SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT: II

MICROBIOLOGY AND CELL BIOLOGY-SBTA1103

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY SCHOOL OF BIO AND CHEMICAL ENGINEERING

SBTA1101	MICROBIOLOGY AND CELL BIOLOGY	L	T	P	Credits	Total Marks
		3	*	0	3	100

COURSE OBJECTIVE

- The course aims to develop skills of the Students in the area of Microbiology and Cell biology particularly to identify microbes, structure, metabolism and Cell Signaling pathways

UNIT 1 CLASSIFICATION AND MULTIPLICATION **9 Hrs.**

Overview of history of Microbiology- Classification of Microbes - Systems of classification, Numerical taxonomy, Identifying characters for classification, General properties and principles of classification of microorganisms Structural organization and multiplication of bacteria, viruses, algae and fungi.

UNIT 2 MICROBIAL NUTRITION, GROWTH AND METABOLISM **9 Hrs.**

Nutritional requirements of bacteria and different media used for bacterial culture; growth curve. Mathematical nature and expression of microbial growth and different methods to quantitate bacterial growth, aerobic and anaerobic bioenergetics and utilization of energy for biosynthesis of important molecules.

UNIT 3 CONTROL OF MICROORGANISMS **9 Hrs.**

Definition of sterilization, Physical and chemical control of microorganisms; host-microbe interactions; antibacterial, anti-fungal and anti-viral agents, mode of action and resistance to antibiotics; clinically important microorganisms.

UNIT 4 CELL ORGANELLES **9 Hrs.**

Evolution of cell; Cell as a unit of living organism, evolution and structure of prokaryotic cell, evolution of eukaryotic cell - Structural and functional features of eukaryotic cell: cell organelles; endoplasmic reticulum, golgi complex, lysosomes, vacuoles, peroxisomes, mitochondria, chloroplast, cytoskeleton, microtubules, nucleus, extracellular matrix etc.

UNIT 5 CELL CYCLE AND APOTOSIS **9 Hrs.**

Cell cycle - An overview of cell cycle; Components of cell cycle control system; Intracellular and Extra-cellular control of cell division, Programmed cell death (Apoptosis), intrinsic & extrinsic pathways of cell death, Apoptosis in relation with Cancer, Viral disease (AIDS) & Organ transplant

Max.45 Hrs.

COURSE OUTCOMES

On completion of the course, student will be able to

CO1 - Familiar with overview and scope of microbiology.
CO2 - Explore the systemic classification of microbes.
CO3 - Study the methods for cultivation of organisms.
CO4 - Understand the basic principles of cellular components.
CO5 - Study the cell cycle principle.
CO6 - Understand the application of microbiology and cell biology in biotechnology.

TEXT / REFERENCE BOOKS

1. Berg, Jeremy M., John L. Tymoczko, Lubert Stryer, J.M. Berg, J.L. Tymoczko and L. Stryer, Biochemistry International version, 2002.
2. Nelson D.L., Lehninger A.L. & Cox, M.M., Lehninger principles of Biochemistry, Macmillan, 2008.
3. Moat A.G., Foster J.W. & Spector, M. P. (Eds.), Microbial Physiology, John Wiley & Sons, 2003.
4. Alberts, Bruce, Dennis Bray, Karen Hopkin, Alexander D. Johnson, Julian Lewis, Martin Raff, Keith Roberts and Peter Walter. Essential Cell Biology, Garland Science, 2015.
5. Karp G., Cell and Molecular Biology: concepts and experiments, John Wiley & Sons, 2009.
6. Robertis De., Cell and Molecular Biology, 1987.
7. Lodish H., Berk A., Darnell J.E., Kaiser C.A., Krieger M., Scott M.P., Bretscher A., Ploegh H. and Matsudaira P., Molecular Cell Biology, Macmillan, 2008.

UNIT: II
MICROBIAL NUTRITION, GROWTH AND METABOLISM

NUTRITIONAL REQUIREMENT OF BACTERIA

- Nutrition is substances used in biosynthesis and energy production and therefore are required for all living things.
- Bacteria, like all living cells, require energy and nutrients to build proteins and structural membranes and drive biochemical processes.
- Bacteria require sources of carbon, nitrogen, phosphorous, iron and a large number of other molecules.
- Carbon, nitrogen, and water are used in the highest quantities.
- The nutritional requirements for bacteria can be grouped according to the carbon source and the energy source.
- Some types of bacteria must consume pre-formed organic molecules to obtain energy, while other bacteria can generate their own energy from inorganic sources.

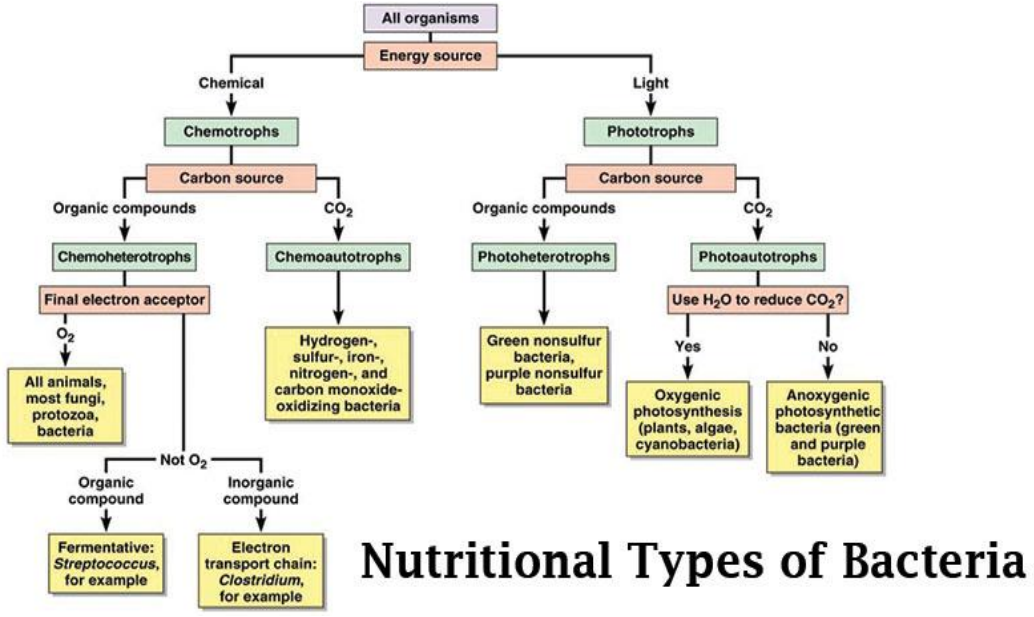


Fig: 2.1 Nutritional types of bacteria

Nutritional Types of Bacteria

On the basis of **energy source** organisms are designated as:

Phototrophs:

- The organisms which can utilize light as an energy source are known as phototrophs. These bacteria gain energy from light.

Chemotrophs:

- These bacteria gain energy from chemical compounds. They cannot carry out photosynthesis.

On the basis of **electron source** organisms are designated as:

Lithotrophs:

- Some organisms can use reduced organic compounds as electron donors and are termed as Lithotrophs.
- They can be Chemolithotrophs and Photolithotrophs

Organotrophs:

- Some organisms can use organic compounds as electron donors and are termed as organotrophs.
- Some can be Chemoorganotrophs and Photoorganotrophs.

Thus, bacteria may be either:

- **Photo-lithotrops:** These bacteria gain energy from light and use reduced inorganic compounds such as H_2S as a source of electrons. eg: *Chromatium okeinii*.
- **Photo-organotrophs:** These bacteria gain energy from light an d use organic compounds such as Succinate as a source of electrons.eg; *Rhodospirillum*.
- **Chemo-lithotrophs:** These bacteria gain energy from reduced inorganic compounds such as NH3 as a source of electron eg; *Nitrosomonas*.
- **Chemo-organotrophs:** These bacteria gain energy from organic compounds such as glucose and ammino acids as a source of electrons.eg; *Pseudomonas pseudoflora*.
- Some bacteria can live ether chemo-lithotrophs or chemo-organotrophs like *Pseudomonas pseudoflora* as they can use either glucose or H2S as electron source.

On the basis of **carbon source** bacteria may be:

- All organisms require carbon in some form for use in synthesizing cell components.
- All organisms require at least a small amount of CO2.
- However, some can use CO2 as their major or even sole source of carbon; such organisms are termed as Autotrophs (Autotrophic bacteria).
- Others require organic compounds as their carbon source and are known as Heterotrophs (Heterotrophic bacteria).

Autotrophic Bacteria

These bacteria synthesize all their food from inorganic substances (H2O, C02, H2S salts).

The autotrophic bacteria are of two types:

(i) Photoautotrophs

- These bacteria capture the energy of sunlight and transform it into the chemical energy.
- In this process, CO2 is reduced to carbohydrates.
- The hydrogen donor is water and the process produce free oxygen.
- Photoautotroph has Chlorophyll pigment in the cell and its main function is to capture sunlight e.g., *Cyanobacteria*.
- Some photoautotrophic bacteria are anaerobes and have bacteriochlorophyll and bacteriovirdin pigments respectively.

Purple Sulphur Bacteria:

These bacteria have the pigment bacteriochlorophyll located on the intracytoplasmic membrane i.e., thylakoids. These bacteria obtain energy from sulfur compounds e.g., *Chromatiiun. Theopedia rosea, Thiospirilium*.

Green Sulphur Bacteria:

These bacteria use hydrogen sulfide (H2S) as hydrogen donor. The reaction takes place in the presence of light and pigment termed as bacteriovirdin or bacteriopheophytin or chlorobium chlorophyll e.g., *Chlorobium limicola, Chlorobacterium* etc.

These bacteria take hydrogen from inorganic sources like sulphides and thiosulphates. Therefore, these bacteria are also known as photolithographs.

(ii) Chemoautotrophs

- These bacteria do not require light (lack the light phase but have the dark phase of photosynthesis) and pigment for their nutrition.
- These bacteria oxidize certain inorganic substances with the help of atmospheric oxygen.
- This reaction releases the energy (exothermic) which is used to drive the synthetic processes of the cell.

Sulphomonas (Sulphur bacteria):

These bacteria obtain energy by oxidation of elemental sulphur or H2S, e.g., *Thiobacillus, Beggiatoa*.

- Elemental Sulphur Oxidising Bacteria: Denitrifying sulphur bacteria oxidize elemental sulphur to sulphuric acid e.g., *Thiobacillus denitrificans*
  $2S + 2H2O + 3O2 \rightarrow 2H2SO4 + 126$ kcal.
- Sulphide Oxidizing Bacteria: These bacteria oxidizes H2S and release the sulphur e.g., *Beggiatoa*.
  $2H2S + 4O2 \rightarrow 2H2O + 2S + 141.8$ cal

Heterotrophic Bacteria

- The heterotrophic bacteria obtain their-ready made food from organic substances, living or dead.

- Most of pathogenic bacteria of human beings, other plants and animals are heterotrophs.
- Some heterotrops have simple nutritional requirement while some of them require large amount of vitamin and other growth promoting substance. Such organisms are called fastidious heterotrophs.
- Heterotrophic bacteria are of three types:

a. Photoheterotrophs

- These bacteria can utilize light energy but cannot use CO2 as their sole source of carbon.
- They obtain energy from organic compounds to satisfy their carbon and electron requirements. Bacteriochlorophyll pigment is found in these bacteria.
- g., Purple non-sulphur bacteria (*Rhodospirillum*, *Rhodomicrobium*, *Rhodopseudomonas palustris*).

b. Chemoheterotrophs

- Chemoheterotrophs obtain both carbon and energy from organic compounds such as carbohydrates, lipids and proteins. Glucose or Monosaccharide [(CH2O)n] + O2 → CO2 + H2O + Energy

There are three main categories that differ in how chemohetrotrophs obtain their organic nutrients:

(i) Saprophytic bacteria.
(ii) Parasitic bacteria.
(iii) Symbiotic bacteria.

i) Saprophytic bacteria

- Saprophytic bacteria obtain their food from the dead and organic decaying matter such as leaves, fruits, vegetables, meat, animal feces, leather, humus etc.
- These bacteria secrete enzymes to digest the food and absorb it.
- The enzymes secreted to break down the complex compounds such as carbohydrate and protein, into simpler soluble compounds, which are easily absorbed.
- Examples are *Bacillus* mycoides, *B.* ramosus, *Acetobacter* etc.

ii) Parasitic bacteria

- These bacteria obtain their nutrition from the tissues of the hosts on which they grow.
- They may be harmless or may cause serious diseases.
- Parasitic bacteria which cause various diseases in plants and animals are known as pathogens, e.g., *Bacillus* typhosus, *B.* anthracis, *B.tetani. B.diplheriae, B.tuberculosis*, *B.* pneumoniae, *Vibrio* cholerae, *Pseudomonas* citri etc.

iii) Symbiotic bacteria

- Symbiotic bacteria live in close association with other organisms as symbionts.
- They are beneficial to the organisms.
- The common examples are the nitrogen-fixing bacteria, e.g., *Bacillus* radicicola, *B.* azotobacter, *Rhizobium*, *Clostridium*, *Rhizobium spp.*, *B.* radicicolaand *B.* azotobacter.
- These bacteria live inside the roots of leguminous plants.
- These bacteria fix free atmospheric nitrogen into nitrogenous compounds which are utilized by the plants. In return, the plant provides nutrients and protection to the bacteria.

TYPES OF CULTURE MEDIA

Culture media contain nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can't grow in any known culture medium.
Organisms that cannot grow in artificial culture medium are known as obligate parasites. *Mycobacterium leprae*, *rickettsias*, *Chlamydias*, and *Treponema pallidum* are obligate parasites. Bacterial culture media can be classified on the basis of composition, consistency and purpose.

Classification of bacterial culture media on the basis of consistency

1. **Solid medium**

   Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.
2. **Semisolid media**
   Semisolid media are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.
3. **Liquid (Broth) medium**

   These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes

such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth.

Classification of culture media on the basis of composition

1. **Synthetic or chemically defined medium**
   A chemically defined medium is one prepared from purified ingredients and therefore its exact composition is known.
2. **Non synthetic or chemically undefined medium**

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

Synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms.

Classification of Bacterial Culture media on the basis of purpose/ functional use/ application

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1. **General purpose media/ Basic media**

   Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar (NA) are considered as basal medium. These media are generally used for the primary isolation of microorganisms.


Fig: 2.2. Nutrient Agar

3. . Enriched medium (Added growth factors):

Fig: 2.3. Blood Agar

Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. Chocolate agar is also known as heated blood agar or lysed blood agar.

3. Selective and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

a. **Selective medium**

Principle: Differential growth suppression
Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated.

Examples of selective media include:

1. Thayer Martin Agar used to recover *Neisseria gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin.
2. Mannitol Salt Agar and Salt Milk Agar used to recover *S.aureus* contains 10% NaCl.
3. Potassium tellurite medium used to recover *C.diphtheriae* contains 0.04% potassium tellurite.
4. MacConkey's Agar used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.
5. Pseudosel Agar (Cetrimide Agar) used to recover *P. aeruginosa* contains cetrimide (antiseptic agent).
6. Crystal Violet Blood Agar used to recover *S. pyogenes* contains 0.0002% crystal violet.
7. Lowenstein Jensen Medium used to recover *M.tuberculosis* is made selective by incorporating malachite green.
8. Wilson and Blair's Agar for recovering *S. typhi* is rendered selective by the addition of dye brilliant green.
9. Selective media such as TCBS Agar used for isolating *V. cholerae* from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

Fig: 2.4. MacConkey's Agar

b. **Enrichment culture medium**

Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as broth medium. Enrichment media are liquid media that also serves to inhibit commensals in the clinical

specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to recover pathogens from fecal specimens.

4. **Differential/ indicator medium: differential appearance:**

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.
Examples of differential media include:

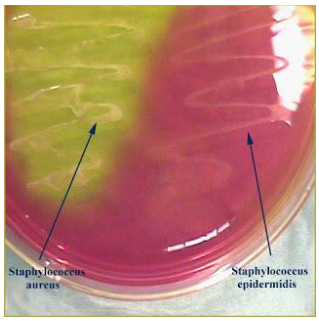


Fig: 2.5 Mannitol salts agar

1. Mannitol salts agar (mannitol fermentation = yellow)
2. Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis)
3. Mac Conkey agar (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies.
4. TCBS (*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose)

5. **Transport media:**

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) aresemi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.

- Cary Blair transport medium and Venkatraman Ramakrishnan (VR) medium are used to transport feces from suspected cholera patients.

- Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery.
- Pike's medium is used to transport streptococci from throat specimens.

6. Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.


Fig: 2.6 Anaerobic Media

Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Robertson Cooked Meat (RCM) medium that is commonly used to grow *Clostridium* spps contains a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.

Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colorless.

7. Assay media

These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

Other types of medium includes;

- Media for enumeration of Bacteria,

- Media for characterization of Bacteria,
- Maintenance media etc.

GROWTH CURVE OF BACTERIA

Growth curve of bacteria is a standard curve which consists of four distinct phases like **log**, **lag**, **stationary** and **death phase** which shows a **Sigmoid growth**. The Growth of bacteria and other organisms is simply referred to as the increase in cell number, cell size and cell mass. The growth of organism influences by many factors like temperature, pH, oxygen requirement, nutrients availability, moisture content etc. Bacteria are the prokaryotic or unicellular organism that most frequently grows by the binary fission.

Phases of the Growth Curve

The different phases of the bacteria during its growth cycle at the given time interval refer to the growth curve of bacteria. The growth curve of bacteria can be

obtained by the following protocol:

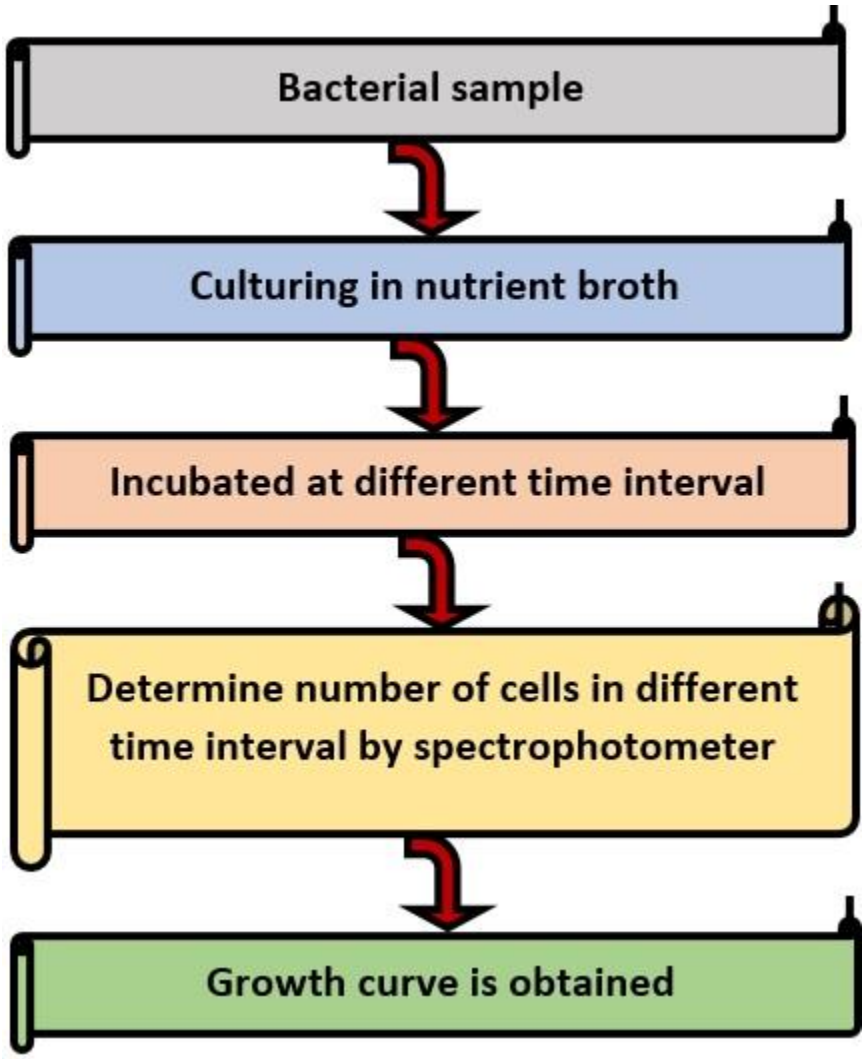


Fig: 2.7. Protocol for Growth curve study

First, take the sample of bacteria.

Then inoculate it into the fresh culture medium (nutrient broth) that contains all the nutrients for the growth.

Then incubate the bacteria for at time intervals and determine the count of bacterial cells at that given time interval by the use of a spectrophotometer.

After noting down the readings a standard graph is prepared between the numbers of bacterial cell vs time interval

And in this way, a standard growth curve is obtained.

There are distinct four phases of the growth curve of bacteria.

- Lag phase
- Log phase
- Stationary phase
- Death phase

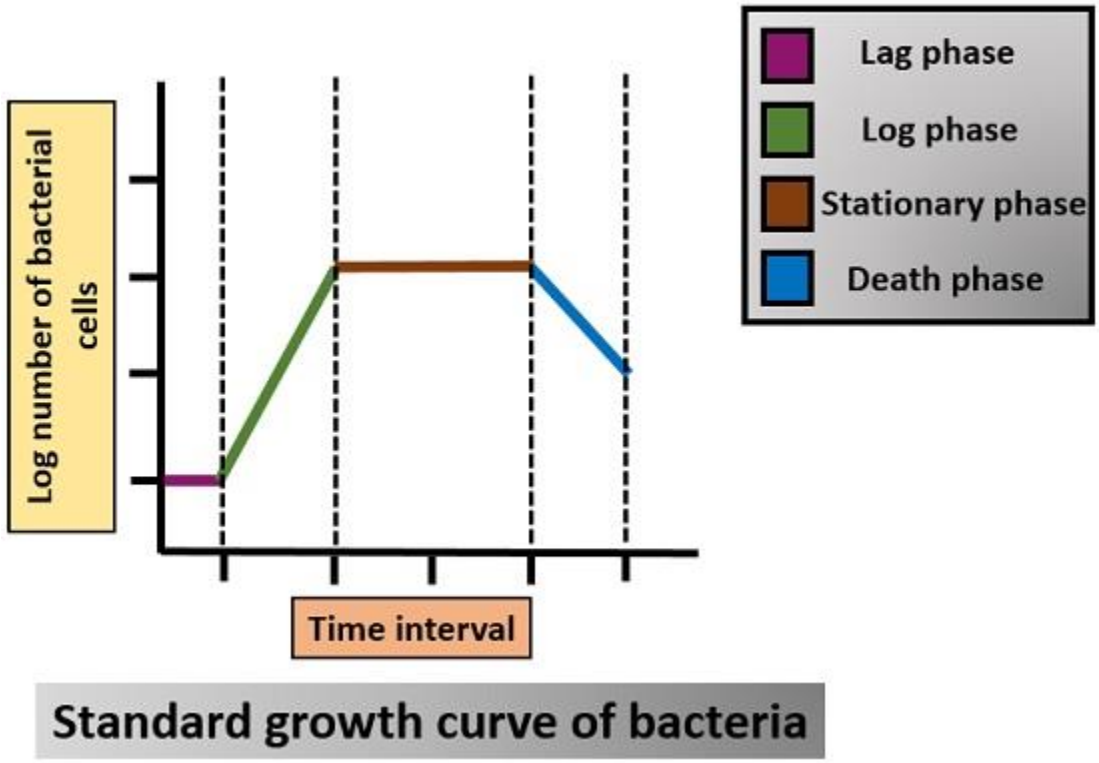


Fig: 2.8 Standard growth curve of bacteria

Lag Phase

This phase is the "**Adaptation** or **Adjustment phase**", where microorganisms adapt themselves to the new environment of the growth medium. Microorganisms retain in this phase for a short period of time (1hr- several days). In this, there is no increase in cell number. The microorganisms only grow in size. In the lag phase, microorganisms release some metabolites:

For adaptation.

To restore the spent material.

No increase in cell, when cells are introduced into fresh media Reasons

(a) Cells may be depleted of a variety of factors that may need to be resynthesized
(b) Medium may be different that previous one and thus new enzymes may be needed for growth

(c) Cells may be injured and need time to recover

Log Phase

This phase also refers to "**Exponential** or **Logarithmic phase**". This phase shows an intense metabolic activity of microorganisms by producing primary metabolites. These metabolites enhance the growth rate i.e. an increase in cell number as well as cell size. It is a period of rapid growth. In this phase, microorganisms are resistant to adverse conditions:

Antibiotic

Radiation

Here, the number of cells produced > the number of cells dying.

Stationary Phase

This phase also refers to "**Stagnant phase**". During this phase, population size is in a state of equilibrium. Cell division begins to slow down. Overall, there is no increase in cell number and cell mass. Factors that slow down the process of cell division can be due to:

- Limited nutrient availability
- Accumulation of toxic product
- An acidic PH of media
- Low oxygen availability

Here, the number of cells produced = the number of cells dying.

Death Phase

This phase also refers to "**Decline phase**", which is the last stage of the growth curve.

In this, the population size decreases at the logarithmic rate.

Death phase is characterized by the loosing of cell division ability of microorganisms.

Here, the number of cells produced < the number of cells dying.

To mathematically express the growth of bacteria, there is a relationship that exists between the initial number of cells present in the log phase and the final number of cells present after the log phase in the cell culture which can be expressed as:

$\frac{dN}{dt} \propto N$

$\frac{dN}{dt} = kN$

k = growth rate
- constant
- rate of cell growth

$\frac{dN}{N} = k\,dt$

$\int$ = Limits $N_0 \rightarrow N_t$

$t_0 \rightarrow t_t$

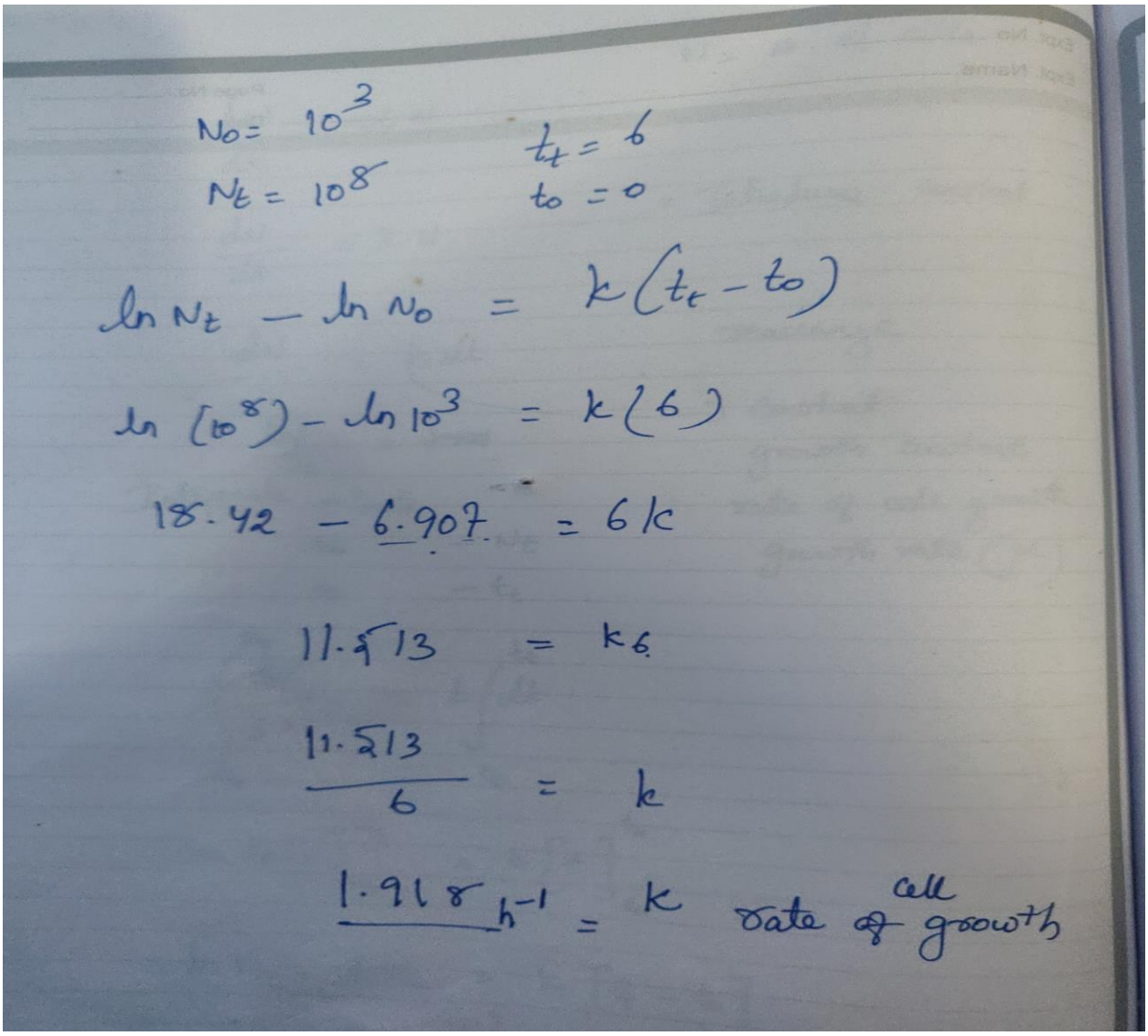
$$\int_{N_0}^{N_t} \frac{dN}{N} = k \int_{t_0}^{t_t} dt$$

$$\ln [N]_{N_0}^{N_t} = k [t]_{t_0}^{t_t}$$

$$\ln N_t - \ln N_0 = k [t_t - t_0]$$

where N_0 – No. of cells (initially) at t_0

N_t = No of cells at time t_t



Important terms to remember:

Generation time: It defines the total time that is required by the bacteria to double its population. Generation time denotes by 'g'. Mathematically it is expressed as:

g =t/n

where **t**: Time is taken by the individual bacterium for the cell division into two. And **n**: Number of generations.

Growth rate: It defines the number of generation of bacteria per hour. Growth rate denotes by 'R'. Mathematically it is expressed as:

R=n/t

Where, **n**: Number of generation
And **t**: cell division of bacteria per hour.

Measurement of microbial growth

A. Total cell number by direct counting

1. Counting chambers (Fig. 6-4):

Special slides with a chamber that holds a known volume and contains an etched grid in the bottom for counting microbes are counted and normalized per ml based on the chamber volume.

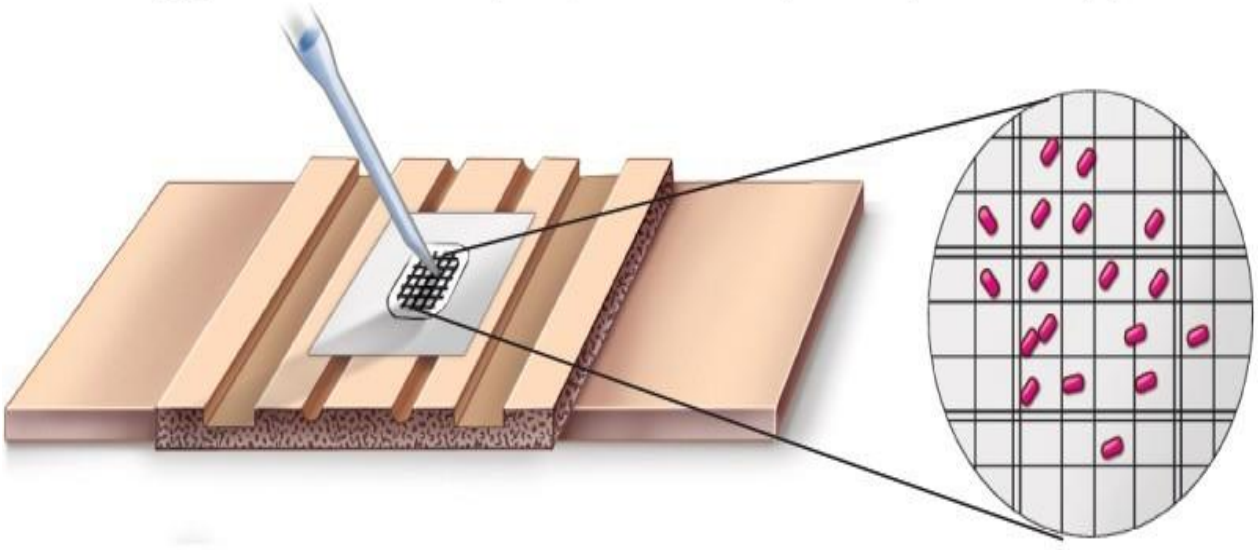


Fig: 2.9. Counting chamber

a) Pros: Easy, inexpensive, and quick

b) Cons: Cannot tell live from dead; need population >106/ml; precision is difficult to achieve due to small sample

2. Coulter counter:

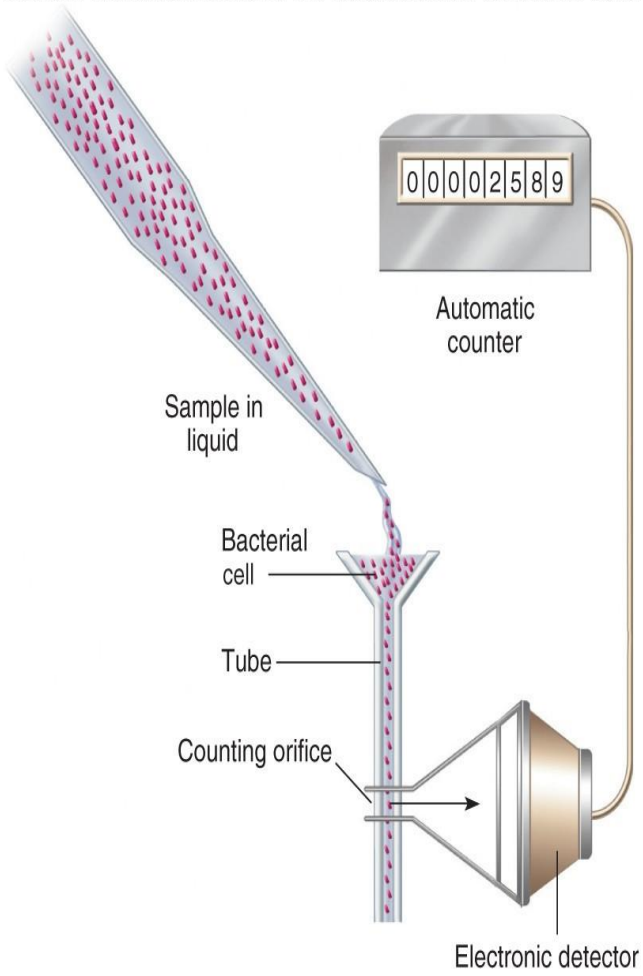


Fig: 2.10. Coulter counter

- Such as Coulter counter
- microbial suspension forced through small hole or orifice
- movement of microbe through orifice impacts electric current that flows through orifice
- cannot distinguish living from dead cells
- quick and easy to use

a) Pros: Easy, inexpensive, and quick

b) Cons: Cannot tell live from dead; only for larger microbes

3. Filter and stain systems (Fig. 6-7, 8):

Filter aliquot of a sample through a membrane filter which retains the bacteria bacteria on filter are stained with fluorescent dye bacteria are then counted using a fluorescence microscope (some stains can differentiate live from dead)

B. Viable cell counting techniques

1. Plate aliquot of liquid culture on solid media count colony forming units (CFU). Assumption is that each cell in the aliquot can form one CFU on the solid media.

a) Pros: Easy; high sensitivity

b) Cons: Have to do several dilutions, many plates; Need correct media; clumps of cells will only give one CFU

c) Calculation of colonies in sample:

colony forming units (ml plated) (dilution plated)

= total CFU/ml ~ total bacteria /ml

2. Filter techniques:

Filter aliquot of a sample through a membrane filter which retains the bacteria filter is placed on agar medium each cell grows into a colony that can be counted This technique is frequently used to sample water supplies; agar medium that filter is placed on can be selective for certain kinds of bacteria.

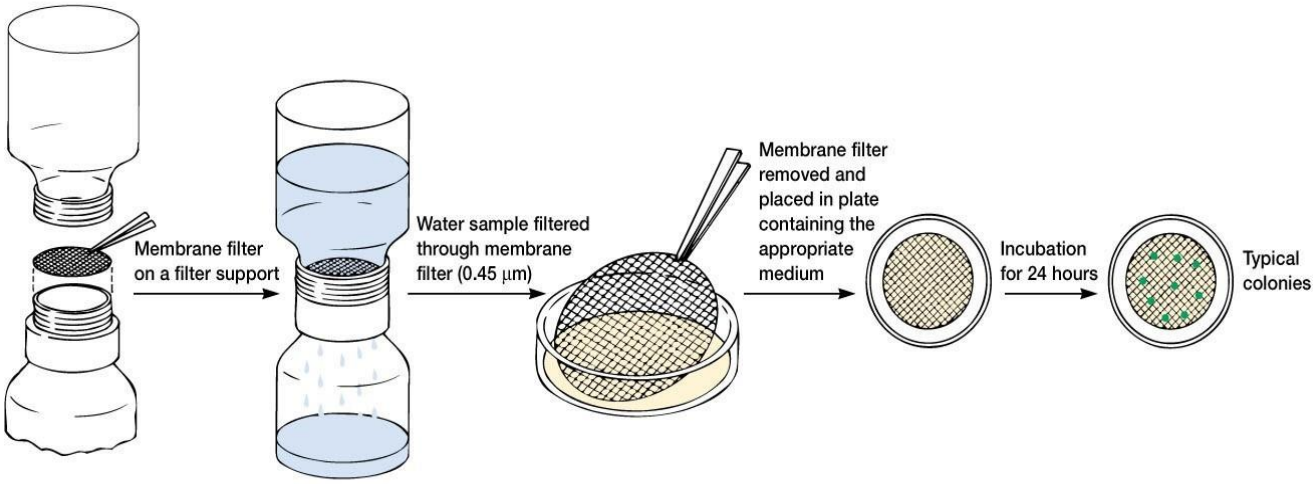


Fig: 2.11 Membrane filter technique for measurement of growth

C. Measurement of cell mass

Increase in cell number leads to an increase in cell mass and can be considered as the marker of population growth.

Methods- 1. Dry weight-

Cells grown in liquid media are centrifuged, dried in the oven and weighed

Good for filamentous fungi.

Disadvantages

a. time consuming and not sensitive

b.bacteria being small several hundred milliliter of culture required

2. Turbidity measurement using spectrophotometer

a) The spectrophotometer measures the turbidity of a sample and generates a value called optical density (OD).

b) Turbidity is a measure of the light absorption by particles in a sample (i.e. microbes in media). Within limits, the light absorbed by a sample is proportional to the concentration of light absorbing materials.

c) Since the OD of a culture increases as the number of organisms increases in a culture, the OD can be used to indirectly calculate the number of microbes. d) Pros: very easy once relationship between # of cells and OD reading is determined for an organism

e) Cons: Samples must contain at least 107 – 109 bacteria per ml; does not indicate whether cells are viable

3. Measurements of cell components

a) Determination of nitrogen content

The major constituent of cell material is protein, and since nitrogen is a characteristic part of proteins, one can measure a bacterial population or cell crop in terms of bacterial nitrogen.

Bacteria average approximately 14 percent nitrogen on a dry weight basis, although this figure is subject to some variation introduced by changes in culture conditions or differences between species**.**

To measure growth by this technique, you must first harvest the cells and wash them free of medium and then perform a quantitative chemical analysis for nitrogen.

Bacterial nitrogen determinations are somewhat laborious and can be performed only on specimens free of all other sources of nitrogen.

Furthermore, the method is applicable only for concentrated populations. For these and other reasons, this procedure is used primarily in research**.**

III. Continuous culture

A. Maintenance of a culture in constant environmental conditions through continual provision of nutrients and removal of wastes.

Useful for:

1. Study in a certain growth phase

2. Study under low nutrient concentrations

3. Evolution studies

B. The chemostat

1. Apparatus that feeds sterile media into a culture at the same rate in which it is removed

2. Essential nutrient is limiting so that flow rate determines growth rate

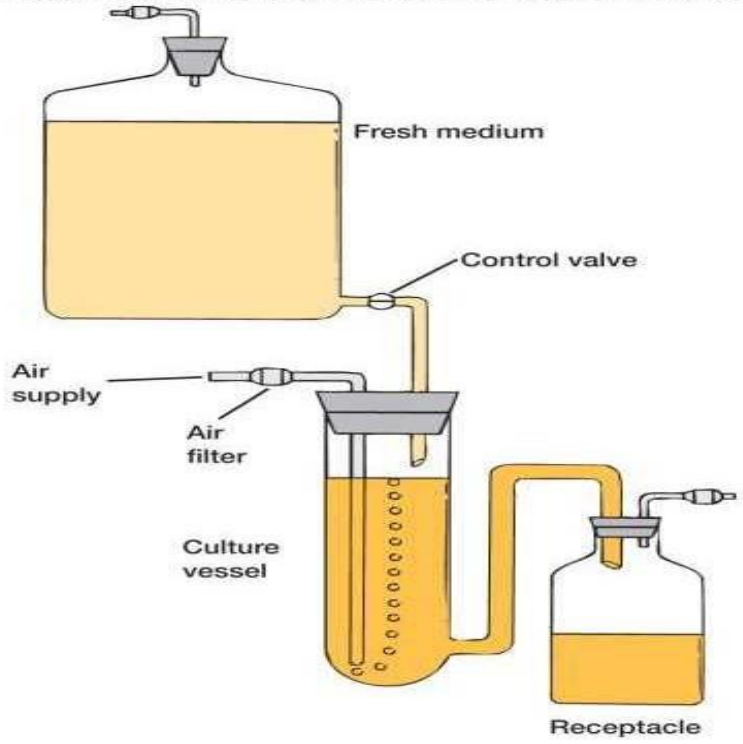


Fig: 2.12 Chemostat

C. Turbidostat

1. Flow rate into the system is adjusted to maintain preset turbidity (cell density).

2. No limiting nutrient

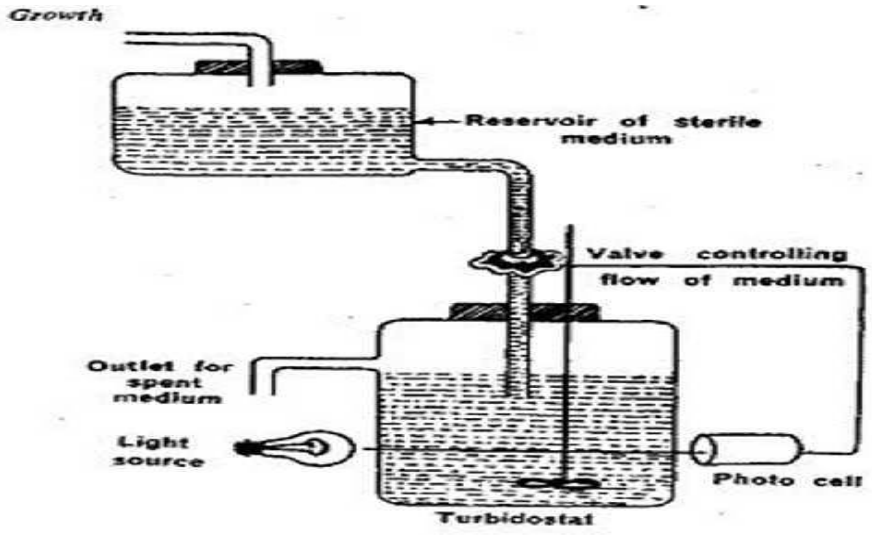


Fig: **2.13 Turbidostat**

AEROBIC AND ANAEROBIC BIOENERGETICS IN BACTERIA

Respiration is of two types, aerobic respiration, and anaerobic respiration.

Aerobic Respiration: It is the process of cellular respiration that takes place in the presence of oxygen gas to produce energy from food. This type of respiration is common in most of the plants and animals, birds, humans, and other mammals. In this process, water and carbon dioxide are produced as end products.

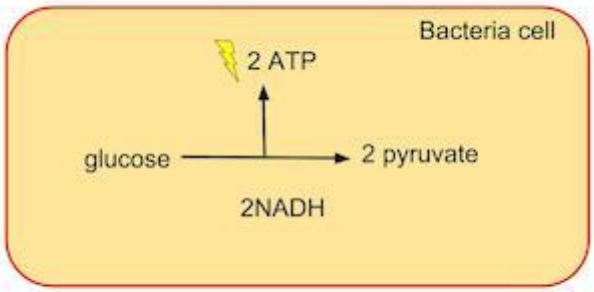
Anaerobic Respiration: It is a process which takes place in the absence of oxygen gas. In this process, the energy is obtained by the breakdown of glucose in the absence of oxygen. One of the best examples of anaerobic respiration is the process of fermentation in yeast.

Aerobic Respiration Diagram

Aerobic respiration makes more energy and takes place in the cytoplasm and the cell wall of bacteria. It includes 3 steps

1. Glycolysis

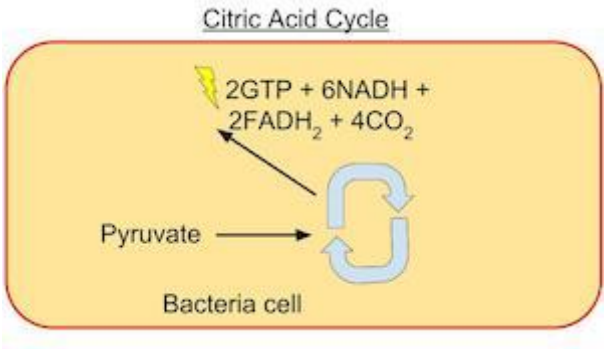
The first step in aerobic respiration is glycolysis, which occurs in the cytoplasm. In glycolysis, glucose in the cell is broken down. In the process, electrons from the original glucose molecule are released and collected by electron carrier molecules called NAD+, which, once they have the electron, are made into NADH. This step makes a little energy, called ATP, but not much. The broken down glucose molecule that results is called pyruvate, which is needed for the next step.



Glycolysis in bacteria

Citric Acid Cycle

The next step is the citric acid cycle, where pyruvate goes through a series of chemical reactions and is converted to other molecules. This happens in the mitochondria of eukaryotic cells, but bacteria don't have mitochondria, so we're still in the cytoplasm. This process releases more electrons, and electron carrier molecules NAD+ and FADH rush in to grab them, which turns them into NADH and FADH2. This step also makes a little ATP (but not a lot) and releases carbon dioxide.



Citric acid cycle in bacteria

Oxidative Phosphorylation

After the citric acid cycle, the cell gathers all its electron carriers to dump their electrons off at the plasma membrane in a process called oxidative phosphorylation, the last step of respiration. This area of the plasma membrane is called the mesosome and has lots of folds to give the cell more space to do respiration. The electron carriers unload their electrons one at a time into a chain of proteins, called the electron transport chain.

Each protein in the chain likes electrons more than the protein before it, so the electrons keep moving towards the next protein. As each protein gets the electrons, they pump hydrogen ions into the periplasm (the space between the membrane and the cell wall). The cell wall acts as an outer barrier, allowing the hydrogen ions to build up.

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This high H+ in the periplasmic space creates a potential difference in the cytoplasm and periplasmic space, which will create a proton motive force. So the H+ ion move through the membrane found Adenosine triphosphate (ATP) synthases are multi-subunit protein complexes that use an electrochemical proton

motive force across a membrane to make the cell's supply of ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi).

Aerobic respiration is the process of utilisation of oxygen to breakdown glucose, amino acids, fatty acids to produce ATP.

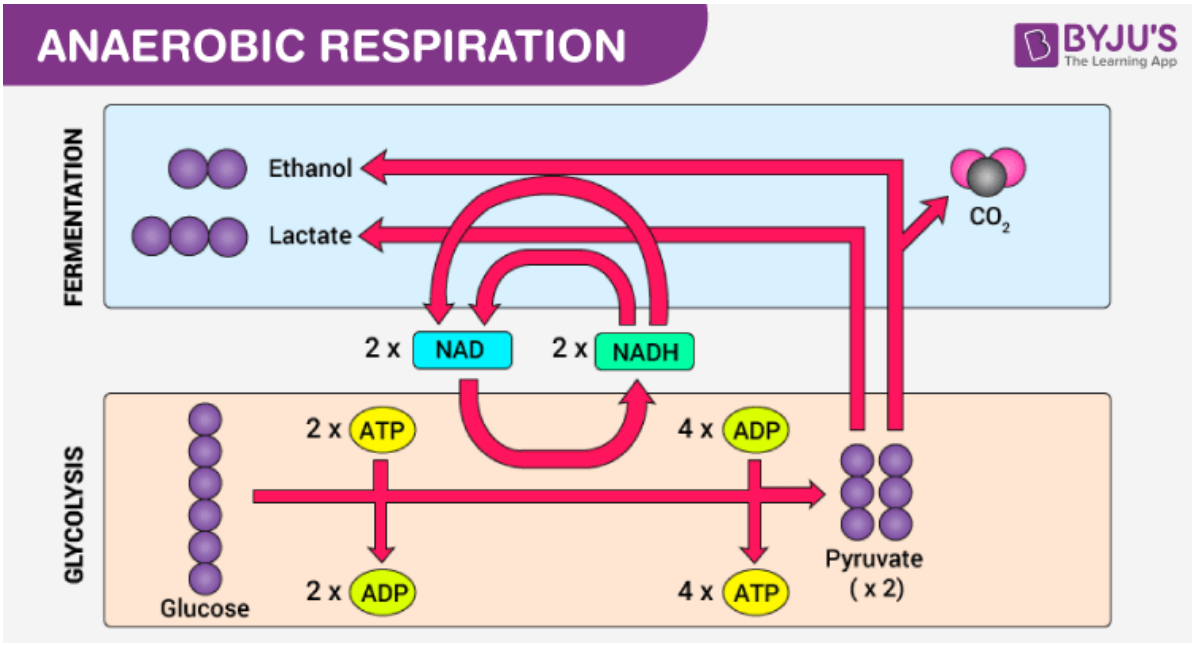
The pyruvate is then converted into acetyl CoA in the mitochondrial matrix.

The Kreb's cycle occurs twice per glucose molecule.

The protein complexes are arranged on the inner mitochondrial matrix so that the electrons pass from one reacting molecule to the other. This is known as the electron transport chain.

ATP synthase produces ATP from ADP and inorganic phosphate

Anaerobic respiration



Anaerobic means "without air". Therefore, this type of cellular respiration does not use oxygen to produce energy. Sometimes there is not enough oxygen around for some organisms to respire, but they still need the energy to survive. Due to

lack of oxygen, they carry out respiration in the absence of oxygen to produce the energy they require, which is referred to as anaerobic respiration. Anaerobic respiration usually occurs in lower plants and microorganisms. In the absence of oxygen, the glucose derived from food is broken down into alcohol and carbon dioxide along with the production of energy.

Glucose → Alcohol + Carbon dioxide + Energy

Glucose → Lactic acid + Energy

Anaerobic respiration produces a relatively lesser amount of energy as compared to aerobic respiration, as glucose is not completely broken down in the absence of oxygen.

Fermentation – Anaerobic Respiration

Anaerobic respiration is a type of cellular respiration where respiration takes place in the absence of oxygen. Fermentation is an anaerobic pathway- a common pathway in the majority of prokaryotes and unicellular eukaryotes. In this process, glucose is partially oxidised to form acids and alcohol.

In organisms like yeast, the pyruvic acid formed by partial oxidation of glucose is converted to ethanol and carbon dioxide (CO_2). This anaerobic condition is called alcoholic or ethanol fermentation. The whole reaction is catalyzed by the enzymes, pyruvic acid decarboxylase and alcohol dehydrogenase. In certain bacteria and animal muscle cells, under anaerobic conditions, the pyruvic acid is reduced to lactic acid by lactate dehydrogenase. This is called lactic acid fermentation. The end products of these anaerobic pathways make them hazardous processes. For example, a concentration of alcohol above 13 percent produced by yeast cells could kill themselves.

In the alcoholic and lactic acid fermentation, $NADH+H^+$ is the reducing agent which is oxidized to NAD^+. The energy released in both the processes is not much and the total sum of ATP molecules produced during fermentation is two, which is very less as compared to aerobic respiration. However, this is commercially employed in the food and beverage industries, and pharmaceutical industries.

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLGOY

UNIT: I

MICROBIOLOGY AND CELL BIOLOGY-SBTA1103

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY SCHOOL OF BIO AND CHEMICAL ENGINEERING

SBTA1101	MICROBIOLOGY AND CELL BIOLOGY	L	T	P	Credits	Total Marks
		3	*	0	3	100

COURSE OBJECTIVE

- The course aims to develop skills of the Students in the area of Microbiology and Cell biology particularly to identify microbes, structure, metabolism and Cell Signaling pathways

UNIT 1 CLASSIFICATION AND MULTIPLICATION **9 Hrs.**

Overview of history of Microbiology- Classification of Microbes - Systems of classification, Numerical taxonomy, Identifying characters for classification, General properties and principles of classification of microorganisms Structural organization and multiplication of bacteria, viruses, algae and fungi.

UNIT 2 MICROBIAL NUTRITION, GROWTH AND METABOLISM **9 Hrs.**

Nutritional requirements of bacteria and different media used for bacterial culture; growth curve. Mathematical nature and expression of microbial growth and different methods to quantitate bacterial growth, aerobic and anaerobic bioenergetics and utilization of energy for biosynthesis of important molecules.

UNIT 3 CONTROL OF MICROORGANISMS **9 Hrs.**

Definition of sterilization, Physical and chemical control of microorganisms; host-microbe interactions; antibacterial, anti-fungal and anti-viral agents, mode of action and resistance to antibiotics; clinically important microorganisms.

UNIT 4 CELL ORGANELLES **9 Hrs.**

Evolution of cell; Cell as a unit of living organism, evolution and structure of prokaryotic cell, evolution of eukaryotic cell - Structural and functional features of eukaryotic cell: cell organelles; endoplasmic reticulum, golgi complex, lysosomes, vacuoles, peroxisomes, mitochondria, chloroplast, cytoskeleton, microtubules, nucleus, extracellular matrix etc.

UNIT 5 CELL CYCLE AND APOTOSIS **9 Hrs.**

Cell cycle - An overview of cell cycle; Components of cell cycle control system; Intracellular and Extra-cellular control of cell division, Programmed cell death (Apoptosis), intrinsic & extrinsic pathways of cell death, Apoptosis in relation with Cancer, Viral disease (AIDS) & Organ transplant

Max.45 Hrs.

COURSE OUTCOMES

On completion of the course, student will be able to

CO1 - Familiar with overview and scope of microbiology.
CO2 - Explore the systemic classification of microbes.
CO3 - Study the methods for cultivation of organisms.
CO4 - Understand the basic principles of cellular components.
CO5 - Study the cell cycle principle.
CO6 - Understand the application of microbiology and cell biology in biotechnology.

TEXT / REFERENCE BOOKS

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2. Nelson D.L., Lehninger A.L. & Cox, M.M., Lehninger principles of Biochemistry, Macmillan, 2008.
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5. Karp G., Cell and Molecular Biology: concepts and experiments, John Wiley & Sons, 2009.
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UNIT: 1

CLASSIFICATION AND MULTIPLICATION

A BRIEF HISTORY OF MICROBIOLOGY

Microbiology has had a long, rich history, initially centered in the causes of infectious diseases but now including practical applications of the science. Many individuals have made significant contributions to the development of microbiology.

Early history of microbiology. Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, and an English scientist named **Robert Hooke** made key observations. He is reputed to have observed strands of fungi among the specimens of cells he viewed. In the 1670s and the decades thereafter, a Dutch merchant named **Anton van Leeuwenhoek** made careful observations of microscopic organisms, which he called animalcules. Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria.

After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high. In those years, scientists debated the theory of spontaneous generation, which stated that microorganisms arise from lifeless matter such as beef broth. This theory was disputed by **Francesco Redi,** who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies. An English cleric named **John Needham** advanced spontaneous generation, but **Lazzaro Spallanzani** disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

Louis Pasteur and the germ theory. Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine “sick,” then perhaps they could cause human illness.

Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of swan-necked flasks filled with broth. He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would not), and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the germ theory of disease, which states that microorganisms are the causes of infectious disease.

Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist Robert Koch provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the

bacilli invariably caused anthrax. The procedures used by Koch came to be known as Koch's postulates (Figure). They provided a set of principles whereby other microorganisms could be related to other diseases.

The development of microbiology. In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a Golden Age of Microbiology during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.

Despite the advances in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after World War II, the antibiotics were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis, and many other diseases declined with the use of antibiotics.

Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the electron microscope was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

Modern microbiology. Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.

One of the major areas of applied microbiology is biotechnology. In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clotdissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

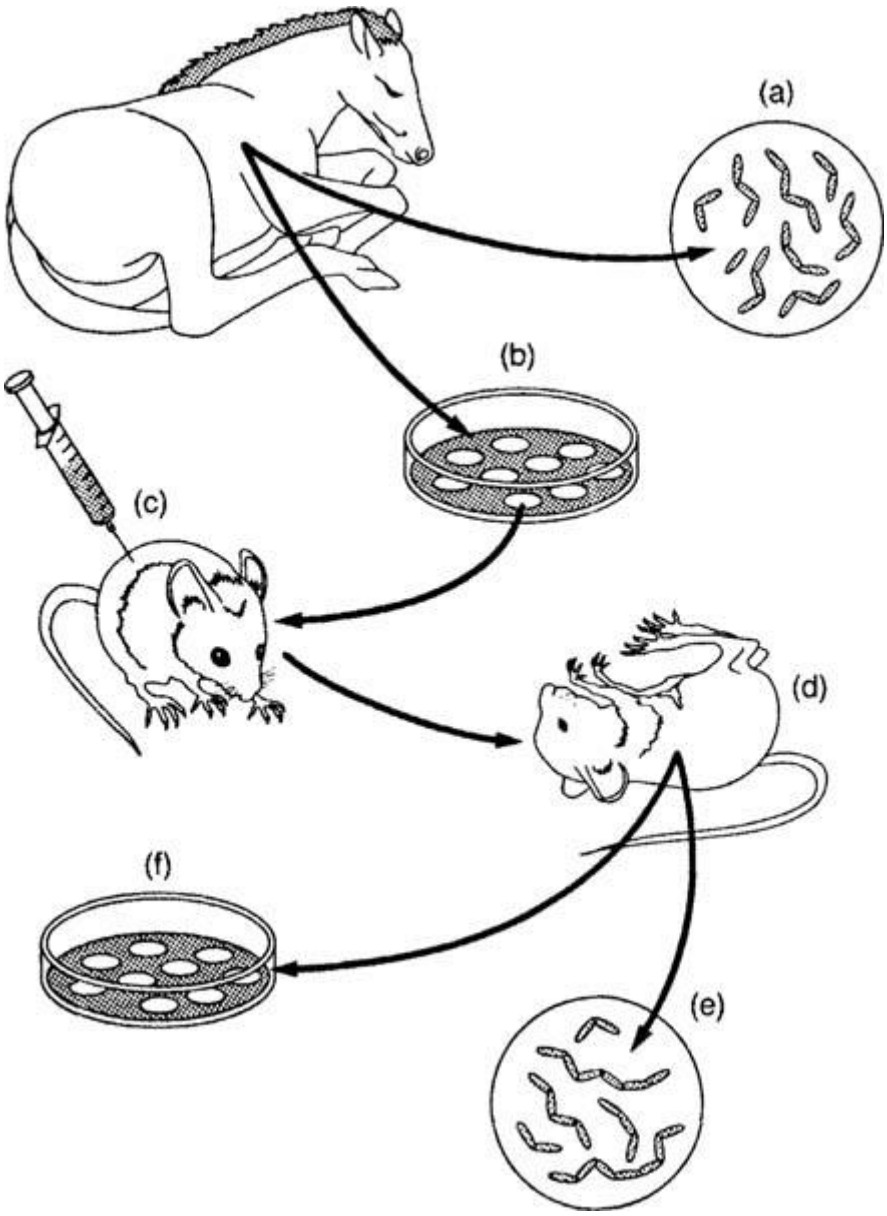

Fig: 1.1 Koch's Postulates

The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

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MICROBIAL CLASSIFICATION AND TAXONOMY

- Taxonomy
    - Science of biological classification
    - Consists of three separate but interrelated parts
        - Classification – arrangement of organisms into groups (taxa, sing.taxon)
        - Nomenclature – assignment of names to taxa
        - Identification – determination of taxon to which an isolate belongs

SYSTEMS OF CLASSIFICTION

A Swedish naturalist named **Carolus Linnaeus** is considered the **'Father of Taxonomy'** since 1700s*His two most important contributions to taxonomy were:

- A hierarchical classification system
- The system of **binomial nomenclature**

He proposed that there were three broad groups, called **kingdoms**, into which the whole of nature could fit.

These kingdoms were **animals, plants, and minerals.**

Binomial nomenclature meant naming species in 2 words : genus , followed by species.

1. TWO KINGDOM OF CLASSIFICATION

- The two kingdom classification system was given by **Carlous Linaaeus**

in 1758.

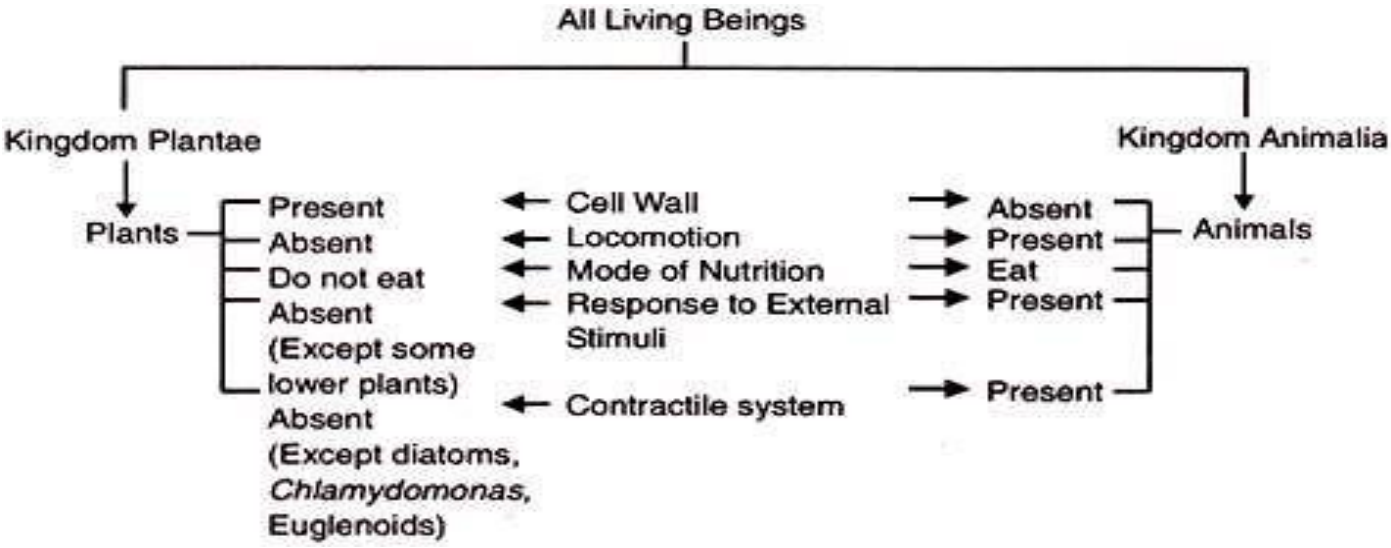

Fig: 1.2. Carlous Linaaeus Two kingdom of classification

2. THREE KINGDOM OF CLASSIFICAITON

Ernst Haeckel modified the 2 kingdom and gave the 3 kingdom of classification

Kingdom Animalia (Multicelluar)

Kingdom Plantae (Multicelluar)

Kingdom Protista (Unicelluar)

3. FOUR KINGDOM OF CLASSIFICATION

* The development of optic and electronic microscopy showed important differences in cells, mainly according to the presence or absence of distinct nucleus, leading *Édouard Chatton* to distinguish organisms in prokaryotes (without a distinct nucleus) and eukaryotes (with a distinct nucleus) in a paper from 1925.

Based on it, Copeland proposed a four-kingdom system, moving prokaryotic organisms, bacteria and "blue-green algae", into the kingdom Monera.

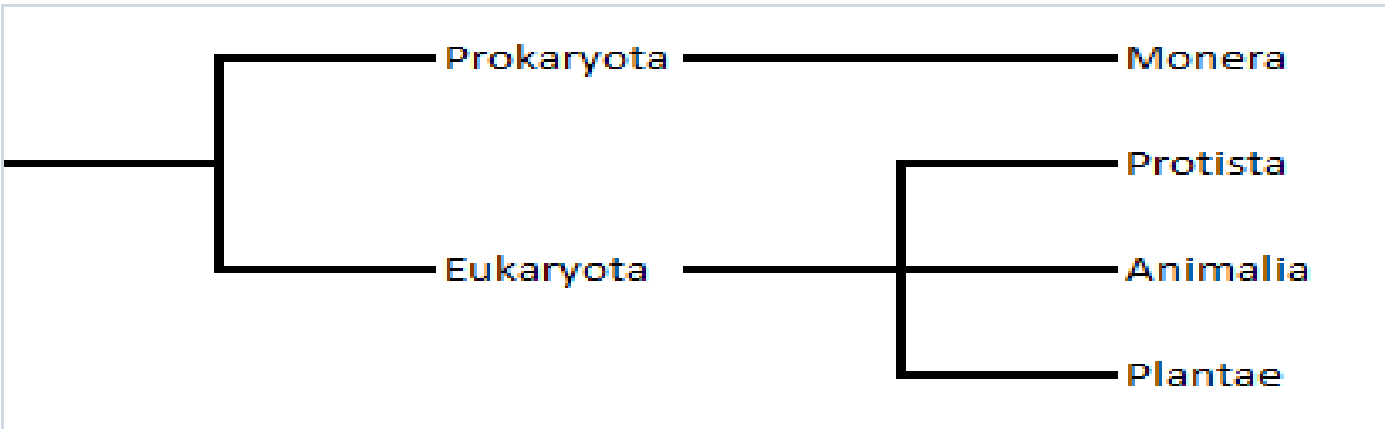

Fig: 1.3. Four Kingdom of classification

4. FIVE KINGDOM OF CLASSIFICATION

The position of fungi was not well established, oscillating between kingdoms Protista and Plantae.

So, in 1969, Robert Whittaker proposed a *fifth kingdom* to include

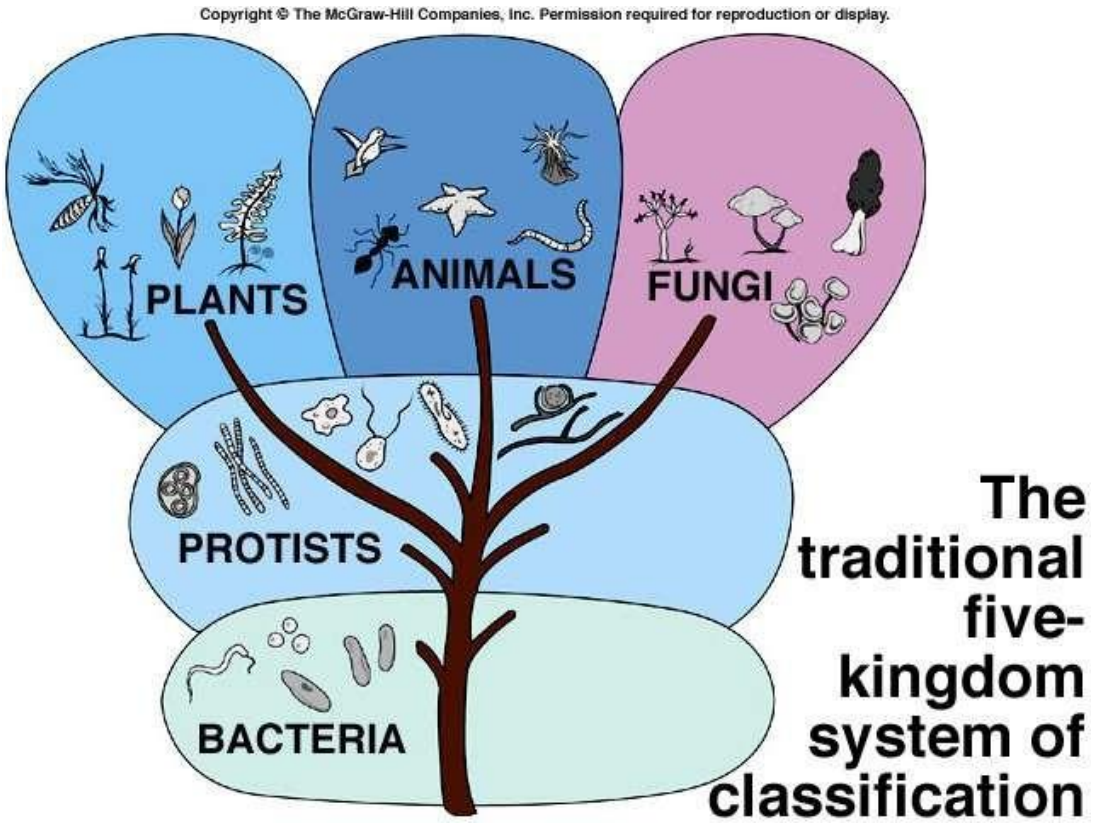

Fig: 1.4. Whittaker Fiver Kingdom

Property	Monera	Protista	Fungi	Plantae	Animalia
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cell organization	Mostly unicellular	Mostly unicellular	Multicellular and unicellular	Mostly Multicellular	Mostly Multicellular
Cell wall	Present in most	Present in some: absent in others	Present	Present	absent
Nutritional class	Phototrophic,heterotrophic or chemoautotrophic	Heterotrophic and phototrophic	Heterotrophic	phototrophic	Heterotrophic
Mode of nutrition	Absorptive	Absorptive or ingestive	Absorptive	Mostly Absorptive	Mostly ingestive
Motility	Motile or non motile	Motile or nonmotile	Nonmotile	Mostly nonmotile	Mostly Motile

Table: 1.1. Characters used for classification of organism in FIVE kingdom concept

5. THREE DOMAINS OF LIFE

The three-domain system is a biological classification introduced by Carl Woese in 1977 that divides cellular life forms into **archaea, bacteria, and eukaryote** domains.

In particular, it emphasizes the separation of prokaryotes into two

groups, originally called Eubacteria (now Bacteria) and Archaebacteria (now Archaea).

*Woese argued that, on the basis of differences in **16S rRNA genes**, these two groups and the eukaryotes each arose separately from *an ancestor with poorly developed genetic machinery, often called a progenote.*

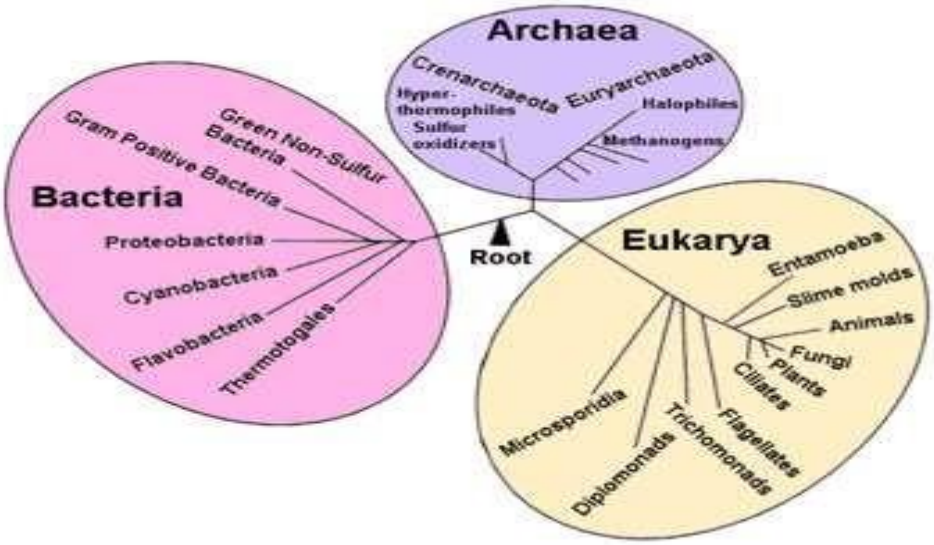

Fig: 1.5 Three Domains of Life

METHODS OF CLASSIFICATION

1.Natural Classification

Natural Classification arranges organisms into groups whose members share many characteristics

- First such classification in 18th century developed by Linnaeus based on anatomical characteristics
- this approach to classification does not necessarily provide information on evolutionary relatedness in microbes

2. Polyphasic classification

- Polyphasic Taxonomy is used to determine the genus and species of a newly discovered procaryote
- incorporates information from phenetic (phenotypic) and phylogenetic analysis

3. **Phenetic classification**
    - groups organisms together based on mutual similarity of phenotypes
    - can reveal evolutionary relationships, but not dependent on phylogenetic analysis
    - E.g because motility and flagella are always associated in particular organisms, it is reasonable to suppose that flagella is involved in some types of motility

4. **Phylogenetic classification**

- Phylogenetic, also called phyletic classification systems
- Phylogeny is based on evolutionary development of a species
- usually based on direct comparison of genetic material and gene products
    - this approach is widely accepted
    - large databases exist for rRNA sequences

5. **Numerical Taxonomy**

A scientist may determine many characteristics (usually 100-200) for each strain studied, giving each characteristics equal weight. Then using a computer the % of similarity (%s) of each strain to every other stain is calculated

For any 2 strain %S= NS

NS+ND (Both positive and negative)

NS: No of similar characteristics

ND: No of different characteristics

DEFINING PROCARYOTIC SPECIES

- The basic taxonomic group in microbial taxonomy is the species.
- Cannot use definition based on interbreeding because procaryotes are asexual.
- A prokaryotic species is collection of strains that share many stable properties and differ significantly from other groups of strains.
- Also suggested as a definition of species as a collection of organisms that share the same sequences in their core housekeeping genes (genes required to code for products needed by cells)-bases on sequence data.

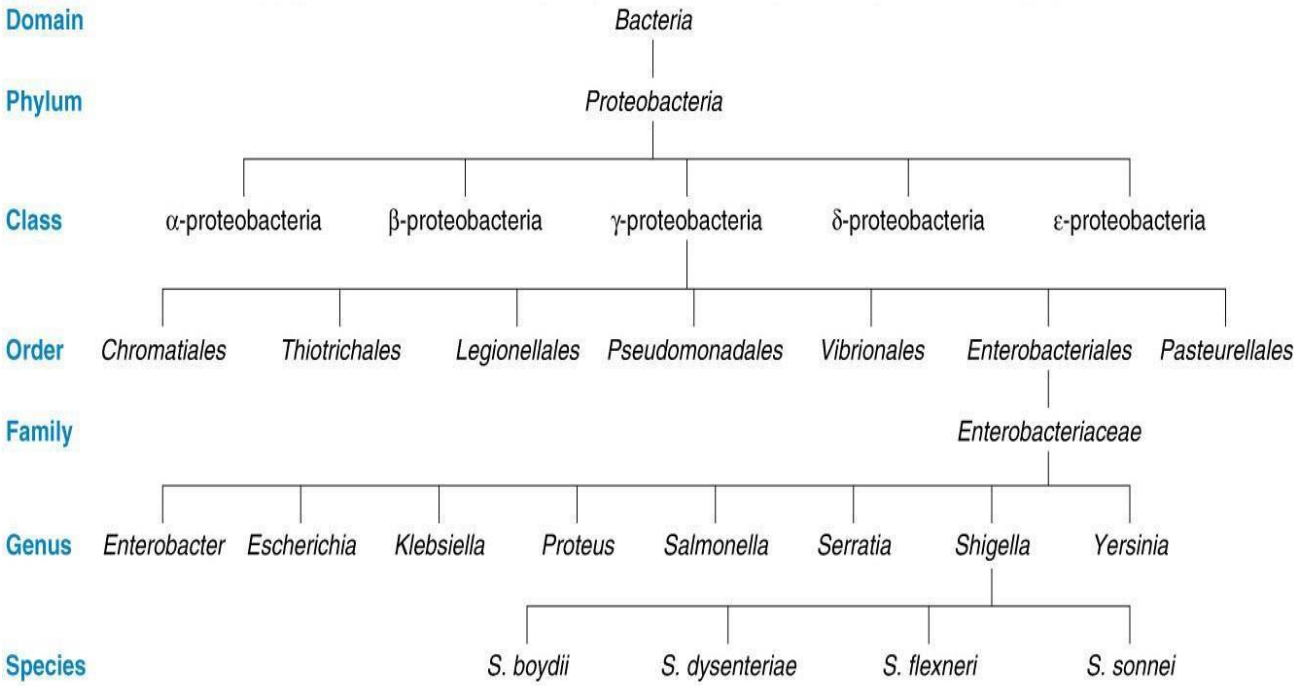

Fig 1.6 Hiearchical arrangement in Taxonomy

Strains

- Descended from a single, pure microbial culture
- Strains vary from each other in many ways
  - Biovars – differ biochemically and physiologically
  - Morphovars – differ morphologically
  - Serovars – differ in antigenic properties

Type strains

- Usually one of first strains of a species studied
- Often most fully characterized
- Not necessarily most representative member of species

Genus

- Genus- well-defined group of one or more strains
- Clearly separate from other genera

- Often disagreement among taxonomists about the assignment of a specific species to a genus

BINOMIAL SYSTEM OF NOMENCLATURE

Binomial system was devised by Carolus Linnaeus

- Each organism has two names
    - genus name – italicized and capitalized (e.g., *Escherichia*)
    - species epithet – italicized but not capitalized (e.g., *coli*)
- can be abbreviated after first use (e.g., *E. coli)*
- a new procaryotic species cannot be recognized until it

has been published in the *International Journal of Systematic and Evolutionary Microbiology*

TECHNIQUES FOR DETERMINING MICROBIAL TAXONOMY AND PHYLOGENY

Classical characteristics

- morphological
- Physiological and metabolic
- Ecological
- Genetic

Molecular characteristics

- nucleic acid base composition
- nucleic acid hybridization
- nucleic acid sequencing
- genomic fingerprinting
- amino acid sequencing

Morphological features used in classification

The morphological features are important in microbial taxonomy in 2 reasons

1. structural features depend on the expression of many genes.

2. Morphological similarity help to find out phylogenetic relatedness

Table 17.2 Some Morphological Features Used in Classification and Identification

Feature	Microbial Groups
Cell shape	All major groups[a]
Cell size	All major groups
Colonial morphology	All major groups
Ultrastructural characteristics	All major groups
Staining behavior	Bacteria, some fungi
Cilia and flagella	All major groups
Mechanism of motility	Gliding bacteria, spirochetes, protists
Endospore shape and location	Endospore-forming bacteria
Spore morphology and location	Bacteria, protists, fungi
Cellular inclusions	All major groups
Colony color	All major groups

[a]Used in classifying and identifying at least some bacteria, archaea, fungi, and protists.

Table: 1.2 Morphological features used in classification and Identification

Physiological and metabolic characteristics

- These characters are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Since proteins are gene products, analysis of these characteristics provide indirect comparison of microbial genomes. The characters include
- Carbon and nitrogen source utilization
- Cell all constituents,
- Energy source
  Fermentation products,

General nutritional types

- Growth temp optimum and range,
- Luminescence
- Mechanism of energy conversion

- Motility
- Osmotic tolerance
- Oxygen relationship
- P H optimum and growth range
- Photosynthetic pigments
- Salt requirement and tolerance
- Secondary metabolites formed
- Sensitivity to metabolic inhibitors and antibiotics
- Storage inclusions

Ecological Characteristics

- Life-cycle patterns
- Symbiotic relationships
- Ability to cause disease
- Habitat preferences
- Growth requirements of temp, OXYGEN, pH,

Genetic Analysis

- Study of chromosomal gene exchange by transformation and conjugation
- plasmids can be used for the analysis of phenotypic traits

Molecular characteristics

- **Nucleic Acid Base Composition**

Determine the G + C content

$$\textbf{Mol\% (G + C)} = \frac{(G + C)}{G + C + A + T} \times 100$$

Where G=Guanine, C=Cytosine, A=adenine and T=Thymine (nucleotide are the DNA base)

- **The G+ C content is often estimated by determining the melting temperature ($T_{m)}$ of the DNA**
- **Higher G + C gives a higher melting temperature**

Nucleic Acid Hybridization

Nucleic Acid Hybridization

- **measure of sequence homology (molecular relatedness)**

Common procedure for hybridisation:

- **I**solated DS DNA from a new isolated want to identify and also isolate from DS DNA from type stain
- Denatured at temp above Tm (Melting point) of DNA
- Get SS DNA and are mixed
- Temperature is bring down below Tm point (30-50C)
- SS DNA which having more or less similar sequence but not identical are start to pair and form unstable hybrid
- When bring the temperature to 10-15 C the strand which are having similar sequence pair and form stale hybrid

Nucleic Acid Sequencing

- most powerful and direct method for comparing genomes
- sequences of 16S (procaryotes) and 18S (eucaryotes) ribosomal RNA (rRNA) are used most often in phylogenetic studies
- complete chromosomes can now be sequenced and compared

Comparative Analysis of 16S rRNA Sequences:

- Oligonucleotide signature sequences are short conserved sequences specific for a phylogenetically defined group of organisms
- either complete or, more often, specific rRNA fragments
- can be compared
- when comparing rRNA sequences between 2 organisms, their relatedness is represented by an association coefficient or S_{ab} value
- the higher the S_{ab} value, the more closely related the organisms

RNA Sequencing are as follows

- ❖ 5S and 16S rRNA s is isolated form 50S and 30S subunits respectively
- ❖ Purified the r RNA and radiolabeled

- ❖ Treated with the enzyme T1ribonucleases, which cleaves it into fragments
- ❖ The fragments are separated
- ❖ All fragments composed of at least 6 nucleotides
- ❖ The sequences of corresponding 16S r RNA fragments from different prokaryotes are then aligned and compared using a computer and association coefficient are calculated.

Complete r RNA sequencing by following method

- RNA is isolated and purified
- The reverse transcriptase is used to make complementary DNA (c DNA) using primers that are complementary to conserved r RNA sequences.
- By PCR the c DNA is amplified
- Then the c DNA is sequenced
- This will give the sequence details of r RNA.

Determine species identity

DNA sequences can also be used to determine species strains in addition to genus

- It requires analysis of genes that evolve more quickly than rRNA encoding genes
- Multilocus sequence typing (MLST), the sequencing and comparison of 5 to 7 housekeeping genes instead of single gene is done.
- This is to prevent misleading results from analysis of one gene.

Genomic Fingerprinting

- Genomic Finger Printing also used for microbial classification and determination of phylogenetic relationships
- Genomic Finger Printing does not involve nucleotide sequencing
- Can be used because of multicopies of highly conserved and repetitive DNA sequences present in most gram-negative and some gram-positive bacteria
- Multicopies can be obtained by Polymerase chain reaction using restriction enzymes

Amino Acid Sequencing

- the amino acid sequence of a protein is a reflection of the mRNA sequence and therefore of the gene which encodes that protein
- amino acid sequencing of proteins such as cytochromes, histones and heat-shock proteins has provided relevant taxonomic and phylogenetic information

- cannot be used for all proteins
- compare protein mass spectra

Assessing Microbial Phylogeny

- evolutionary relationships represented using phylogenetic trees
- A phylogentic tree is a graph which connects nodes and branches

Bacterial cell Structure and Function

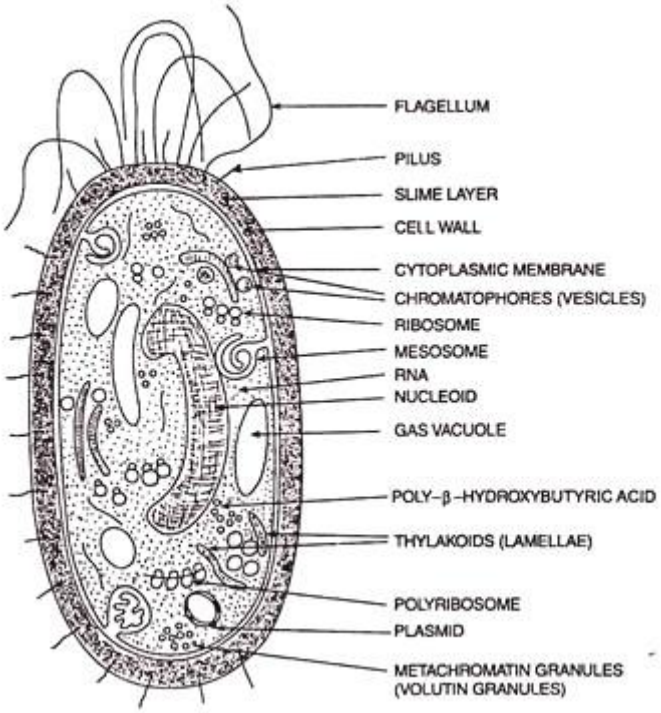

Fig: 1.7 Ultrastructure of bacterial cell

Smaller and simpler in structure than eukaryotic cells, with no recognizable organelles.

- All of the activities performed by organelles also take place in bacteria, but they are not carried out by specialized structures.
- The small size, simple design, and broad metabolic capabilities of bacteria allow them to grow and divide very rapidly and to inhabit and flourish in almost any environment.
- They were first seen under a microscope by Anton van Leeuwenhoek in 1676.
- As microscopes have improved, scientists have come to understand

Organized into 3 categories :

- Internal Structures: Cytoplasm, nucleoid, bacterial chromosome, plasmid, ribosomes, and storage granules
- Cell envelope: cell membrane, peptidoglycan cell wall or an outer lipid membrane (only found in Gram-negative cells)
- External structures (appendages & coverings): flagella, fimbriae, sex pilus and glycocalyx

Intracellular structures Cytoplasm

- Chromosome
- Plasmid
- Ribosomes
- Inclusion bodies
- Cytoplasm
- Chromosome
- Plasmid
- Ribosomes
- Inclusion bodies

Cytoplasm

- Portion of the cell that lies within the PM
- substances within the plasma membrane, excluding the genetic material.
- Gel-like matrix composed of mostly water(4/5 th), enzymes, nutrients, wastes, and gases
- Contains cell structures - ribosomes, chromosome, and plasmids , as well as the components necessary for bacterial metabolism.
- It is relatively featureless by electron microscope - although small granules can be seen.
- carries out very important functions for the cell -growth, metabolism, and replication .

Constituents

- Proteins including enzymes
- Vitamins
- Ions
- Nucleic acids and their precursors
- Amino acids and their precursors
- Sugars, carbohydrates and their derivatives
- Fatty acids and their derivatives

Nucleoid

- Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus.
- The nucleoid is a region of cytoplasm where the chromosomal DNA is located.
- It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found.

Plasmids

- small extra-chromosomal DNA
- contain genes for antibiotic resistance or virulence.
- Structure Similar to most bacterial chromosomes, but considerably smaller.
- plasmids are covalently closed circular DNA
- In a few species linear plasmids have been found.
- Size : Chromosomal DNA is typically about 4000 kb,
- plasmid DNA ranges from 1-200 kb.
- Number of plasmids: 1-700 copies of plasmid in a cell.

Plasmid Function

- The function of plasmids is not always known, but they are not normally essential for survival of host, although their presence generally gives the host some advantage.
- Antibiotic resistance - Some plasmids code for proteins that degrade antibiotics-a big advantage for pathogens.
- Some encode for proteins which confer virulence factors on the host. For example- *E. coli* plasmid Ent P307 codes for an enterotoxin which makes *E. coli* pathogenic.
- Conjugative plasmids - These allow exchange of DNA between bacterial cells.
- Plasmids and the associated traits can be transferred between bacteria, even from one bacterial species to another.
- Plasmids are not involved in reproduction.
- Plasmids replicate independently of the chromosome.
- Plasmids are passed to other bacteria by two means.

For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission.

- **Other types of plasmids:**,form tube like structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information.

- Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts.
- The ability to insert specific genes into plasmids have made them extremely useful tools in the area of genetic engineering/RDNA Technology .

Ribosomes- protein synthesis machinery

- Consists of RNA and protein
- Abundant in cytoplasm
- Often grouped in long chains called polyribosomes.
- give the cytoplasm of bacteria a granular appearance in EM.
- smaller than the ribosomes in eukaryotic cells-but have a similar function
- Bacterial ribosomes have sedimentation rate of 70S; their subunits have rates of 30S and 50S.
- The unit used to measure sedimentation velocity is Svedberg

- They translate the genetic code from the molecular language of nucleic

acid to that of amino acids—the building blocks of proteins.

- Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure.
- Bacterial ribosomes are never bound to other organelles as they sometimes are bound to the endoplasmic reticulum in eukaryotes, but are free-standing structures distributed throughout the cytoplasm.
- There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.
- Streptomycin binds 70S ribosome and stops protein synthesis but it can not bind 80S ribosome of eukaryotes and thereby eukaryotic cell remains unaffected.

Bacterial Chromosome - Genophore

- The bacterial chromosome consists of a single, circle of deoxyribonucleic acid.
- DNA is double stranded- two strands line up antiparrallel to each other and the bases are linked together with hydrogen bonds.

- It includes most of the genetic material of the organism .

- Unlike the DNA in eukaryotic cells, which resides in the nucleus, DNA in bacterial cells is not sequestered in a membrane-bound organelle but appears as a long coil distributed through the cytoplasm.

- In many bacteria the DNA is present as a single, circular chromosome and in some cases the DNA is linear rather than circular.
- some bacteria may contain two chromosomes
- As in all organisms, bacterial DNA contains the four nitrogenous bases adenine (A), cytosine (C), guanine (G), and t
- The amount of DNA in bacterial chromosomes ranges from 580,000 base pairs in *Mycoplasma gallinarum* to 4,700,000 base pairs in *E. coli* to 9,140,000 base pairs in *Myxococcus xanthus*.
- As in all organisms, bacterial DNA contains the four nitrogenous bases adenine (A), cytosine (C), guanine (G), and t

Inclusion bodies

- **Inclusion bodies:** Bacteria can have within their cytoplasm a variety of small bodies collectively referred to as inclusion bodies.
- Some are called granules and other are called vesicles.
- Inclusions are considered to be nonliving components of the cell that do not possess metabolic activity and are not bounded by membranes.
- The most common inclusions are glycogen, lipid droplets, crystals, and pigments.

Granules:

Densely compacted substances without a membrane covering.

- Nutrients and reserves may be stored in the cytoplasm in the form of glycogen, lipids, polyphosphate, or in some cases, sulfur or nitrogen for later use.
- Each granule contains specific substances, such as glycogen (glucose polymer) and polyphosphate (phosphate polymer, supplies energy to metabolic processes).
- Sulfur bacteria contains reserve granules of sulfur.
- These granules are depleted in starvation.
- Some aquatic photosynthetic bacteria and cyano bacteria have rigid gas-filled vacuoles and it helps in floating at a certain level - allowing them to move up or down into water layers with different light intensities and nutrient levels.

- Some magnetotactic bacterium, eg. Aquaspirillium magnetotacticum , stores Magnetitite (Ferric oxide). The presence of such magnetic inclusions enables these bacteria to responds to magnetic fields.

Microcompartments

- Microcompartments are widespread, membrane- bound organelles that are made of a protein shell that surrounds and encloses various enzymes.
- Carboxysomes are protein-enclosed bacterial microcompartments that contain enzymes involved in carbon fixation.
- Magnetosomes are bacterial microcompartments, present in magnetotactic bacteria, that contain magnetic crystals.

Cell Envelope

- Plasma Membrane
- Periplasmic Space
- Cell Wall
- Outer membrane

Plasma Membrane

- Phospholipid bilayer surrounding the cytoplasm and regulates the flow of substances in and out of the cell.
- Consists of both lipids and proteins.
- Protects the cell from its surroundings.
- Selectively permeable to ions and organic molecules and controls the movement of substances in and out.
- numerous proteins moving within or upon this layer are primarily responsible for transport of ions, nutrients and waste across the membrane.

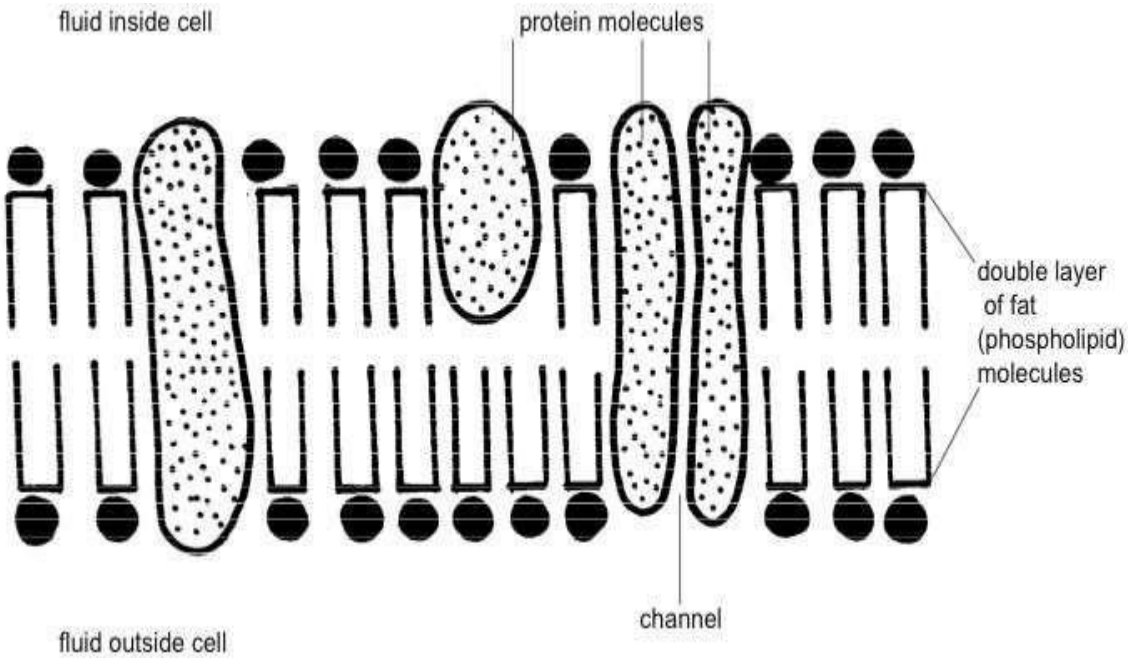

Fig: 1.8 Structure of bacterial cell membrane

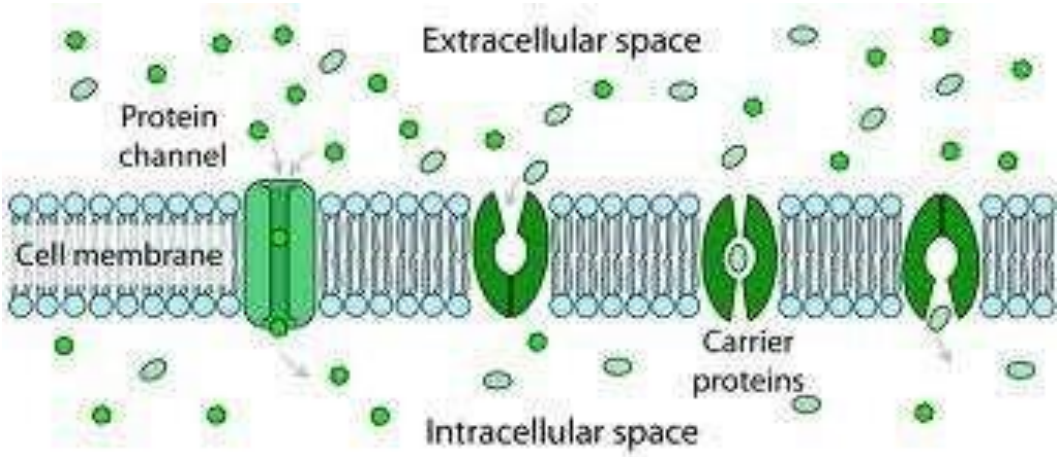

Fig: 1.9 Structure of bacterial cell membrane with protein channels

Periplasmic space

- **Gram-negative bacteria** : space between the cytoplasmic membrane and the cell wall and space found between cell wall and the outer membrane

- Periplasm may constitute up to 40% of the total cell volume in G-ve species.
- **Gram-positive bacteria** : space between the cytoplasmic membrane and the cell wall.
- The periplasm is filled with water and proteins and is reminiscent of the cytoplasm.
- However periplasm contains proteins and other molecules distinct from those in the cytoplasm because the membrane prevents the free exchange between these two compartments.
- Periplasmic proteins have various functions in cellular processes including: transport, degradation, and motility.
- Periplasm controls molecular traffic entering and leaving the cell.

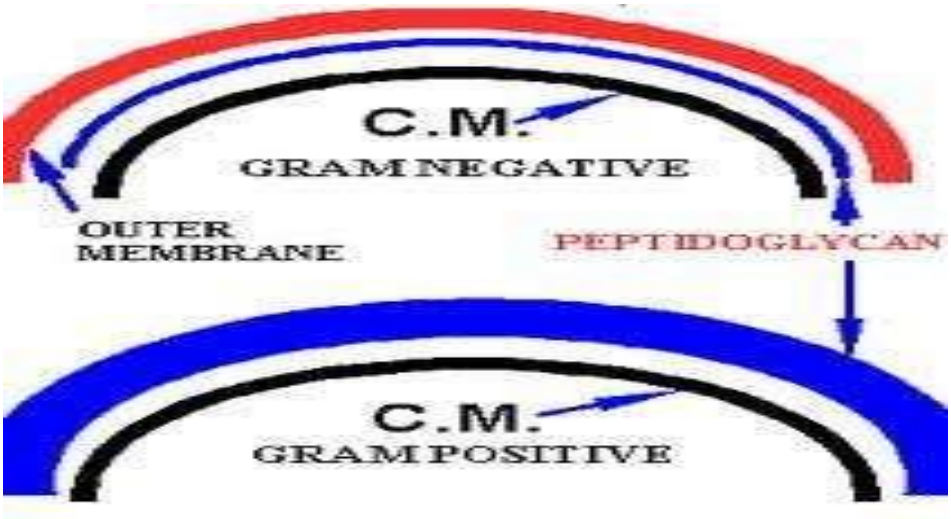

Fig: 1.10 Periplasmic space

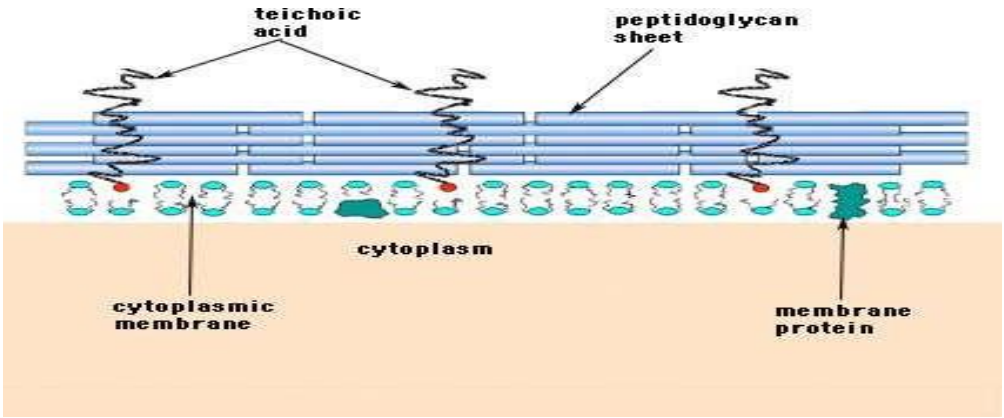

Fig: 1.11 Gram positive cell wall

Gram-positive Cells

- G+ve bacteria possess thick cell wall containing many layers of peptidoglycan and teichoic acids.
- In G+ ve cells, peptidoglycan is the outermost structure and makes up as much as 90% of the thick compact cell wall.

Gram-negative Cell wall

- G-ve bacteria have relatively thin cell wall consisting of few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins
- Peptidoglycan makes up only 5 – 20% of the cell wall and is not the outermost layer, but lies between the plasma membrane and an outer membrane.

Gram Staining

- Developed in 1884 by Danish scientist Christian Gram.
- It is a differential stain.
- In this, bacteria are first stained with crystal violet, then treated with a mordant - a solution that fixes the stain inside the cell.
- Bacteria are then washed with a decolorizing agent, such as alcohol, and counterstained with safranin, a light red dye.
- **Gram-positive bacteria** are those that are stained dark blue or violet by Gram staining.
- **Gram-negative bacteria** cannot retain the crystal violet stain, instead take up the counterstain and appearred or pink.
- The walls of gram-positive bacteria have more peptidoglycans than do gram-negative bacteria. Thus, gram-positive bacteria retain the original violet dye and cannot be counterstained.

Cell wall

- If the bacterial cell wall is entirely removed, it is called a **protoplast** while if it's partially removed, it is called a **spheroplast**.
- Antibiotics such as penicillin inhibit the formation of peptidoglycan cross-links in the bacterial cell wall.

- The enzyme **lysozyme**, found in human tears, also digests the cell wall of bacteria and is the body's main defense against eye infections.

outer membrane

- Similar to the plasma membrane, but is less permeable .
- This membrane has tiny holes or openings called **porins**.
- **Porins** block the entrance of harmful chemicals and antibiotics, making G-ve bacteria much more difficult to treat than G+ve cells.
- Composed of lipopolysaccharides (LPS).
- LPS is a harmful substance classified as an endotoxin.
- Lipopolysaccharides, which acts as an **endotoxin**, are composed of polysaccharides and **lipid A** (responsible for much of the toxicity of G-ve bacteria).
- These differences in structure can produce differences in antibiotic susceptibility
- Ex: vancomycin can kill only Gram +ve bacteria and is ineffective against Gram -ve pathogens, such as *Haemophilus influenzae* or *Pseudomonas aeruginosa.*

External structures

- Flagella
- Pili/fimbriae
- Capsule/slime layer
- **Flagella Singular: flagellum**
- Long, whip-like semi-rigid cylindrical structures that aids in cellular locomotion
- Function much like the propeller on a ship.
- about 20 nm in diameter and up to 20 micromts in length.
- Diameter of a prokaryotic flagellum is about 1/10 th of that of eukaryotic.
- Flagella are driven by the energy released by the transfer of ions down an electrochemical gradient across the cell membrane.
- Made up of protein subunits called flagellin.
- Each flagellum is attached to cell membrane with the help of proteins other than flagellin.
- The basal region has a hook like structure and a complex basal body. The basal body consists of a central rod or shaft surrounded by a set of rings.

- Bacterial spp differ in the number and arrangement of flagella on their surface.
- Bacteria may have one, a few, or many flagella in different positions on the cell.
- **Monotrichous** - single flagellum
- **amphitrichous** a flagellum at each end **lophotrichous -** clusters of flagella at the poles of the cell
- **peritrichous** - flagella distributed over the entire surface of the cell .
-

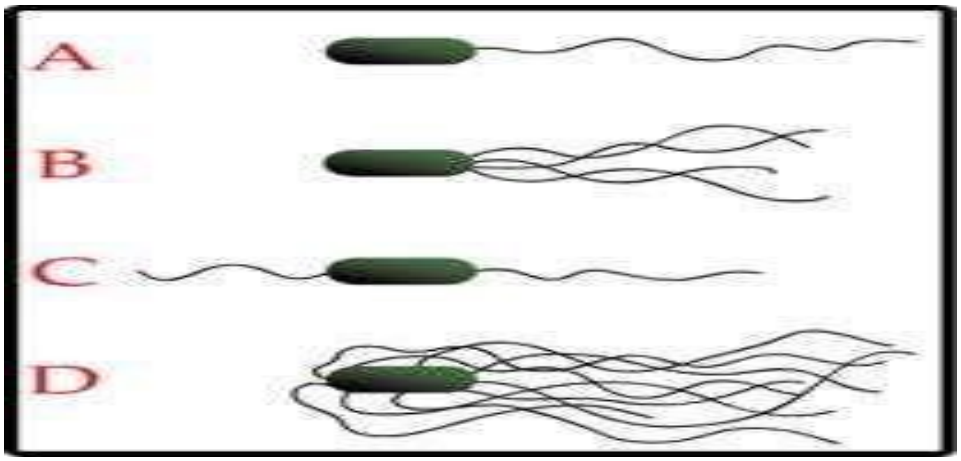

Fig: 1.12. Types of Flagella arrangement in bacteria

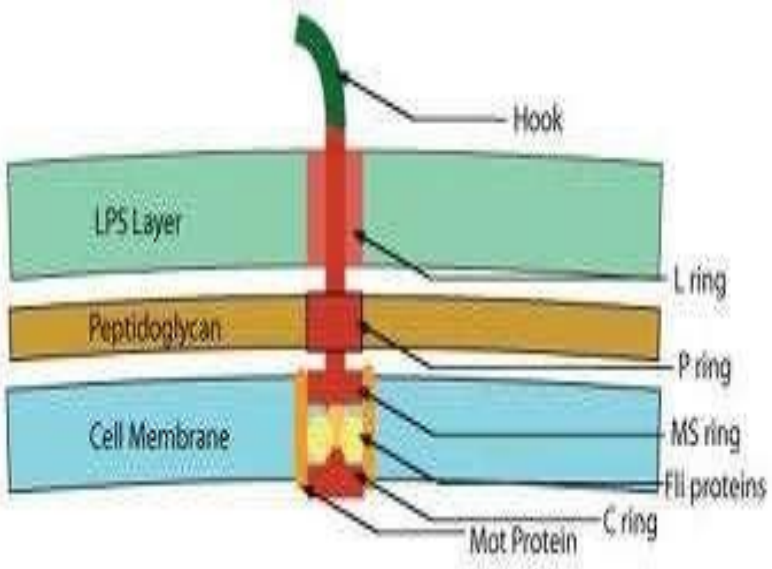

Fig:1.13. Structure of flagella in Gram negative cell

Motile bacteria are attracted or repelled by certain stimuli in behaviors called taxis: these include chemotaxis, phototaxis, and magnetotaxis.

- The flagella beat in a propeller-like motion to help the bacterium move toward nutrients; away from toxic chemicals; towards the light (photosynthetic cyanobacteria).
- Prokaryotes exhibit a variety of movements:

move , swim ,tumble ,glide, swarm in response to environmental stimuli.

FIMBRIAE AND PILI

- Hollow, hair like structures made of protein
- Involved in attachment to solid surfaces or to other cells and are essential for the virulence of some bacterial pathogens.
- Fimbriae fine filaments of protein just 2–10 nm in diameter and up to several micrometers in length.
- They are distributed over the surface of the cell, and resemble fine hairs when seen under the electron microscope.

Pili: (*sing*. pilus)

Pili are cellular appendages, slightly larger than fimbriae

- Involved in attachment to surfaces.
- Specialized pili, the sex pili, allows the transfer of genetic material from one bacteria to another in a process called conjugation where they are called conjugation pili or "sex pili".
- type IV pili - generate movement.
- Helps in colonization and pathogenicity

Glycocalyx :

sticky coating produced by many bacteria covering the surface of cell.

- The glycocalyx is composed of polysaccharides (sugars) and proteins.
- The bacterial glycocalyx has 2 forms
- a highly structured rigid capsule
- a disorganised loose slime layer -
- Capsules are found on many pathogenic bacteria

The glycocalyx has several functions including:

- protection, attachment to surfaces and formation of biofilms.
- The glycocalyx helps protect the bacteria cell by preventing immune cells from attaching to it and destroying it through phagocytosis.

REPRODUCITON

Type of Asexual reproduction in bacteria : Budding Fragmentation Binary Fission

Budding:In this case small bud farmation from a parental cell. And daughter cell , finally gets separated from the parent cell.

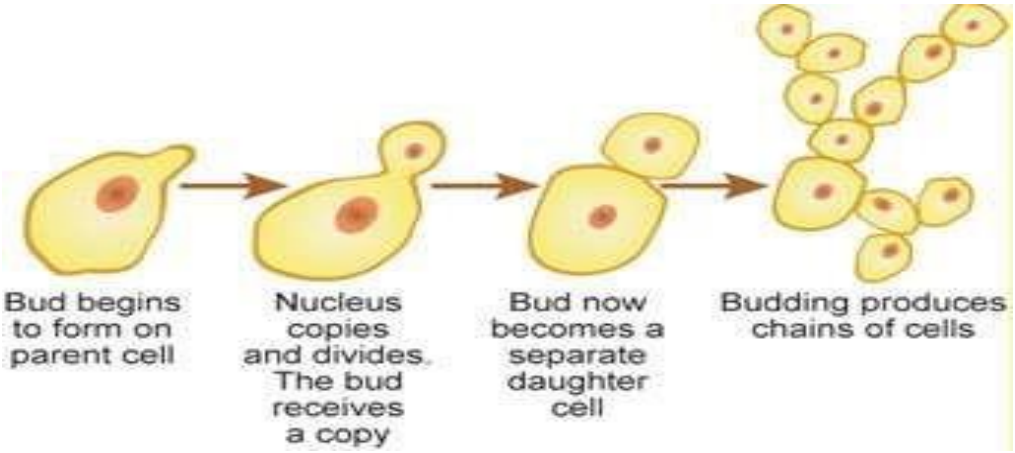

Fig.1.14. Bacterial reproduction by budding

FRAGMENTATION: This is a form of reproduction where a new organism grows from a fragment of parent cell.

Each fragment develops into in a fully grown individual.

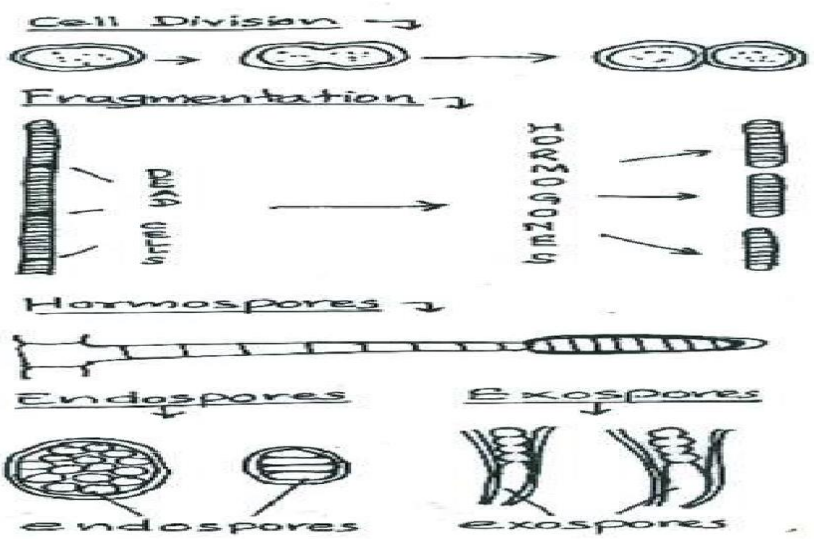

Fig: 1.15. Bacterial reproduction by fragmentation

Binary Fission – It is division of a single cell into two or more cells,and the regeneration of those cells into separate entities resembling the original cell.

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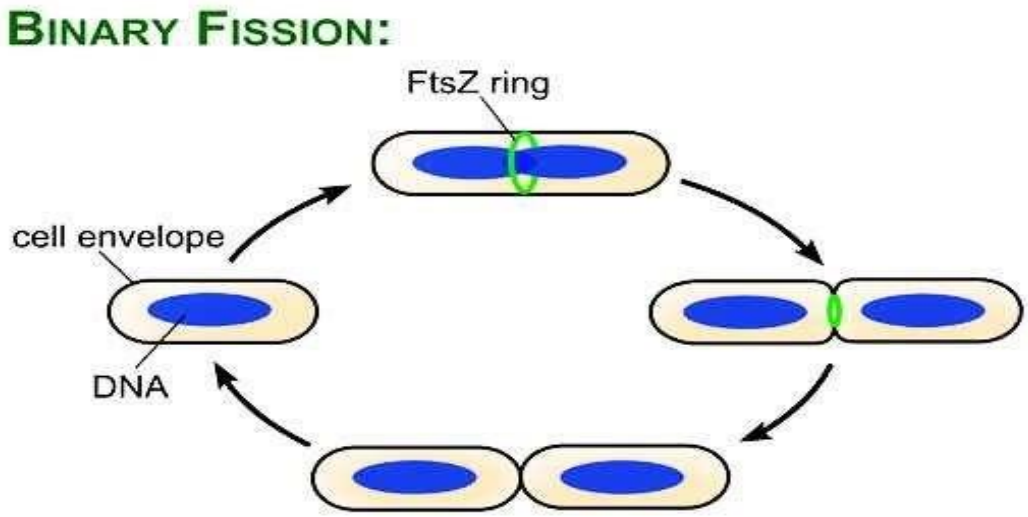


Fig: 1.16. Bacterial reproduction by Binary fission

VIRAL STRUCTURE AND REPLICATION

Viruses are noncellular genetic elements that use a living cell for their replication and have an extracellular state. Viruses are ultramicroscopic particles containing nucleic acid surrounded by protein, and in some cases, other macromolecular components such as a membranelike envelope.

Outside the host cell, the virus particle is also known as a **virion.** The virion is metabolically inert and does not grow or carry on respiratory or biosynthetic functions.

At present, there are no technical names for viruses. International committees have recommended genus and family names for certain viruses, but the process is still in a developmental stage.

Viruses vary considerably in size and shape. The smallest viruses are about 0.02 μm (20 nanometers), while the large viruses measure about 0.3 μm (300 nanometers). Smallpox viruses are among the largest viruses; polio viruses are among the smallest.

Viral structure. Certain viruses contain ribonucleic acid (RNA), while other viruses have deoxyribonucleic acid (DNA). The nucleic acid portion of the viruses is known as the**genome.** The nucleic acid may be single-stranded or double-stranded; it may be linear or a closed loop; it may be continuous or occur in segments.

The genome of the virus is surrounded by a protein coat known as a **capsid**, which is formed from a number of individual protein molecules called **capsomeres.** Capsomeres are arranged in a precise and highly repetitive pattern around the nucleic acid. A single type of capsomere or several chemically distinct types may make up the capsid. The combination of genome and capsid is called the viral **nucleocapsid.**

A number of kinds of viruses contain **envelopes.** An envelope is a membranelike structure that encloses the nucleocapsid and is obtained from a host cell during the replication process. The envelope contains viral-specified proteins that make it unique. Among the envelope viruses are those of herpes simplex, chickenpox, and infectious mononucleosis.

The nucleocapsids of viruses are constructed according to certain symmetrical patterns. The virus that causes tobacco mosaic disease, for example, has **helical symmetry.** In this case, the nucleocapsid is wound like a tightly coiled spiral. The rabies virus also has helical symmetry. Other viruses take the shape of an icosahedron, and they are said to have **icosahedral symmetry.** In an icosahedron, the capsid is composed of 20 faces, each shaped as an equilateral triangle (Figure 1). Among the icosahedral viruses are those that cause yellow fever, polio, and head colds.

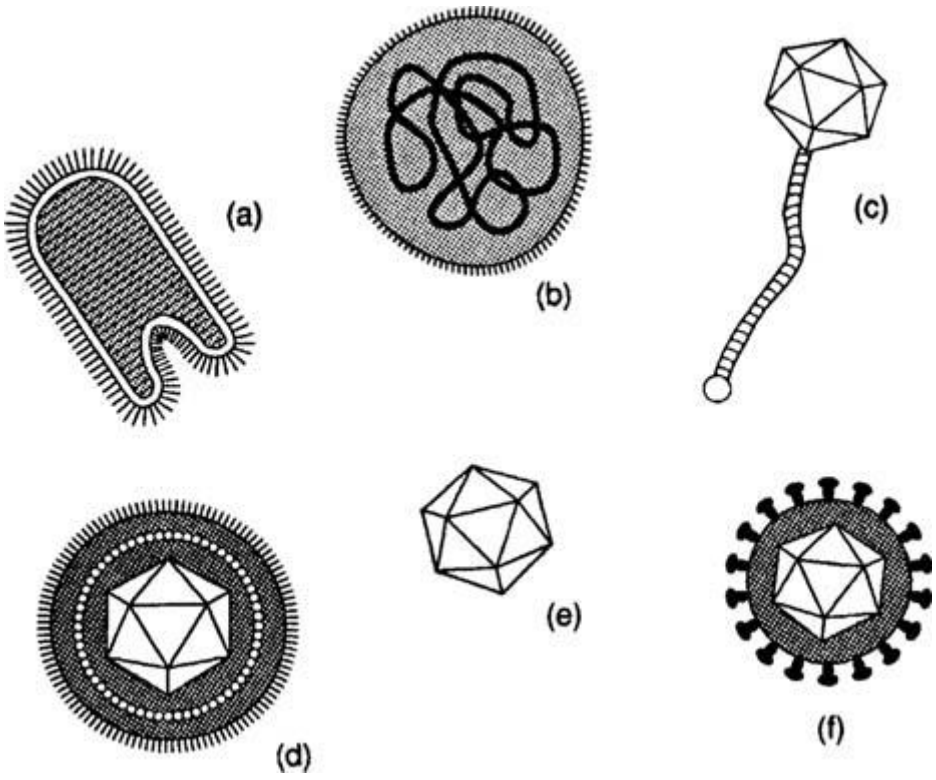


Fig: 1.17. Morophology of viruses. (a) The helical virus of rabies. (b) The segmented helical virus of influenza. (c) A bacteriophage with an icosahedral head and helical tail. (d) An enveloped icosahedral herpes simplex virus. (e) The unenveloped polio virus. (f) The icosahedral human immunodeficiency virus with spikes on its envelope.

The envelope of certain viruses is a lipid bilayer containing glycoproteins embedded in the lipid. The envelope gives a somewhat circular appearance to the virus and does not contribute to the symmetry of the nucleocapsid. Projections from the envelope are known as **spikes.** The spikes sometimes contain essential elements for attachment of the virus to the host cell. The virus of AIDS, the human immunodeficiency virus, uses its spikes for this purpose.

Bacteriophages are viruses that multiply within bacteria. These viruses are among the more complex viruses. They often have icosahedral heads and helical tails. The virus that attacks and replicates in *Escherichia coli* has 20 different proteins in its helical tail and a set of numerous fibers and "pins." Bacteriophages contain DNA and are important tools for viral research.

Viral replication. During the process of **viral replication**, a virus induces a living host cell to synthesize the essential components for the synthesis of new viral particles. The particles are then assembled into the correct structure, and the newly formed virions escape from the cell to infect other cells.

The first step in the replication process is **attachment.** In this step, the virus adsorbs to a susceptible host cell. High specificity exists between virus and cell, and the envelope spikes may unite with cell surface receptors. Receptors may exist on bacterial pili or flagella or on the host cell membrane.

The next step is **penetration** of the virus or the viral genome into the cell. This step may occur by phagocytosis; or the envelope of the virus may blend with the cell membrane; or the virus may "inject" its genome into the host cell. The latter situation occurs with the bacteriophage when the tail of the phage unites with the bacterial cell wall and enzymes open a hole in the wall. The DNA of the phage penetrates through this hole.
The **replication** steps of the process occur next. The protein capsid is stripped away from the genome, and the genome is freed in the cell cytoplasm. If the genome consists of RNA, the genome acts as a messenger RNA molecule and provides the genetic codes for the synthesis of enzymes. The enzymes are used for the synthesis of viral genomes and capsomeres and the assembly of these components into new viruses. If the viral genome consists of DNA, it provides the genetic code for the synthesis of messenger RNA molecules, and the process proceeds.

In some cases, such as in HIV infection (as discussed below), the RNA of the virus serves as a template for the synthesis of a DNA molecule. The enzyme reverse transcriptase catalyzes the DNA's production. The DNA molecule then remains as part of the host cell's chromosome for an unspecified period. From this location, it encodes messenger RNA molecules for the synthesis of enzymes and viral components.

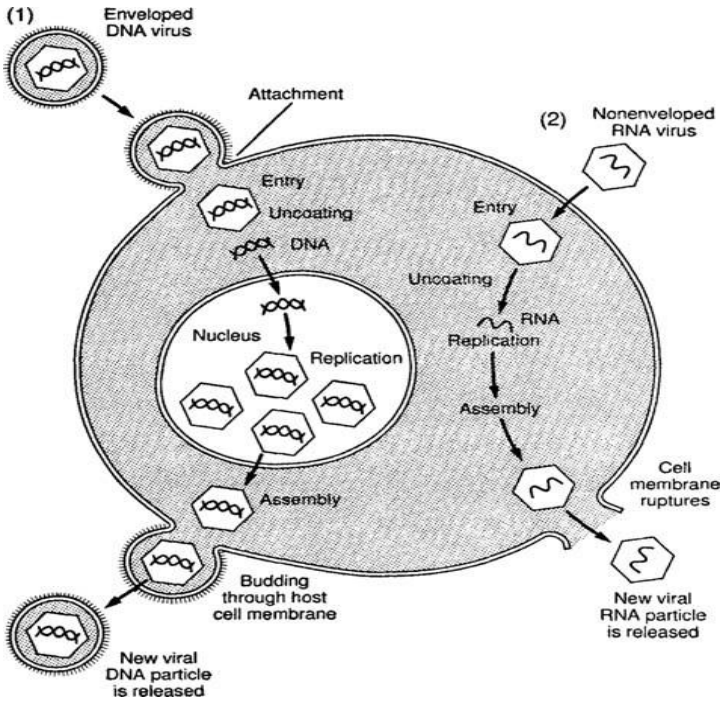


Fig:1.18. A generalized representation of the replication of two viruses. Replication of a DNA virus is shown in (1); replication of an RNA virus is displayed in (2).

For the **release** of new viral particles, any of a number of processes may occur. For example, the host cell may be "biochemically exhausted," and it may disintegrate, thereby releasing the virions. For enveloped viruses, the nucleocapsids move toward the membrane of the host cell, where they force themselves through that membrane in a process called **budding.** During budding, a portion of cell membrane pinches off and surrounds the nucleocapsid as an envelope. The replication process in which the host cell experiences death is called the **lytic cycle** of reproduction. The viruses so produced are free to infect and replicate in other host cells in the area.

Lysogeny. Not all viruses multiply by the lytic cycle of reproduction. Certain viruses remain active within their host cells for a long period without replicating. This cycle is called the **lysogenic cycle.** The viruses are called **temperate viruses**, or **proviruses**, because they do not bring death to the host cell immediately.

In lysogeny, the temperate virus exists in a latent form within the host cell and is usually integrated into the chromosome. Bacteriophages that remain latent within their bacterial host cell are called **prophages.** This process is a key element in the recombination process known as **transduction.**

An example of lysogeny occurs in **HIV infection.** In this case, the human immunodeficiency virus remains latent within the host T-lymphocyte. An individual whose infection is at this stage will not experience the symptoms of AIDS until a later date.

FUNGI

- ***Mykes*** (Greek word) : Mushroom
- Fungi are eukaryotic protista; differ from bacteria and other prokaryotes.
    1. Cell walls containing **chitin** (rigidity & support), mannan & other polysaccharides
    2. Cytoplasmic membrane contains ergosterols
    3. Possess true nuclei with nuclear membrane & paired

Chromosomes

1. Cytoplasmic contents include mitochondria and endoplasmic reticulum
2. Divide asexually, sexually or by both
3. Unicellular or multicellular

Fungal cell structure

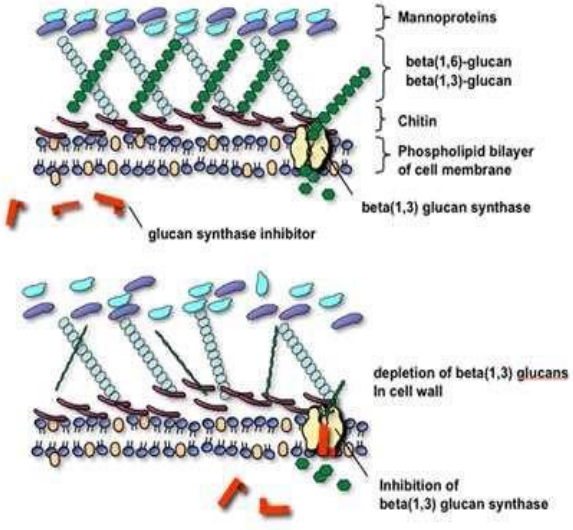


Fig: 1.19. Fungal cell wall structure

- Cell wall consists of chitin not peptidoglycan like bacteria
- Thus fungi are resistant to antibiotics as penicillins
- Chitin is a polysaccharide composed of long chain of n- acetylglucosamine.
- Also the fungal cell wall contain other polysaccharide, β- glucan, which is the ***site of action of some antifungal drugs.***
- Cell membrane consist of ergosterol rather than cholesterol like bacterial cell membrane
- Ergosterol is the site of action of antifungal drugs, amphotericin B & azole group

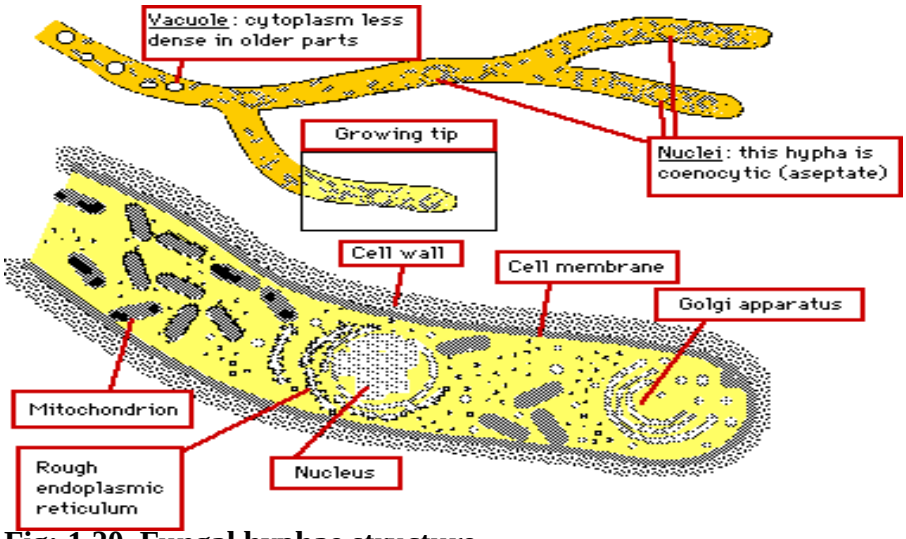


Fig: 1.20. Fungal hyphae structure

Fig: 1.21. Mold

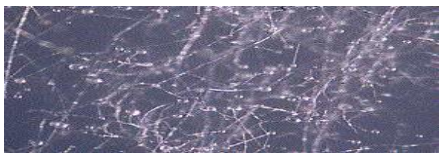
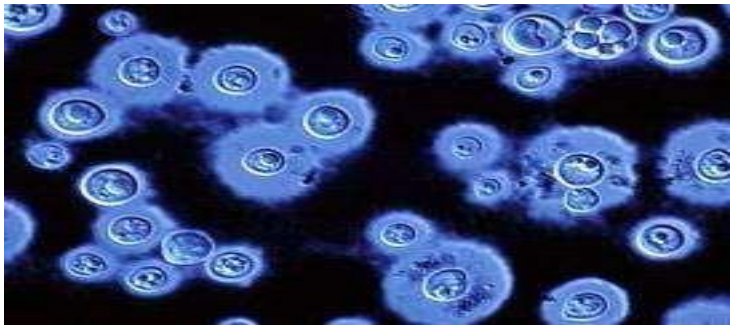


Fig: 1.22.Yeast



Many pathogenic fungi are **dimorphic**, forming hyphae at ambient temperatures but yeasts at body temperature.

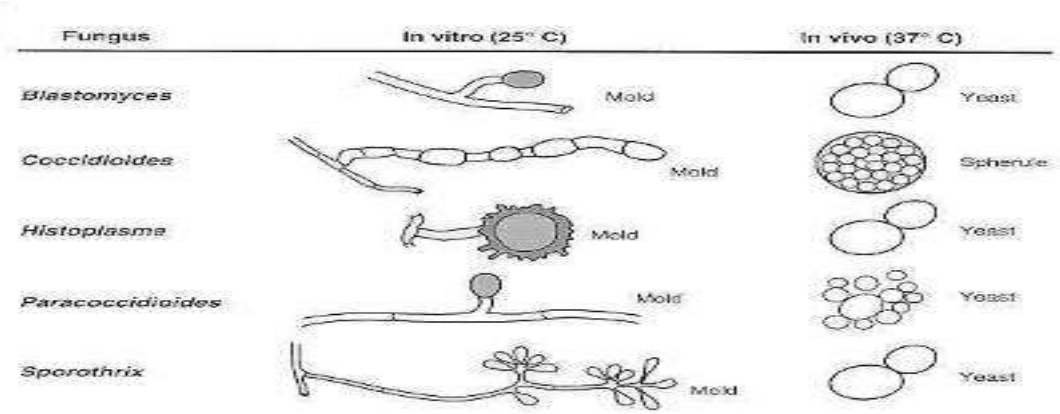


Fig: 1.23. Dimorphic Fungal Species

- **Yeast** :- Unicellular budding yeast
- **Hypha** :- Elongation of apical cell produces a tubular, thread like structure called hypha. Hyphae may be septate or nonseptate.
- **Mycelium** :- Tangled mass of hyphae is called mycelium. Fungi producing mycelia are called molds or filamentous fungi.

Mycelium

- Mass of branching intertwined hyphae
  a. Vegetative Mycelium- hyphae that penetrate the supporting medium and absorb nutrients
  b. Aerial Mycelium- hyphae projects above the surface of medium and bearr the

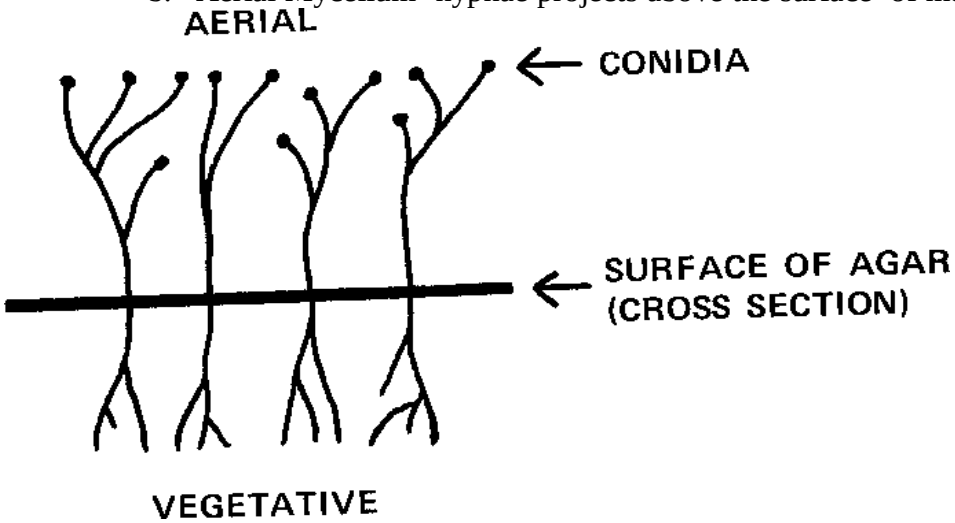


Fig: 1.24. Fungal mycelium

Vegetative types

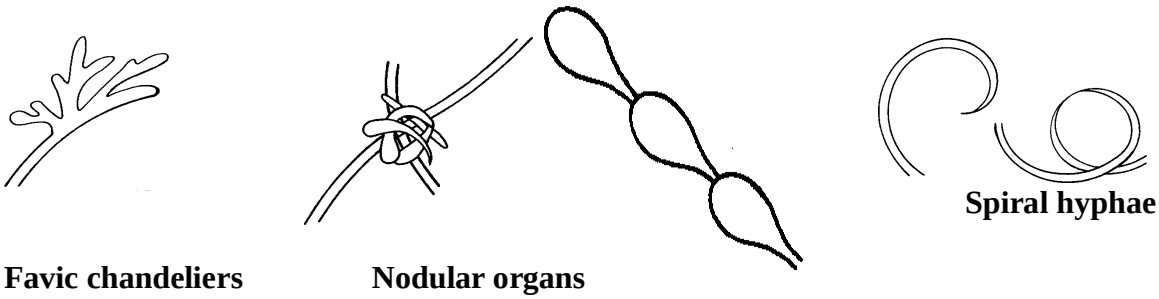


Fig: 1.25 Fungal hyphe different morphology

Classification of fungi

1. Morphological
2. Systematic classification

Morphological classification

1. Yeasts
2. Yeast-like fungi
3. Filamentous fungi (molds)
4. Dimorphic fungi

Yeasts

- These occur in the form of round or oval bodies which reproduce by an asexual process called budding in which the cell develops a protuberance which enlarges and eventually separates from the parent cell.
- Yeasts colonies resemble bacterial colonies in appearance and in consistency
- Examples are- *Saccharomyces cerevisiae, Cryptococcus neoformans*

Yeast form

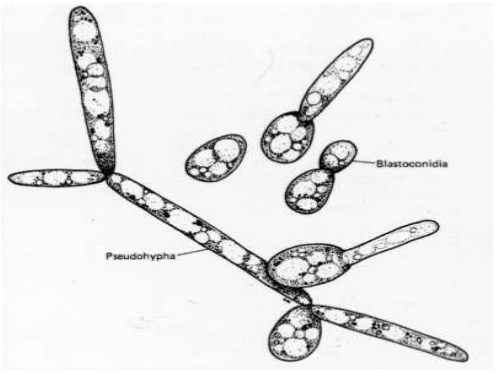


Fig: 1.26: Yeast form fungi

Most fungi are obligate or facultative aerobes. Reproduction is the formation of new individuals having all the characteristics typical of a species. The fungi reproduce by means of asexual and sexual or parasexual reproduction. Asexual reproduction is sometimes called somatic or vegetative and it does not involve union of nuclei, sex cells or sex organs. The union of two nuclei characterizes sexual reproduction.

Yeast-Like

Yeast like fungi grow partly as yeast and partly as elongated cells resembling hyphae. The latter form a pseudomycelium.

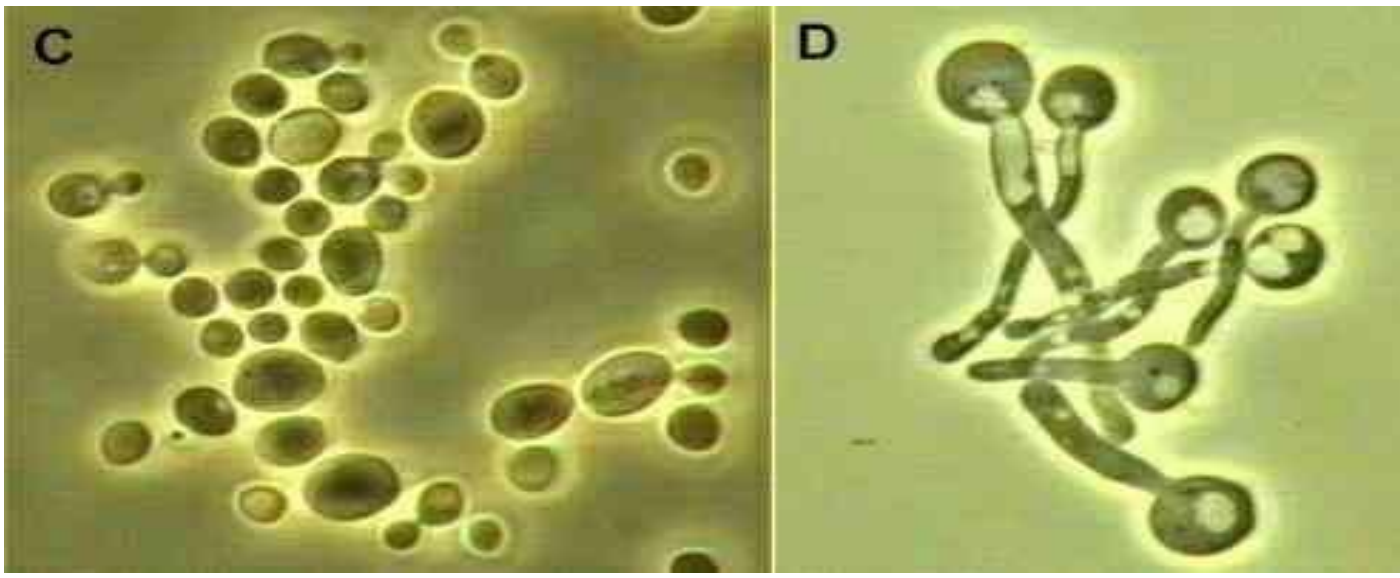


Fig: 1.27. C.Yeast D. Yeast like fungi

Molds or Filamentous Fungi

- The basic morphological elements of filamentous fungi are long branching filaments or hyphae, which intertwine to produce a mass of filaments or mycelium Colonies are strongly adherent to the medium and unlike most bacterial colonies cannot be emulsified in water
- The surface of these colonies may be velvety, powdery, or may show a cottony aerial mycelium.
- Reproduce by the formation of different types of spores
- Example: *Dermatophytes, Aspergillus, Penicillium, Mucor, Rhizopus*

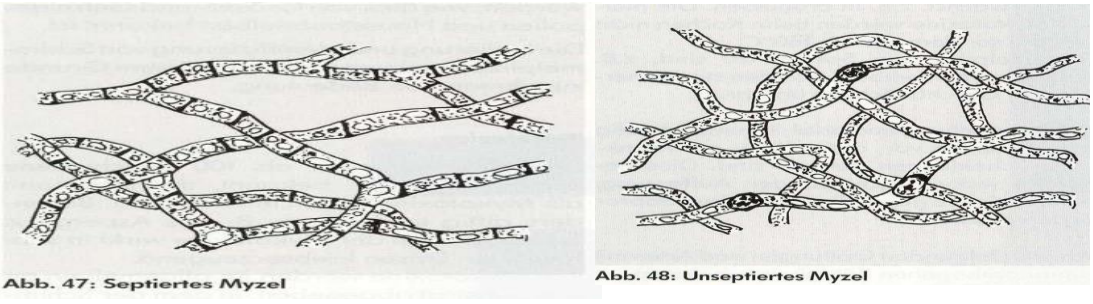


Fig: 1.28. Septate mycelium aseptate mycelium

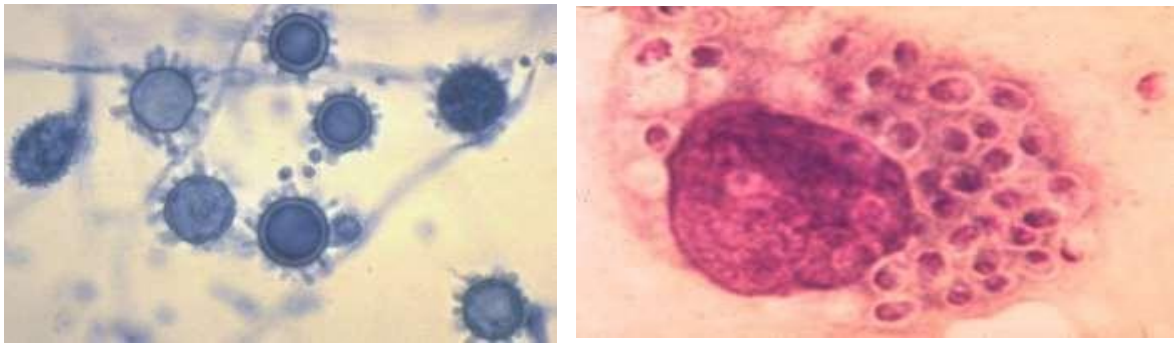
Dimorphic Fungi

- These are fungi which exhibit a yeast form in the host tissue and in vitro at 37^0C on enriched media and mycelial form in vitro at 25^0C

Examples:

Histoplasma capsulatum Blastomyces dermatitidis Coccidioides immitis Paracoccidoides brasiliesis Penicillium marneffei Sporothrix schenckii

- *Histoplasma capsulatum* - Dimorphism Filamentous mold in environment
  - Thin septate hyphae, microconidia, and tuberculate macroconidia (8-14 µm)
- Budding yeast (2-4 µm) in tissue
  - Dimorphic transition is thermally dependent and reversible (25°C « 37°C).



A B.

FIG: 1.29. A. Micro- and macroconidia B. Yeast within histiocyte

Systematic classification

- Based on sexual spores formation: 4 classes
- Zygomycetes
- Ascomycetes
- Basidiomycetes
- Deuteromycetes (fungi imperfectii)

All the first three reproduce sexually

Zygomycetes

- Lower fungi
- Broad, nonseptate hyphae
- Asexual spores -

Sporangiospores: present

within a swollen sac- like

structure called

Sporangium

- **Examples:** Rhizopus, Absidia, Mucor
- Lower fungi
- Broad, nonseptate hyphae
- Asexual spores -

Sporangiospores: present

within a swollen sac- like

structure called

Sporangium

- **Examples:** Rhizopus, Absidia, Mucor

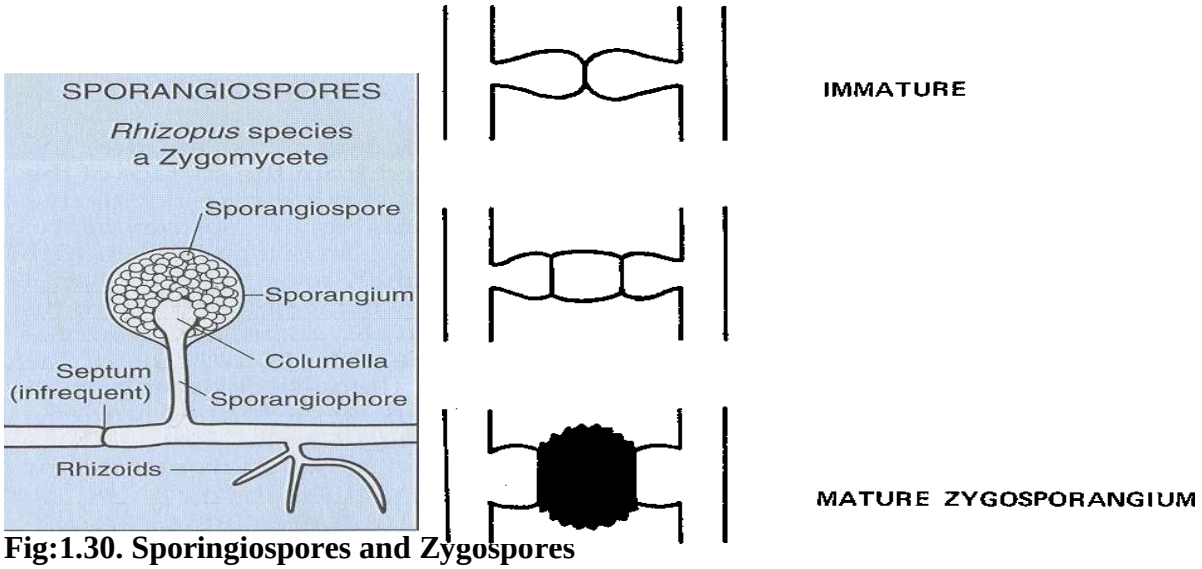


Fig:1.30. Sporingiospores and Zygospores

Ascomycetes

- Sexual spores called ascospores are present within a sac like structure called Ascus.
- Each ascus has 4 to 8 ascospores
- Includes both yeasts and filamentous fungi

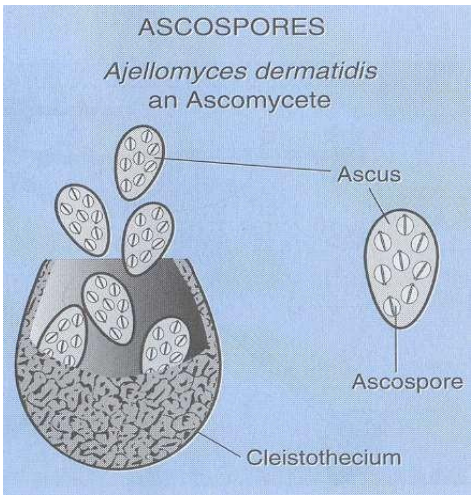


Fig: 1.31. Ascospores of fungi

- Narrow, septate hyphae
- **Asexual spores are called conidia borne on conidiophore**
- Examples: Penicillium, Aspergillus

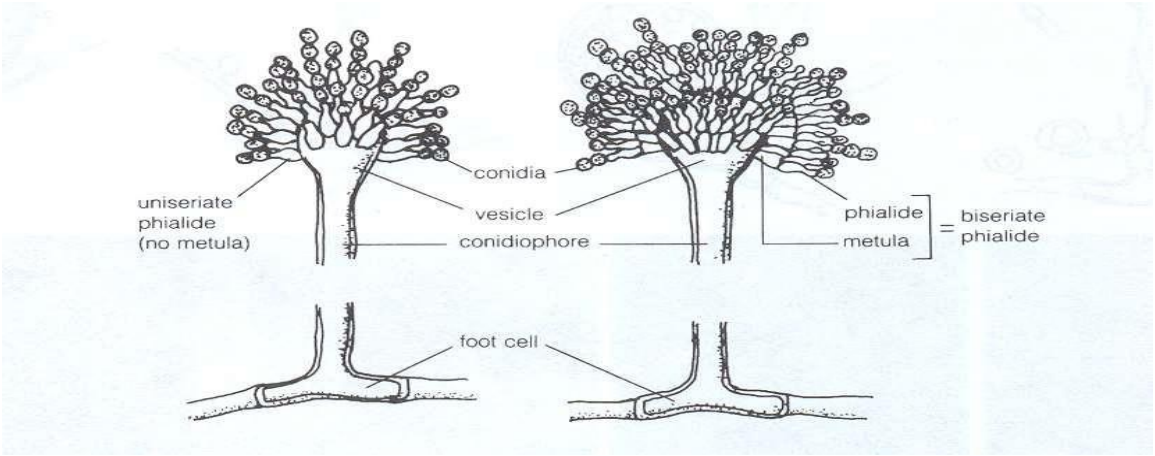


Fig: 1.32. Conidiospores

Basidiomycetes

Sexual fusion results in the formation of a club shaped organ called base or basidium which bearspores called basidiospores

Examples: *Cryptococcus neoformans*, mushrooms

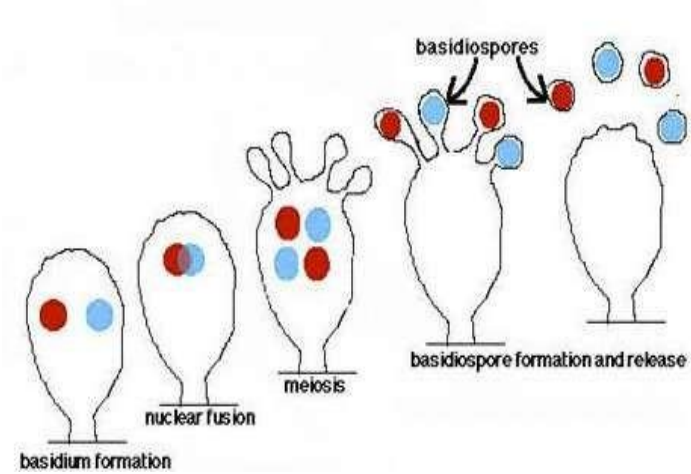


Fig:1.33. Basidiospores formation in fungi

Deuteromycetes or Fungi imperfectii

- Group of fungi whose sexual phases are not identified
- Grow as molds as well as yeasts
- Most fungi of medical importance belong to this class
- Examples: *Coccidioides immitis, Paracoccidioides brasiliensis, Candida albicans*

Reproduction and sporulation

Types of fungal spores

1. **Sexual spores**
2. **Asexual spores**

Sexual spores

- **Sexual spore is formed by fusion of cells and meiosis as in all forms of higher life**
- **Ascospores**
  - **Ascus**
  - **Ascocarp**
- **Basidiospores**
- **Zygospores**

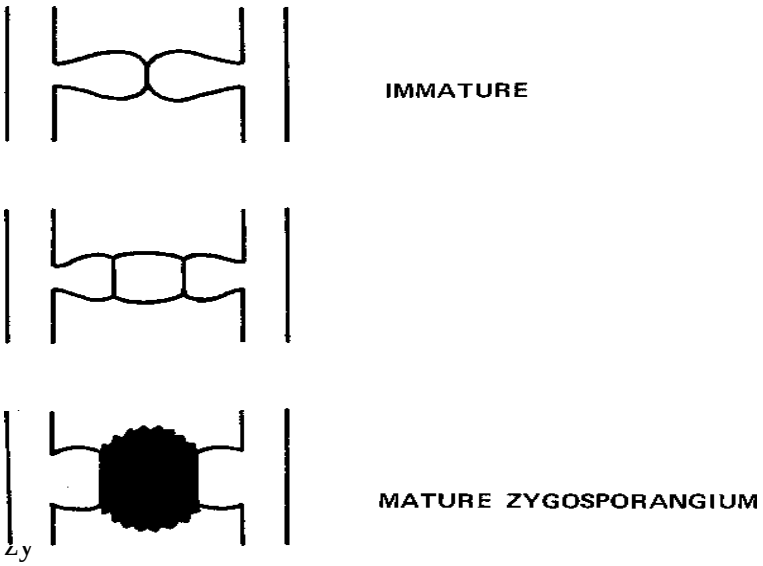


Fig: 1.34. Zygospore formation in fungi

REPRODUCTION IN FUNGI

ASEXUAL REPRODUCITON

Asexual spores

- These spores are produced by mitosis

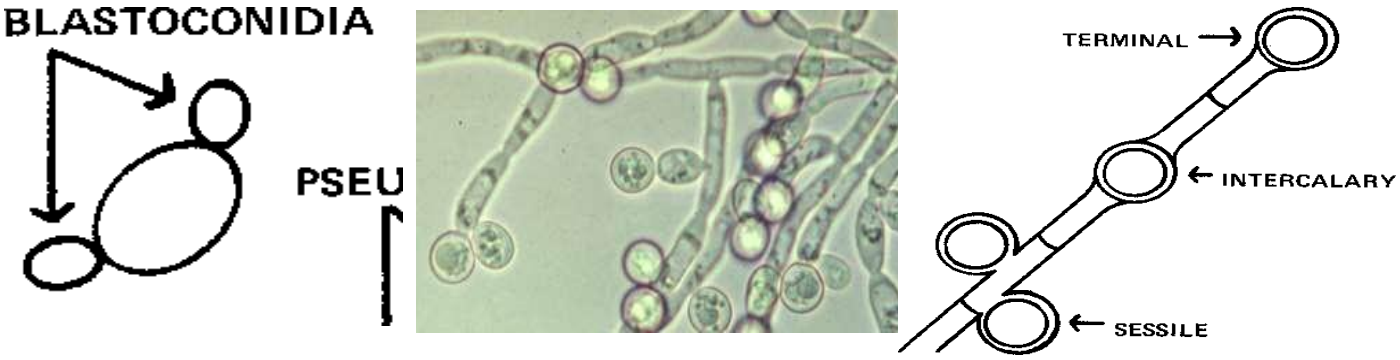
1. Vegetative spores
2. Aerial spores

Vegetative spores

- **Blastospores:** These

are formed by budding from parent cell, as in yeasts

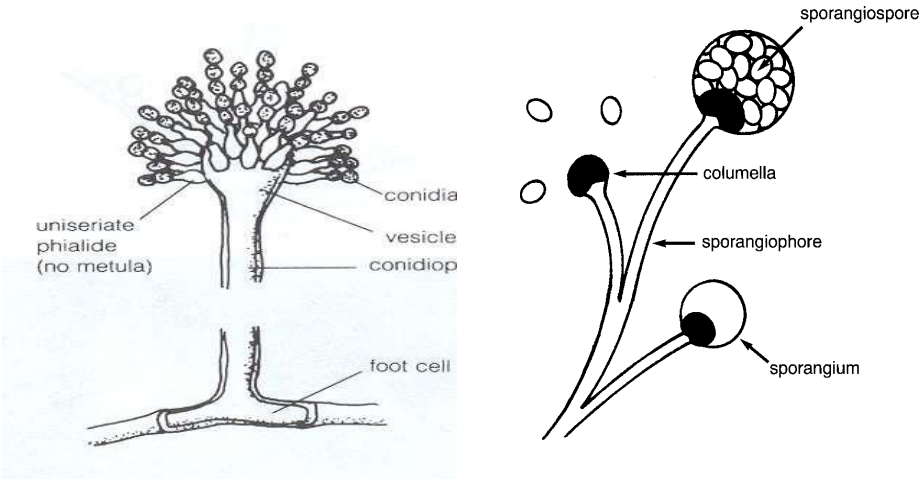
- **Arthrospores –** formed by segmentation & condensation of hyphae
- **Chlamydospores –** thick walled resting spores developed by rounding up and thickening of hyphal segments.



a **b** **c**

Fig: 1.35. a. Blastoconidia, b. Arthrosproes, c. Chlamydospores

Aerial spores



- a b

Fig: 1.36. a. Condiospores b. Sprongiospore

- Microconidia - Small, single celled
- Macroconidia – Large and septate and are often multicellular

ALGAE STRUCTURE AND REPRODUCTION

They can be defined as the small autotrophs that fail to show any cellular differentiaton & their sex organs are unicellular & if multicellular all cells are fertile

DISTINGUISHING FEATURES:

- They are photoautotrophs
- They primarily inhabit aquatic habitats
- The vegetative body does not show any differentiation into various tissue systems
- They show progressive complexity in reproduction
- They do not develop embryo after fusion of gamates during sexual reproduction
- Range in size from microscopic to single celled organisms to large seaweed
- Many species occur as single cells others as multicellular
- Algal cells are eucaryotic
- Study of algae is called phycology
- Cellwall is thin and rigid
- Motile algae such as euglena have flexible cell

  membrane called periplasts
- Cell walls of many algae are surrounded by a flexible gelatinous outer matrix
- A discrete nucleus is present
- Inclusions like starch granules, oil droplets and vacuoles are present
- Chlorophyll and other pigments are present
- Chloroplasts may occur one,two or many per cell they may be ribbon like ,bar like ,net like,or as discrete discs

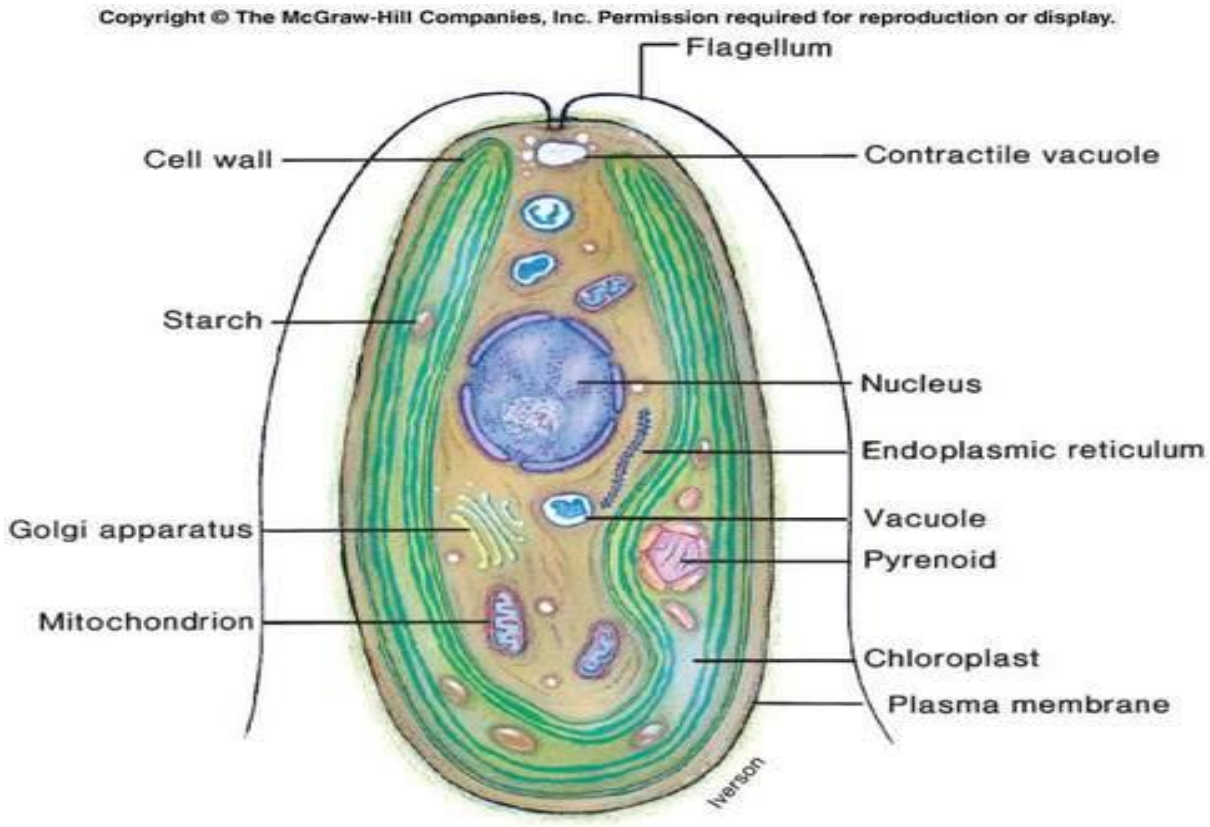


Fig: 1.37. ALGAE CELL STRUCTURE

GENERAL CHARACTERISTICS

- Thallus organisation
- Cell structure
- Algal flagella
- Algal pigments
- Algal nutrition
- Food reserves
- Reproduction

1) THALLUS ORGANISATION

a)Unicellular algae:

- single cells, motile with flagellate (like *Chlamydomonas and Euglena*) or nonmotile (like *Diatoms*).

Euglenophyta, Chlorophyta containg mostly unicellular algae that occur often in fresh water.

Diatoms, unicellular algae that have siliceous cell walls.

Dinoflagellates (Red algae), unicellular flagellated algae, with some that are armored with cellulose

Fig: 1.38. Unicellular algae

b)Colonial algae: Motile or non motile algae may form a colony by aggregation of the products of cell division with in a mucillagenous mass.

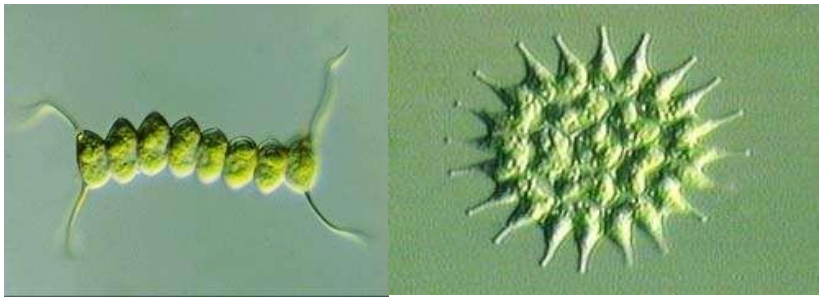


Fig: 1.39. Colonial algal morphology

c. **Coenobial :**

The colony is formed with a definite shape, size and arrangement of cells.

Ex: *volvox*

- **Palmelloid :**

Irregular arrangement of cells varying in number

,shape and size.

Ex: *Chlamydomonas , Tetraspora*

- **Dendroid:**

Looks like microscopic tree due to union of mucilagenous threads present at base of each cell.

Ex: *Chrysodendron*

- **Rhizopodial colony:**

Cells are united through rhizopodia Ex: *Chrysidiastrum*

c)Filaments algae

- Daughter cells remain attached after cec)Filaments algae:

- ll division and form a cell chain
- Adjacent cells share cell wall (distinguish them from linear colonies!)
- May be unbranched (uniseriate

such as *Zygnema* and *Ulthrix*) or branched (regular mutiseriate such as *Cladophora* or unreguler mutiseriate such as *Pithophora*

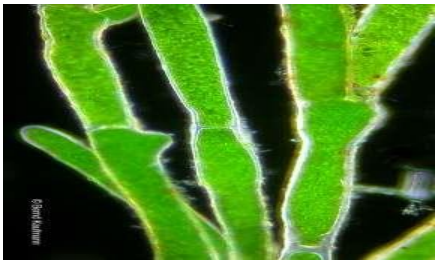


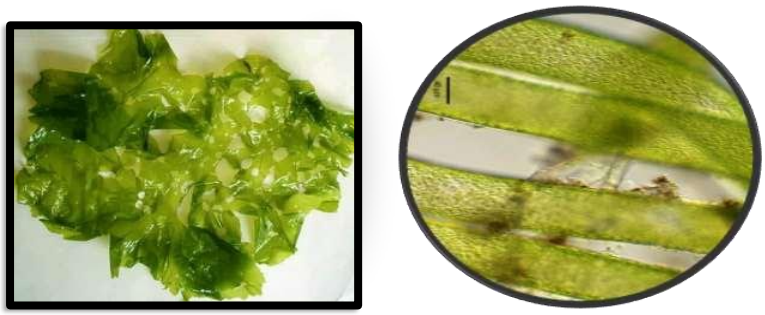
Fig: 1.40. Filamentous algal Morphology

d) **Coenocytic or siphonaceaous:**

one large, multinucleate cell without cross walls such as *Vaucheria*

e) **Parenchymatous:**

mostly macro-scopic algae with tissue of undifferentiated cells and growth originating from a meristem with cell division in three dimensions such as *Ulva*



A B

Fig: 1.41. A. Parenchymatous algal morphology, B. Coenocytic algal morphology

CELL STTUCTURE

- Eukaryotic characterised by presence of well organised nucleus and membrane bound organelles like plastids ,mitochondria and Golgi bodies

- An intermediate form called mesokaryotic occurs in Dianophyceae which shows both eukaryotic (nucleus with nuclear membrane & chromosomes) and prokaryotic characters(basic proteins are absent)
- Some do not has true cell wall Ex: euglena, gymnodinium & possess a membrane called pellicle around cytoplasm
- Motile flagella possess a pigmented spot known as eye-spot or stigma(swimming) - photoreceptive organelle , help algae in response to light
- Cell wall is with mixed carbohydrates and substances like alginic acid , fucoidin , fucin & hemicelluloses present
- Mitochondria, Golgi complex , Endoplasmic reticulum present.

ALGAL FLAGELLA

- Found in all algae except Rhotophyceae
- The main function is motility
- They are of 2 types
- Whiplash or acronematic-possess smooth surface
- Tinsel or pleuronematic-covered by fine filamentous appendages called as mastigonemes or flimmers
- Tinsel is divided into 3 types
    - **Pantonematic**-mastigonemes arranged in two opposite rows or radially
    - **Pantocronematic**-Pantonematic flagellum with a terminal fibril
    - Stichonematic-mastigonemes develop only on one side of the flagellum

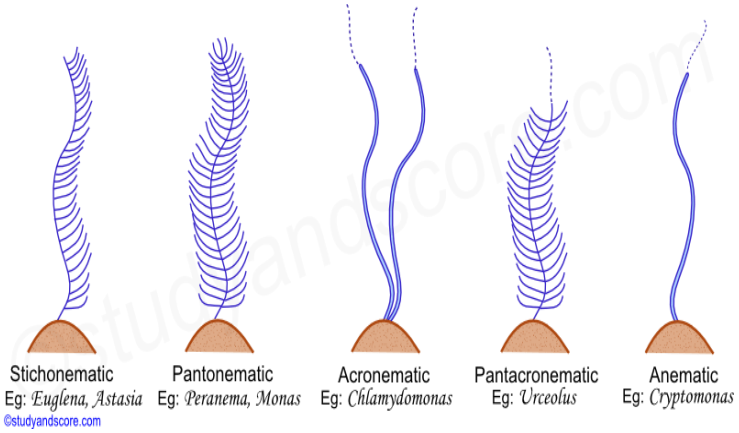


Fig: 1.42. Flagella in Algae with different mastigonemes arrangement

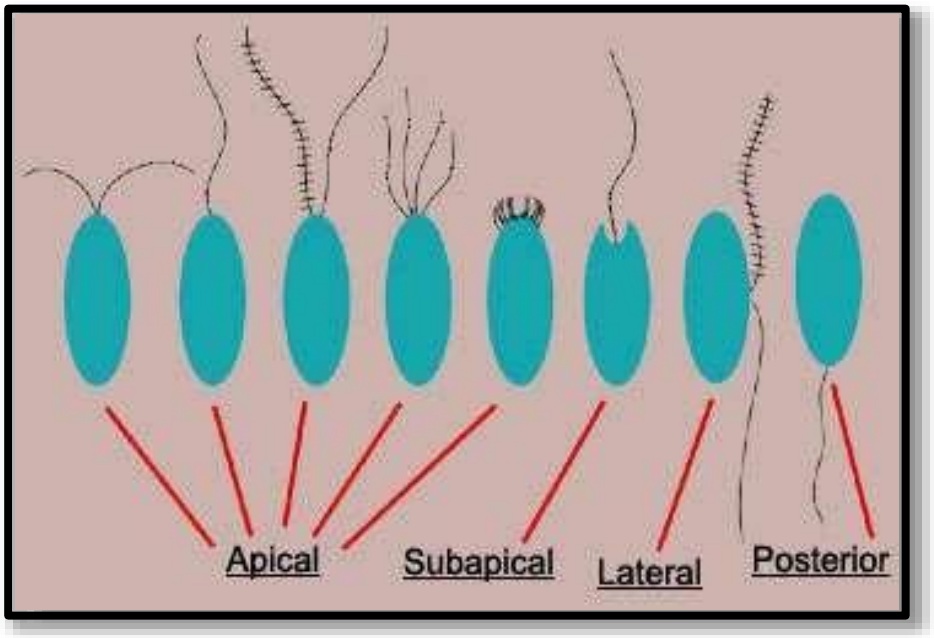


Fig: 1.43. Different locations of flagella arrangement in algal cell

4)ALGAL PIGMENTS

Distinct chlorplast, nuclear region and complex organelles.

- Thylakoids are grouped into grana

Pyrenoids are centers of carbon dioxide fixation within the chloroplasts of algae. Pyrenoids are not membrane bound organelles, but specialized area in plastid that contain high of ribulose-5 biphosphate carboxylase/oxygenase necessary in photosynthesis for carbon fixation and thus sugar formation starch, a storage form of glucose, is oftn found around pyrenoids.

- The pigments are within membrane bound organelles called plastids
- May be leucoplasts (colourless plastids) or chromoplasts (coloured plastids)
- Chromoplasts- contain chlorophyll a and b
- Chromatophores -contain only chlorophyll a
- Types - Chlorophylls(5), xanthophylls(20), carotenes(5) and phycobillins (7)
- Chlorophyll a present in all
- Xanthophylls(yellow/brown) present in chlorophyceae and pheophyceae

- B carotene present in most algae
- Phycobillins are water soluble red(phycoerythrin) and blue(phycocyanin) confined to rhodophyceae

5)ALGAL NUTRITION

- Photo autotrophic and synthesis their own food from carbondioxide and water
- Aquatic forms obtain carbon dioxide and water by diffusion and osmosis
- Aerials obtain water from damp substratum and carbon dioxide from air
- They also synthesis oil and protiens from carbohydrates

6)FOOD RESERVES

- Food materials accumulated as polysaccharides
- True starch-seen in two algal divisions chlorophyta and charophyta
- Floridean starch- found in rhodophyta
- Laminarin- found in brown algae
- Paramylon- found in euglenoids
- Leucosin-peculiar to xanthophyta , bacillariophyta & chrysophyta
- Fats occur as reserved food in appreciable amounts in the cells of xanthophyta , bacillariophyta & chrysophyta

7)REPRODUCTION IN ALGAE

There are three common methods of reproduction found in algae.

1. Vegetative reproduction
2. Asexual reproduction
3. Sexual reproduction

1. Vegetative reproduction

The vegetative reproduction in algae includes those methods of propagation in which portion of the plant body become separated off to give rise to individuals. Vegetative reproduction take place by different methods.

(i) By cell division:

- The mother cells divide and the daughter cells are produced, which become new plants.
- It is sometime known as Binary Fission.
- This type of reproduction is found in Diatoms , Euglena .

(ii) Fragmentation:

- The plant body breaks into several parts or fragments and each such fragment develops into an individual.
- This type of vegetative reproduction is commonly met within filamentous forms, e.g., Ulothrix, Spirogyra, etc.
- The fragmentation of colonies also takes place in several blue green algae, e.g.Aphanothece, Nostoc, etc.

(iii) Hormogone formation:

- When the trichome's break in small pieces of two or more cells, such pieces are called 'hormogones'
- In some Blue green algae the fragments undergoes a gliding movement which are called 'Hormogones'.
- Each hormogone develops into a new plant, e.g., Oscillatoria, Nostoc, etc

(iv) Hormospores or hormocysts:

- Such multicellular spore-like structure function as perennating bodies called " hormospores " or " hormocyasts " .
- They are thick-walled hormogones, and produced in some drier conditions.

(vi) Tubers:

- Usually these bodies are rounded and filled up with abundance of starch.
- Each body may give rise to a new plant, e.g., Chara.

(v) By adventitious thalli:

- Certain special structures of thalli are formed which help in vegetative reproduction. The well known propagula of Bryopsis, Sphacelaria are good examples

(vii) Bulbils:

- Small bud-like structures. Usually develop on the rhizoids of Chara are called bulbils.
- Each such bulbil may develop into a new plant.

(viii) Akinetes:

- It is the types of reproduction very common in the blue green as well as green algae.
- These akinetes are a type vegetative cell which is thick walled and will overcome the unfavourable condition.
- Sometimes they are formed in chain.
- Each akinete may develop into a new plant.
- This type of reproduction is found in Oedogonium, Ulothrix, etc.

ix) Adventitious Branches

- Adventitious Branches are formed in some large thalloid forms of algae.
- These branch when get detached from the parent thallus develops into new plant .
- Adventitious branch like protonema formed on the internodes of chara .

- E.g Dictyota , Fucus .

2.ASEXUAL REPRODUCTION

- Asexual reproduction is a mode of reproduction by which offspring arise from a single organism, and inherit the genes of that parent only.
- it is reproduction which almost never involves ploidy or reduction.
- The offspring will be exact genetic copies of the parent, except in the specific case of automixis .
- A more stringent definition is agamogenesis which is reproduction without the fusion of gametes.
- Usually the protoplast of a cell divides into several protoplasts and there after they escape from the mother and develop into new plants.
- Asexual reproduction is the primary form of reproduction for single-celled organisms such as the archaebacteria , eubacteria, and protists .
- Many plants and fungi reproduce asexually as well.
- Asexual reproduction take place by a variety of spore formed in different Algae. they include……

i. By zoospores

- The zoospores are formed from certain older cells of the filaments.
- The cytoplasm divides to form zoospores which are escaped from the mother cell.
- They are always formed in favourable conditions.
- The zoospores are always motile.
- The zoospore are naked protoplasmic bodies which move by mean flagella or cillia .
- They may be (i) biflagellate, (ii) tetraflagellate, and (iv) compound zoospores.
- E.g Oedogoniales , Vaucheriaceae

v (ii) **By aplanospores**:

- When motile phase of zoospores is eliminated, the bodies are called aplanospores.
- The aplanospore are produce when there is a lack of sufficient water.
- These are covered by a thin wall but do not possess flagella like the zoospores.
- The also germinate directly to give rise to new plant .

v (iii) **By hypnospores** :

- Actually they are very thick-walled aplanospores and develop only in adverse conditions.
- In comparatively drier situation the content of mother cell round off and secrete a thick wall around them , to tide over the unfavourable condition.
- These thick walled structure called resting spore or hypnospores .
- Sometime the entire cell as such become thick-walled to form an akinete .
- They are usually produced at the approach of dry and hot weather .

- On the approach of favourable condition they germinate directly to produce a new plant or form zoospores.
- e.g., Pediastrum, Vaucheria.

v (iv) **Palmella stage**:

- The approach of dryness as when the plants are left on the moist bank by receding water of the ponds the cells of many algae continue to divide but their contents are not liberated.
- The mother wall becomes gelations thus forming a mass or colony of rounded cells which lie embedded in a jelly like substance formed from the cell walls.
- On the return of favourable condition the cell come out either as zoospore or as aplanospores.
- The germination to produce normal plant .
- e.g Ulothrix etc

v (v) **Autospores**:

- They are just like aplanospores except that they are smaller in size.
- They resemble in shape to mother cell except in size.
- Each autospore gives rise to a new plant.
- Such autospores are reported from many Chlorococcales.
- E.g ,Scenedemus etc.

v (vi) **Endospores**:

- In many blue green algae and Bacillariophyceae, the endospores are formed within the cells.
- The endospore forming cell behaves as a Sporangium .
- On the approach of favourable conditions, each endospore develops in a new individual.

v (vii) **Cysts** :

- These are thick walled spores formed during unfavourable conditions or even when food supply is abundant.
- During their formation as in vacheria the thallus becomes many septate and each chamber thus formed produce a thick walled cysts.

- sometime the cysts many be formed in rhizoides as in botrydium when they are called rhizocysts .

- Sometime a cyst may divide further to form a number of microcysts

4. Sexual reproduction

- The first fossilized evidence of sexual reproduction in eukaryotes is from the Stenian period, about 1 to 1.2 billion years ago.
- These reasons include fighting the accumulation of deleterious mutations, increasing rate of adaptation to changing environments, dealing with competition or as an adaptation for repairing DNA damage and masking deleterious mutations.
- While these ideas about why sexual reproduction has been maintained are generally supported, the ultimate size of the population determines if sexual reproduction is entirely beneficial.
- Larger populations appear to respond more quickly to benefits obtained through sexual reproduction than smaller population sizes.
- a basic advantage for sexual reproduction in slowly reproducing complex organisms, exhibiting characteristics that depend on the specific environment that the given species inhabit, and the particular survival strategies that they employ .
- It is greatly advanced method of reproduction

Conditions for sexual reproduction:

- (a) The sexual reproduction takes place after considerable accumulation of food material and the climax of vegetative activity is over.
- (b) The bright light is the major factor for the production of the gametes.
- (c) A suitable pH value is required.
- (d) The optimum temperature is necessary.

Sexual reproduction are three main types,

- isogamy
- heterogamy
- Aplanogamy or conjugation

(i) **Isogamy**:

- The fusion of similar motile gametes is found in many species.
- Usually the gametes taking part in fusion come from two different individuals or filaments, sometimes these gametes come from two different cells of the same filament.
- they cannot be classified as "male" or "female." Instead, organisms undergoing isogamy are said to have different mating types, most commonly noted as "+" and "-" strains

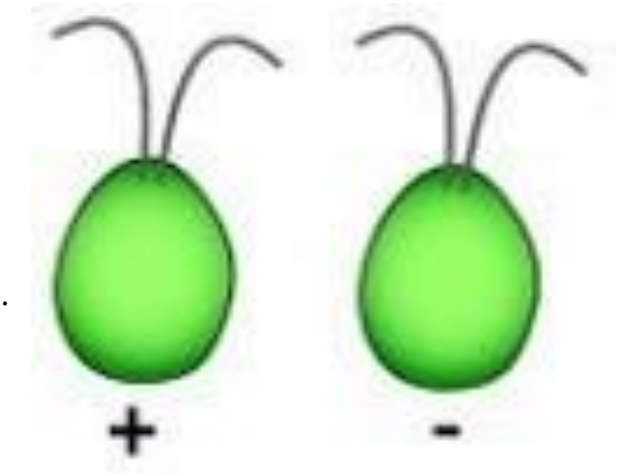


Fig: 1.44. Isogamy gametes

- although in some species there are more than two mating types .
- Fertilization occurs when gametes of two different mating types fuse to form a zygote.

v (ii) **Heterogamy**:

- The fusion of dissimilar gametes is called heterogamy.
- There are two main types ,
  - (a) Anisogamy:
  - (b) Oogamy:

Ø (a) **Anisogamy**:

- The fusing gametes are similar in appearance and are motile but are different physiologically or in size.
- The smaller gamete is considered to be male (sperm cell), where as the larger gamete is regarded as female (egg cell).
- Small or microgametes and large or macrogametes .

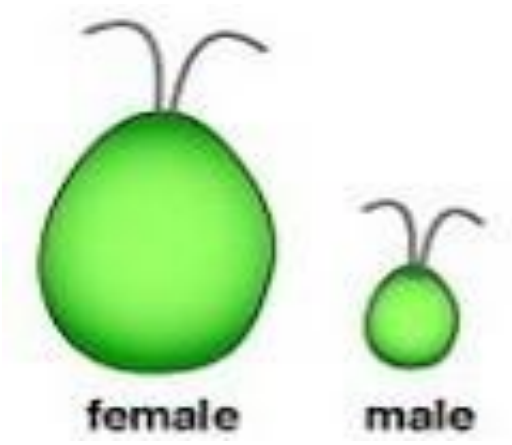


Fig: 1.45. Anisogamy gametes

- There are several types of anisogamy. Both gametes may be flagellated and thus motile.
- Alternatively, neither of the gametes may be flagellated.
- This situation occurs for example in some algae and plants.
- In the red alga Polysiphonia, large non-motile egg cells are fertilized by small, non-motile spermatia.

Ø(b) **Oogamy** :

- In this case, the male antherozoid (male gamete) fuses with the female egg.
- The fusing gametes are different in size and behaviour .
- One of the gamete is small and motile while the other is large and non-motile.

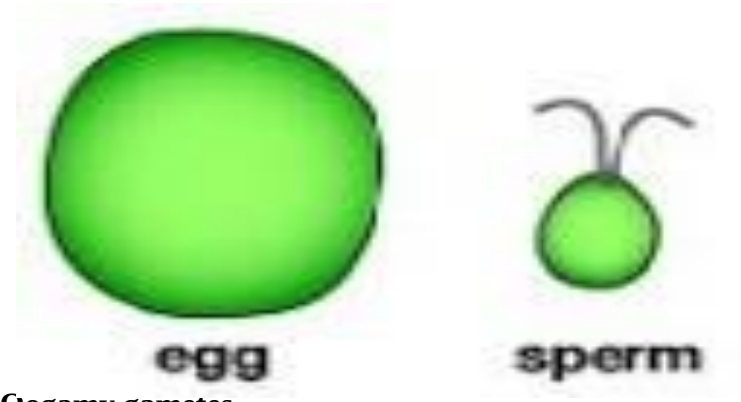


Fig: 1.46. Oogamy gametes

- This types of sexual reproduction is termed as fertilization or oogamyus reproduction and the product is called an oospore.
- This is usually found in higher types of green and brown algae.

E.g ,Vaucharia , Chara etc

v (iii) **Aplanogamy or conjugation**:

- It implies the fusion of two non-flagellate amoeboid gametes (aplanogametes).
- They are morphologically similar but physiologically dissimilar,

e.g., order Conjugales.

- In fresh water algae, the sexual reproduction is best means of perennation because it is followed by the formation of thick-walled zygote or oospore.