MICROBIAL GROWTH

Restricted (due to exhaustion of nutrients, lessened availability of oxygen, accumulation of toxic byproducts of metabolism, etc) vs. unrestricted.

Counting bacteria:

Viable count (dilute the sample, spread a measured aliquot on an agar plate, incubate the petri dish, count colony forming units).

Total count (using a chamber or a particle counter), measures both alive and dead bacteria.

Unrestricted bacterial proliferation mimics an autocatalytic first-order chemical reaction, and it can be described by:

\[
dN/dt = kN \quad (1)
\]

where \( N \) is the concentration of cells, \( t \) the time, and \( k \) a constant of proportionality, called the specific growth constant.

So, \( (1) \) states that the change in the number of cells over time depends on how many cells you start with and on an intrinsic property of the organisms: how fast they can grow and multiply in a given medium. The rate of change (and not the actual number of cells at any given time) depends solely on \( k \).

The dimensions of the specific growth rate are reciprocal time (h\(^{-1}\)). Also, the number of cells \( (N) \) is always expressed in cells/ml.

From \( (1) \),

\[
N = N_0 e^{-kt} \quad (2)
\]

For two time points \( (t_1 \text{ and } t_2) \), equation \( (2) \) becomes,

\[
\ln(N_2/N_1) = k(t_2-t_1) \quad (3), \text{ or}
\]
\[ \log N_2 - \log N_1 = k(t_2 - t_1)/2.303 \quad (4) \]

Equations (3) & (4) describe a straight line. Cultures that obey equation (3) or (4) are said to be growing exponentially.

The doubling time \( t_d \) is the time it takes for \( N_1 \) to become \( 2N_1 \) (i.e. \( N_2 = 2N_1 \)).
At each division, one cell becomes 2, the 2 become 4, etc. The series 2, 4, 8, 16, ..., \( 2^g \) describes the increase in cell numbers.
So, \( g \) is the number of generations. Then, for two time points \( t_1 \) and \( t_2 \),

\[ \frac{N_2}{N_1} = 2^g \quad \text{(or } N = N_0 2^g) \quad (5) \]

Equation (5) works in the same way as the previous ones. From (5),

\[ \ln \left( \frac{N_2}{N_1} \right) = g \ln 2 \quad (6) \]

Now we see that (6) and (3) describe the same thing, thus

\[ g \ln 2 = k(t_2 - t_1) \quad (7) \], and

\[ k = g \ln 2 / (t_2 - t_1) \quad (8) \]

Equation (8) says that the specific growth rate is a function of the number of times the average cells will divide in a given period of time.

- Draw growth curves to illustrate lag, exponential and stationary phases.
- The advantages of working in exponential phase.
- Draw curves for total count and live count.
PROBLEMS

1) Suppose we inoculate 100 ml of broth with 1,000 actively dividing cells each of a bacterial strain A and strain B. A few hours later we observe $5 \times 10^5$ per ml of strain A and $5 \times 10^3$ per ml of strain B. What are the relative growth rates of the two strains?

If we use equation (3), then

For strain A we have: $\ln(5 \times 10^5/10) = k_A(t_2-t_1)$, and (1-1)

For strain B we have: $\ln(5 \times 10^3/10) = k_B(t_2-t_1)$ (1-2)

If we call $k_A = x k_B$, then (1-2) becomes:

$\ln(5 \times 10^3/10) = k_A(t_2-t_1)/x$, or

$\ln(5 \times 10^3/10) x = k_A(t_2-t_1)$ (1-3)

From (1-1) and (1-3),

$\ln(5 \times 10^5/10) = \ln(5 \times 10^3/10) x$, or

$x = \ln(5 \times 10^5/10)/ \ln(5 \times 10^3/10)$, or

$x = 10.82/6.21 = 1.75$

So, strain A growth rate is 1.75 that of strain B.

2) How long will it take for a single bacterium that divides once every 20 min, to give rise to a population of 6 billion?

Again, we need to use equation (3). Before doing so, the relationship between $t_d$ (doubling or generation time) and $k$ (the specific growth constant) has to be established (again using (3), and substituting $N_2 = 2N_1$):

$\ln(N_2/N_1) = k(t_2-t_1)$, or

$\ln(2N_1/N_1) = k t_d$, or

$t_d = \ln 2/k$, or $k = \ln 2/t_d$.

Then, we have:

$\ln(6 \times 10^9/1) = (\ln 2/0.333h) t_x$, or

$22.51 = (0.693/0.333h) t_x$, or

$t_x = 22.51/2.10 = 10.7h$

So, bacteria divide very fast!!
3) How long will it take for an animal that gives birth once every year (to one baby), to give rise to a population of 6 billion? (Assume that the baby reaches reproductive maturity immediately, so within a year it will give birth to a new baby).

As in Problem 2,
\[\ln\left(\frac{6 \times 10^9}{1}\right) = \left(\frac{\ln 2}{8,760}\right) t_x,\]
\[22.51 = \left[\frac{0.693}{8,760}\right] t_x,\]
\[t_x = \frac{22.51}{0.0000791} = 284,541.99 \text{ hr} = 32.48 \text{ years}\]

This is 26,567 times longer than the time it will take the bacterium to reach the same population size (which is exactly the ratio of their doubling times/specific growth rates). Then why in problem 1 we didn’t get 0.01, but instead we got 0.57? (the above statement applies only if we start with one individual).

4) Same as problem 3, but assume the animal divides once every 100 years.
Answer: \(2.84 \times 10^7\) hr, or 3,240 yr.
**Mutation rate**: the probability with which a particular mutational event takes place per biological entity (virus, cell, individual) per generation.

So, the frequency of mutants and mutation rate, are a function of cell proliferation parameters.

Some equations, assuming that:

1) A given mutation rate is constant.
2) The growth rates of mutant and wild type are the same.
3) The rate of back mutations is insignificant.
4) The frequency of mutants is low (i.e. there are a lot more wild type individuals than mutants).

The Poisson equation describes the probability distribution of random events -- for example, the probability that random mutations in a cell will affect ("hit") a particular gene ("target").

\[ P_x = \frac{h^x e^{-h}}{x!} \]

where:

- \( P_x \) = probability that a target will have exactly \( x \) hits
- \( h \) = average number of hits per target

The simplest way of determining \( P_x \) is to determine the frequency of zero events and plug this value into the Poisson equation:

\[ P_0 = \frac{h^0 e^{-h}}{0!} = \frac{1 \cdot e^{-h}}{1} = e^{-h} \]

\[ \therefore h = -\ln P_0 \]

The mutation rate is the number of mutations per cell division. Because the cell population is so large, the number of cell divisions may be approximately equal to the number of cells in the population (N).
This approach is only OK if we start with very few cells and examine the results from very large cultures.

If one samples a culture at any given time, it will include not only the new mutants that arose in the last generation, but the ones from the previous generation AND THEIR DESCENDANTS.

The number of new mutants at each generation EQUALS the number of mutants that descended from mutants that arose in the previous generation!!!

If we include the number of generations (g) that have elapsed, and if we assume that every generation contributes equally to the total number of mutants, the equation above becomes

\[ a = \frac{h}{N} / g N_g \]
PROBLEMS

1) To determine the frequency of Str^R mutants a fluctuation test was done with 50 tubes each containing 10^8 cells and 42 of the tubes contained no mutants. Use the Luria-Delbruck calculation to determine the mutation rate to Str^R.

Answer:

First calculate the average number of hits per cell
\[ h = -\ln \left( \frac{42}{50} \right) = -\ln(0.84) = 0.17 \]

Then divide the average number of hits per cell by the number of cells in the population
\[ a = \frac{h}{N} = \frac{0.17}{10^8} = 1.7 \times 10^{-9} \]

2) Liquid bacterial growth medium in a sterile tube was inoculated with actively multiplying bacteria. Call the time of inoculation time – zero. The culture was assayed for the number of bacteria each hour thereafter. The data are tabulated below:

<table>
<thead>
<tr>
<th>Time</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.0x10^5</td>
</tr>
<tr>
<td>1</td>
<td>8.0x10^5</td>
</tr>
<tr>
<td>2</td>
<td>2.0x10^6</td>
</tr>
<tr>
<td>3</td>
<td>5.3x10^6</td>
</tr>
<tr>
<td>4</td>
<td>1.4x10^7</td>
</tr>
<tr>
<td>5</td>
<td>3.6x10^7</td>
</tr>
<tr>
<td>6</td>
<td>9.4x10^7</td>
</tr>
<tr>
<td>7</td>
<td>2.5x10^8</td>
</tr>
</tbody>
</table>

a) Plot the log of the number of bacteria versus time.
b) What is the doubling time of the culture?
c) Suppose there were NO mutants (of a specific type) in the starting inoculum (time zero). When the number of bacteria reached 1.2x10^6 the frequency of mutants was measured and found to be 10^-4. What is the value of the mutation rate?
d) How many mutant cells would you expect to find when the number of cells reached 2.4x10^6?
a) 

\[ y = 0.4165x + 5.4772 \quad R^2 = 1 \]

b) From a) we got the slope = 0.4172. To get \( k \), however, one needs to convert from log to ln. So, \( k = 0.4172 \times 2.303 = 0.96 \)

Since \( \ln 2 = kt_d \),
then \( t_d = 0.693/0.96 = 0.72 \text{ h} \)

c) One can use \( h/N_g = \alpha g \) and solve for \( \alpha \).

The frequency of mutants \([h/N_g]\) is \( 10^{-4} \) however, we need to calculate \( g \).

Then, from \( \ln(N_2/N_1) = g \ln 2 \), and for the two time points when the cultures were at \( 3.0 \times 10^5 \) (\( N_1 \)) and when they reached \( 1.2 \times 10^6 \) (\( N_2 \)), we get:

\[ \ln(1.2 \times 10^6 / 3.0 \times 10^5) = g \cdot 0.693, \text{ or} \]
\[ g = 1.3863 / 0.693 = 2 \]

So, \( \alpha = 10^{-4}/2 = 5 \times 10^{-5} \)
d) If we use \( h = g \alpha N_g \) and solve for \( h \), and realizing that \( g \) increased by just 1 (from 2 to 3), we have:

\[
\text{number of mutants} = 3.5 \times 10^{-5} \times 2.4 \times 10^6 = 360.
\]