The Survival of Bacteria under Starvation Conditions: a Mathematical Expression of Microbial Death

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Microbial death was studied under starvation conditions. The kinetics of cell death were described by $dN/dt = \kappa N^{\alpha+1}$, where N is the number of viable cells at time t , and κ and α are constants concerning growth or death of bacteria. Death in the decline phase of the culture also fitted the above equation, except for irregular oscillations with relatively short periods. This suggested that bacterial death in the decline phase was mainly caused by the same factor(s) as death under starvation conditions.

INTRODUCTION

Most textbooks of microbiology state that the kinetics of death of bacterial populations is exponential (Stanier *et al.*, 1976; Salle, 1973; Rahn, 1945), but other authors present several examples of non-exponential survival curves (Yanagita, 1981; Wilson & Miles, 1946; Moats, 1971). Fluctuations in the number of viable cells in the decline phase were suggested to result from a sudden growth of mutants resistant to the adverse conditions, at the expense of nutrient released from lysed cells. The reason for the gradual decrease of death rate of the population was the same (Yanagita, 1981). Rahn (1930) pointed out the non-exponential death of bacteria: the death rate decreased and the survival curve lagged below the expected straight line in semi-logarithmic plots. However, Rahn (1945) rejected this view. Moats (1971) proposed a mathematical method of calculating the kinetics of thermal death of bacteria which was based on the 'multiple-target theory'. Peled *et al.* (1977) presented an improved theory and applied it to describe the kinetics of death of starved yeast cells.

In the present study the death of bacteria under starvation conditions, and in the decline phase in batch culture, was investigated; an equation is proposed which fits the experimental data.

METHODS

Bacterial strains. *Escherichia coli* IFM 3293, *Serratia marcescens* IFM 3248, *Salmonella typhimurium*, *Proteus morganii* IFM 3201 and *Staphylococcus aureus* 209p were used.

Growth and starvation conditions. All organisms were preincubated in nutrient broth (3.0 g meat extract, 10.0 g peptone and 5.0 g NaCl per litre of distilled water, pH 7.0) for 12 h at 35 °C, then cultured at 35 °C in nutrient broth in 250 ml culture bottles for 24 h on a reciprocal shaker at 120 r.p.m. The bacteria were harvested by centrifuging at 2500 *g*; after being washed three times in 0.07 M-phosphate buffer (pH 7.0), they were resuspended in the same buffer to a final density of 2×10^8 to 2×10^9 bacteria ml⁻¹ and incubated under the conditions used for growth. We regarded this incubation as starvation (Druilhet & Sobek, 1975; Boylen & Mulks, 1977; Thomas & Batt, 1968).

Counting method. Viability was assessed by plate counts of bacteria diluted in 0.85% NaCl solution. The counts were made after incubation at 25 °C for 48 h.

RESULTS

The multiplication rate of bacteria at a given time was assumed to be proportional to the number of cells present at that time, as represented by the equation

$$dN/dt = \kappa N \quad (1)$$

where N is the number of cells at time t , and κ is the growth constant. The solution of the equation (1) at the initial condition, $N(t_0) = N_0$, is

$$N = N_0 e^{\kappa(t-t_0)} \quad (2)$$

Equations (1) and (2) represent the exponential growth phase of bacteria when $\kappa > 0$ and the stationary phase when $\kappa = 0$. Although, according to many textbooks, equations (1) and (2) represent exponential death when $\kappa < 0$, in practice the process of bacterial death is not always exponential (Yanagita, 1981). In equation (1) κ is constant and independent of N . However, the value of κ should depend on the value of N at a given time; that is, κ is a function of N , $\kappa = \kappa(N)$. So from equation (1), the equation

$$dN/dt = \kappa(N)N \quad (3)$$

may be obtained.

When $\kappa(N) = \kappa - \lambda N$ ($\kappa > 0$, $\lambda > 0$), the solution of equation (3) at the initial condition, $N(t_0) = N_0$, is

$$N(t) = \frac{\kappa N_0 e^{\kappa t}}{\lambda N_0 e^{\kappa t} + (\kappa - \lambda N_0) e^{\kappa t_0}}$$

This is Verhulst-Pearl's logistic equation which fits the growth of bacteria, yeast, *Paramecium* and *Drosophila* well (Pielou, 1969).

To describe the death of bacteria in batch culture, we propose that

$$\kappa(N) = \kappa N^\alpha \quad (4)$$

where κ and α are constants. This hypothesis is one of the simplest forms of the function $\kappa(N)$, in which the death rate decreases gradually with N in the case of $\kappa < 0$ and $\alpha > 0$. From equations (3) and (4), the following equation was obtained:

$$dN/dt = \kappa N^\alpha N = \kappa N^{\alpha+1} \quad (5)$$

This equation could be used to predict the kinetics of death under starvation conditions and in the decline phase. Its solution at the initial condition, $N(t_0) = N_0$, is

$$N(t) = \frac{N_0}{(1 - \alpha \kappa (t - t_0) N_0^\alpha)^{1/\alpha}} \quad (6)$$

The constants κ and α must be determined in order to apply equations (5) and (6) to the experimental data. From equation (4), putting $t = t_0$, the death rate at time t_0 is

$$-\kappa(N_0) = -\kappa N_0^\alpha$$

This equation is approximately equal to the mean value of the death rates in the short interval between two points before and after time t_0 ; as a result,

$$d = -\kappa N_0^\alpha$$

is obtained, where d is the mean value of the death rates. The values of N were determined by plate count, the value of d was determined at two intervals before and after time t_0 , and the value κ can be determined if α is known. The value of α is not easily obtained since it seems to vary with the species of bacteria and culture conditions. The most suitable value of α for the experimental data was determined by the method of least-squares.

The values of the parameters for equation (5) under starvation conditions, and the correlation coefficients between logarithms of data and the corresponding calculated values, are shown in Table 1. The values of α were within a relatively narrow range (0.17–0.25), and may have an unknown biological meaning. Correlation coefficients were close to 1, and the theoretical survival curves calculated from equations (5) and (6) fitted the plate count data well (Fig. 1a).

Fig. 1. Survival of *E. coli* starved in phosphate buffer (a), and the decline phase of *Staphylococcus aureus* in nutrient broth (b). —, Curve calculated from equation (6); ----, curve calculated from Moats' theory; O, experimental values.

Table 1. Values of the parameters for equation (5) under starvation condition and correlation coefficients (C) between experimental data and theoretical values

Bacterium	α	d	N_0^*	$\kappa^\dagger$	C
<i>Escherichia coli</i>	0.22	0.022	2.3×10^9	-2.0×10^{-4}	0.990
<i>Serratia marcescens</i>	0.17	0.038	6.2×10^8	-1.2×10^{-3}	0.998
<i>Salmonella typhimurium</i>	0.22	0.020	2.9×10^9	-2.1×10^{-4}	0.991
<i>Proteus morganii</i>	0.25	0.071	8.1×10^8	-4.2×10^{-4}	0.990

* Initial number of bacteria at time t_0 .

$\dagger \kappa = -d/N_0^*$.

Table 2. Values of the parameters for equation (5) in the decline phase and correlation coefficients (C) between experimental data and theoretical values

Bacterium	α	d	N_0^*	$\kappa^\dagger$	C
<i>Escherichia coli</i>	0.26	0.022	3.5×10^9	-7.3×10^{-5}	0.970
<i>Serratia marcescens</i>	0.22	0.061	2.0×10^9	-5.5×10^{-4}	0.973
<i>Staphylococcus aureus</i>	0.25	0.024	6.5×10^8	-1.5×10^{-5}	0.956
<i>Staphylococcus aureus</i> ‡	0.29	0.053	8.0×10^9	-7.1×10^{-5}	0.971
<i>Salmonella typhimurium</i> §	0.20	0.024	2.3×10^9	-3.2×10^{-4}	0.961

* Initial number of bacteria at time t_0 .

$\dagger \kappa = -d/N_0^*$.

‡ Incubated at 27.5 °C.

§ Data from Wilson & Miles (1946).

Fig. 1 (b) shows the number of surviving cells in the decline phase and the theoretical survival curves calculated from equations (5) and (6). The experimental data fitted the theoretical survival curves, although the fit was better under starvation conditions than during the decline phase. The irregular oscillations with relatively short periods, which were observed in the decline phase of all strains, did not occur under starvation conditions. The values of the parameters for equation (5) in the decline phase and the correlation coefficients are shown in Table 2; the values of α in the decline phase of each strain are similar to those under starvation conditions (Table 1). The correlation coefficients, however, were lower than those under starvation conditions.

DISCUSSION

The differential equation (5) and its solution (6) quantitatively predicted the survival under starvation conditions during the period 600–1200 h. Moats (1971) proposed the multiple-target theory of thermal death of bacteria and inferred a series of equations on the fraction of survivors. Moats' main equation was

$$(d/\sigma)^2 = N(e^{-2\kappa t} - 2e^{-(\kappa t + \kappa t_{50})} + e^{-2\kappa t_{50}})/(e^{-\kappa t} - e^{-2\kappa t})$$

where d/σ is the ratio of deviation from the mean to standard deviation (obtained from a table of the normal probability integral) for the fraction of survivors at time t ; t_{50} is the time at which the fraction of survivors is 0.5; N is the number of critical sites in each cell and κ is the rate constant of inactivation of critical sites. Peled *et al.* (1977) stated that the kinetics of death under starvation conditions are similar to those of heat-treated cells, and proposed an improved equation. Fig. 1 (*a*) indicates the numbers of survivors of *E. coli* under starvation conditions, and shows that the theoretical curve obtained from Moats' equation fits the experimental data from the initiation of starvation well until about 400 h, after which the difference between them increases rapidly. Similarly in Fig. 1 (*b*), which shows the number of survivors of *Staphylococcus aureus* in the decline phase, the difference between the two curves becomes significant after 200 h. The calculated survival curves obtained from equation (6) fit the experimental results better than those obtained from Moats' equation (Fig. 1).

The parameter α may contain some unknown factors, for example, temperature, starvation condition, population heterogeneity (Rahn, 1930), and change of the components of the starvation medium by metabolic products or autolysis of cells (Yanagita, 1981). The value α will be explained by further investigations involving other species of bacteria and conditions of starvation.

We hypothesize that the main factor causing bacterial death in the decline phase is the same as that which causes death under starvation conditions, since the shapes of the survival curves in the two conditions are similar. The oscillations with relatively short periods during the decline phase suggest that a fraction of the population, perhaps comprised of mutants which are resistant to the adverse conditions, grows suddenly during the death process.

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