compared with that of bright-field microscopy. The disadvantage of this method is light passes around rather than through the organisms, making it difficult to study their internal structure.

Fluorescence microscope

When ultraviolet or short-wavelength or invisible light falls on a fluorescent substance, the wavelength of the invisible light increases, so that it becomes luminous and is said to fluoresce. If tissues, cells or bacteria are stained with a fluorescent dye and are examined under the microscope with ultraviolet light instead of ordinary visible light, they become luminous and are seen as bright objects against a dark background.

Electron microscope

The greatly increased resolving power of the electron microscope (EM) has enabled scientists to observe the detailed structures of prokaryotic and eukaryotic cells. The superior resolution of the EM is due to the fact that electrons have a much shorter wavelength than the photons of white light. In EM, a beam of electrons is employed instead of the beam of light used in the optical microscope.

The resolution that can be obtained with EM is hundred times more than that of the light microscope.

SHAPE OF BACTERIA

Bacteria exist in different shapes as under (Fig. 2.1):

1. **Cocci** (from *kokkos* meaning berry) are round or oval cells.
2. **Bacilli** (from *baculus* meaning rod) are rod or stick-shaped. The ends may be square or rounded. The bacilli with tapered, pointed ends are termed fusiform. In some of the bacilli the length of the cells may be equal to width. Such bacillary forms are known as **coccobacilli**. The latter have to be carefully differentiated from cocci.
3. **Vibrios** are curved or comma-shaped rods.
4. **Spirilla** are non-flexuous spiral forms with one to three fixed curves in their rigid bodies.
5. **Spirochaetes** (from *spira* meaning coil and *chaite* meaning hair) are slender and flexuous spiral forms.
6. **Mycoplasmas** are cell wall deficient organisms. Therefore, they do not possess stable morphology. They occur as round or oval bodies or as interlacing filaments.

GROUP PATTERNS

The most frequent method of reproduction among bacteria is asexual binary fission, that is, each cell splits in half, forming two new cells. As they increase in number they form distinct groups. Cocci that split along one plane only tend to arrange themselves in pairs (**diplococci**) or in chains (**streptococci**). When the division occurs alternatively in each of two planes, groups of four (**tetrads**) or eight (**octads**) are formed. Haphazard splitting in several planes results in the formation of clusters of cocci (Fig. 2.1).

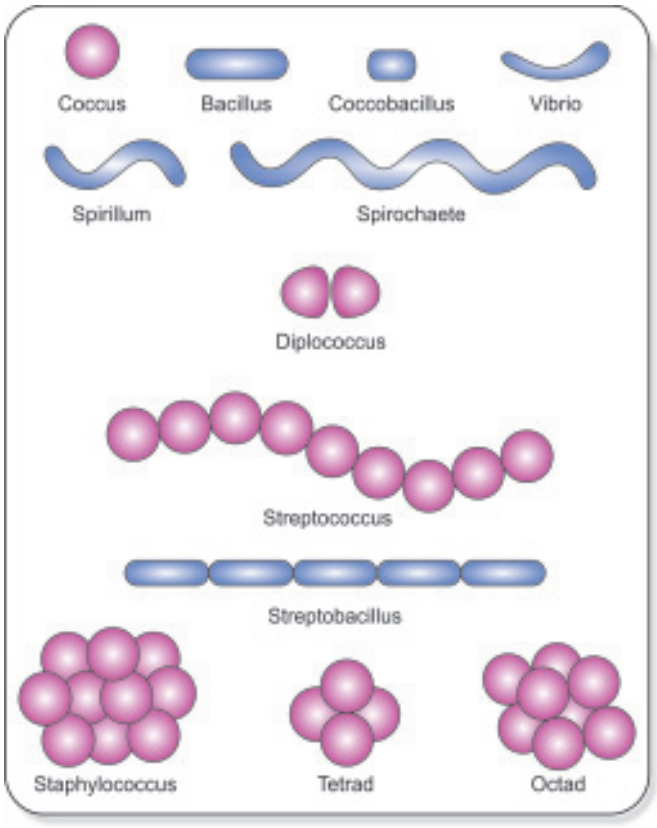

Fig. 2.1. Shapes and group patterns of bacteria.

Bacilli split only across their short axes, therefore, the patterns formed by them are limited. They may appear as end to end pairs (**diplobacilli**), or chains (**streptobacilli**) (Fig. 2.1). In some instances, there occurs incomplete separation of the daughter cells after binary fission. The bacilli remain attached to each other at various angles, resembling the letters V or L. This is called **Chinese letter arrangement** and is characteristic of *Corynebacterium diphtheriae.*

ANATOMY OF A BACTERIAL CELL

The principal structure of a bacterial cell is shown in Fig. 2.2. The interior of the cell, the protoplast, is differentiated into cytoplasm and nuclear material. Cytoplasm is bounded by a thin, elastic and semipermeable cytoplasmic membrane. Outside this lies cell wall, which gives the bacterium its shape and rigidity. Cell wall, in many bacteria, is enclosed by a protective gelatinous covering layer called capsule. Many bacteria also possess flagella which are the organelles of motility and some species have fimbriae (pili) too.

Bacterial cell wall

- It is a complex rigid structure which gives bacteria their definite shape.
- It is permeable to passage of liquid nutrient material into the cell, and to outward passage of substances produced within the cell.

3

Growth and Nutrition of Bacteria

Bacteria reproduce by a process called binary fission, in which a parent cell divides to form a progeny of two cells. This results in a logarithmic growth rate – one bacterium will produce 16 bacteria after four generations.

Generation time

The time required for a bacterium to give rise to two daughter cells is known as **generation time**. Under constant conditions, the generation time for any organism is quite reproducible, but differs greatly among different bacteria. The fastest growing bacteria have generation time of 15–20 minutes under optimum growth conditions. Many bacteria, however, have generation times of hours or even days. In *Escherichia coli* it is 20 minutes, in tubercle bacilli it is 20 hours and in lepra bacilli it is 20 days.

1. Total count

This is total number of bacteria present in a specimen irrespective of whether they are living or dead. This is done by counting the bacteria under microscope using counting chamber and by comparing the growth with standard opacity tubes.

2. Viable count

This measures only viable (living) cells which are capable of growing and producing a colony on a suitable medium.

BACTERIAL GROWTH CURVE

When a bacterium is inoculated into a suitable culture medium and incubated, its growth follows a characteristic course. If both total and viable counts are made at different intervals and plotted in relation to time, then a characteristic growth curve is obtained. A typical growth curve contains four major phases (Fig. 3.1).

1. Lag phase

When bacteria are seeded into fresh medium, multiplication usually does not begin immediately. The period between inoculation and beginning of multiplication is known as lag phase. **During this period the organisms adapt themselves to growth in fresh medium and increase in size and metabolic activity. Therefore, lag phase is regarded as a period not of rest but of intense metabolic activity**.

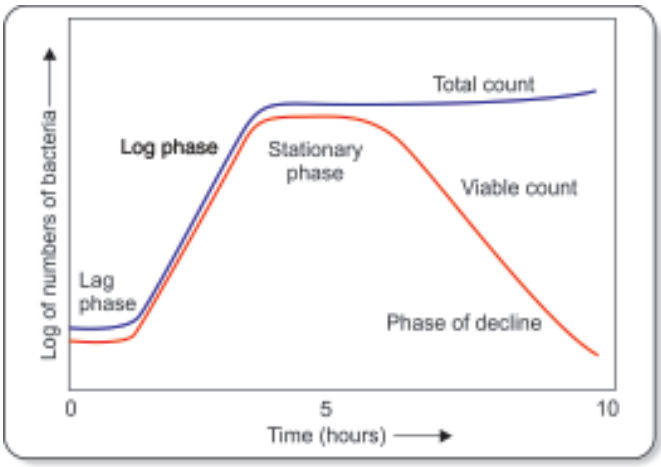

Fig. 3.1. Bacterial growth curve.

2. Log or exponential growth phase

During this phase the bacteria are multiplying at their maximum rate and their number increases exponentially or by geometric progression with time. If logarithm of bacterial count is plotted against time a straight line is obtained. **In the log phase, the bacterial cells are smaller and stain uniformly.** Exponential phase is of limited duration because of:

- exhaustion of nutrients,
- accumulation of toxic metabolic end products,
- rise in cell density,
- change in pH, and
- decrease in oxygen tension (in case of aerobic organisms).

be stab inoculated on semisolid agar or on Dorset egg medium followed by incubation. When growth appears they can be stored in refrigerator.

Sugar media

For the identification of most of the organisms, sugar fermentation reactions are carried out. Glucose, lactose, sucrose and mannitol are widely used sugars. For the preparation of sugar media, 1% of the concerned sugar is added to peptone water with a suitable indicator. Durham's tube (a small tube) is kept inverted in the tube containing this medium to detect gas production. For fastidious organisms like *C. diphtheriae* and pneumococci, Hiss's serum sugar is used.

Anaerobic media

For the growth of anaerobes, the media used contain reducing substances. These include thioglycollate broth and cooked meat broth. The sterile muscle tissue, in cooked meat broth, contains reducing substances, particularly glutathione, which permit the growth of many strict anaerobes. In addition to its reducing effect, the meat provides a variety of nutritional substances for bacterial growth. In this medium, saccharolytic clostridia rapidly produce acid and gas but do not digest the meat. The cultures may have slight sour smell and the meat is often reddened. The proteolytic clostridia produce blackening of the meat, decomposing it and reducing it in volume with the formation of foul-smelling products.

Preparation of commonly used culture media

Peptone water

This medium is used chiefly as the basis for carbohydrate fermentation media, and for subculture of bacteria for antimicrobial susceptibility testing. It is also used to test for the formation of indole.

Peptone	10 g
Sodium chloride	5 g
Water	1 litre

Dissolve the ingredients in warm water, adjust the pH to 7.4–7.5 and filter. Distribute as required and autoclave it at 121°C for 15 minutes.

Alkaline peptone water

It is prepared in the same manner as above. However, pH is adjusted to 8.6.

Sugar fermentation media (peptone water sugars)

Peptone water is prepared as above, pH is adjusted to 7.8, and 1% Andrade's indicator is added. Steam for 20 minutes at 100°C. Filter and add 1% of different sugars (but in case of dulcitol, 0.5%). Sugars commonly tested are glucose, mannitol, sucrose, lactose and maltose. Add 5 ml each of sugar media in test tubes containing inverted Durham's tubes completely filled with liquid and containing no air bubble. Steam for three successive days at 100°C for 20 minutes. Production of acid is indicated by change in the colour of the medium to red or pink, and gas, if produced, collects in Durham's tube.

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1 M sodium hydroxide has been added to turn the colour of the solution yellow.

Hiss's serum sugar media

These media are used to test the fermentation reactions of nutritionally exacting bacteria such as diphtheria bacilli and streptococci. One part of serum (sheep or ox serum) is mixed with three parts of distilled water. The reaction of the medium is adjusted to pH 7.5, and 5 ml of 0.2% solution of phenol red per 100 ml of the medium is added prior to sterilization. The various sugars are incorporated in the proportion of 1%. Sterilization is done by intermittent steaming at 100°C for 20 minutes each day on three successive days and distribute it aseptically in 2.5 ml amounts in screw-capped 6 ml bottles. Fermentation is indicated by the production of acid, which alters the indicator and causes coagulation of the medium.

Selenite F broth

The selenite in this enrichment medium inhibits coliform bacilli while permitting salmonellae and many shigellae to grow.

Sodium hydrogen selenite, $NaHSeO_3$	4 g
Peptone	5 g
Lactose	4 g
Disodium hydrogen phosphate, $Na_2HPO_4.2H_2O$	9.5 g
Sodium dihydrogen phosphate, $NaH_2PO_4.2H_2O$	0.5 g
Sterile water	1 litre

Dissolve the ingredients with sterile precautions and distribute the yellowish solution in about 10 ml amounts in screw-capped containers. Steam at 100°C for 20 minutes (once only). Do not autoclave, because excessive heating spoils the medium. A slight red precipitate may form but it is of no consequence. The pH of the medium should be 7.1.

Care must be taken in the preparation and use of the medium, because selenium salts are toxic and teratogenic, and volatile derivatives, including hydrogen selenide, are toxic when inhaled.

Tetrathionate broth

The tetrathionate formed by the chemical action of sodium thiosulphate and iodine is inhibitory to coliform organisms and permits the growth of salmonellae.

Thiosulphate solution

Sodium thiosulphate	24.8 g
Sterile water	100 ml

Mix the salt and water and steam for 30 minutes at 100°C.

Assimilation media for yeasts

Assimilation is the utilization of carbon (or nitrogen) source by a microorganism in the presence of oxygen. A positive reaction is indicated by the presence of growth or a pH shift in the medium. **Since all yeasts assimilate glucose, it acts as a positive control.**

1. Carbon assimilation medium

Yeast nitrogen base	6.7 g
Appropriate pure carbohydrate	5 g
Distilled water	100 ml

Heat to dissolve. Sterilize by Seitz or membrane filtration. Add 0.5 ml of the solution to 4.5 ml of sterile distilled water in screw cap tubes. Store in refrigerator. These may be used for one month.

2. Nitrate assimilation medium

Yeast carbon base	11.7 g
Potassium nitrate	0.78 g
Distilled water	100 ml

Warm gently to dissolve. Sterilize by Seitz or membrane filtration. Add 0.5 ml of medium to 4.5 ml of sterile distilled water in screw-cap tubes. Store in refrigerator. These may be used for one month.

Test procedure

Make a suspension of the yeast in sterile distilled water. This suspension should not exceed the turbidity of McFarland No. 1 standard. Add 0.1–0.2 ml of the yeast suspension to each tube of medium. Include a tube of yeast nitrogen base without any carbon source, and a tube of yeast carbon base without potassium nitrate, as controls for carryover. Incubate tubes at the yeast's optimal temperature. Examine cultures over a period of 7–14 days for dense turbidity caused by growth. The negative-control tubes without a carbon or nitrogen source should show no growth.

Auxanographic plate method

1. Carbon assimilation tests

Yeast nitrogen base	0.67 g
Noble or washed agar	20 g
Distilled water	1000 ml

Dispense in 20-ml quantities into 18 × 150 mm screw-cap tubes. Autoclave at 121°C for 15 minutes. Allow to harden as butts. Store in refrigerator.

Test procedure

- Melt a tube of nitrogen base medium in a boiling-water bath. Allow to cool to 47–48°C.
- With a sterile cotton-tipped applicator, make a heavy suspension of a 24- to 72-hour yeast culture in 4 ml of sterile distilled water. The density of the suspension should be equal to that of a McFarland No. 4 or 5 standard.
- Pour the yeast suspension into the tube of molten yeast nitrogen base agar. Mix thoroughly by inverting tube several times.
- Pour the yeast-agar mixture into a sterile 15 × 150 mm petri plate. Allow to solidify at room temperature.
- Place carbohydrate-containing disks, evenly spaced, on the plate.
- Incubate at 30°C for 18–24 hours and then examine for growth around each disk. Growth around a disk indicates that the yeast assimilates that sugar.

2. Nitrate assimilation tests

Medium

Yeast carbon base	12 g
Noble or washed agar	20 g
Distilled water	1000 ml

Tube in 20-ml aliquots and autoclave at 121°C for 15 minutes. Store in refrigerator.

Peptone solution for positive control

Peptone	10 g
Distilled water	100 ml

Sterilize by filtration and store in refrigerator

Test procedure

- Melt a tube of yeast carbon base medium in a boiling-water bath. Allow to cool to 47–48°C.
- Make an aqueous solution suspension of the yeast to a density equal to a McFarland No. 1 standard.
- Add 0.1 ml of yeast suspension to the tube of medium. Mix thoroughly.
- Pour the yeast-agar mixture into a sterile petri plate. Allow to solidify at room temperature.
- Place approximately 1 mg of potassium nitrate crystals on agar surface away from the centre of the plate.
- Place about 0.1 ml of peptone solution (positive control) on agar surface opposite potassium nitrate site.
- Incubate at 30°C for 48–96 hours.

For test to be valid growth must occur in the peptone area. If growth is seen in the "peptone area", examine for growth in the potassium nitrate area (growth indicates assimilation of potassium nitrate).

ENVIRONMENTAL FACTORS INFLUENCING GROWTH

Oxidation-reduction (Redox) potential

On the basis of the influence of oxygen on growth and viability, the bacteria are divided into two categories – aerobes and anaerobes:

- **Aerobes** require oxygen for their growth. They may be obligate aerobes like *Pseudomonas aeruginosa* which can