# COMPLEX DYNAMICS IN BACTERIUM–PHAGE INTERACTIONS

Heidi Kristensen, Lars Risbo, Erik Mosekilde
Physics Laboratory III
The Technical University of Denmark
2800 Lyngby, Denmark

Jacob Engelbrecht
Institute of Dairy Science
Royal Veterinary and Agricultural University
1870 Frederiksberg C, Denmark

Abstract

In order to examine different strategies in the search for more resistant bacterial cultures, we have simulated a variety of growth, mutation, competition and selection processes that may arise in interacting populations of bacteria and phages. Our model considers a culture containing several variants of the same bacterium, each sensitive to attacks from a specific phage. The culture is growing in a chemostat with a continuous supply of nutrients. Surplus bacteria and vira are removed through dilution. Depending on the rate of dilution, the model exhibits a stable equilibrium, self–sustained oscillations, quasi–periodic behavior, deterministic chaos, or extinction of certain species. The model can also be used to describe evolutionary changes in the composition of the microbiological system.

## Introduction

Interacting populations of bacteria and phages (i.e., vira) play an important role in many microbiological applications. The homogeneous bacterial cultures used in modern cheese production, for instance, are often quite sensitive to attacks from phages, and considerable efforts are invested into the search for more resistant cultures. As a means to evaluate different strategies for this search, we have developed a model which can be used to simulate a variety of different processes that may arise in interacting populations of bacteria and phages.

The model considers a population containing several variants of the same bacterium, each being sensitive to attacks from particular phages. In such an attack, the phage will adsorb to the bacterial surface and attempt to transfer its DNA into the cell. This can lead to (i) a lytic response in which the virus programs the bacterial cell to replicate the phage DNA, (ii) a lysogenic response in which the viral DNA is inserted into the bacterial DNA with the result that the cell becomes partly resistant to new attacks, or (iii) a lethal response in which the bacterial cell is killed before additional vira can be produced. It is also possible that the attack can fail, the penetrating phage being destroyed by the bacterial restriction enzymes, or that the cell spontaneously mutates into a cell with a different resistance. Under stress, a lysogenic bacterium may again release its viral DNA, providing in this way for the possibility of a renewed infection.

To enable us to study evolutionary processes, we have adapted the general modeling framework proposed, for instance, by Bruckner, Ebeling and Scharnhorst (1989). In this framework one considers an enumerable set of fields (populations) each characterized by

the properties of its occupying elements (individuals). Within and between these fields a variety of processes can take place, including spontaneous generation, self–reproduction, error production, death of elements, and transitions from one field to another. Mutation represents an example of a process by which individuals of one population are transformed into individuals of another population. Infection, by which an individual is transferred from exposed to infectious, is another example.

Since the first occupation of a new field necessarily starts with very few individuals, a discrete, stochastic approach is needed to simulate evolutionary dynamics. A similar approach is also appropriate for many epidemic problems where an infection may start by the random contamination of a single individual. However, the transition probabilities associated with the various processes need not be linear. Processes that involve individuals from two different populations typically contain bi–linear terms, and higher order terms may become significant in the presence of heavily populated fields. The presence of such terms allows the systems to exhibit highly nonlinear dynamic phenomena such as multistability, self–sustained oscillations, quasi–periodic behavior, deterministic chaos, and simultaneously existing periodic or chaotic orbits.

Deterministic chaos and other highly nonlinear dynamic phenomena have previously been reported for a variety of different predator–prey models. Of particular interest to the present study are investigations of complex dynamic phenomena in the interaction between HIV and the immune system (Anderson and May, 1989), and studies of chaos in models of childhood diseases (Olsen, Truty and Schaffer, 1988). Compared with previous work on bacterium–phage interactions (Levin 1986 and 1988), our model distinguishes itself by considering a more complex situation involving several variants of the same bacterium. At the same time we combine the stochastic description of fields with few elements with the nearly deterministic description of heavily populated fields.

## The Simple Model

In the first version of our model we consider a bacterial population consisting of two variants: unmodified bacteria which are relatively sensitive to phage attacks, and modified bacteria for which the infection probability is many times as small. The modified bacteria are assumed to be produced by mutation of unmodified cells, and the reverse process in which modified cells lose their resistance to phage attacks is also accounted for. This is illustrated in the flow diagram of figure 1. Except for such reverse mutations, the modification is inherited by daughter cells. The reproduction rates for the two types of bacteria are taken to be 0.05/min and 0.047/min, respectively. This implies that the resistance to phage attacks is "payed for" by a slightly slower rate of reproduction.

The culture is assumed to grow in a chemostat with a continuous supply of nutrients. With increasing bacterial populations, the growth rates are reduced because of decreasing resource availability. This decrease is described by a logistic growth correction which is assumed to depend on the total bacterial population. In addition there is a continuous wash out of bacteria by dilution. In the absence of vira, the population of unmodified bacteria will therefore outgrow and suppress the population of modified cells, and a stable equilibrium exists in which the population of unmodified bacteria is controlled by the supply of nutrients and the rate of dilution.

From time to time, a virus particle may enter the system. If the virus succeeds in infecting a bacterial cell, it will program the cell to reproduce the viral DNA. This leads to a rapid multiplication while at the same time the cell is depleted of some of its

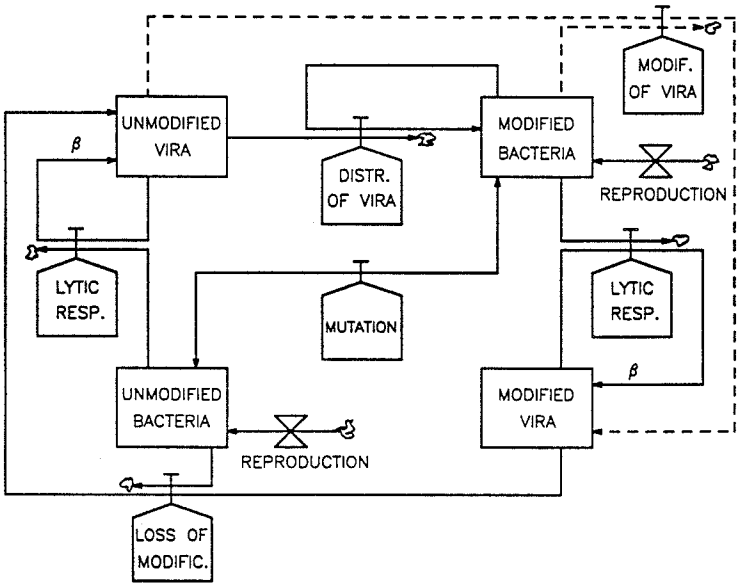


Figure 1. In its simplest form, our model considers a population consisting of two variants of the same bacterium: unmodified cells which have the advantage of a faster reproduction rate, and modified cells which are significantly less sensitive to phage attacks. There is a small probability that a viral DNA– molecule penetrates the restriction system of a modified cell. This will give rise to the reproduction of modified vira that can infect other modified cells.

essential compounds. At the end the cell disintegrates (lyses), and a burst of vira is liberated. The burst size $\beta$ is typically of the order of 100. If, on the other hand, the cell is resistant to the viral attack, it will destroy the intruding DNA by means of its restriction/modification system. This involves the combined action of modification enzymes that encode all DNA molecules produced by the cell, and restriction enzymes that destroy uncoded DNA.

In a few cases, a virus particle may succeed in penetrating the restriction system and infecting a modified bacterial cell. The new vira which are produced in this way will be encoded in accordance with the modification system of the cell. As a result these vira can infect other modified cells. Of course, a modified virus can also infect an unmodified cell. However, the vira produced in such a process have lost their modification.

To allow us to describe processes in which very few individuals are involved, the model equations are formulated in terms of discrete, stochastic transitions. At the same time, we have adopted a generic representation which allows us to describe many different problems, depending upon the choice of parameters. Thus, the bacterial populations are represented by a vector $POP_i$, with $i = 1, 2 - - -$ n identifying the particular variant. Similarly, the populations of phages are represented by the vector $PHA_j$, where $j = 1, 2 - - -$ k may encompass a large number of different species. In the present case $i = j = 1$ for unmodified bacteria and vira, and $i = j = 2$ for modified bacteria and vira, respectively.

The lytic reaction in which a phage of type $j$ infects a cell of type $i$ to produce $\beta$ new vira of type $i$ may be represented as

$$POP_i, PHA_j, PHA_i \rightarrow POP_i - 1, PHA_j - 1, PHA_i + \beta$$

where, as previously noted, $\beta$ is the burst size. Assuming that the chemostat is effectively stirred such that the populations are homogeneously distributed over the available volume, the intensity of the above process may be expressed as

$$W_{lytic} = B_{ij} \times POP_i \times PHA_j$$

Here, the rate constants

$$B_{11} = B_{12} = B_{22} = 2 \cdot 10^{-7}/\text{min}$$

for unrestricted lytic response, and

$$B_{21} = 5 \cdot 10^{-11}/\text{min}$$

for the infection of a modified cell by an unmodified virus. This implies that a modified cell is 4000 times as resistant as an unmodified cell towards attacks from unmodified vira.

The parameters $B_{ij}$ may be considered the basic rate constants of the model relative to which many of the other rate constants are scaled. The actual magnitudes of $B_{ij}$ primarily depend upon the diffusion constants for the vira and upon the probabilities that a phage adsorbs to a cell. This probability again depends upon the density of specific receptors on the cell surface.

Destruction of vira by resistant bacteria is expressed by

$$POP_i, PHA_j \rightarrow POP_i, PHA_j - 1.$$

The intensity of this process is

$$W_{destruction} = C_{ij} \times POP_i \times PHA_j$$

with

$$C_{21} = 2 \cdot 10^{-7}/\text{min}$$

for attacks of unmodified vira on modified bacteria. In the other cases where the cell has no particular resistance towards the intruding vira we have taken

$$C_{11} = C_{12} = C_{22} = 10^{-8}/\text{min}.$$

Thus, in these cases, about 5% of the vira are destructed.

Mutation processes are expressed by

$$POP_i, POP_j \rightarrow POP_i - 1, POP_j + 1.$$

The intensity is here

$$W_{mutation} = M_{ij} \times POP_i$$

with $M_{12} \cong M_{21} = 5 \cdot 10^{-4}$/min. This implies that approximately 1% of all bacterial reproductions leads to a mutation.

The resource limited bacterial growth processes are described by

$$POP_i \longrightarrow POP_i + 1$$

with

$$W_{growth} = A_j \times POP_j \times \left[1 - \Sigma_i POP_i / POPM\right].$$

As previously noted, the rate constants are taken to be $A_1 = 0.050$/min and $A_2 = 0.047$/min, respectively. In the simulations to be presented here we have taken POPM = 3000. Thus, due to limited resources, the total bacterial population $\Sigma_i POP_i$ is restricted to 3000 cells. Because of the simultaneous removal of cells through dilution, the actual bacterial population will not attain this value.

The dilution process is described by

$$POP_i \longrightarrow POP_i - 1$$

with the intensities

$$W_{removal} = POP_i \times DFLOW$$

or

$$W_{removal} = PHA_j \times DFLOW.$$

Here, DFLOW is a control parameter which is varied from simulation to simulation. A typical value is DFLOW = 0.02/min, corresponding to a time of occupancy in the chemostat of 50 min. The bacterial growth rates of 0.05 and 0.047/min correspond to doubling times of the order of 30 min.

Finally, the spontaneous contamination of the chemostat by unmodified vira is expressed by

$$PHA_j \longrightarrow PHA_j + 1$$

with

$$W_{spontaneous} = G_j$$

Here, $G_1 = 0.1$/min, and $G_2 = 0$.

The various processes occur with vastly different intensities and also with intensities that vary orders of magnitude over time. Reproduction of bacterial cells and replication of vira

in lytic reactions thus occur at much higher rates than the processes by which bacteria and vira are modified. To attain a reasonable dynamic range in the stochastic model, we have represented the various transition rates as Poisson processes. This implies that the probability that k transitions of a given type will take place during the time increment DT is given by

$$p_k = \frac{(W \cdot DT)^k}{k!} \exp\{-W \cdot DT\}$$

with W being one of the above calculated intensities. In this way we can simulate the model with significantly larger DT than it would otherwise be possible. For very fast processes we have replaced the Poisson distribution by a normal distribution with the appropriate mean and variance.

Simulation Results

Our simulations with the simple 2 × 2 population model were all performed with similar initial conditions. We start with 5 unmodified bacterial cells and 0 modified cells. The initial population of unmodified vira is assumed to be $10^5$ min × DFLOW. For DFLOW = 0.040/min, this corresponds to a viral population of 4000 particles. In addition there is a random contamination of the chemostat corresponding to an average inflow of 0.1 unmodified virus per minute. The simulation period is 10,000 min, or approximately 1 week.

Figure 2 shows the results obtained for a dilution rate of DFLOW = 0.040/min. This corresponds to an average time of occupancy for cells and vira in the chemostat of 25 min. With this relatively high rate of dilution, the presence of vira has little significance. As a consequence of their higher rate of reproduction, the unmodified bacteria outgrow the

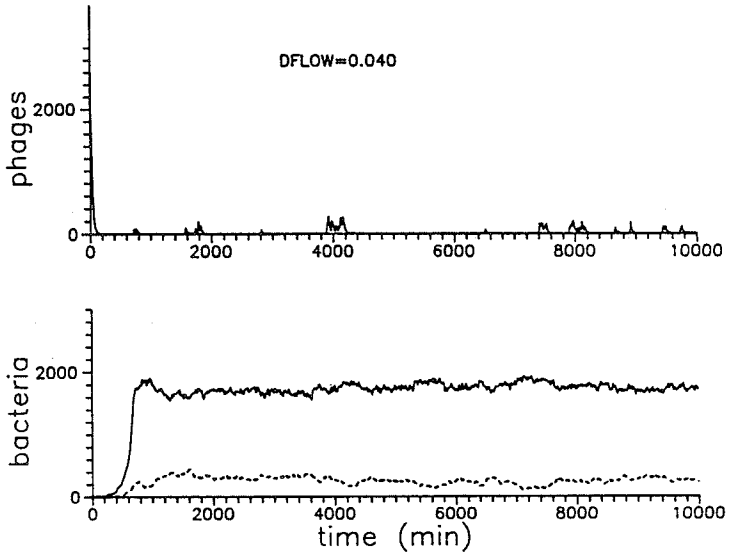


Figure 2. With a rate of dilution of DFLOW = 0.040/min, the viral populations are suppressed, and the population of unmodified cells dominates.

population of modified cells to reach a stable saturation level of approximately 1700 cells. By mutation, a population of approximately 200 modified cells is maintained. The initial population of unmodified phages is almost completely washed out, before a significant bacterial population is established. From time to time, minor epidemics of attacks by unmodified vira break out. These epidemics never develop into anything significant, however, and they rapidly die out again.

A somewhat lower rate of dilution allows the phages to establish a major attack on the population of unmodified bacteria. This is illustrated in figure 3 for DFLOW = 0.030/min. The reduction in the population of unmodified bacteria brought about by the infection gives a better chance to the modified bacteria. On the other hand, the growth of this population curbs the viral infection, and the population of unmodified bacteria soon reassumes its dominance. Somewhat later, a new infection occurs. The bacterial population is still stable against viral infections, however, and these infections occur as a purely stochastic phenomenon, since very few viral particles are present in the intermediate periods.

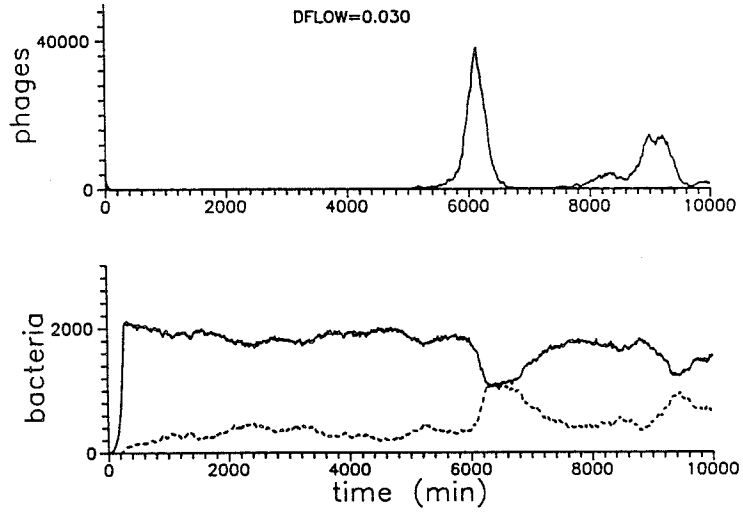


Figure 3. With a somewhat lower rate of dilution (DFLOW = 0.030/min), the phages can establish major attacks on the population of unmodified cells. The bacterial population is still stable against viral infections.

With further reduction in the rate of dilution, the phage attacks become more severe, and also more regular. This is illustrated in figure 4 where DFLOW = 0.0225/min. Viral populations of the order of 50 – 100,000 particles are now attained in the infections. Each time, however, the growth of the population of modified bacteria brought about by the infection kills off the vira, and one can envisage that the system will develop a regular oscillatory mode with a period of the order of 3000 min. This period is controlled mainly by the time it takes for the population of unmodified bacteria to recover after an infection, i.e., by the time it takes the bacterial population to forget the resistance developed during the infection.

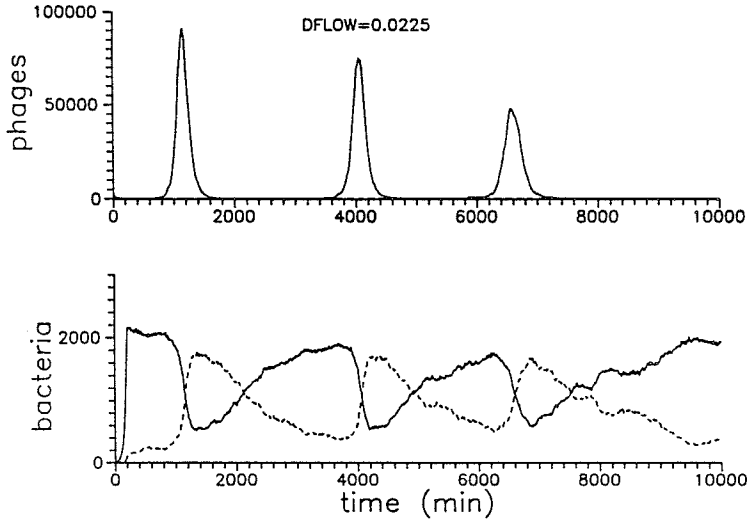


Figure 4. With DFLOW = 0.0225/min, the infections become more severe but also more regular in their appearance. Each time, however, the induced shift in the composition of the bacterial population brings the infection to an end.

If the rate of dilution is further reduced, a qualitative shift in the behavior of the model tends to occur as modified vira now appear in the system. Hereafter, the cells are no longer capable of killing off the phages, and a predator–prey relation between modified vira and modified bacteria develops. The appearance of modified vira is conditioned by the simultaneous existance of large populations of unmodified vira and of modified

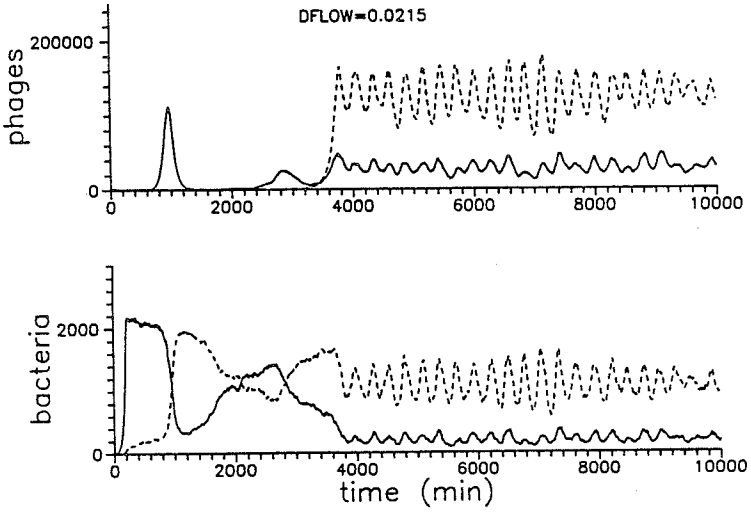


Figure 5. With DFLOW = 0.0215/min, modified vira tend to appear in the system. Hereafter, the cells are no longer capable of killing off the phages, and an oscillatory predator–prey relation between modified vira and modified bacteria develops.

bacteria. In figure 5, where DFLOW = 0.0215/min, modified vira emerge during the second infection, i.e. at about time 3000 min. From then on, the system enters a self–sustained oscillatory behavior with a period of approximately 200 min. It is interesting to note that the two bacterial populations vary in phase, and that the same is true for the two viral populations. However, the populations of unmodified bacteria and vira remain low.

If DFLOW is further reduced, modified vira emerge even earlier. This is illustrated in figure 6 for DFLOW = 0.015/min. At the same time, the intensity of the oscillations between modified bacteria and vira becomes higher, and the oscillations become more regular. The period of these oscillations also increases slightly. With a rate of dilution as low as 0.007/min, the population of modified vira becomes strong enough to kill off the bacterial population (see figure 7). Thereafter, the vira can no longer multiply, and the viral population is soon washed out of the chemostat.

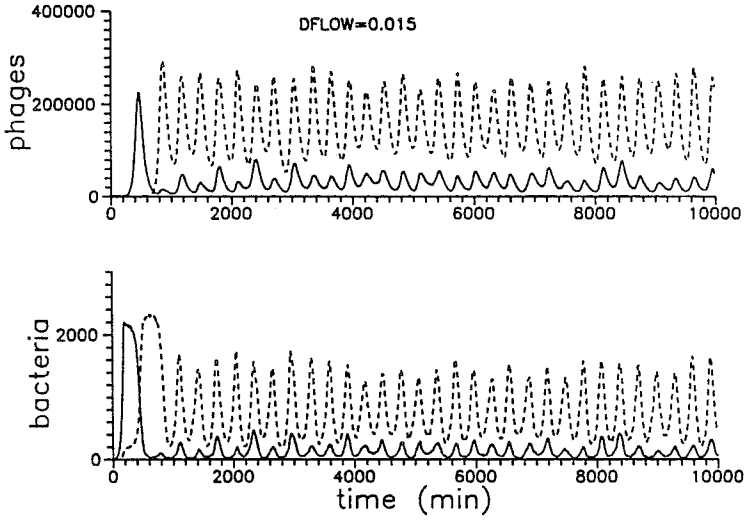


Figure 6. With DFLOW = 0.015/min, modified vira emerge already in the first infection, and the oscillatory behavior associated with the interaction between modified vira and modified cells becomes more intense.

For comparison with the above simulations which were performed with a continous dilution, figure 8 shows the results of a simulation in which a small sample (2%) of the existing culture is taken every 400 min and allowed to regrow in an otherwise uncontaminated chemostat. This resembles the manner in which bacterial cultures as applied, for instance, in cheese production have been maintained for decades or maybe even centuries. In the simulation shown in figure 8, modified vira never emerge, and we see a modulation of the composition of the bacterial population in response to more or less random viral infections. Due to the stochasticity involved, with the same basic parameters other simulations may result in the development of modified vira, yielding a completely different behavior of the system.

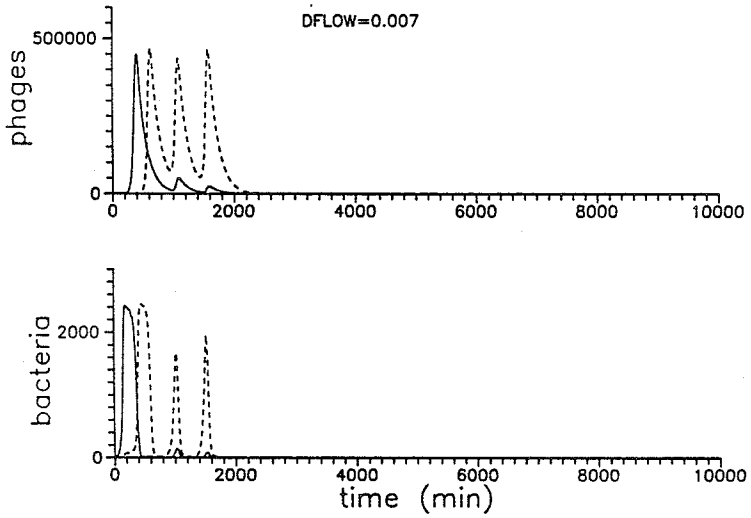


Figure 7. With DFLOW = 0.007/min, the population of modified vira becomes strong enough to kill off the bacterial populations. Hereafter, the vira can no longer replicate, and they too, soon disappear.

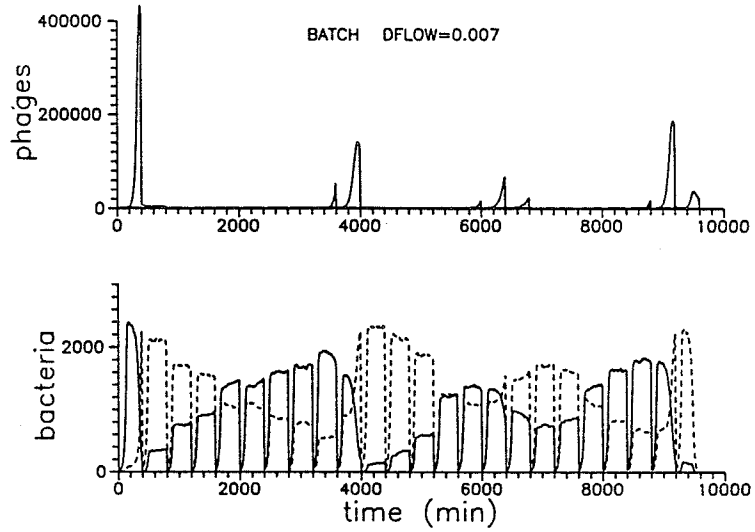


Figure 8. In this simulation, a small sample of 2% of the culture is taken out every 400 min and allowed to grow in another chemostat. Observe the modulation in the composition of the bacterial population in response to viral infections.

## Discussion

As illustrated by the above simulations, the rate of dilution is a significant control parameter which shifts the balance between vira and bacteria. From a physical point of view, dilution is a source of dissipation in the system, and low rates of dilution therefore

predispose the system for unstable behavior.

At high dilution rates, the vira are effectively suppressed, and unmodified bacteria dominate. At lower dilution rates, the virus population becomes strong enough to disturb the competition between rapidly growing, relatively sensitive cells and the less rapidly reproducing, modified bacteria. In a certain range of parameters, the presence of vira thus increases the diversity of the bacterial population. Besides the rate of dilution, other significant control parameters are the bacterial growth rates, the supply of substrate, the burst size, the adsorption rate of vira to the cell surface, the mutation rates, and the probability that an unmodified virus can penetrate the restriction system of a modified cell.

We have also performed a series of simulations with a slightly different model in which three variants of the same bacterium are considered. Each variant is assumed to be sensitive to a particular phage and resistant to the phages which attack the other variants. In this model we have accounted for the replication delay associated with a lytic response. On the other hand, mutation between the 3 bacterial variants is neglected. Finally, the supply of nutrients has been increased, and saturation now occurs at populations of the order of $10^6$ cells. Thus, in practice the model is deterministic.

The behavior of this 3 × 3 system is very similar to the simple migration model considered by Sturis and Mosekilde (1988). If a particular bacterial variant happens to become more frequent than the others, and if the growth rates are similar, this variant will outgrow and start to suppress the others. However, this makes the population more sensitive to infections by the phage which attacks the predominant variant. As a result, a significant reduction of this population may occur, and another variant may start to dominate. This cyclic behavior may produce a self–sustained oscillation between the bacterial variants. With sufficient symmetry, the system will show two coexisting solutions: one which rotates between the variants in the order 1⟶2⟶3, and one which rotates in the opposite direction 1⟶3⟶2. The solution that the system will choose in a given situation depends upon the initial conditions. It is interesting to note, however, that the boundary between those initial conditions which give one solution and those which lead to the other is likely to be fractal. Thus, the behavior of the system is extremely sensitive to the initial conditions.

Figure 9 shows the phase plots of two such coexisting periodic solutions. As a control parameter is changed, the system passes through a series of qualitative changes by which various quasi–periodic and periodic solutions are produced. In certain parameter ranges, coexisting with the periodic or quasi–periodic solutions one also finds chaotic solutions. This type of solution is illustrated in the phase plot of figure 10 which was obtained with the same parameter values as figure 9, only with a different set of initial conditions.

By virtue of the positive feedback associated with reproduction processes, because of the time lags associated with incubation and maturation delays, and because of the nonlinear interaction terms, population dynamics involving more than a few species is almost bound to produce complex modes of behavior. As illustrated by the above simulations, even a system with only 3 bacterial and 3 viral populations can produce almost any kind of complex behavior, including coexisting periodic and chaotic solutions.

Because of their short generation times, microbiological systems lend themselves very directly to studies of these types of behavior. Such systems can also be prepared in a variety of well–controlled conditions. The selective pressure provided by the presence of vira plays an important role for the evolutions of new bacterial variants. This evolution

again influences the direction in which new vira develop. Interacting populations of vira and bacteria are therefore also a natural subject for studies of evolutionary dynamics.

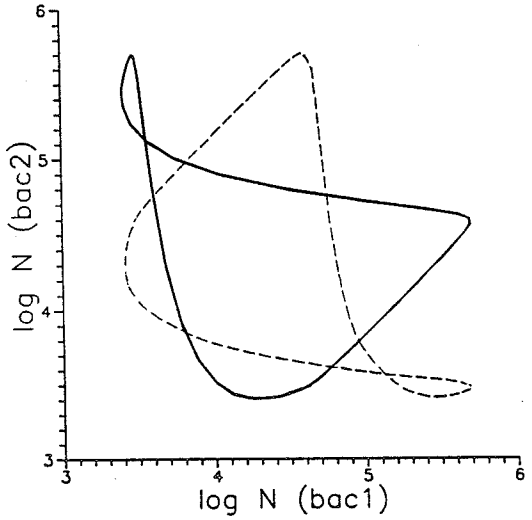


Figure 9. A model with three bacterial variants each sensitive to a particular phage can produce coexisting self–sustained oscillations. Which of the two solutions a or b the system chooses depends upon the initial conditions in an extremely critical manner.

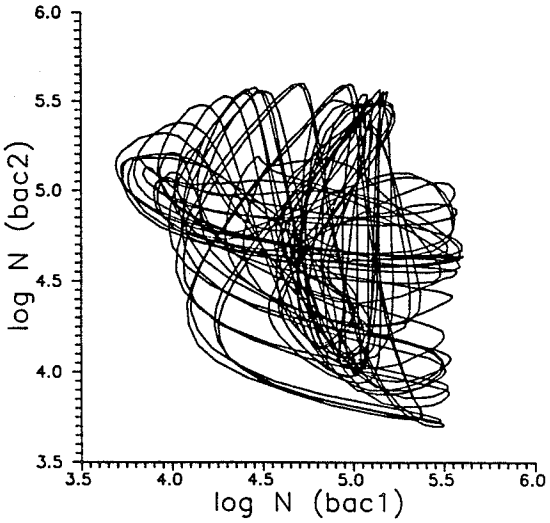


Figure 10. Phase plot of a chaotic solution which exists for the same parameter values as the periodic solutions in figure 9.

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