Programme – B.Pharm
Course- Pharmaceutical Microbiology
Course Code – BP 303 T
Year – 2nd
Sem – 3rd
Unit – I (Part II)
Topic- Introduction to Microbiology
Sub- Topic- Introduction to Prokaryotes & Eukaryotes, Morphological classification of Bacteria, Growth curve, isolation & preservation methods for pure cultures.

Prokaryotes:

Greek word **'pro'** means **before** and **'karyon'** means **nut** or **kernel**, referring to the cell nucleus. Prokaryotes are organisms without a cell nucleus, or any other membrane bound organelles. Most prokaryotes are unicellular, but some prokaryotes are multicellular.

Examples: Blue-green algae (cyanobacteria), bacteria, archaea etc.

Figure: Classification of Bacteria

• **Phenotypic classification**

Morphological
Anatomical
Staining
Cultural characteristics
Nutrition
Environmental factors
Biochemical reactions
Antigenic structure

• **Genotypic classification**

DNA-DNA hybridization
G+C content

Morphological classification

• Bacteria can be classified into **six** major groups on morphological basis.

1. TRUE BACTERIIA

• **Coccii** – These are spherical or oval cells. On the basis of arrangement of individual organisms they can be described as

– **Monococci** (Cocci in singles) – *Monococcus* spp.
– **Diplococci** (Cocci in pairs) – *Streptococcus pneumoniae*
Staphylococci (Cocci in grape-like clusters) *Staphylococcus aureus*
– **Streptococci** (Cocci in chains) – *Streptococcus pyogenes*
– **Tetrad** (Cocci in group of four) - *Micrococcus* spp.
– **Sarcina** (Cocci in group of eight)

• **Baciillllii** – These are rod-shaped bacteria. On the basis of arrangement of organisms, they can be described as

– **Diplobacilli**
– **Streptobacilli**
– **Palisades**
– **Chinese-letter form**
– **Coccobacilli**
– **Comma-shaped**

2. ACTIINOMYCETES

These are rigid organisms like true bacteria but they resemble fungi in that they exhibit branching and tend to form filaments.
They are termed such because of their resemblance to sun rays when seen in tissue sections.

3. Spirochaettes

These are relatively longer, slender, non-branched microrganisms of spiral shape having several coils.

4. Mycoplasmas

These bacteria lack in rigid cell wall & are highly pleomorphic & of indefinite shape.
They occur in round or oval bodies and in interlacing filaments.

5. Ricketssiae and Chllamydiae

These are very small,obligate parasites, & at one time were considered closely related to the viruses. Now, these are regarded as bacteria.

Bacterial Growth Curve

During typical bacteria growth (growth cycle) bacteria cell divide by binary fission and their mass and number increase in an exponential manners. Bacterial growth in culture can be separated into at least four distinct phases.

1. Lag phase

This is period of intense physiologic adjustment involving the induction of new enzymes and the synthesis and assembly of ribosome. In lag phase and during this phase there occur

1. increase in size of cells
2. increase in metabolic rate
3. adaptation to new environment and necessary enzymes.

The length of lag phase depend upon

a. Type of bacteria.
b. Better the medium, shorter the lag phase.
c. The phase of culture from which inoculation in taken
d. Size or volume of inoculum.
e. Environmental factors like temperature.

2. Logarithmic (Exponential) phase

In logarithmic phase the bacterial cell start dividing and their number increase by geometric progression with time. During this period: -

a. bacteria have high rate of metabolism
b. bacteria are more sensitive to antibiotics
c. rate of penetration of the medium it depends on the concentration of material in the media

3. Stationary phase

In stationary phase after some time a stage comes when rate of multiplication and death becomes almost equal it may be due to: -

a. depletion of nutrient
b. accumulation of toxic products and sporulation may occur during this stage.

4. Decline or death phase

In decline (death) phase, during this phase population decreases due to death of cells the factors responsible are: -

a. nutritional exhaustion
b. toxic accumulation
c. autolysis enzymes

Pure Culture Technique

Culture : Act of cultivating microorganisms or the microorganisms that are cultivated
Mixed culture : more than one microorganism
Pure culture : containing a single species of organism

A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample many times before inoculating the new medium.

Why important ?

Pure cultures are important in microbiology for the following reasons-

1.Once purified, the isolated species can then be cultivated with the knowledge that only the desired microorganism is being grown.

2. A pure culture can be correctly identified for accurate studying and testing, and diagnosis in a clinical environment.

3. Testing/experimenting with a pure culture ensures that the same results can be achieved regardless of how many time the test is repeated.

ISOLATION TECHNIQUE OF PURE CULTURE

•Cultures composed of cells arising from a single progenitor
•Progenitor is termed a CFU
•Aseptic technique prevents contamination of sterile substances or objects
•Common isolation techniques
-Streak plate method
-Pour plate method
-Spread plate method
-Roll tube method

1.Streak plate method

• Streaking is the process of spreading the microbial culture with an inoculating needle on the surface of the media.
• Sterilize the inoculating needle by flame to make red hot and allow it to cool for 30 seconds.
• The sample is streaked in such a way to provide series of dilution.
• purpose- thin out inoculum to get separate colonies.
• sub culturing can be done by streaking well isolated colonies from streak plate to new plate.

2. Pour plate method

• The bacterial culture and liquid agar medium are mixed together.
• After mixing the medium, the medium containing the culture poured into sterilized Petri dishes (petri plates), allowed solidifying and then incubated.
• After incubation colonies appear on the surface.

DISADVANTAGES-

1. Microorganism trapped beneath the surface of medium hence surface as well as subsurface Colonies are developed which makes the difficulties in counting the bacterial colony.
2. Tidious and time consuming method, microbes are subjected to heat shock because liquid mediummaintainedat45℃.
3. Unsuitable- Psychrophile

3. Spread plate method

• This is the best method to isolate thepure colonies.
• In this technique, the culture is not mixed with the agar medium. Instead it is mixed with normal saline and serially diluted.
• 0.1 ml of sample taken from diluted mixture, which is placed on the surface of the agar plate and spread evenly over the surface by using L shaped glass rod called spreader.
• Incubate the plates
• After incubation, colonies are observed on the agar surface.

ADVANTAGES

1. It is a simple method.
2. In this method only surface colonies are formed.
3. Micro-organisms are not exposed to higher temperature.

4. Micromanipulator method

Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture.This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation. The single cell of microbe sucked into micropipette and transferred to large amount of sterile medium.

ADVANTAGES

The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species.

DISADVANTAGES

The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled person.

PRESERVATION OF PURE CULTURE

To maintain pure culture for extended periods in viable condition without any genetic change is referred as Preservation.

During preservation most important factor is to stop microbial growth or at least lower the growth rate.

Due to this toxic chemicals are not accumulated and hence viability of microorganism is not affected.

Objectives of preservation

1. To maintain isolated pure culture for extended periods in a viable conditions.
2. To avoid contamination
3. To restrict Genetic Mutation

Why to Preserve Bacteria?

In nature there are only 1% bacteria which is pathogenic and harmful to Animalia and Plantae.

99% of bacterial populations are of economic importance for human beings and plants.

In soil for nutrient uptake in food industry, in sewage treatment, in medical industry.

So the preservation of bacteria is one of the most profitable practice economically as well as environmentally.

Preservation methods of Bacteria

1. Periodic transfer to fresh medium
2. Storage at low temperature
3. Storage in sterile soil
4. Preservation by overlaying culture with mineral oil
5. Lyophillization or freeze drying

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