**Bacterial Growth and Nutrition**

 **T**he most common mean of bacterial reproduction is binary fission and its mathematical representation is 1→21→22→23→24………..2n. Few bacterial species reproduce by budding or by fragmentation.

**Bacterial Growth Curve:**

If a single bacterium is inoculated into a flask of liquid culture medium and incubated bacterium will undergo binary fission and period of rapid growth will ensure. If we use theoretical number of bacteria which should be present at various interval of time and plot data as number of bacteria versus time, growth curve will be obtained.

**Phases of growth curve:**

**Lag phase:**

Addition of inoculum to a medium is not followed immediately by doubling of population instead population remain unchanged. In broth some cells actually die from shock of transfer or inability to adapt to new environment. Cells are physiologically active they increase in size the organism are metabolizing but there is lack in cell division. It can last for 1 hour or several days. At the end of lag phase each organism divide.

**Logarithmic ∕Exponential ∕ Log phase:**

During this period cell divide steadily at constant rate, so result is a straight line in growth curve.

Generation time is the time required for a population to double. It can be determined from number of bacteria (n) that occurs in particular time interval (t).

$$g=t∕n$$

Note all bacteria have same generation time. For E-coli generation time is 15-20 minutes. And for certain other bacterial species it may be in hours. The generation time is useful in determining the amount of time after which the symptoms of disease appear in infected individual. Faster division times often means a shorter incubation period.

**Growth Rate:**

 Number of generation per hour is called growth rate. It is represented by “R”. It is reciprocal of generation time “g”.

$$R=n∕t$$

During log phase of growth micro-organism are particularly susceptible to adverse conditions, radiations and many anti-microbial drugs.

**Stationary Phase:**

The logarithmic phase of growth begins to tapper off after several hours in a gradual fashion represented by transition from a straight line through a curve to another straight line. Population remains constant because reproduction rate is balanced by equivalent death rate.

Reason of this phase is:

 1. Production of toxic products during growth.

2. Termination of some nutrients.

3. Variation in pH of the medium.

**Decline ∕Death Phase:**

 After stationary phase bacteria may die faster than new cells are produced. Reasons are:

1. Depletion of essential nutrients.
2. Accumulation of inhibitory products i.e. acids.

**Factors Affecting Bacterial Growth**:

**T**he growth rate of bacteria depends upon following factors:

1. **Bacterium species:**

Various bacterial species have different generation time. For E-coli generation time is 15-20 minutes and for some mycobacterium species generation time is 20 hours.

1. **Chemical composition of medium:**

 The growth of bacteria depends upon the chemical composition of the media. Medium that fulfills all nutritional requirement of bacteria provide best opportunity for growth.

**Example:** E-coli generation time in milk is 12.5 minutes and in broth media 17 minutes.

1. **Physical Requirements**

**Temperature:**

All processes of growth are dependent upon chemical reactions and rate of these reactions is influenced by temperature.

The minimum growth temperature is the lowest temperature at which the species will grow. The optimum growth temperature is the temperature at which the species grows best. The maximum growth temperature is the highest temperature at which growth is possible

**Classification of bacteria on the basis of temperature requirement:**

1. **Psychrophiles (cold loving microbes):**

They are able to grow at 0oC or lower though they best grow at higher temperature. Optimum temperature for these bacteria is 15oC or lower.

**Example:**  *Vibrio psychroerythrus*

Minimum temperature is -1oC

Optimum temperature is 15oC

Maximum temperature is 20oC.

**Psychrotrophs or Psychrotolerant;** Organisms of this type are much more common than psychrophiles and are the most likely to be encountered in low-temperature food spoilage because they grow fairly well at refrigerator temperatures. We will use the term psychrotrophs, which food microbiologists’ favor, for this group of spoilage. Psychrotrophs actually do not grow well at low temperatures, except in comparison with other organisms; given time, however, they are able to slowly degrade food.

**2. Mesophiles (Moderate temperature loving microbes):**

 They grow best with a temperature range of approximately 25-40oC.

**Example:** All pathogenic bacteria of human and warm blooded animals. They grow best at 37oC.

**3. Thermophiles (heat loving microbes):**

 They grow best at temperature above 45oC. They are present in hot springs can be contaminant in dairy products because they survive pasteurization temperature .They are little threat to human.

**Example:** For *Thermus aquaticus* optimum temperature is70-72oC.

1. **Hyper thermophiles:**

Some microbes’ members of Archae have growth temperature of 80oC or higher called hyper-thermophiles.

**Gaseous requirements for bacterial growth:** Principal gases that affect bacterial growth are oxygen and carbon dioxide.

Classification of bacteria on the basis of gaseous requirements:

1. **Aerobic bacteria:** These bacteria require oxygen for their growth and can grow well incubated in the air atmosphere i.e. 21% oxygen.

Toxic forms of oxygen are Superoxide radicals and peroxide radicals .

e.g. Pseudomonas species: in these bacteria, presence of enzymes super oxide dimustase and catalase allows toxic form of oxygen to be neutralized.

2) **Anaerobic bacteria:** These bacteria don’t use oxygen to obtain energy. Oxygen is toxic for them and they cannot grow when incubated in air atmosphere.

e.g. Clostridium species.

Anaerobic bacteria can be further divided into following types:

1. **Tolerant anaerobes***:* Can tolerate low levels of oxygen because they have super Oxide dimustase enzyme to neutralize toxic form of oxygen.
2. **Strict anaerobes***:* Cannot tolerate even low levels of oxygen.

3) **Microaerophilic bacteria:** they require low levels of oxygen for growth. Cannot tolerate the level of oxygen present in the atmosphere.

4) **Facultative anaerobes:** they have both aerobic and anaerobic growth. However, they show greater growth in presence of oxygen. e.g. E.coli. but when oxygen is absent they switch to anaerobic metabolism.

**Cultivation of aerobic and anaerobic bacteria**

**Aerobic bacteria:**we grow aerobic or facultative anaerobes in tubes or small flasks under normal atmospheric conditions, however, when to grow bacteria large quantity, increase in exposure of medium to atmosphere is required. It is increased by displaying medium in shallow layer or increase aeration by shaking the inoculated liquid culture.

**Anaerobic bacteria***:* Strict anaerobes can be grown by taking special precautions to exclude all atmospheric oxygen from medium by following methods:

* Use of reduced medium.
* During preparation, culture medium is boiled for several minutes to deprive of most of the dissolved oxygen.
* A reducing agent e.g. cystine is added in the medium to deprive off dissolved oxygen
* By using anaerobic chambers or jars.

 **The p H requirements for bacterial growth:**

Bacterial classification on the basis of p H requirement is given below:

**Acidophiles:** Acid tolerant bacteria are called acidophile. They grow best at p H below 5. They are valuable in food and dairy industry. The active culture in cup of yogurt are actually acidophile bacterial species.

**Neutrophiles**: Majority bacterial species are neutrophiles. They grow in p H range of 6.5-7.5. Most bacteria are neutrophiles.

**Alkilophiles:** They grow best at pH above 9 .

**Nutritional requirements for bacterial growth:**

All forms of life, from microorganisms to human being share certain nutritional requirements for their growth and normal functioning.

**Source of energy:** All organisms require a source of energy. Some depend upon chemical compounds for their energy and are termed as chemotrophs. e.g. *E.coli*. Other can utilize radiant energy and are called phototrophs. e.g. *Chromatium okenii*

**Source of electron:** All organisms require a source of electron for their metabolism. Some can use inorganic compounds as electron donor termed as lithotrophs. e.g. *Chromatium okenii*. Others use organic compounds as electron donors and are termed as organotrophs.

 **Carbon source**: Carbon is about half of the dry weight of bacterial cell. Carbon is required for synthesizing cell components. Some bacteria use carbon dixide as their major carbon source. Such organisams are termed as autotrophs. e.g. *Desulphovibrio desulphuricans.* Other organisms require organic compounds as their carbon source, they are termed as heterotrophs. e.g. E.coli.

**Nitrogen source:** Nitrogen makes 14% of dry weight of bacterial cell. All organisms require nitrogen to synthesize their cell components such as DNA and RNA. Forms of nitrogen used by bacteria are:

1. Atmospheric nitrogen
2. Inorganic nitrates and nitrites.
3. Organic compounds containing nitrogen.

**Oxygen source**: oxygen can be obtained from

1. atmospheric oxygen
2. water
3. Component atom of various nutrients.

**Sulphur source:** Sulphur is required for synthesis of sulphur containing amino acids. Bacteria can utilize:

1. organic sulphur
2. inorganic sulphur
3. elemental sulphur

**Phosphorous source:** Phosphorous is supplied in the form of phosphate. It is essential component of nucleic acids, phospholipids and teichoic acid.

**Metal ions**: All living organisms require certain metal ions like K+ , Ca+2, Mg+2 , Fe+2, Zn+2, Cu+2.

**Vitamins:** They function as co enzymes for several enzymes.

**Water:** In case of bacteria all nutrients must be in aqueous solution before they enter the cell. Water is also required as chemical reactant for many hydrolytic reactions to be carried out by cell.

To grow bacteria successfully, laboratory workers must provide proper kind of medium and appropriate set of physical conditions.

**Gowth Characteristics of Bacteria**

To determine the growth characteristics of bacterial strain, it is necessary to observe the features of colony and Broth culture.

**Colony Characteristics**

1. **Colony size**

Colony range in size from extremely pin point measuring only a fraction of mm in diameter to large colonies measuring 5 – 10 mm in diameter.

1. **Shape (Form) of colonies**

Bacterial colonies have different shapes and forms.

* Circular
* Irregular
* Rhizoid
1. **Margin of Colonies**

Outer edge or periphery of bacterial colony may take one of the several different patterns depending upon species

1. **Entire margin**

Sharply defined even margin

1. **Filamentous Margin**

Thread Like Margin

1. **Lobate Margin**

Marked indentation

1. **Undulate Margin**

Wavy indentation

1. **Serate Margin**

Tooth like appearance



1. **Elevations in Colony**

The degree to which a colony is raised on agar surface is called elevations in colony

1. **Flat colony**

Elevation not visible

1. **Raised colony**

Slightly eleveated

1. **Convex colony**

Dome shape elevation

1. **Umbonate colony**

Raised with elevated convex centeral region

1. **Optical Features**
2. Colonies may be opaque i.e. no light transmission
3. Colonies may be transparent i.e. full light transmission
4. Colonies may be translucent i.e**.** partial light transmission
5. **Chromogenesis ( Pigmentation)**

Some bacterial species produce and retain water insoluble pigments and thus causing colonies to become coloured

Example

* ***Serratia marcescens*** : It produce red color colonies.
* ***Micrococcus luteus*** : It produces yellow color colonies.
1. **Consistency**

It can be determined by touching a transfer needle to a colony. Some species have colonies having **butter like consistency**. Other have **Rubbery , Brittle or Powder like consistency.**

**Characteristics of Broth Culture**

1. **Amount of Growth**

Growth may be scant, moderate of abundant.

1. **Distribution of Growth**

Grwoth may be unifromly distributed through out the medium or alternatively ot may be confined to the surface called Pellicle or may accumulate as sediment

**Bacterial Isolation Techniques**

1. **Streak Plate Technique**

Also called dilution technique or separation technique

* It is a rapid qualitative isolation method. By means of a wire loop a portion of mixed culture is placed on the surface of agar medium.
* Flame the loop, cool it and then streak across the surface of area 1. This process thin out bacteria on agar surface.
* Now again reflame and cool the loop turn petri dish at 90 degree angle and touch the loop to the corner of culture in area 1 and drag several time across agar in area 2.
* Reflame and cool the loop and again turn perti dish at 90 degree angle and streak in area 3.
* Without reflaming the loop again turn petri dish at 90 degree angle and drag culture from corner of 3 to area 4.
* Incubate for 24 hours

Subculturing of a colony from a single streak plate does not assure purity for this reason it is advisable to streak a culture several times on media in order ti assure purity.

**Plate Counts**

The most frequently used method of measuring bacterial populations is the plate count. An important advantage of this method is that it measures the number of viable cells. One disadvantage may be that it takes some time, usually 24 hours or more, for visible colonies to form. This can be a serious problem in some applications, such as quality control of milk, when

it is not possible to hold a particular lot for this length of time.

Plate counts assume that each live bacterium grows and divides 10 produce a single colony. When a plate count is performed, it is important that only a limited number of colonies develop in the plate. When too many colonies are present, some cells are overcrowded and do not develop; these conditions cause inaccuracies in the count. The U.S. Food and Drug Administration convention is to count only plates with 25 to 250 colonies, but many microbiologists prefer plates with 30 to 300 colonies. To ensure that some colony counts will be within this range, the original inoculum is diluted several times in a process called serial dilution

Pour Plates and Spread Plates. A plate count is done by either the **pour plate** method or the **spread plate** method

1. **Spread Plate Technique**

Mixed culture is diluted in a series of tube containing a sterile liquid usually water. A sample of liquid removed from each tube placed on the surface of agar plate and spread evenly by a bent glass rod. After incubation in at least one plate the number of bacteria will be low to allow development of well separated colonies.

**Advantage**

Only surface colonies will develop

1. **Pour Plate Method**

**Method 1**: In pour plate method mixed culture is diluted and added directly in tubes of molten agar medium (Cooled to 45 degree). The inoculated medium is than dispensed in to petri dishes allowed to solidfy and then incubated.

**Method 2**: Molten agar is poured in petri dishes containing a specified amount of diluted sample. After addition of agar, cover is placed and plate is gentally rotated in circular motion to achieve uniform distribution of microorganisms.

**Disadvantage**

Surface and subsurface colonies develop by this method.