Development of Phage Populations in a Bacterial Culture: a Mathematical Model

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Phage Yield, Infection, Computer Simulation

A mathematical model for the interaction kinetic in phage-bacterium culture is proposed. An appropriate analytical relationship between phage and bacterial concentrations has been derived. Characteristic kinetic constants have been obtained by comparing the experimental growth curves with computer-simulation analysis. The model allows also to evaluate the phage-yield as a function of the initial concentrations.

Mathematical Approach

Mathematical model

As far as we know a mixed population of phage and its host bacterium hasn't ever been exhaustively studied from a kinetical point of view.

Such an approach is important for at least two reasons. First it may suggest the most appropriate values of initial concentration of bacteria and phages in order to obtain a massive production of phage. On the other hand a satisfactory mathematical model allows to calculate, as a function of time, the concentrations of the two interacting populations; comparison to experimental results gives therefore opportunity to obtain the values of essential parameters of the system, like adsorption probability, lag time and burst size.

Purpose of the present work is not to introduce a mathematical model based on a system of hypothesis commonly valid for many of the most studied phages. We must however keep in mind that every couple phage-host bacterium has usually peculiar characteristics which affect the kinetic of the system.

It isn't therefore possible to build up a mathematical model valid in any case, even if in general it is possible to use in various cases the system of equations we shall obtain by introducing suitable modifications in the model.

The assumptions made are in part of biological character (so restricting the variety of the systems to which the model may be referred) and in part have the purpose to simplify the mathematical problem through a suitable approximate description of some aspects of the processes [1–3]. They are the following:

a) The growth rate α is a constant; bacterial growth is therefore considered only in exponential phase.
b) The absorption of the phage in the host follows a kinetic of first order both for phages and host concentrations. This hypothesis, as Krueger and Delbrück have shown [4, 5], is valid for a very large range of concentrations.
c) The infection probability of a bacterium K is considered as constant. In fact the absorption rate depends [6, 7] upon the chemicophysical conditions of the growth medium; in particular on the ion concentrations in solution.
d) We do not distinguish between adsorption probability and infection probability, *i. e.* we suppose that all the phages, which are adsorbed on a cell wall, begin a lytic cycle. Then we, in general, exclude from our considerations the lysogeny; but an extension of the system of equations to this case is quite simple.
e) The burst size is considered as constant; the constant λ which appears in the equations, is an appropriate mean value of the real burst sizes varying from bacterium to bacterium.
f) The lag time τ is considered as constant. This is the most crucial hypothesis concerning the ability of the model to reproduce experimental results.

Assuming the distribution of lag times to be gaussian, the number of bacteria which will lyse at

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time t is proportional to the integral

$$\int_0^t K\, n(x) N(x) \exp\left[-\frac{(x-t+\tau)^2}{\sigma^2}\right] \mathrm{d}x .$$

This integral can be approximated by

$$K\, n(t-\tau) N(t-\tau)$$

only if we do not make a great error when $n(x)$ and $N(x)$ are substituted by their values at the maximum of the gaussian. This happens only if $\sigma \ll \tau$, the characteristics variation time of n and N.

Let us consider, under these hypothesis, the situation at the time t of a bacterial culture infected at $t = 0$ by a virulent phage.

Let us suppose, for sake of simplicity, that both, bacteria and phages belong to pure strains, which derive from one clone, and that the possible rise of mutants has a negligible effect on the growth of the mixed culture.

The variations of the concentrations of bacteria (N), phages (n) and infected bacteria (N_f) at time t will be expressed by the equations:

$$\begin{aligned}
\frac{\mathrm{d}N(t)}{\mathrm{d}t} &= \alpha N(t) - K\, n(t) N(t), \\
\frac{\mathrm{d}n(t)}{\mathrm{d}t} &= K \lambda\, n(t-\tau) N(t-\tau) \\
&\quad - K\, n(t) N(t) - K\, n(t) N_f(t), \\
\frac{\mathrm{d}N_f(t)}{\mathrm{d}t} &= K\, n(t) N(t) - K\, n(t-\tau)\, N(t-\tau). \qquad (1)
\end{aligned}$$

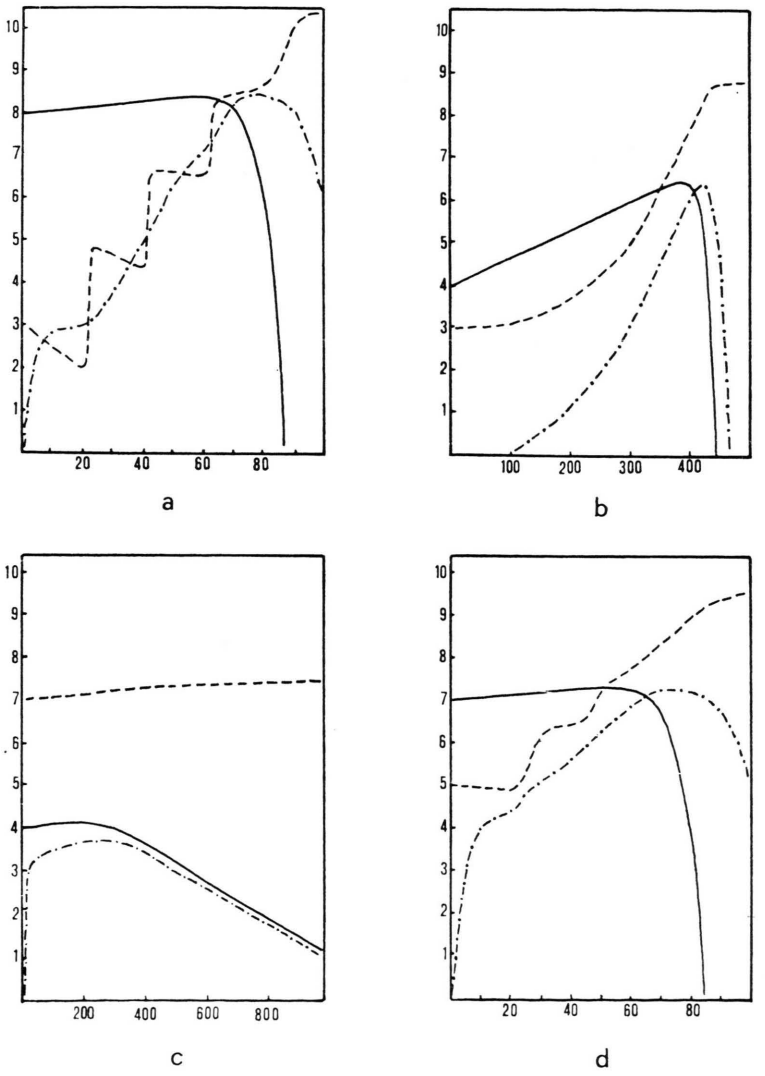

Fig. 1. Theoretical population-curves of bacteria N (——), phages n (— — —) and infected bacteria N_f (— · — · —). In ordinates the logarithm of populations and in abscissa the time in minutes. a) $N_0 = 10^8$ bact/ml, $n_0 = 10^3$ phag/ml, b) $N_0 = 10^4$ bact/ml, $n_0 = 10^3$ phag/ml, c) $N_0 = 10^4$ bact/ml, $n_0 = 10^7$ phag/ml, d) $N_0 = 10^7$ bact/ml, $n_0 = 10^5$ phag/ml.

Numerical solution of the system

The equations (1) are very difficult to integrate by an analytical way. We can obtain [8] a relation between the variables $N(t)$, $n(t)$ by introducing the approximation

$$\frac{1}{\tau} N(t)\, n(t) - N(t-\tau)\; n(t-\tau) \cong \frac{\mathrm{d}}{\mathrm{d}t}\, n(t)\, N(t). \tag{2}$$

This isn't of any practical utility.

Solution of system (1) has been obtained in a numerical way by means of the relation

$$f(t+\Delta t) = f(t) + f'(t)\,\Delta t \tag{3}$$

with an integration step $\Delta t = 6$ sec.

In Fig. 1 some theoretical population-curves, as calculated from Eqn (1), have been drawn corresponding to different values of initial populations n_0 and N_0.

The values $\lambda = 150$, $\alpha = 0.015\ \mathrm{min}^{-1}$, $\tau = 20$ min, $K = 10^{-9}\ \mathrm{cm}^3\ \mathrm{min}^{-1}$ have been chosen, which may be considered appropriate for the most commonly used phage-bacterium system [9].

Materials and Methods

The validity of the described mathematical model has been tested experimentally for the four systems described in Table I. The test consists of determining experimentally the functions $n(t)$ $N(t)$ and loocking for the values of the constants α, λ, τ, K, which best fit the experimental results by means of Eqn (1). Generally the values so obtained are not far from those obtained independently by classical experiments.

Table I. Phage-bacterium systems and growth conditions

Bacterium	Phage	Medium	Reference
E. coli B	T4rII	M9S	10
E. coli B/5	T2r$^+$	M9S	11
E. coli CC17	ϕX174	TPG3A	12
E. coli M72	$\lambda N_9 Ai_8$	TB+M	13

M: maltose 0.2%.

The phage-bacterium systems and experimental conditions are described in Table I.

During bacterial growth aliquot have been periodically withdrawn to determine the bacterial concentration by means of optical density. At the prefixed value of bacterial concentration a suitable dose of phage suspension has been added. From this time the yield of the bacterial and phage concentration has been followed by means of standard techniques.

Results

A) Experimental results and determination of the constants of the systems

In Figs 2 – 5 the results of infection with the different phage stocks have been reported. From the curves it is possible to obtain the values of characteristic constants of the kinetics of infection. Let's subscript the index 1, 2, 3, 4, to values that correspond to different systems.

a) Growth rate. From the first Eqn (1), when $K n \ll \alpha$ we have

$$\mathrm{d}N/\mathrm{d}t \cong \alpha N \tag{4}$$

and

$$\log N = \log N_0 + \alpha t. \tag{5}$$

The method of least square allows to get the best slope of the straight line that represents bacterial growth in semilog paper and to obtain α:

$$\begin{aligned} \alpha_1 &= (0.015 \pm 0.02)\ \mathrm{min}^{-1}, \\ \alpha_2 &= (0.014 \pm 0.02)\ \mathrm{min}^{-1}, \\ \alpha_3 &= (0.015 \pm 0.02)\ \mathrm{min}^{-1}, \\ \alpha_4 &= (0.014 \pm 0.02)\ \mathrm{min}^{-1}. \end{aligned} \tag{6}$$

b) Burst size. The ratio between maximum phage and bacterial concentration is a good excess evaluation of the burst size.

In our case we have:

$$\begin{aligned} \lambda_1 &= \frac{n_1\,\mathrm{max}}{N_1\,\mathrm{max}} = 75 \pm 10, \\ \lambda_2 &= \frac{n_2\,\mathrm{max}}{N_2\,\mathrm{max}} = 180 \pm 20, \\ \lambda_3 &= \frac{n_3\,\mathrm{max}}{N_3\,\mathrm{max}} = 150 \pm 15, \\ \lambda_4 &= \frac{n_4\,\mathrm{max}}{N_4\,\mathrm{max}} = 100 \pm 10. \end{aligned} \tag{7}$$

c) Lag time. Lag time has been obtained by looking for the time when the phage concentration equals the number of phages absorbed in the first five minutes multiplied by the burst size

$$\begin{aligned} \tau_1 &= (16 \pm 2)\ \mathrm{min}, \\ \tau_2 &= (23 \pm 3)\ \mathrm{min}, \\ \tau_3 &= (24 \pm 4)\ \mathrm{min}, \\ \tau_4 &= (65 \pm 5)\ \mathrm{min}. \end{aligned} \tag{8}$$

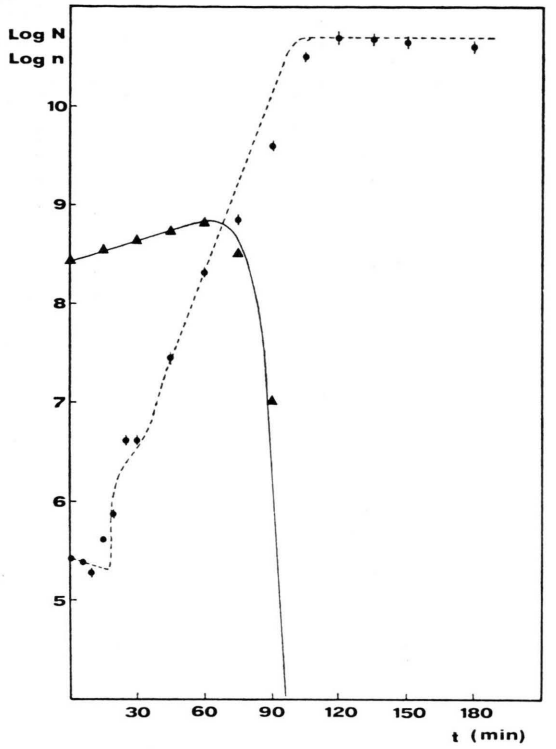

Fig. 2. Comparison between theoretical curves and experimental data for a system *E. coli* B (——) T4r II (– – –) when the parameters assume the values of Table II.

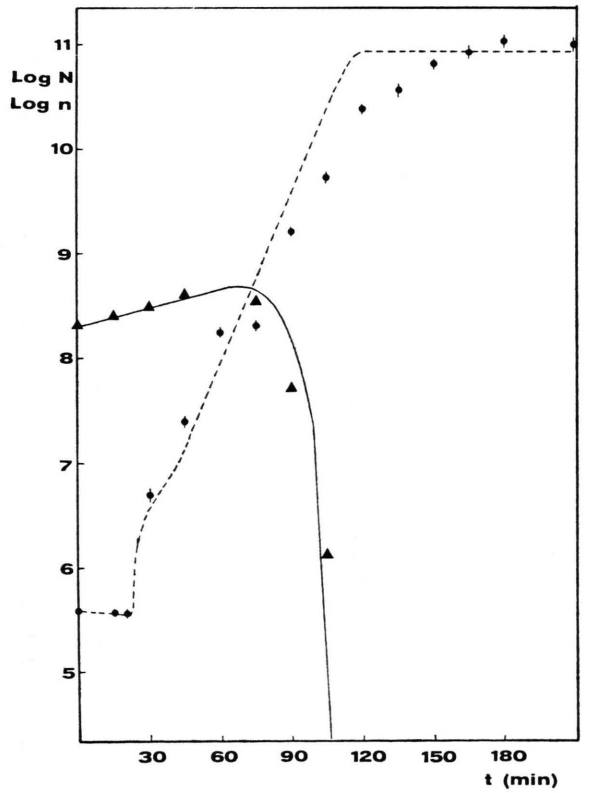

Fig. 3. Comparison between theoretical curves and experimental data for a system *E. coli* B/5 (——) T2r+ (– – –) when the parameters assume the values of Table II.

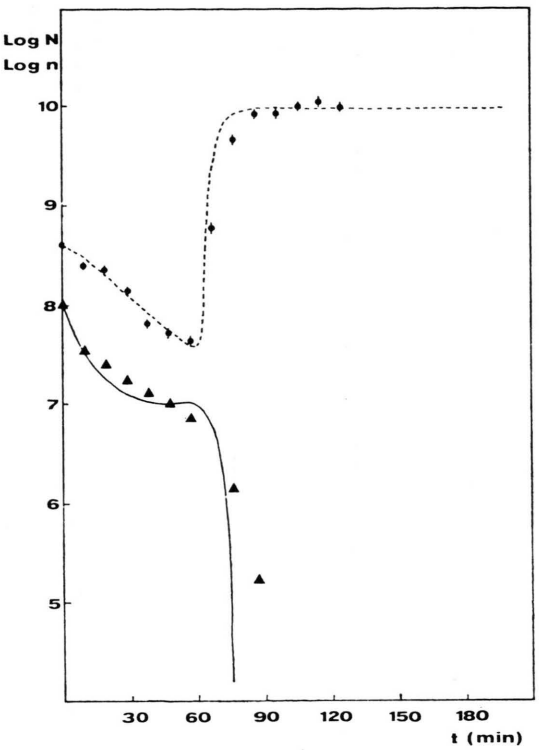

Fig. 4. Comparison between theoretical curves and experimental dato for a system *E. coli* CC17 (——) ϕX 174 (– – –) when the parameters assume the values of Table II.

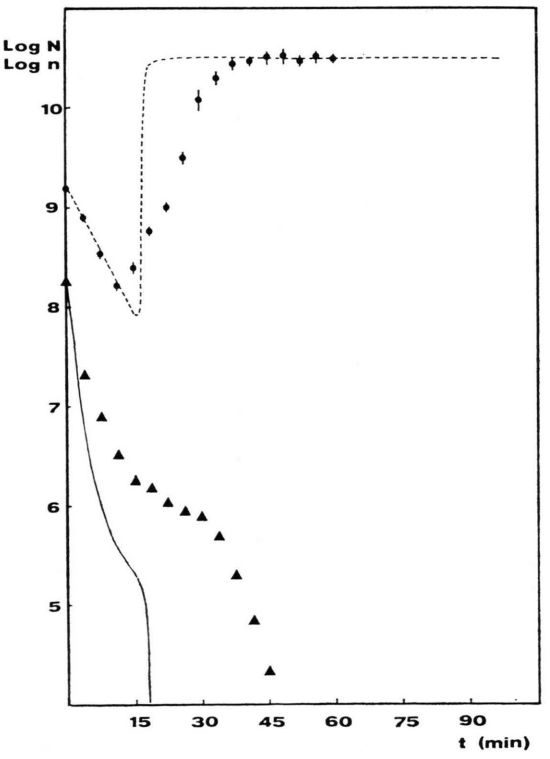

Fig. 5. Comparison between theoretical curves and experimental data for system *E. coli* M 72 (——) $\lambda N_9 Ai_8$ (– – –) when the parameters assume the values of Table II.

d) Adsorption probability. It is possible to estimate this parameter from phage disappearance in the first minutes of infection. By the third Eqn of system (1), pointing out that $K n \ll \alpha$, we have for K the equation

$$K = \frac{\alpha}{N_0[\exp(\alpha t_2) - \exp(\alpha t_1)]} \log \frac{n(t_1)}{n(t_2)},$$

$$K_1 = (5 \pm 3) \times 10^{-11}\ \mathrm{cm^3\ min^{-1}},$$
$$K_2 = (3 \pm 2) \times 10^{-11}\ \mathrm{cm^3\ min^{-1}},$$
$$K_3 = (8 \pm 2) \times 10^{-10}\ \mathrm{cm^3\ min^{-1}},$$
$$K_4 = (5 \pm 2) \times 10^{-10}\ \mathrm{cm^3\ min^{-1}}. \qquad (9)$$

e) Infection constant. By the first Eqn (1) we have at the maximum ($\mathrm{d}N/\mathrm{d}t = 0$)

$$k = \alpha / n.$$

Then in the first two experimental situations we can compute the infection constants and compare them with those computed in the previous way

$$k_1 = \alpha_1 / n_1 = (6 \pm 1) \times 10^{-11}\ \mathrm{cm^3\ min^{-1}},$$
$$k_2 = \alpha_2 / n_2 = (7 \pm 1) \times 10^{-11}\ \mathrm{cm^3\ min^{-1}}.$$

Results are not far from (9). Then we can assume that the two constants have the same values with good approximation.

B) Comparison between experimental and theoretical results

We can have an estimation of the values of the constants with a best fit method by assuming the above values as starting values and by finding those which inserted in Eqn (1) minimize the parameter $\Sigma i \frac{|x_i - c_i|}{\mathrm{Max}(x_i, c_i)}$ where x_i and c_i are experimental and calculated values of the concentrations at the time t_i. The results are reported in Table II.

In the first experiment (system *E. coli* B-T4rII) theoretical results are in good agreement with experimental data when the parameters assume the values:

$$\lambda_1 = 70, \quad \tau_1 = 16\ \mathrm{min}, \quad K_1 = 5 \times 10^{-11}\ \mathrm{cm^3\ min^{-1}},$$
$$\alpha_1 = 0.016\ \mathrm{min^{-1}}$$

not far from those previously obtained. In Fig. 2 the theoretical previsions are compared with the experimental results. The disagreement is more evident in the last part of the curve representing phage population as a function of time. The experimental results seem to point out a delay of ~ 8 minutes in the lysis with respect to the mathematical model prediction.

A similar comparison has been made for the second system (*E. coli* B/5-T2r+). As it is shown in Table II and in Fig. 3 the experimental data differ from the theoretical prediction in a much more appreciable way than in the previous case, especially for high values of $n(t)$. Furthermore experimental results point out a delay in the latent period when the multiplicity of infection grows up. We had to expect a result of this kind because the mathematical model doesn't account for lysis inhibition. The good agreement we have in the first period of infection, when this phenomena doesn't take place, is however a positive fact, because it points out that the model differs from experimental results only when there are present those phenomena which are not explicitly accounted for by the model. In the last two systems (*E. coli* CC17-ϕX174 and *E. coli* M72-$\lambda N_9 Ai_8$) as indicated in Table II and in Figs 4, 5, the agreement is not so good, probably because in the actual situation, high multiplicity of infection and single burst, the lysis inhibition and indetermination in lysis time become important.

Table II. Comparison between experimental data and best fit values of the parameters.

System		α [min^{-1}]	λ	τ [min]	K [cm^3 min^{-1}]
E. coli B-T4rII	exp.	0.015	75	16	5×10^{-11}
	comp.	0.016	70	16	5×10^{-11}
E. coli B/5-T2r+	exp.	0.014	180	23	3×10^{-11}
	comp.	0.015	175	24	3×10^{-11}
E. coli CC17-ϕX174	exp.	0.015	150	24	8×10^{-10}
	comp.	0.016	140	18	8×10^{-10}
E. coli M72-$\lambda N_9 Ai_8$	exp.	0.014	100	65	5×10^{-10}
	comp.	0.015	95	68	3×10^{-10}

exp.: obtained from experimental curves.
comp.: calculated by use of the mathematical model.

From these observations comes out that the model hasn't only a foretelling validity but it can even be a useful tool of research to point out anomalies of behaviour of particular systems.

C) Phage yield

Our model easily allows to obtain information concerning the yield of phage production with given initial conditions. In the following calculations we took into account the experimental result that bacterial lysis stops when bacterial metabolism is halted in the saturation phase. We imposed that $n(t)$, $N(t)$, $N_f(t)$ will remain constant when the bacterial concentration reach sufficient high values by exhaustion of nutrition or for other chemico-physical reasons to prevent growth. Such value, depending from experimental conditions, has been taken equal to 10^9 bacteria/cm^3.

Curves of Fig. 6 show the log of the yield $\left(\log \frac{n_f\, i\, n}{n_0}\right)$ as a function of the initial bacterial concentration with different initial phage concentration for a peculiar choice of the constants α, K, λ, τ.

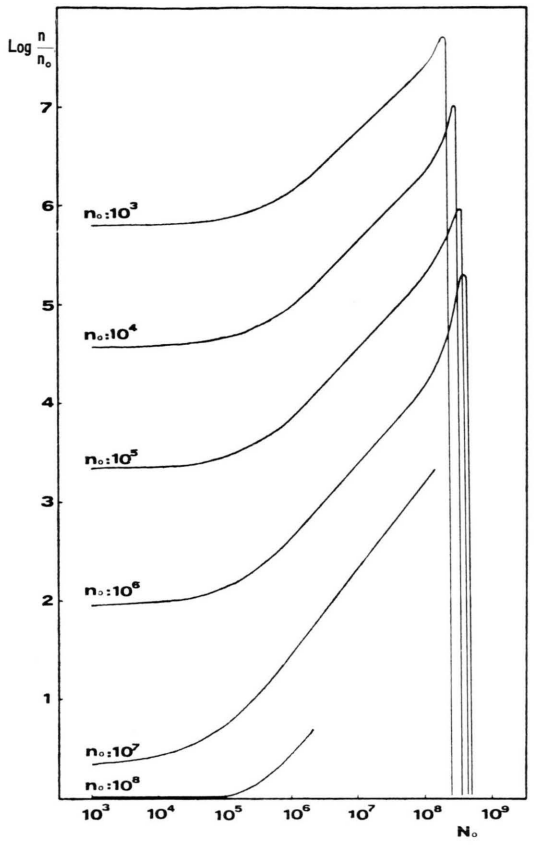

Fig. 6. Phage yield from various initial concentration of phages and bacteria.

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