CULTIVATION AND GROWTH OF BACTERIA

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Content

1. Nutritional Requirements
2. Bacteriological media
3. Physical conditions required for the growth
4. Bacterial reproduction
5. Growth curve of bacteria

1. Nutritional Requirements

- Bacteria require **adequate nutrition, optimum pH, temperature and oxygen** for the growth and multiplication.
- Suitable artificial media containing sources of **carbon, nitrogen, hydrogen, oxygen, phosphorous** and other elements such as **sodium, potassium, magnesium and iron** in a very small amounts have been used for the cultivation of micro-organisms in the laboratory.

- Bacteria can be classified depending on nutritional requirements

1. **Source of energy:**

- Bacteria which derive their energy from sunlight are called **phototrophs**. Eg: Rhodospirillum rubrum.
- Bacteria which derive their energy from chemical reactions are called **chemotrophs**. Eg: Escherichia coli.

2. Source of electrons:

- All microbes require a source of electrons for their metabolism.
- Bacrteia which use reduced inorganic compounds as electron donors are called **lithotrophs**. Eg: Pseudomonas pseudoflava.
- Some other species which use organic compounds as electron donors are called **organotrophs**. Eg: E. coli.

3. Source of carbon

Microbes requires carbon for synthesizing the cell components.

Some species use CO_2 as the major source of carbon. These micro organisms are called **auto-trophs.** eg: Nitrifying bacteria.

Other species requires organic compounds as the source of carbon, such species are called **heterotrophs**. Eg: E.coli.

4. Nitrogen:

- Bacteria can use nitrogen from the atmosphere or from inorganic compounds such as nitrates, nitrites, ammonium salts or organic compounds such as aminoacids.
- Nitrogen is the major component of protein and nucleic acid.

5. Sulphur:

- Sulphur is needed for the synthesis of aminoacids. Eg: Cystine, methionine, etc.

6. Phosphorous:

- Phosphorous is an essential component of nucleotides, nucleic acid, phospholipids etc.

7. Mineral salts, Growth factor or bacterial vitamins and water are also essential for bacterial growth.

BACTERIOLOGICAL MEDIA

- Media are the artifically prepared material of various nutrients for the growth and multiplication of microorganisms.

Characteristics of media

- All media must provide carbon source, nitrogen source, minerals and other growth factors.
- All media to be used must be initially free from microorganisms. So that it must be sterilized before use.

Common ingredients of media

1. **Water**

- Source of hydrogen and oxygen is used as diluent.

2. **Electrolytes**

- Sodium chloride and other electrolytes.

3. **Peptone**

- It is a complex mixture of partially digested proteins from animals or vegetable source.
- The main constituents are protease, amino acids, inorganic salts which includes phosphate, potassium and magnesium and growth factor includes nicotinic acid and riboflavin.
- Peptone mainly supplies nitrogenous material and also acts as a buffer.
- Peptone is stored in a tightly closed container because it is hygroscopic and becomes sticky when exposed to air.

4. Yeast extract and Meat extract (YEME)

- YEME media contains protein degradation products, carbohydrates, inorganic salts and certain other growth factors.
- These are used for enriching culture media.

5. Blood

- It enriches media, usually 5-10 % defibrinated horse or sheep blood is used.

6. Agar

- Agar is a long chain polysaccharide obtained from seaweeds algae.
- Agar is a mixture of two polysaccharides such as aggarose (70%) and agropectin (30%).
- It also contains calcium, chloride, magnesium, sulphate, iron etc.
- Agar is now commonly used in the preparation of solid media.

Properties of agar are as follows:

- It acts as good solidifying agent
- Bacteriologically inert.
- It is stable or firm at different temperatures used for incubation.
- It melts at 95 to 98 °C and remains liquid upto 40 to 42 °C .
- It gets solidified below 40 °C .
- Easily available and economical.

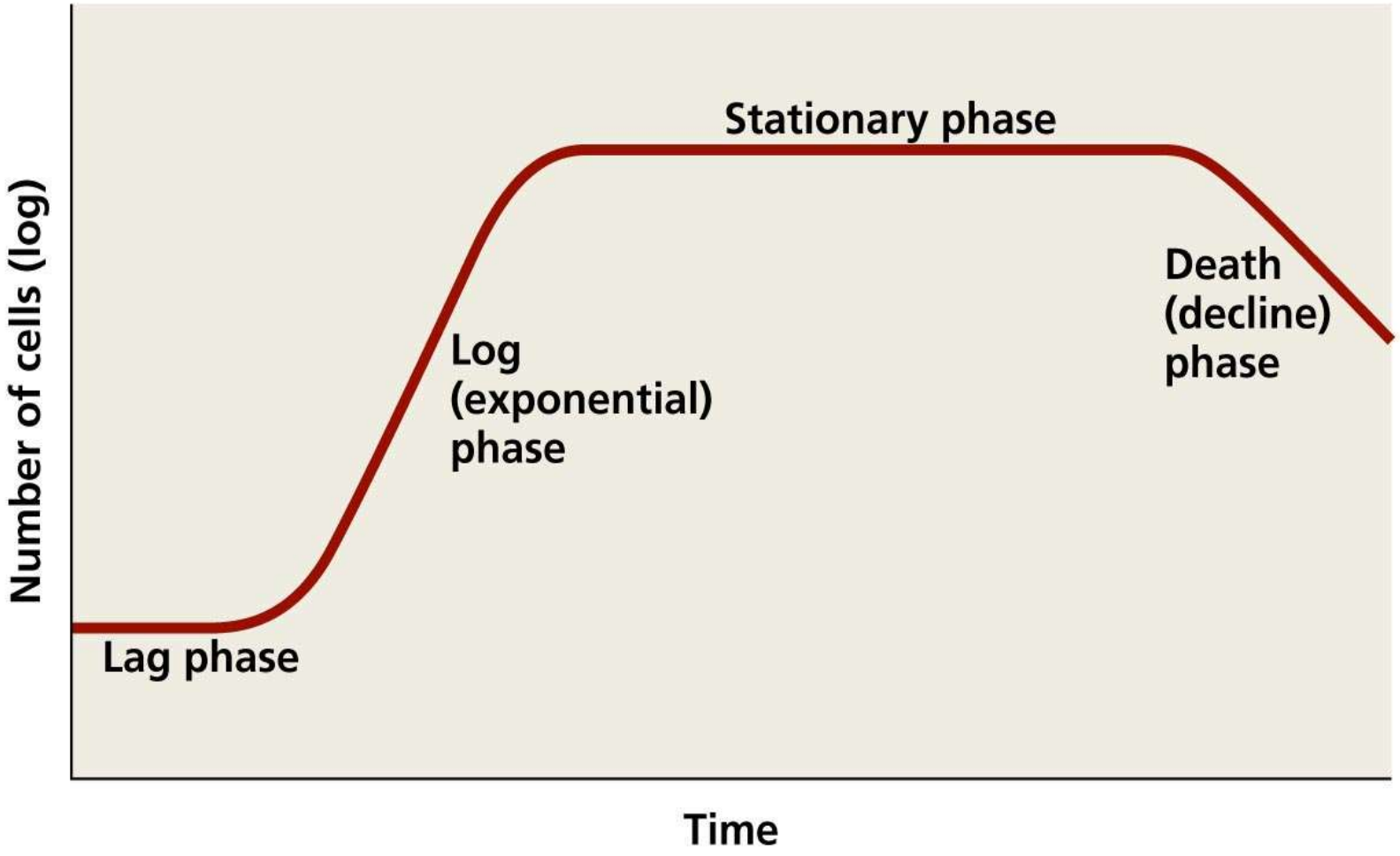
GROWTH CURVE OF BACTERIA

- Normal growth curve of bacteria can be determined by inoculating a small number of bacterial cells into a suitable culture media and counting the bacteria at regular interval.
- When the logarithms of the viable cells are plotted against time on graph paper, it gives a typical curve called as bacterial growth curve or growth cycle of bacteria.

The resulting curve has four distinct phases

1. Lag phase
2. Log or logarithmic or exponential phase
3. Stationary phase
4. Death or decline phase

GROWTH CURVE OF BACTERIA



1. **Lag phase**

- When bacteria are inoculated into a fresh medium, the microbial population remains constant. The period between inoculation and the beginning of multiplication is known as the lag phase.
- In this phase , bacterial cells adjust itself to adopt the new environment.
- During this phase the size of the cell is increased.

2. Log phase

- During this phase the cell divides steadily at a constant rate and the log of the number of cells plotted against time results in a straight line.
- The bacteria multiplies at their maximum rate and their number increases exponentially with time.

3. Stationary phase

- In this phase a constant high population of cells is maintained by a balance between cell division and cell death.
- The rate of multiplication is reduced because depletion of nutrients, accumulation of toxic waste products, very high concentration of cell and low oxygen level.
- During this stage food material gets consumed
- Bacterial count at this stage shows no change.

4. Death or decline phase

- This is the final phase of the bacterial growth, death occurs due to depletion of nutrients and the accumulation of toxic by-products.
- The bacteria becomes old and are unable to reproduce.

Between each of these phase, there is a small curved portion called the transitional period.

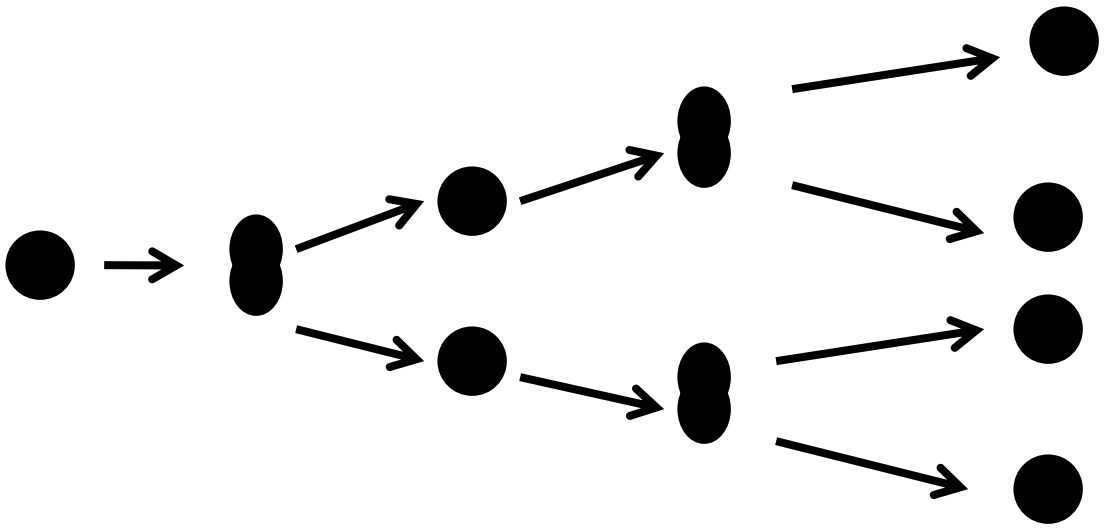
Bacterial Reproduction

- Bacteria reproduce asexually as well as sexually.
- A bacterial reproduction takes place by the following method:

1. Binary fission
2. Budding
3. Fragmentation
4. Formation of conidiospores or sporangiospores

1. Binary fission

- Micro-organisms multiply by asexual process of cell fission .
- Most bacteria multiply by transverse binary fission, that is division into two equal cells.



2. Budding

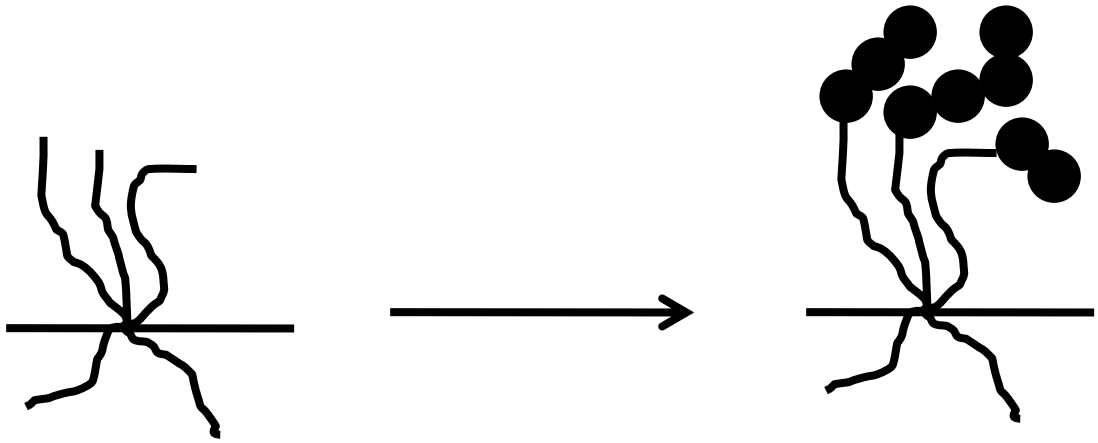
- A process in which a small bud develops at one end of the cell is called budding.
- This bud enlarges and eventually develops into a new cell which separates from the parents
- Eg: Rhodopseudomonas acidophila.

3. Fragmentation

- Bacteria may grow or reproduce by fragmentation of the filaments into small bacillary or coccoid cells.
- Each filament grow and forms new cells.
- Eg: Nocardia species.

4. Formation of conidiospores or sporangiospores

- Streptomyces and related bacteria produce many spores per organism.
- Each spore develop and forms new cells.



QUANTITATIVE MEASUREMENT OF BACTERIAL GROWTH

1. **Indirect method (measurement of cell mass)**
1. Cell volume method
2. Dry weight method
3. Chemical method
4. Turbidimetric method

2. Direct microscopic count (measurement of cell number)

1. Colony counting method
2. Breed's method
3. Membrane filter technique
4. Electron coulter counter

Indirect method (measurement of cell mass)

1. **Cell volume method**

- Take 10 ml of liquid culture in a calibrated centrifuge tubecalled Hopkin's tube.
- The suspension is centrifuged under defined conditions, the pellet settles down and the volume of wet pellet is directly measured.

Indirect method (measurement of cell mass)

2. Dry weight method

- This method is not suitable for bacteria.
- It is widely used for measuring the growth of moulds.
- The suspension is centrifuged under defined conditions.
- The pellet settles down, is further washed, free from liquid medium, dried in desiccator and weighed accurately.

Indirect method (measurement of cell mass)

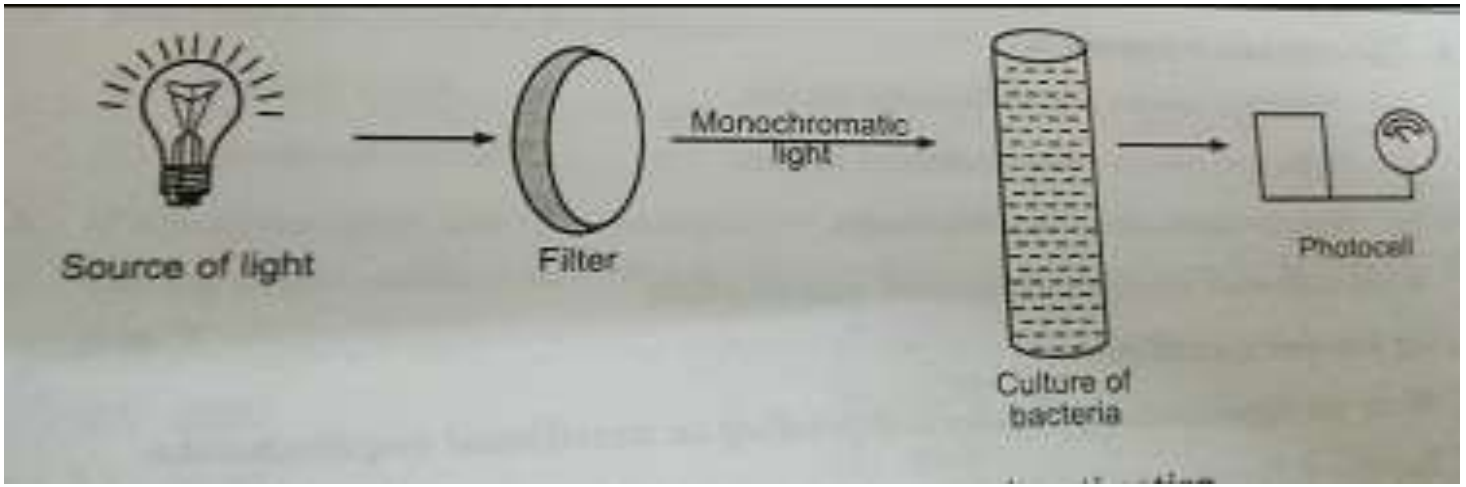
3. Chemical method

- It is estimated by measuring the amount of certain chemical compounds such as protein, free amino acids, phosphorous, DNA and RNA etc.
- This are always constant in living cells.

Indirect method (measurement of cell mass)

4. Turbidimetric method

- In this method the growth of bacteria is measured by volume of turbidity formed.
- This technique is based on the amount of light absorbed through a suspension of bacteria.
- Light absorbance is directly proportional to turbidity of the medium.



Direct microscopic count (measurement of cell number)

1. **Colony counting chamber**

- In this method, special counting chamber (petroff-Hauser slide) is used.
- Here a measured volume of bacterial suspension is spread over the measured area of slide and cells are counted by mounting the slide under the microscope.
- The total number of bacteria can be counted from the proportion of total volume known.

Direct microscopic count (measurement of cell number)

2. Breed's method

- Here a known volume of bacterial suspension is spread over an exact area of the slide for eg : 2 cm^2 are used.
- The suspension is dried, fixed and stained with methylene blue.
- The cells are counted in known proportion of total area.

Direct microscopic count (measurement of cell number)

3. Membrane filter technique

- This technique is used when the turbidity is very low in a suspension of microorganisms.
- The suspension is poured in a porous membrane and the micro organisms are filtered.
- The number of bacteria collected on the filter is stained and the membrane is dried and treated with immersion oil to make it transparent.
- The stained organisms are counted under a microscope and calculated from the filtration area of the membrane.

Direct microscopic count (measurement of cell number)

4. Electronic coulter counter

- This method is used to identify the exact total counts in a few seconds.

PHYSICAL CONDITIONS REQUIRED FOR GROWTH OF BACTERIA

- Apart from the type of media for bacterial growth many physical conditions of the environment are very much essential for the optimum growth.
- Some of these conditions are:

1. Temperature
2. pH
3. Gaseous requirements
4. Osmotic pressure
5. light

1. Temperature

- Temperature is one of the most important physical factor that has the great influence on the growth of the micro organisms.
- The temperature that allows for rapid growth during a short period of time is known as the **optimum growth temperature.**
- The highest temperature at which micro-organisms shows growth is known as the **maximum growth temperature.**
- The lowest temperature at which micro-organisms shows growth is known as the **minimum growth temperature.**

- Based on temperature tolerance, bacteria can be classified into following categories:

1. **Psychrophiles**

- They are able to grow at 0°C but have optimum temperature of 15°C or lower.

2. **Mesophiles**

- They are able to grow between the range of 20 to 40°C.
- All bacteria that are pathogenic for humans or warm blooded animals are mesophiles.

3. **Thermophiles**

- They are able to grow between the range of 20 to 40°C.

2. pH

Each microbial species has definite pH range for growth and multiplication of micro-organisms.

1. **Acidophiles**

- These bacteria grow best in a pH range between 1 to 6.5. Eg: lactobacillus acidophiles.

2. **Neutrophiles**

- These bacteria grow best in a narrow pH range between 6.5 to 7.5 Eg: E.coli, salmonella typhi etc.

3. **Alkalophiles**

- These bacteria grow best in a pH range between 7.5 to 14. eg: Vibrio cholerae.

3. Gaseous Reqiurements

1. Aerobic bacteria

- These bacteria require oxygen for growth and energy.
- E.g: E.coli.

2. Anaerobic bacteria

- These bacteria do not use oxygen for growth and energy.
- E.g: clostridium species.

3. Facultatively anaerobic bacteria

- These bacteria do not require oxygen for growth but if oxygen is available, is used for energy production.
- These bacteria grow in both aerobic and anaerobic conditions.
- E.g Pseudomonas species.

4. **Microaerophilic bacteria**

- These bacteria require low levels of oxygen for growth but cannot tolerate the levels of oxygen present in air atmosphere.
- Eg: lactobacillus plantarum.

4. Osmotic Pressure

- Bacteria are more tolerant to osmotic variations because of mechanical strength of the cell wall.
- They can grow in media with widely varying contents of salts, sugar and other solutes.
- Sudden exposure of bacteria to solutions of high salt concentrations may cause plasmolysis. Hence, 0.5% NaCl is added to almost all culture media to make the environment isotonic.

5. light

- Darkness is usually favourable for the growth and viability of all microorganisms.
- They are sensitive to ultraviolet radiation, direct light and other radiation.

Thank you