PHYSIOLOGY OF BACTERIA (BACTERIAL GROWTH AND NUTRITION)

Generation Time

Time required for a bacterium to give rise to two daughter cells under optimum conditions.

• For *Escherichia coli* and most of the other pathogenic bacteria, -it is 20 minutes;

• For *Mycobacterium tuberculosis*: It is 14 hours

• For *Mycobacterium leprae*: It is 12–13 days

Bacterial Count

• **Total count:**

Indicates the total number of bacteria (live or dead) in the specimen. This is done by counting the bacteria under the microscope using a counting chamber.

• **Viable count:**

Measures the number of living (viable) cells in the given specimen. A viable count may be obtained by:

- **Dilution method:**
  In **dilution method**, several tubes with liquid culture media are incubated with varying dilutions of sample and the viable count is calculated from the number of tubes showing bacterial growth. This method is widely used in microbiological testing of water for presumptive coliform count in drinking water.
- **Pour plate method (best method):**
  - In the **plate method**, a sample is diluted and small volume of it is spread on the surface of an agar plate. The number of colonies that grow after a suitable incubation time indicates
- Surface viable count by spreading method
- Surface viable count by Miles and Misra method.

Bacterial Growth Curve:

When a broth culture is inoculated with a small bacterial inoculum, the population size of the bacteria increases showing a classical pattern. The bacterial growth curve shows the following four distinct phases:

1. Lag phase: After a liquid culture broth is inoculated, the multiplication of bacteria does not start immediately. It takes some time to multiply. The time between inoculation and beginning of

multiplication is known as lag phase. In this phase, the inoculated bacteria become acclimatized to the environment, switch on various enzymes, and adjust to the environmental temperature and atmospheric conditions.

During this phase, there is an increase in size of bacteria but no appreciable increase in number of bacterial cells. The cells are active metabolically. The duration of the lag phase varies with the bacterial species, nature of culture medium, incubation temperature, etc. It may vary from 1 hour to several days.

2. Log phase: This phase is characterized by rapid exponential cell growth (i.e., 1 to 2 to 4 to 8 and so on). The bacterial population doubles during every generation. They multiply at their maximum rate. The bacterial cells are small and uniformly stained. The microbes are sensitive to adverse conditions, such as antibiotics and other antimicrobial agents.

3. Stationary phase: After log phase, the bacterial growth almost stops completely due to lack of essential nutrients, lack of water oxygen, change in pH of the medium, etc. and accumulation of their own toxic metabolic wastes. Death rate of bacteria exceeds the rate of replication of bacteria. Endospores start forming during this stage. Bacteria become Gram variable and show irregular staining. Many bacteria start producing exotoxins.

4. Decline phase: During this phase, the bacterial population declines due to death of cells. The decline phase starts due to

(*a*) accumulation of toxic products and autolytic enzymes and

(*b*) exhaustion of nutrients. Involution forms are common in this stage.

The ***continuous culture*** is a method of culture useful for industrial and research purpose. This is achieved by using a special device for replenishing nutrients and removing bacterial population continuously so that bacteria growth is not inhibited due to lack of nutrients or due to accumulation of toxic bacterial metabolites.

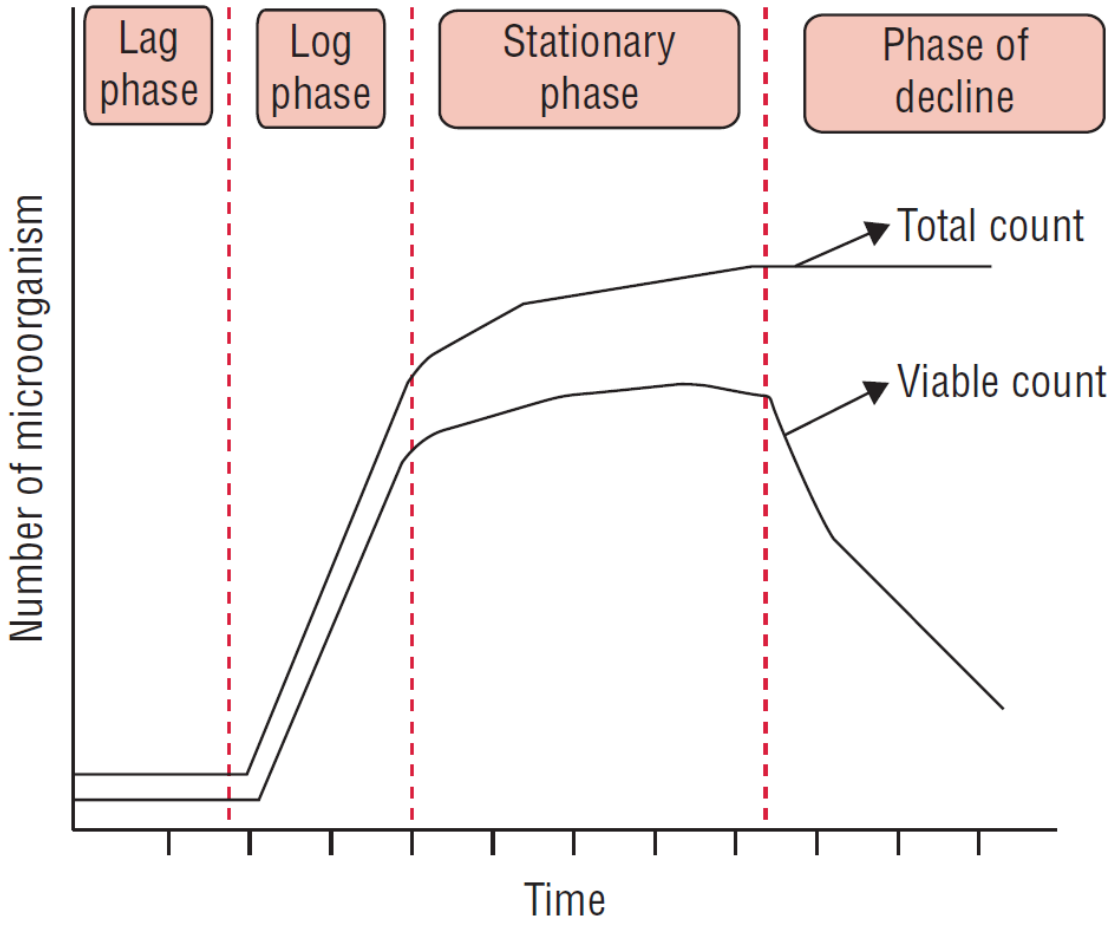

Fig: Bacterial Growth Curve

Factors Affecting Growth of Bacteria

1. Oxygen:

On basis of their oxygen requirements bacteria are classified as:

• **Obligate aerobes:** Grow only in the presence of oxygen (e.g. *Pseudomonas, M. tuberculosis, Bacillus, Brucella,* and *Nocardia)*

• **Facultative anaerobes:** They are aerobes that can also grow anaerobically (e.g. most of the pathogenic bacteria, e.g. *E.coli, S.aureus,* etc).

• **Facultative aerobes:** They are anaerobes that can also grow aerobically (e.g. *Lactobacillus*)

• **Microaerophilic bacteria:** Can grow in the presence of 5–10% of oxygen (e.g. *Campylobacter, Helicobacter, Mycobacterium bovis*).

• **Obligate anaerobes:** Grow only in the absence of oxygen. Oxygen is lethal to these bacteria (e.g. *Clostridium)*
• **Aerotolerant anaerobe:** They can tolerate oxygen for some time, but do not use it (*Clostridium histolyticum).*

2. Carbon dioxide: Capnophilic bacteria need 5–10% of CO2. E.g.*Brucella abortus, Streptococcus pneumonia,* etc.

3. Temperature: Based on optimal temperature needed for growth, bacteria can be grouped into:

• **Psychrophiles:** Grow below 20°C, e.g. saprophytes.
• **Mesophiles:** Grow between 25°C and 40°C, e.g. most of the pathogenic bacteria
• **Thermophiles:** Grow above of 55°C - 80°*C,* e.g. *Bacillus stearothermophilus*

4. pH: Most pathogenic bacteria grow between pH 7.2 - pH 7.6**.** Very few bacteria (lactobacilli) can grow at acidic pH below pH 4, while bacteria such as *Vibrio cholerae* are capable of growing at alkaline pH (8.2–8.9).

5. Light: Bacteria (except phototrophs) grow well in dark. They are sensitive to ultraviolet rays and other radiations in light. Photochromogenic mycobacteria produce pigments only on exposure to light.

6. Osmotic pressure: Microbes obtain almost all their nutrients in solution from surrounding water. Hence factors such as osmotic pressure and salt concentration of the solution affect the growth of bacteria. Bacteria by virtue of mechanical strength of their cell wall are able to withstand a wide range of external osmotic variations. Organisms requiring high osmotic pressures are called osmophilic bacteria. Sudden exposure of bacteria to hypertonic solution may cause osmotic withdrawal of water, leading to osmotic
shrinkage of the protoplasm (***plasmolysis***). On the other hand, sudden transfer of bacteria from concentrated solution to distilled water may cause excessive imbibition of water leading to swelling and bursting of cell (***plasmoptysis***).

7. Other factors: Mechanical and sonic Stresses, Moisture, and desiccation.