Lec.3 Classification of bacteria

Bacterial classification depends on the following characteristics:

1. Morphology and arrangement
2. Staining
3. Cultural characteristics
4. Biochemical reactions
5. Antigenic structure
6. Base composition of bacterial DNA

**Morphology and staining of bacteria are the commonly used characteristics to classify bacteria:

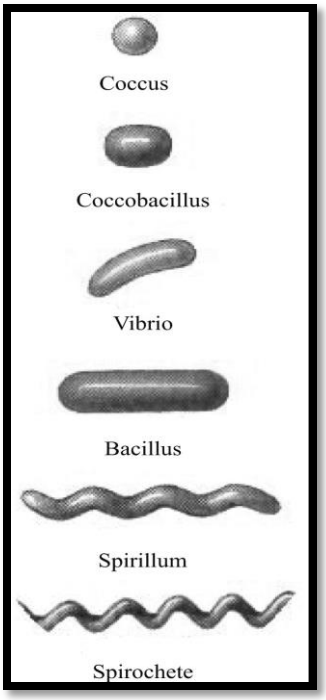
➢ Morphology of bacteria

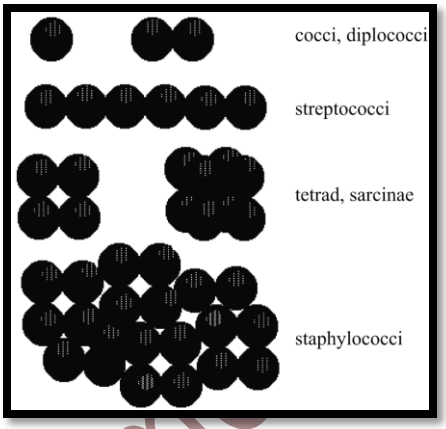
Depending on their morphology (shape), bacteria are classified into several varieties:

1. Cocci (from kokkos meaning berry) are spherical or oval cells
2. Bacilli (from baculus meaning rod) are rod shaped cells

3. Vibrios are comma shaped curved rods and derive their name from their characteristics vibratory motility.

4. Spirilla are rigid spiral forms.
5. Spirochetes (from speira meaning coil and chaite meaning hair) are flexuous spiral forms
6. Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus)
7. Mycoplasmas are bacteria that are cell wall deficient (lackcell wall) and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.





Bacteria sometime show characteristic cellular arrangement or grouping. According to the plane of cellular division, **cocci may be arranged in pairs (diplococci)**, **chains (streptococci)**, **groups of four (tetrads) or eight (sarcina)**, or **grape like clusters (staphylococci).**

➢ Stained Preparations

Live bacteria do not show the structural detail under the light microscope due to lack of contrast. Hence staining techniques are used to produce colour contrast. Routine methods of staining of bacteria involve dying and fixing smears – procedures that kill them.

Bacteria have an affinity to basic dyes due to acidic nature of their protoplasm. The commonly used staining techniques are:

1- Simple Stains

Dyes such as methylene blue or basic fuchsin are used for simple staining. They provide colour contrast, but impart the same colour to all bacteria.

2- Negative Staining

Bacteria are mixed with dyes such as Indian ink or nigrosin that provide a uniformly coloured background against which the unstained bacteria stand out in contrast. Very slender bacteria like spirochetes that cannot be demonstrated by simple staining methods can be viewed by negative staining.

3- Impregnation Methods

Cells and structures too thin to be seen under ordinary microscope may be rendered visible if they are impregnated with silver on the surface. These are used for demonstration of spirochetes and bacterial flagella.

4- Differential Stains

These stains impart different colours to different bacteria or bacterial structures, the two most widely used differential stains are the Gram stain and Acid fast stain.

The gram stain was devised by histologist **Christian Gram as a method of staining bacteria in tissues.

Gram positive cells are simpler chemical structure with a acidic protoplasm.It has a thick peptidoglycan layer. Teichoic acids are intertwined among the peptidoglycan and the **teichoic acids** are the major surface antigen determinants

Gram negative cells are more complex, they are rich in lipids. The membrane is bilayered as phospholipids, proteins and lipopolysaccharide.

Lipopolysaccharides (LPS) are also known as endotoxin. Gram negative cells have a peptidoglycan layer which is thin and formed by just one or two molecules. No Teichoic acids are found in the cell wall of Gram negative bacteria.

The Outer membrane has Lipopolysaccharide channels with porins which transfer the solutes across. Lipoprotein cross link outer membrane and peptidoglycan layer Gram reaction may be related to the permeability of the bacterial cell wall and cytoplasmic membrane to the dye-iodine complex, the Gram-negative, but not the Gram-positive cells, permitting the outflow of the complex during decolourisation.

**Gram staining is an essential procedure used in the identification of bacteria and is frequently the only method required for studying their morphology.

The acid fast stain was discovered by **Ehrlich, who found that after staining with aniline dyes, tubercle bacilli resist decolourisation with acids. The method as modified by Ziehl and Neelsen, is in common use now.

- **Growth and multiplication of bacteria**

→Bacteria divide by binary fission and cell divides to form two daughter cells. Nuclear division precedes cell division and therefore, in a growing population, many cells having two nuclear bodies can be seen.

→ Bacterial growth may be considered as two levels, increase in the size of individual cells and increase in number of cells.

→ Growth in numbers can be studied by **bacterial counts** that of total and viable counts. :

1- The **total count** / gives the number of cells either living or not.
2- The **viable count** / measures the number of living cells that are capable of multiplication.

➢ Bacterial Growth Curve

When bacteria is grown in a suitable liquid medium and incubated its growth follows a definite process. If bacterial counts are carried out at intervals after inoculation and plotted in relation to time, a growth curve is obtained. The curve shows the following phase:

(i) Lag phase

Immediately following innoculation there is no appreciable increase in number, though there may be an increase in the size of the cells. This initial period is the time required for adaptation to the new environment and this lag phase varies with species, nature of culture medium and temperature.

(ii) Log or exponential phase

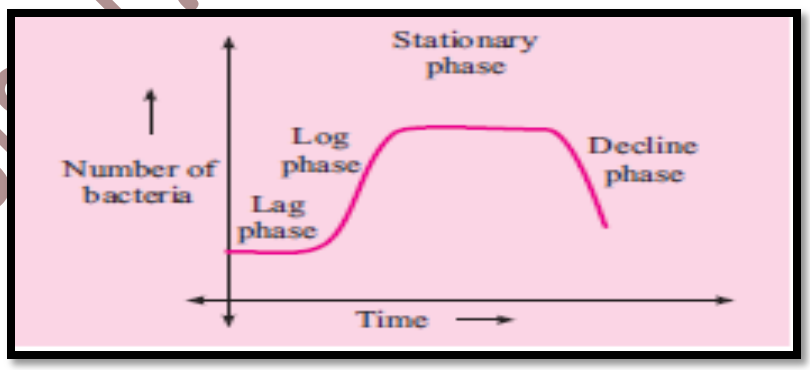
Following the lag phase, the cell starts dividing and their numbers increase exponentially with time.

(iii) Stationary phase

After a period of exponential growth, cell division stops due to depletion of nutrient and accumulation of toxic products. The viable count remains stationary as an equilibrium exists between the dying cells and the newly formed cells.

(iv) Phase of decline

This is the phase when the population decreased due to cell death.



The various stages of bacterial growth curve are associated with morphological and physiological alterations of the cells.

- The maximum cell size is obtained towards the end of the lag phase.
- In the log phase, cells are smaller and stained uniformily.
- In the stationary phase, cells are frequently gram variable and show irregular staining due to the presence of intracellular storage granules. Sporulation occurs at this stage. Also, many bacteria produce secondary metabolic products such as exotoxins and antibiotics.
- Involution forms are common in the phase of decline.

➢ Factors that affect the growth of bacteria

Many factors affect the generation time of the organism like temperature, oxygen, carbon dioxide, light, pH, moisture, salt concentration.

1- Nutrition

The principal constituents of the cells are water, proteins, polysaccharides, lipids, nucleic acid and mucopeptides. For growth and multiplication of bacteria, the minimum nutritional requirement is water, a source of carbon, nitrogen and some inorganic salts.

****Bacteria can be classified nutritionally**, based on their energy requirement and on their ability to synthesize essential metabolites:

1- **phototrophs bacteria** :which derive their energy from sunlight.
2- **chemotrophs bacteria:** obtain energy from chemical reactions.
3- **Autotrophs bacteria**: which can synthesize all their organic compounds.
4- **Heterotrophs bacteria:** unable to synthesize their own metabolites are.

2- Oxygen

Depending on the influence of oxygen on growth and viability, bacteria are divided into **aerobes** and **anaerobes**.

1-Aerobic bacteria: require oxygen for growth. They may be :

a-obligate aerobes like cholera, vibrio, which will grow only in the presence of oxygen

b- facultative anaerobes which are ordinarily aerobic but can grow in the absence of oxygen.

**Most bacterial of medical importance are facultative anaerobes.

2-Anaerobic bacteria: such as clostridia, grow in the absence of oxygen and the obligate anaerobes may even die on exposure to oxygen. **Microaerophilic bacteria** are those that grow best in the presence of low oxygen tension.

3- Carbon Dioxide

All bacteria require small amounts of carbon dioxide for growth. This requirement is usually met by the carbon dioxide present in the atmosphere. Some bacteria like Brucella abortus require much higher levels of carbon dioxide.

4- Temperature

Bacteria vary in their requirement of temperature for growth. The temperature at which growth occurs best is known as the **optimum temperature**.

1- **mesophilic bacteria:**which grow best at temperatures of 25-40°C.
2- **Psychrophilic bacteria:** grow best at temperatures below 20°C.
3- **Thermophiles bacteria:** grow best at high temperatures, 55-80°C (non pathogenic bacteria)

The lowest temperature that kills a bacterium under standard conditions in a given time is known as **thermal death point**.

5- Moisture and Drying

Water is an essential ingredient of bacterial protoplasm and hence drying is lethal to cells. The effect of drying varies in different species.

6- Light

Bacteria **except** phototrophic species grow well in the dark. They are sensitive to ultraviolet light and other radiations. Cultures die if exposed to light.

7- H-ion concentration

Bacteria are sensitive to variations in pH. Each species has a pH range, above or below which it cannot survive and an optimum pH at which it grows best. Majority of pathogenic bacteria grow best at neutral or slightly alkaline **pH (7.2 – 7.6)**

8- Osmotic Effect

Bacteria are more tolerant to osmotic variation than most other cells due to the mechanical strength of their cell wall.

Sudden exposure to hypertonic solutions may cause osmotic withdrawal of water and shrinkage of protoplasm called **plasmolysis.**