Department of biology

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((Microbial Physiology))

Stage (-3-)

LEC- ((5))

Stages of Microbial growth

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Microbial growth defined as an increase in cellular constituents resulting an increase in a microorganism size, population number or both.

Bacteria reproduce asexually by means of simple transverse binary fission. Their numbers (n) increase logarithmically (n = 2G). The time required for a reproduction cycle (G) is called the generation time (g) and can vary greatly from species to species.

Fast-growing bacteria cultivated in vitro have a generation time of 15–30 minutes. The same bacteria may take hours to reproduce in vivo. Obligate anaerobes grow much more slowly than aerobes; this is true in vitro as well. Tuberculosis bacteria have an in-vitro generation time of 12–24 hours. Of course the generation time also depends on the nutrient content of the medium.

The so-called normal growth curve for bacteria is obtained by inoculating a nutrient broth with bacteria the metabolism of which is initially quiescent, counting them at intervals and entering the results in a semi log coordinate system (Figure. 1) .

The lag phase (A) is characterized by an increase in bacterial mass per unit of volume, but no increase in cell count. During this phase, the metabolism of the bacteria adapts to the conditions of the nutrient medium.

In the following log (or exponential) phase (C), the cell count increases logarithmically up to about 109/ml. This is followed by growth deceleration and transition to the stationary phase (E) due to exhaustion of the nutrients and the increasing concentration of toxic metabolites. Finally, death phase (F) processes begin.

The generation time can only be determined during phase C, either graphically or by determining the cell count (n) at two different times and applying the formula:

$$g = \frac{t_2 - t_1}{\log_2 n_2 - \log_2 n_1}.$$

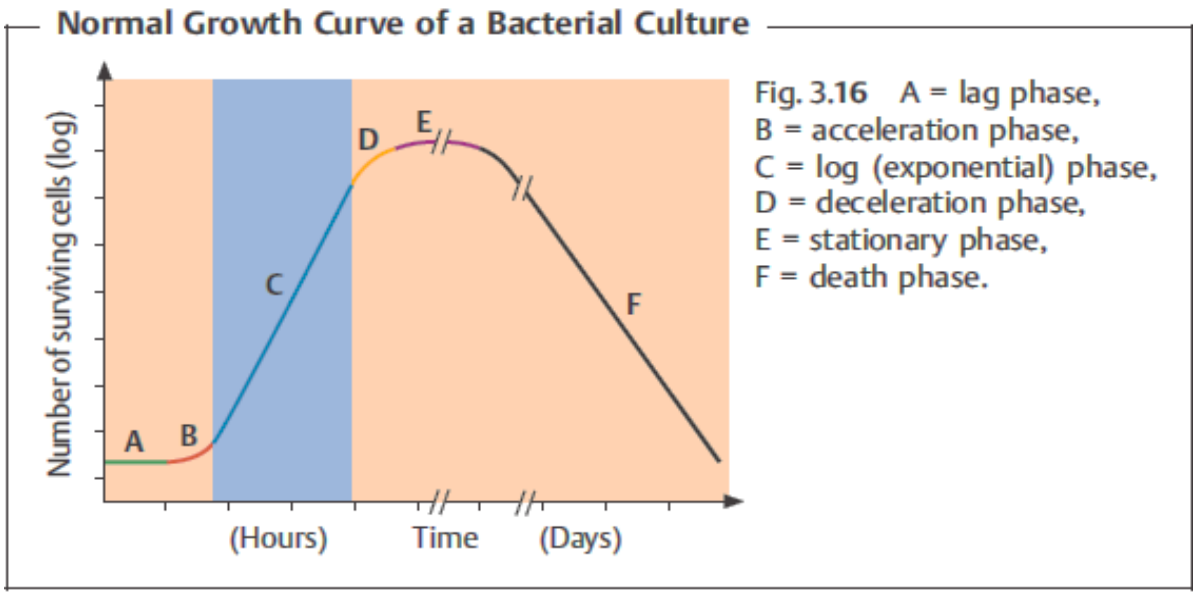

Figure (1) : Phases of the bacterial growth

Methods of measuring microbial growth

Methods of Measuring Microbial Growth : There are different methods of counting microbial growth. These are based on different parameters of cells such as dry-weight and wet-weight measurement, absorbance, cell plate, density, turbidity, ATP measurement, viable count, ATPase activity and use of Coulter counter.

1. Wet Weight Measurement: Measuring cell mass is an easy step of cell growth measurement. A known volume of culture sample from the ferment or is withdrawn and centrifuged , Wet weight of pellets is measured by using pre-weighed filter paper. A pre-weighed filter paper of similar size is used to subtract the weight of wet filter paper. Thus wet-weight of cells is calculated.

2. Dry Weight Measurement: Dry weight measurement of cell material is similar to that of wet weight. Here dry weight of pre-weighed filter paper containing pellets of microbial cells is measured . Dry weight of filter paper is nullified by subtracting the dry weight of only filter paper of similar size. Thus dry weight of microbial cells can be obtained . For example dry weight of about one million cells of *E. coli* is equal to 150 mg. Dry weight of bacterial cells is usually 10-20% of then- wet weight.

3. Absorbance: Absorbance is measured by using a spectrophotometer. Scattering of light increases with increase in cell number. When light is passed through bacterial cell suspension, light is scattered by the cells. Therefore, transmission of light declines. At a particular wavelength absorbance of light is proportional to the cell concentration of bacteria present in the suspension. Thus cell growth of any bacterial suspension at a particular wavelength at different intervals can be measured in terms of absorbance and a standard graph (between absorbance and cell concentration) can be prepared.

4. Cell Count (Manual): Cell growth is also measured by counting total cell number of the microbes present in that sample. Total cells (both live and dead) of liquid sample are counted by using a special microscope glass slide called Petroff-Hausser Counting Chamber. In this chamber a grid is marked on the surface of the glass slide with squares of known area . The whole grid has 25 large squares, a total area of 1 mm2 and a total volume of 0.02 mm3 (1/50 mm). All cells are counted in large square and total number per ml sample is measured. If 1 square contains 12 cells, the total number of cells per ml sample will be: 12 cells x 25 square x

50x103 = 1.5xl07 cells. If there is dilute culture, direct cell counting can be done. However, the cell culture of high density can be diluted. Otherwise clumps of cells would be formed which would create problem in exact counting of bacterial cells.

5. Viable Count: A viable cell is defined as a cell which is able to divide and increase cell numbers. The normal way to perform a viable count is to determine the number of cells in the sample which is capable of forming colonies on a suitable medium. it is assumed that each viable cell will form one colony. Therefore, viable count is often called plate count or colony count. There are two ways of forming plate count.

6. Spread Count Method: A volume of culture (0.1 ml) is spread over the surface of an agar plate by using a sterile glass spreader. The plate is incubated to develop colonies. Then the number of colonies is counted .

Method	Application	Comments
Direct microscopic count	Enumeration of bacteria in milk or cellular vaccines	Cannot distinguish living from nonliving cells
Viable cell count (colony counts)	Enumeration of bacteria in milk, foods, soil, water, laboratory cultures, etc.	Very sensitive if plating conditions are optimal
Turbidity measurement	Estimations of large numbers of bacteria in clear liquid media and broths	Fast and nondestructive, but cannot detect cell densities less than 10^7 cells per ml

Some methods used to measure bacterial growth