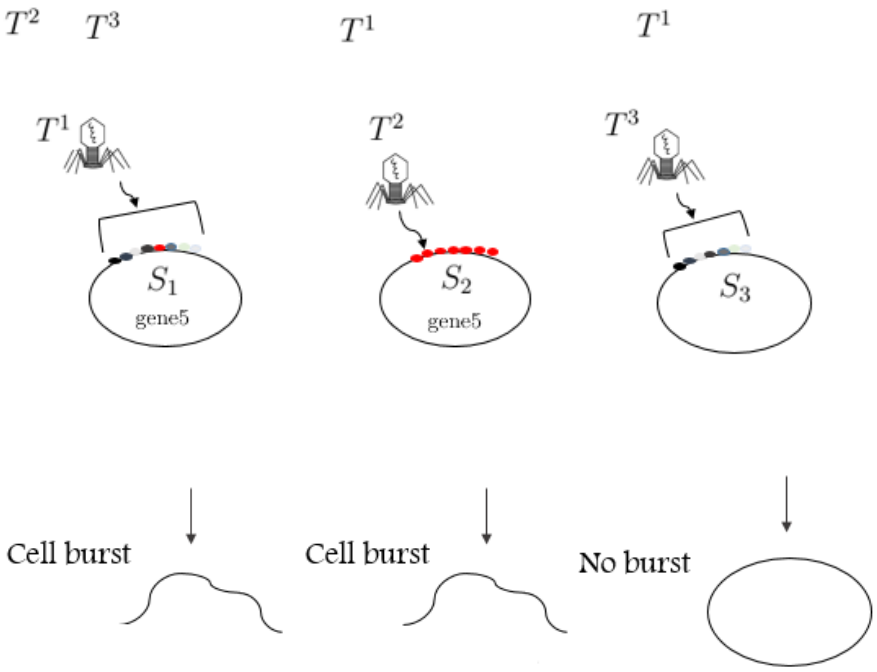

Figure 14: Schematic description of model 2

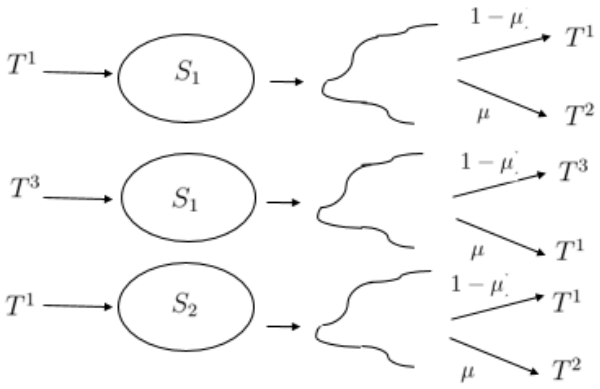

Figure 15: Three possible scenarios where phages T^1, T^3 infect cells and mutate into T^2, T^1 respectively with rate μ or not with $1-\mu$

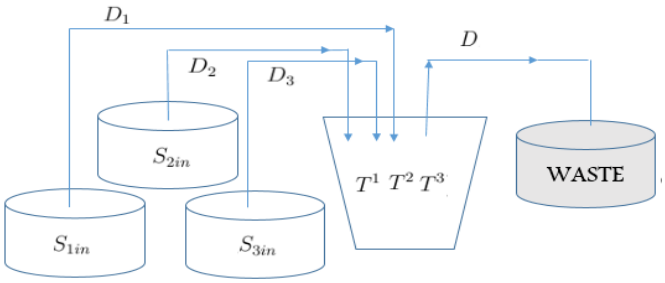

Figure 16: Schematic of chemostat for model 2