Vivekanand College, Kolhapur (Empowered Autonomous)

Department of Microbiology

Power Point Presentation Bank (PPT bank)

(2022-2023)

Sr. No.	Name of Topic	Class	Course
1.	Culture media	B.Sc. I	Basic Biochemistry Paper III
2.	Amylase Fermentation	B.Sc. III	Paper XI Industrial Microbiology
3.	Bacterial Nomenclature (Bacterial Systematics)	B.Sc. I	Paper I History and mile stones in microbiology
4.	Concept of Sterilization (Control of micro organisms)	B.Sc. I	Paper II Basic Techniques in Microbiology
5.	Wine Fermentation	B.Sc. III	Paper XI Industrial Microbiology
6.	Good laboratory Practices	B.Sc. III	Paper XIV Agricultural and Environmental Microbiology
7.	Introduction to Microbiology	B.Sc. I	Paper I History and Mile stones in microbiology

CULTURE MEDIA

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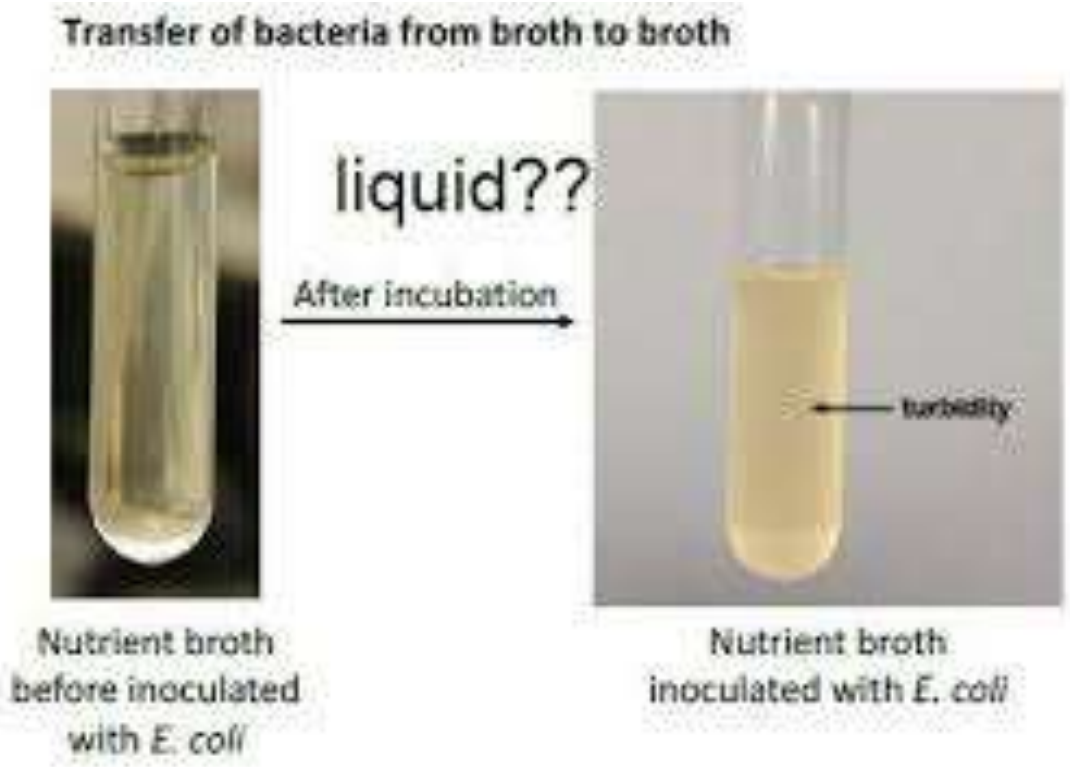
Lab animals

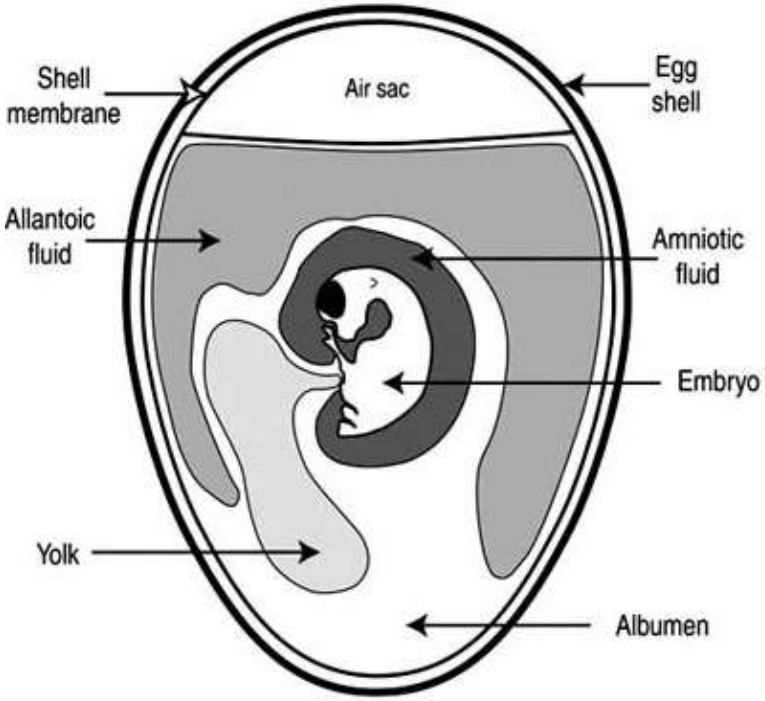
Plants

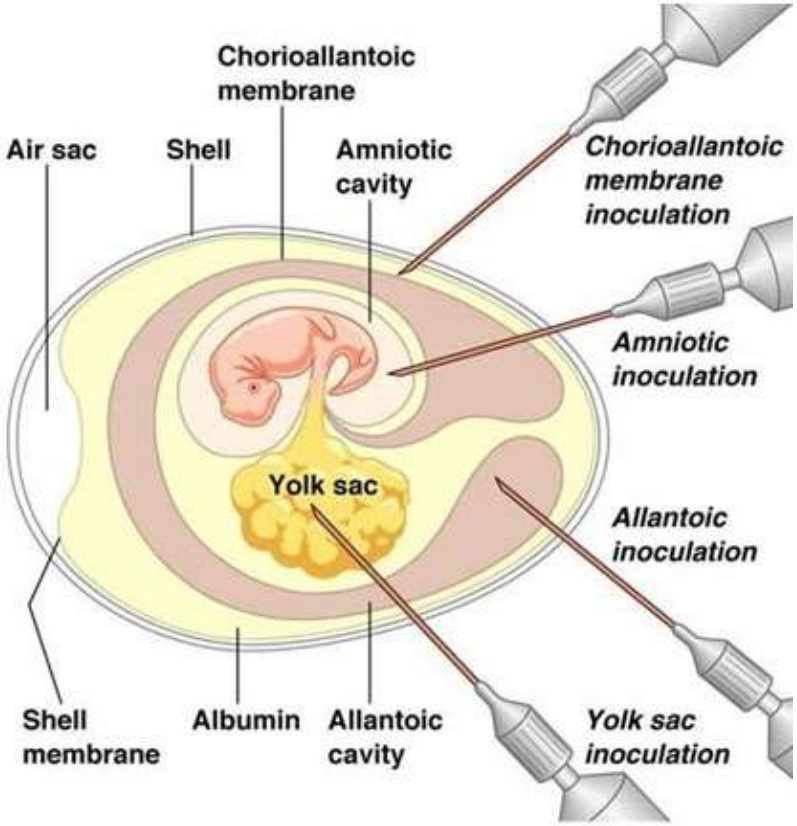
Broth Culture



Pocks formed by bacteriophages

Embryonated egg





Media types

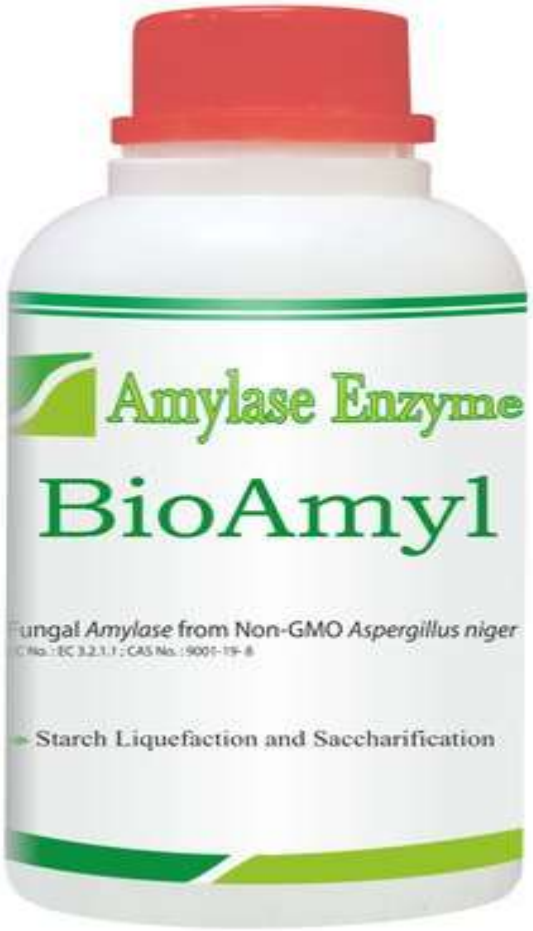
- Natural media

Synthetic media

- Inorganic synthetic media

Winogradsky's media

Amylase fermentation



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INTRODUCTION

Amylases are a complex group of enzymes that hydrolyze polysaccharides like starch and glycogen to glucose.
During the hydrolysis of 1, 4-glycoside, linkages present in above polysaccharides are degraded which result first in the formation of short chain dextrins, then to maltose and glucose. It is produced by plants, animal and microorganisms.

TYPES OF AMYLASE-

1. Alpha-amylase is widespread among living organisms. In the digestive systems of humans and many other mammals. By acting at random locations along the starch chain, α-amylase breaks down long-chain saccharides, ultimately yielding either maltose, dextrose, glucose etc. It acts anywhere on substrate.
2. Beta-amylases are present in yeasts, molds, bacteria, and plants, particularly in the seeds. They are the principal components of a mixture called diastase that is used in the removal of starchy sizing agents from textiles and in the conversion of cereal grains to fermentable sugar.

Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. They are produced by plants, Fungi and bacteria.
Both enzyme are present in seed of plants.

Table 8.4: Important applications of α-amylases

Industry	Source		Applications
	Bacillus	Aspergillus	
Starch industry	+	--	Liquefaction of starch for production of glucose, fructose, maltose
Milling	--	+	Modification of deficient flour
Alcohol	+	+	Liquefaction of starch before the addition of malt for saccharification
Baked goods	--	+	Increase in the proportion of fermentable carbohydrates
Brewing	+	--	Barley preparation, liquefaction of additives
	--	+	Improved fermentability of grains, modification of beer characteristics
Paper	+	--	Liquefaction of starch without sugar production for sizing of paper
Textiles	+	--	Continuous desizing at high temperature
Feed industry	+	--	Improvement of utilization of enzymatically treated barley in poultry and calf raising
Sugar	+	--	Improvement of filterability of cane sugar juice via breakdown of starch in juice
Laundry and detergent	+	--	Increase in cleansing power for laundry soiled with starch, additive in dish washer detergents

3.1. Sources

- There are 2 major reasons for the increasing interest in microbial sources:

1) The growth of microorganisms is rapid and this will in turn speed up the production of enzyme.

- Microorganisms are easy to handle when compared to animals and plants.
- They require lesser space and serve as more cost effective sources.

2) Microorganisms can be easily manipulated using genetic engineering or other means.

- They can be subjected to strain improvement, mutations and other such changes by which the production of α-Amylase can be optimized.
- α-Amylase is produced by several bacteria, fungi and genetically modified species of microbes.

Bacteria which produce α -amylase are
Bacillus subtilis,
B. Cereus
B. amylo- liquefaciens
B. coagulans
B. polymyxa
B. Stearothermophilus
B. Caldolyticus
B. Acidocaldarius
B. subtilis amylosaccharaticus
B. licheniformis,
species of *Lactobacillus, Micrococcus, Pseudomonas, Arthrobacter, Escherichia, Proteus, Thermomonospora* and *Serratia.*

Some α-amylase producing fungi are
species of *Aspergillus, Penicillium, Cephalosporium, Mucor, Candida, Neurospora* and *Rhizopus*

There are mainly two methods which are used for production of α-Amylase using fungal strains on a commercial scale. These are:

1) Submerged fermentation (SMF)

2) Surface fermentation (SF)

Surface culture method

Oldest method
Solid substrate such as wheat bran serve basic component of media
Fungus is cultivated on solid substrate in tray in large chamber or horizontal drums
These chamber trays, drum are washed with detergents, sanitized using sodium hypochlorite
Steaming is also done.
After sterilization of medium (mash), it is cooled to room temperature and inoculated with fungal spore(*Aspergillus* strain)
Inoculated medium is distributed in tray in shallow layers.
Temperature and humidity conditions are controlled by circulation of hot or cool moist air
Maximum enzyme production occurs in 1-7 days

Harvesting-

Medium harvested and enzyme is extracted by Filteration, centrifugation, vacuum evaporation
OR
The wheat bran is directly dried and grinded to obtain enzyme bran.
Water or buffer passes through the bran, which takes enzyme with them.
The extract is collected and enzyme is precipitated using isopropylalcohol .

Substrates Used in SSF

Sugar Cane Bagasse

Tea Waste

Wheat Bran

Saw Dust

Apple Pomace

Coconut oil Cake

Submerged culture method

It gives high yield, using deep tank fermentation

Usually flour starch serves as raw material supplemented with inorganic salt

Addition of stillage , corn steep liquor, yeast extract stimulate amylase production

Calcium carbonate is added to adjust pH .

Addition of salts like Nacl, KCL in trance amount is effective.

pH- 5.0

Medium is sterilized for 1 hour and cooled at room temperature.

It is inoculated with fungal culture (*A .niger* NRRL 337)

and incubated at 30 ºC with continuous shaking for 96 hours

To avoid bacterial contamination ammonium bifluoride or sodium pentachlorophenate.

Harvesting-

First step in harvesting of enzymes from the fermentation broth is removal of insoluble products like microbial cells which is generally carried out by centrifugation.

Most of industrial enzymes are extracellular which remain in the fermentation medium after removal of the biomass.

The biomass is treated with lime to deactivate the microorganisms and stabilize it during storage & then can be used as a fertilizer.

The enzymes remaining in the broth are then concentrated by various methods like evaporation, membrane filtration or crystallization

Industrial production of Bacterial amylase-

It is usually carried out using selected strains of *B subtilis* (or *B amyloliquifacience*)

First used by Underkoffler in 1966

Stock culture is maintained on nutrient agar slants. Properly stored in refrigerators.

Fermentation inoculum is prepared by inoculating stock culture in nutrient broth at 32 C on shaker

This content is used to inoculate seed tank(fermentation media) with 40 gallon of medium.

Seed is grown for 10 hours at 32C with agitation and aeration .

Fermentation medium-

Sterilization at 121 C for 35 min and cooled to 35 C.

Table 24.5:Medium for production of bacterial amylase

COMPONENT	QUANTITY
Soybean meal	1.85 %
Amber BYF autolysed Brewer's yeast fraction	1.50 %
Distiller's dried soluble	0.76 %
Casein hydrolysate	0.65 %
Lactose	4.75 %
$MgSo_4$	0.04 %
Anti foaming agents	0.05 %
Water	90.40 %

Fermentation process:

Seed culture is added to fermentation media and conditions are maintained for production.
After fermentation is started , sample for enzyme are taken aseptically every 4 hour for first 24 hours and then after every 2 hour until process is completed.
Enzyme assay are done for collected sample.
The process get completed within 48 hours.

Recovery of enzyme-

Filteration using diatomaceous earth filter
Filtrate containing enzyme transfer to precipitation tank where enzyme is precipated using alcohol
Precipitate is collected after settling and recovered.
Transfer precipitate to vacuum drier, grind it to fine powder and then packed.

BACTERIAL NOMENCLATURE

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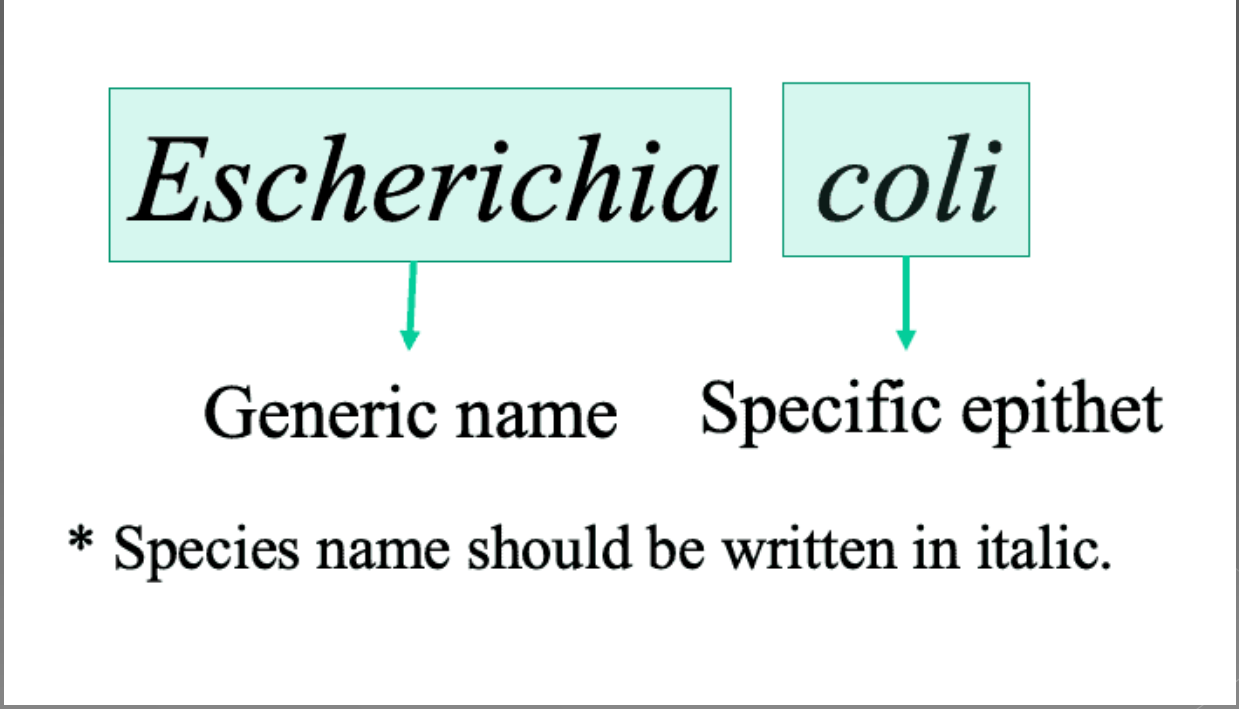
Taxonomic Ranks

- Classification of organism is to arrange them into similar or related groups on the basis of their structural, functional, genetic characters that help us to identify that organism. Identification is a process of characterizing an organism. The nomenclature is the system of assigning the name to the organism on the basis of information collected in respect to the organism.
- Bacterial nomenclature and naming are regulated by the International Code of Nomenclature of Prokaryotes (shortly the **Prokaryotic Code**), but the classification of actual species is not.. For example, the name *Escherichia coli* is regulated by the Prokaryotic Code, but not the properties and taxonomic classification of the species itself. This means that there is no official taxonomy and only names are controlled.

- As for animals and plants, bacterial (and archaeal) nomenclature follows the binomial nomenclature introduced by Carl Linnaeus.
- **How is a species name formed?**
- A species name should have two parts as a binomial system: (1) generic name and (2) specific epithet. This combination represents the 'species name' and it should be unique in the nomenclature system.



- The scientific names of all taxa must be treated as Latin; names of taxa above the rank of species are single words.
- **Names are organized into a hierarchical system**
- The hierarchical system of the official nomenclature is as follows: (only the popular ones are given)
  - **Phylum (or Division):** at present, the phylum rank is not controlled by the Prokaryotic Code. Oren *et al.* proposed to include 'phylum' in the Code, but the proposal was not yet discussed and approved by the International Committee on Systematics of Prokaryotes (ICSP), the body that controls the Prokaryotic Code [Learn more].
  - **Class:** The type of a class is one of the orders. The class is named after the type genus of the type order of the class.
  - **Order**: The type of an order is one of the genera. The order name is named after the type genus of the order. E.g. The order *Pseudomonadales* is named after the type genus *Pseudomonas*.

- **Family**: In general, the family name is named after the type genus of the family, e.g. The family *Pseudomonadaceae* is named after the type genus *Pseudomonas*.
- **Genus**
- **Species**
- **Subspecies**: Subspecies are created only when it is necessary. There is no 'type subspecies' concept in the Code. I think that many subspecies should be re-evaluated using genomics as they are mainly classified by single or a few phenotypes. For example, an important probiotic species, *Lactobacillus delbrueckii* contains six subspecies.

Rank	Suffix	Example
Order	-ales	*Pseudomonadales*
Suborder	-ineae	*Pseudomonadineae*
Family	-aceae	*Pseudomonadaceae*
Genus		*Pseudomonas*
Species		*Pseudomonas aeruginosa*

- These names are given by the scientists who propose the species and assign it to a genus. Naturally, any species can be assigned in this hierarchical system; however, this is not always implemented. Officially, a species must be classified in a known genus but assigning that genus to a family, then an order and so on is optional. Frequently this happens because the taxonomic relationships are ambiguous, so it isn't really clear how to group genera into higher taxonomic ranks. This leaves a lot of chaos in the bacterial taxonomic system and it's particularly bad news for microbiome researchers where investigations are made at the different taxonomic ranks such as family or even phylum
- **Serovar vs. serotype : Serovar** and **serotype** are synonyms and thus, interchangeable terms, but according to the Rules of the Bacteriological Code (1990 Revision), serovar is the preferred term. **Serogroup** is a group of bacteria containing a common antigen. A serogroup may contain several serotypes. Serogroup is not an official designation, but has been used to classify bacteria belonging to the genera *Leptospira, Salmonella, Shigella* and *Streptococcus*.

CONCEPT OF STERILIZATION

METHODS OF STERILIZATION

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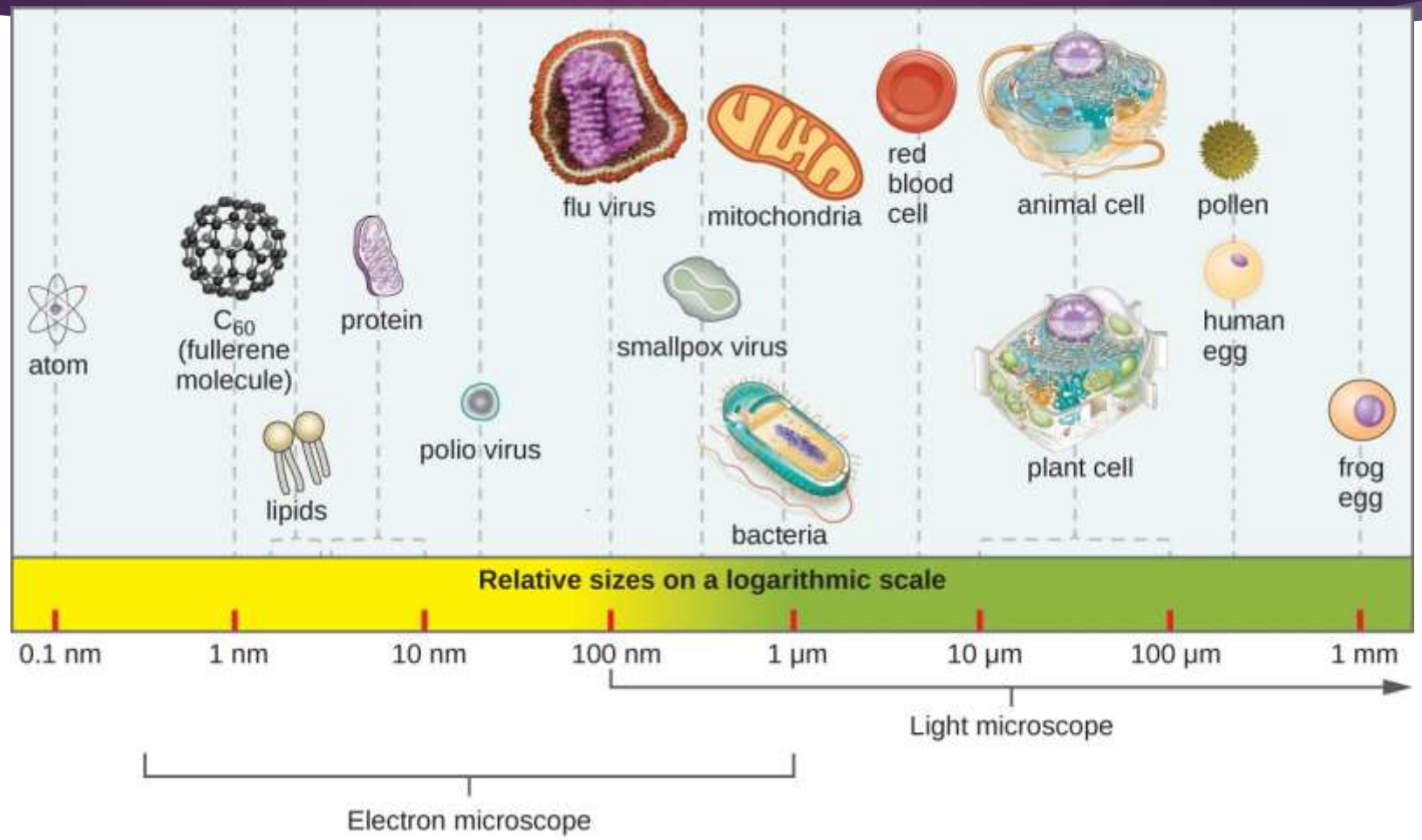
What are micro organisms?

- A micro organism is an organism that can only be seen through a microscope. They include bacteria, fungi, algae, protozoa and viruses. Some microbes have been used by mankind since a long time for the production of milk products like curd, yoghurt and wines from fruit juices. Their existence was brought into light for the first time by the scientist Antony Van Leeuwenhoek and after examining through saliva, urine, cow dung, teeth tartar where he found tiny motile objects he named them as animalcules.
- Microbiology is a branch of life sciences which deals with the study of micro organisms.
- Some micro organisms are important in food fermentation, production of antibiotics, vaccine production, alcohols, acetone, organic acids, vitamins, biofertlizers, biopesticides, probiotics, etc.

- Some micro organisms can cause diseases and their relation to diseases as a causative agent helped us to understand true nature of diseases. They may cause spoilage and poisoning of food which degrades the nutritive quality of the food items.



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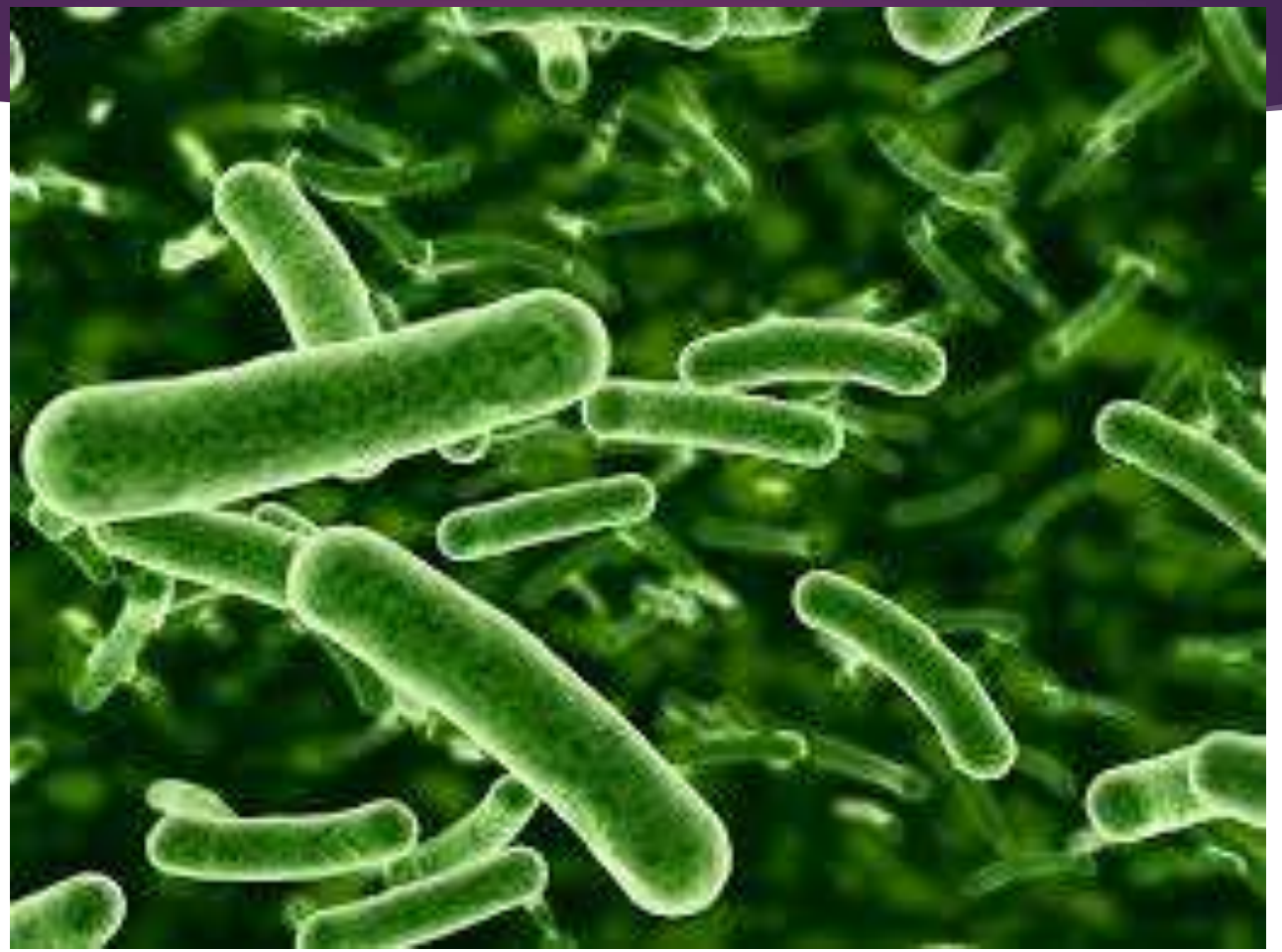
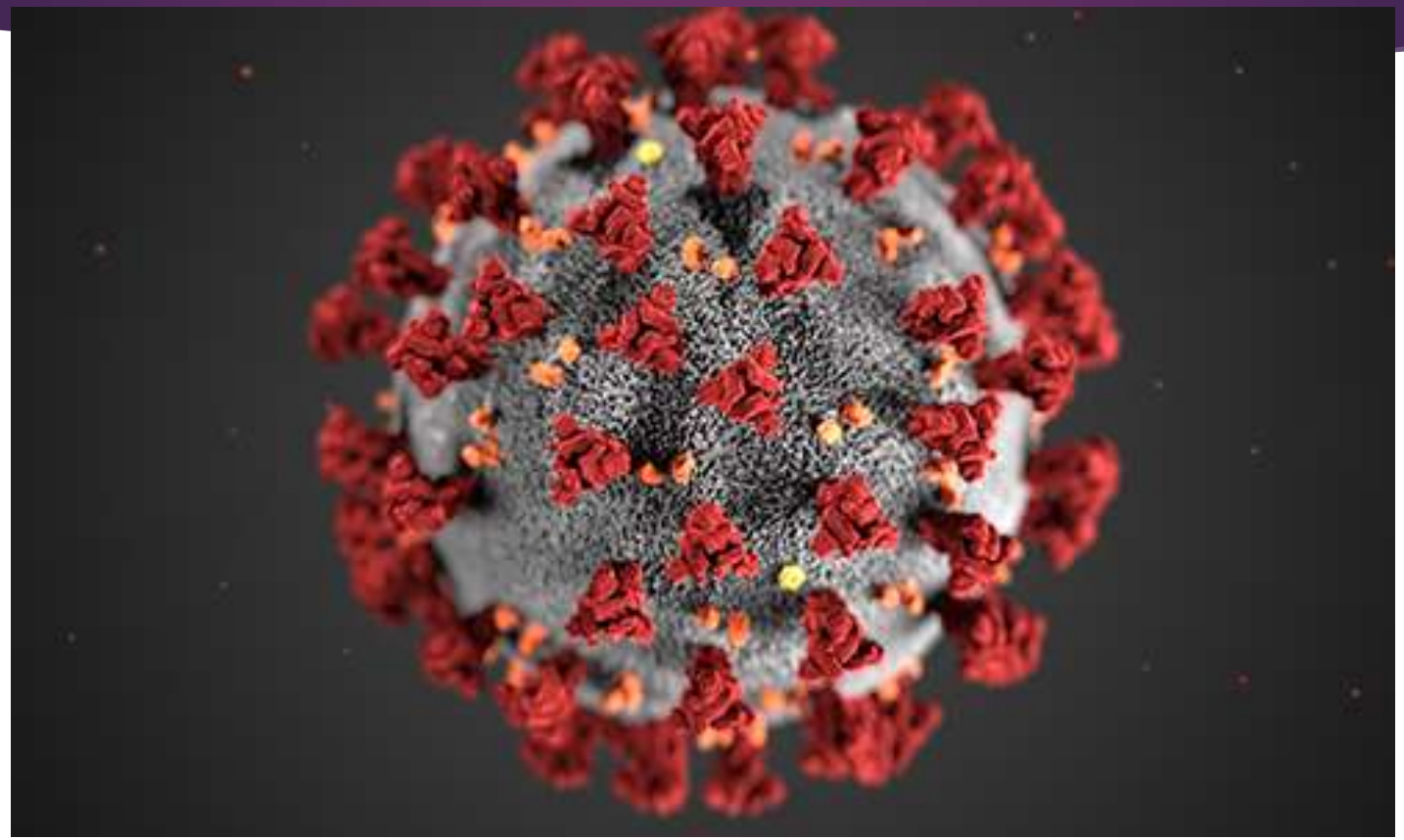


FUNGI

Algae

VIRUSES



PROTOZOA

What is sterilization?

- Micro organisms like bacteria, viruses, fungi, algae, protozoa, etc. are beneficial as well as harmful to mankind and can cause many diseases to man, animals and even plants. Because of the hazardous consequences it is essential to kill such micro organisms or to inhibit their growth.
- Sterilization (Latin: *Sterilis* – Unable to produce offspring or barren)

Sterilization is a process by which all living cells, viable spores, viruses, viroid are either destroyed or removed from an object or habitat.

An object is said to be sterile when it is totally or completely free of viable micro organisms. By sterilization, microbial population is reduced to level that is considered safe by Public Health Standards.

Disinfection:

- It is a process by which killing or inhibition or removal of micro organism that may cause disease. It kills growing forms but not spores which are resistant. Disinfection is carried out by using chemicals and used for non living objects.

Antiseptic

Sterilization by physical agents

- Temperature (Heat):

1) Dry Heat: Sterilization done in absence of water

e.g. HOT AIR OVEN

2) Moist Heat: Sterilization done in presence of water

e.g. AUTOCLAVE

- Fractional Sterilization
- Pasteurization

- DESSICATION
- OSMTIC PRESSURE
- RADIATIONS
- FILTRATION

STERILIZATION BY CHEMICAL AGENTS

- Phenols and phenolic compounds
- Halogen compounds
- Heavy metals
- Gaseous agents

Wine fermentation

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INTRODUCTION

Wine is fermented fruit juice. The most important fruit is *Vitis vinifera* (Vitaceae), but any fruit can be used. Example- orange, cherry, apples, blackberries etc.

Yeasts occur on the skins of most fruits and if the fruits are mashed, the sugar-containing juices begin to ferment.

History and origin-

Winemaking probably began as one of the earliest of human enterprises (8000-3000 B.C.).

The wine grape was domesticated by at least 4000 B.C.

Wine was used for Egyptian worship ceremonies.

Wine only became a popular beverage about 2000- 1000 B.C. in Greece.

About 600 B.C., wine growing reached France. Wine grapes were introduced early into the United States. The Spanish introduced grapes into California in the 1700's.

TYPES OF WINE-

1.Appetizer wine- e.g- sherry , vermouth
2.Red table wine- Red pinot, burgundy
3. White table wine- Pinot, chiandi
4. Desert wine- Tokay, port
5. Sprinkling wine- Champagne, sprinkling burgundy (carbonation is done in bottle)
6. Dry wine- High alcohol content, it contain less sugar
7. Still wine- It do not contain carbon dioxide. Eg- table wine, desert wine

The alcohol concentration varies in different wine. Sprinkling wine, it contains 10-14% of alcohol by volume where as in appetizer and desert wine content is 20% by volume.

The fundamental process is similar in all regions but the taste depends on varieties of grapes, climate and acidity of wine.

Ripe grapes

Transportation

Crushing

Fermentation

Pressing

Filtration

Aging

Bottling

Degustation

Process of wine fermentation

1. The grape- Selection of best variety of grapes and at proper maturity stage is important.

2. Handling of grapes- Careful supervision while selection of grapes, gathering and their transport to clean containers for protecting them from deteriotion.

3. Crushing of grapes- crushing and steamed of grapes is done by machines.
Use of iron , tin or copper container is avoided as they cause clouding in wine. Stainless steel Or nickel vessels should be used . Before crushing they are cooled overnight.

4. Treatment before fermentation-

Grapes contain a variety of microorganisms, moldes , yeast on its surface.

A winer has to ensure that the quality of produced wine should be maintained by killing, Microorganism found on the grapes and using a pure culture of specific yeast.

Metasulphite (2-6 gm./per tone of grape juice) destroy or inhibit the growth of undesirable like acetic acid bacteria, wild yeast, moldes or sulphur gas is passes through must to control microbes.

In few case pasteurization can be done in place of sulphitation.

5.Fermentation

It depends on yeast selected, nutrient in must, sugar concentration, acidity, oxygen supply and Temperature.

S. Cerevisiae var. elliposodeus (1-5%) is added to pasteurized must.

It is added after 6 hours after sulphitation.

A large supply of oxygen is needed for yeast multiplication.

The content is mixed twice a day to facilitate temperature and aeration.

A cap formation (grape skin, pieces of stem) is observed after some time on fermentation vat.

Avoid over aeration of must. Fermentation is carried out at 21-25º C (below 30ºC)

After 3-5 days maximum colour extraction from skin of grapes is done by agitation

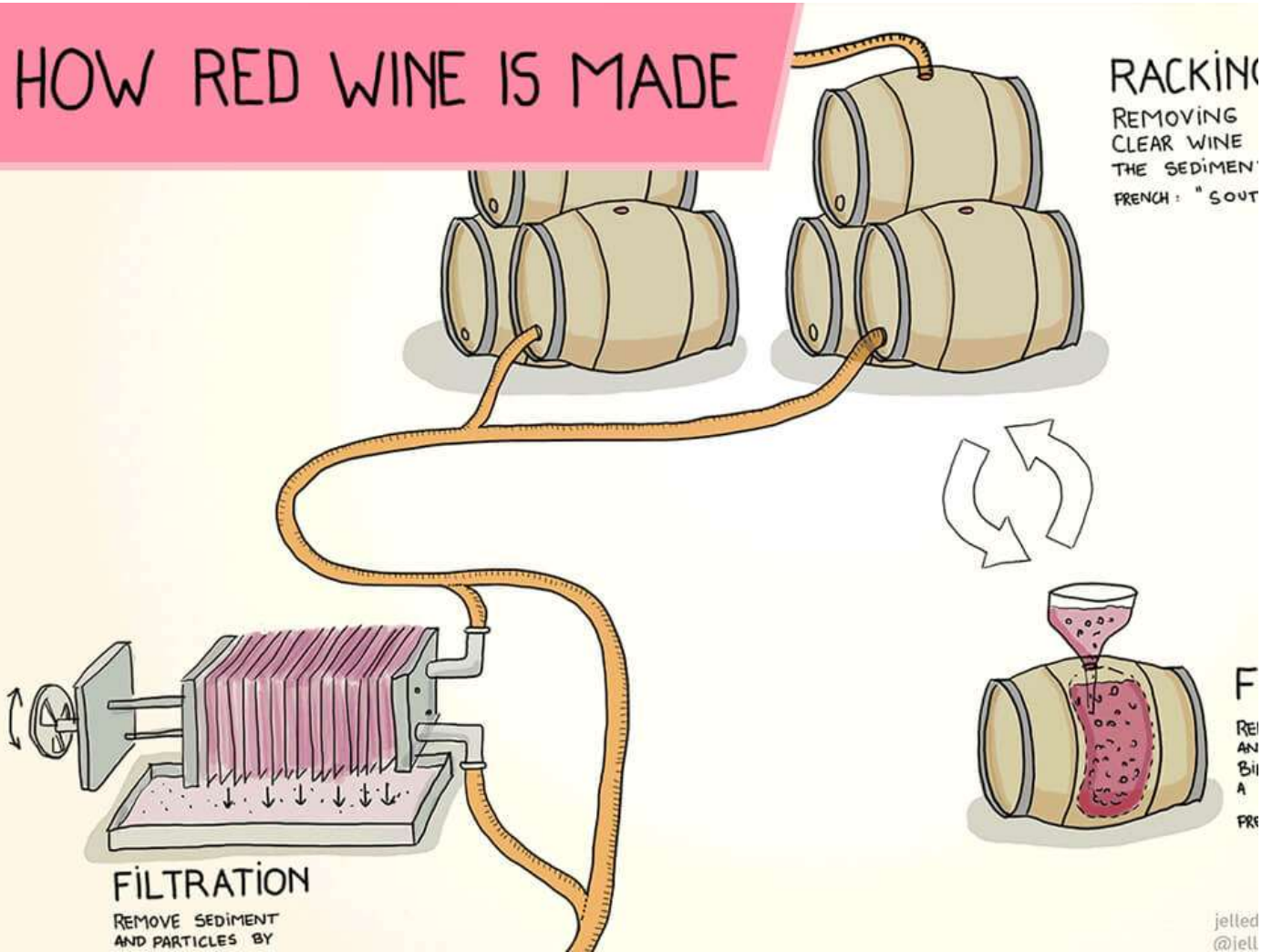
Check sugar concentration (below 0.1% cease process) and alcohol content at regular interval


alamy stock photo

Fermentation will not reach this stage when-
(1) musts of very high sugar content are fermented
(2) alcohol-intolerant strains of yeast are used
(3) fermentations are carried on at too low or high temperatures
(4) fermentation under pressure is practiced.
Fermentation of normal musts is usually completed in 10 to 30 days.

6.Post fermentation-
The free run wine is placed in close storage tank equipped with bunge that allow the excess co2 To escape.

7. Racking- In most cases, the major portion of the yeast cells will soon be found in the sediment, or lees. Separation of the supernatant wine from the lees is called racking.
Potassium bitartarate is found in lees that is less soluble in alcohol than in water and precipitate Out readily at room temperature.
Other proteins, insoluble matter are removed during racking and Filteration.
Also flash pasteurization is carried out cooling at R.T and holding at low temperature for few days. Followed by Filteration.


HOW RED WINE IS MADE
RACKING
REMOVING
CLEAR WINE
THE SEDIMEN
FRENCH: "SOUT
FILTRATION
REMOVE SEDIMENT
AND PARTICLES BY
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8.Storage and ageing-
During this steps two process occurs i.e. clearing of wine and development of flavours .
Storage is done using white oak or red wood. The container are completely filled with wine providing some head space.
To prevent aerobic microbes contamination.
Continuous inspection is done along with racking and refilling.
Flavours development occurs during aging as result of oxidative change and ester formation.
Ester formation is important for aroma development.
Time of aging varies from wine to wine.

9.Clarification-
It occurs naturally over a period of time.
It can be done by finning, Filteration, heating, refrigeration.
Finning agents are casein, tannins, gelatin etc followed by Filteration.

10.Packing- From large oak barrels , it is transferred to bottles or can of small or medium size.
Pasteurization is done for 30 min at 140ºC.

Defects in wine-

Two type of defects are observed-

A) Defect caused by microbes-

1. Disease caused by aerobic microorganism- It is caused by *Mycoderma* and *Acetobacter.*

Acetobacter will produce vinegar from wine in presence of oxygen.

Candia mycoderma form film over the surface of wine and attack the alcohol and acid content in wine.

2. Disease caused by facultative anaerobes and anaerobes-

Tourne disease- Tourne specifies the microorganism or condition produced by bacteria in wine. It is common disease. Acidity in wine increases by silk type of cloudiness appearance.

Anaerobic bacteria inhibited by tanning but strongly by metasulphite or SO2.Maintaing aseptic conditions is important to avoid contamination.

Bitter wine result from contamination of butyric acid bacteria.

B) Defects not caused by microbes-

Defect caused by metals, enzyme or finning agents(gelatin cause clouding of wine)

Iron will cause clouding of wine called ferric casse.

Oxidase cassse is caused by enzyme oxidase produced by certain mold which cause white wine To become brown. Oxidase is inhibited by sulfite gas treatment or pasteurization.

GOOD LABORATORY PRACTICES

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Good Laboratory Practice

- GLP is an FDA regulation. GLP is a formal regulation that was created by FDA (United States Food and Drug Administration) in 1978.
- GLP was first introduced in New Zealand and Denmark in 1972. GLP was instituted in US following cases of fraud generated by toxicology labs in data submitted to the FDA by pharmaceutical companies.
- Definition: "GLP embodies a set of principles that provides a frame work within which laboratory studies are planned, performed, monitored, archived & reported"

- GLP is a formal regulation created by USFDA as these regulations were proposed on November 19, 1976 and designated as a new part of Chapter 21 of the Code of Federal Regulations as 21 CFR part 58 in 1979
- In 1981 an organization named as OECD (Organization for Economic Cooperation and Development) produced GLP principles that are international standards.
- GLP in OECD principles is defined as "a quality system concerned with the organizational process and the conditions under which non clinical health and environmental safety studies are planned, performed, monitored, recorded, archived & reported"

- Why were GLP's created?
- In the early 70's FDA became aware of cases of poor laboratory practice all over the United States.
- They discovered a lot of fraudulent activities and a lot of poor lab practices.
- Examples of some of these poor lab practices found were equipment was not calibrated to standard form therefore it gave wrong measurements.
- Incorrect /inaccurate accounts of the actual lab study.
- Inadequate test systems.

- Purpose:
- GLP is to certify that every step of the analysis is valid or not.
- It assures the quality and integrity of data submitted to FDA in support of the safety of regulated products.
- GLP's have heavy emphasis on data recording, record and specimen retention.

- Principles of GLP:

1. Test Facility Organization and Personnel
2. Quality Assurance Program (QAP)
3. Facilities
4. Apparatus, materials & reagents
5. Test systems
6. Test & reference substances
7. Standard Operation Procedures(SOP)
8. Performance of the study
9. Reporting of study results
10. Storage and retention of record and materials

1. Test Facility Organization and Personnel

Study Personnel Responsibilities

- Should have the knowledge of the GLP principles
- Access to the study plan and appropriate SOP's
- Comply with the instructions of the SOP's
- Record raw data
- Study personnel are responsible for the quality of their data
- Exercise health precautions to minimize risk
- Ensure the integrity of the study

2. Quality Assurance Program

Responsibilities of a QA personnel

- Access to the updated study plan and SOP's
- Document verification of the compliance of study plan to GLP principles
- Three types of inspection

  -Study based inspections

  -Facility based inspections

  -Process based inspections
- Inspection of the final reports for accurate and full description
- Report the inspection results to management
- Statements

3. Facilities

- Suitable size, construction and location
- Adequate degree of separation of the different activities
- Isolation of test systems and individual projects to protect from biological hazards
- Suitable rooms for the diagnosis, treatment and control of diseases
- Storage rooms

4. Apparatus, materials and reagents

- Apparatus of appropriate design and adequate capacity
- Documented inspection, cleaning, maintenance and calibration of apparatus
- Apparatus and materials not to interfere with the test systems
- Chemicals, reagent and solutions should be labeled to indicate identity, expiry and specific storage instructions

5. Test systems

- Physical and chemical test systems
- Biological test systems
- Record of source, data of arrival and arrival conditions of test systems
- Proper identification of test systems in their container or when removed
- Cleaning and sanitization of containers
- Pest control agents to be documented

6. Test and reference items

- Receipt, handling, sampling and storage
- Characterization
- Known stability of test and reference items
- Stability of the test item in its vehicle (container)
- Experiments to determine stability in tank mixers used in the field studies
- Samples for analytical purposes for each batch

7. Standard Operating Procedure

- Written procedures for a laboratory program
- They define how to carry out protocol specified activities
- Most often written in a chronological listing of action steps
- They are written to explain how the procedures are supposed to work

SOP's

- Routine inspection, cleaning, maintenance, testing and calibration
- Actions to be taken in response to equipment failure
- Keeping records, reporting, storage, mixing and retrieval of data
- Definition of raw data
- Analytical methods

8. Performance of the study

- Prepare the study plan
- Content of the study plan
- Identification
- Records
- Dates
- Reference to test methods
- Information concerning the sponsor and facility
- Conduct of the study

9. Reporting of study results

- Information on sponsor test and facility
- Experimental starting and completion dates
- A quality assurance program statement
- Description of materials and test methods
- Results
- Storage (sample, reference items, raw data, final reports

10. Storage and Retention of Records and Materials

- The plan study, raw data and samples
- Inspection data and master schedules
- SOP's
- Maintenance and calibration data
- If any study material is disposed off before expiry the reason to be justified and documented
- Index of materials retained

What Good Laboratory Must Contain?

- Area should be free from smoke, smell, dust, etc.
- Ensure good ventilation, proper illumination and prefer natural light
- Air condition the lab with humidity control
- Enough space for measuring and testing instrument
- Proper arrangement of testing
- Take care of all safety points including proper earthing as well as fire safety
- Avoid uncleanable spots in floor, wall, ceiling
- Establish proper areas for storage of incoming samples as well as test completed samples
- Also provide sample collection place as well as packing and disposal of tested samples

Do this for GLP

- Keep the things at its location after use
- Store heavy things at bottom and if possible on trollies
- Give name of location to everything
- Follow ‘Everything has a place and everything at its place’ principle
- Prepare location list and display it
- Put ladders for things stored on top
- Identify everything with its name/ purpose
- Follow ‘FIFO’ to prevent old accumulation for laboratory chemicals

Benefits of good laboratory practices

- It will give better image of company as a Quality producer in Global market
- Provide hot tips on analysis of data as well measure uncertainty and perfect record keeping
- Provide guideline for doing testing and measurement in detail
- Provide guidelines and better control for maintenance of instruments, environment control, preservation of test records, etc.

Conclusion

- Gives better image of company as a Quality producer in Global market provide hot tips on analysis of data as well as measure uncertainty and perfect record keeping and guideline for doing testing and measurement in detail.
- Finally GLP provide guidelines and better control for maintenance of instruments, environment control, preservation of test records, etc.

Introduction to Microbiology

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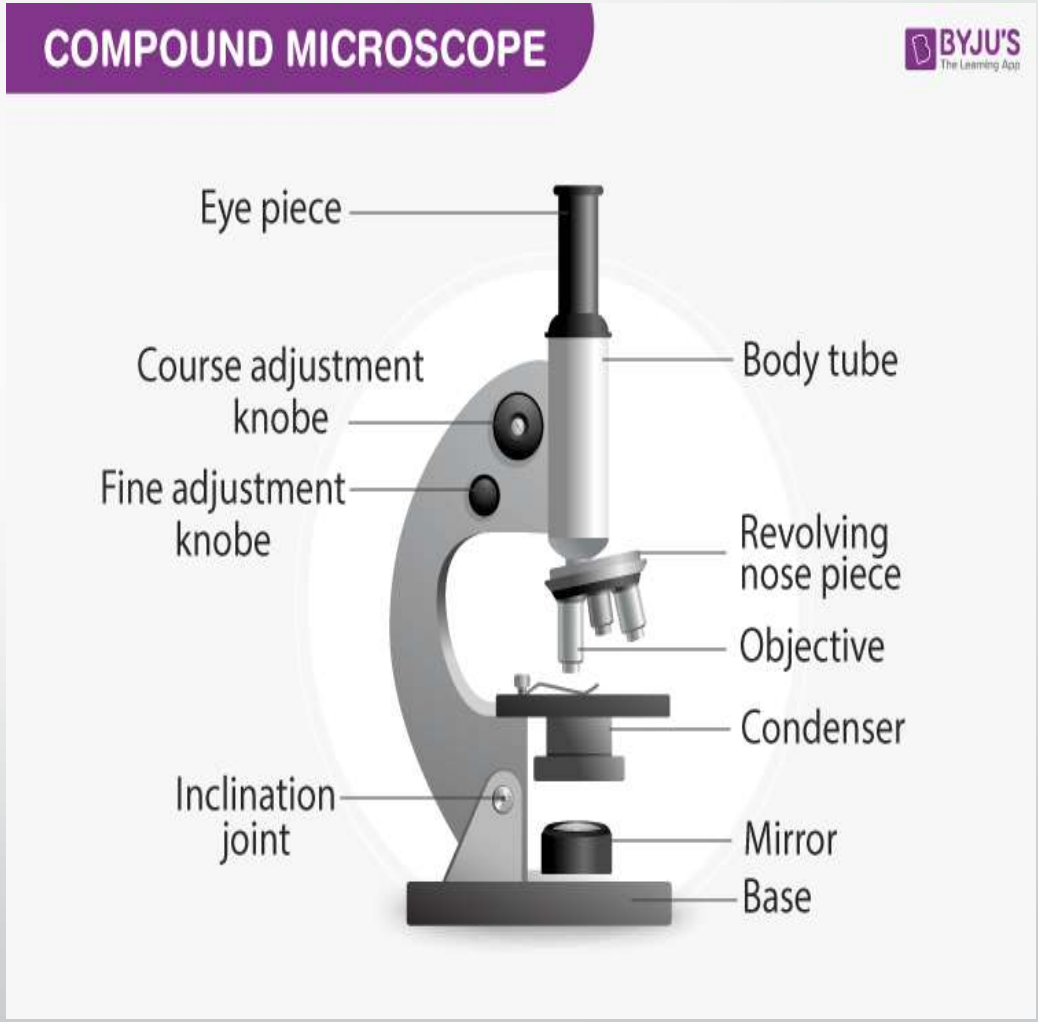
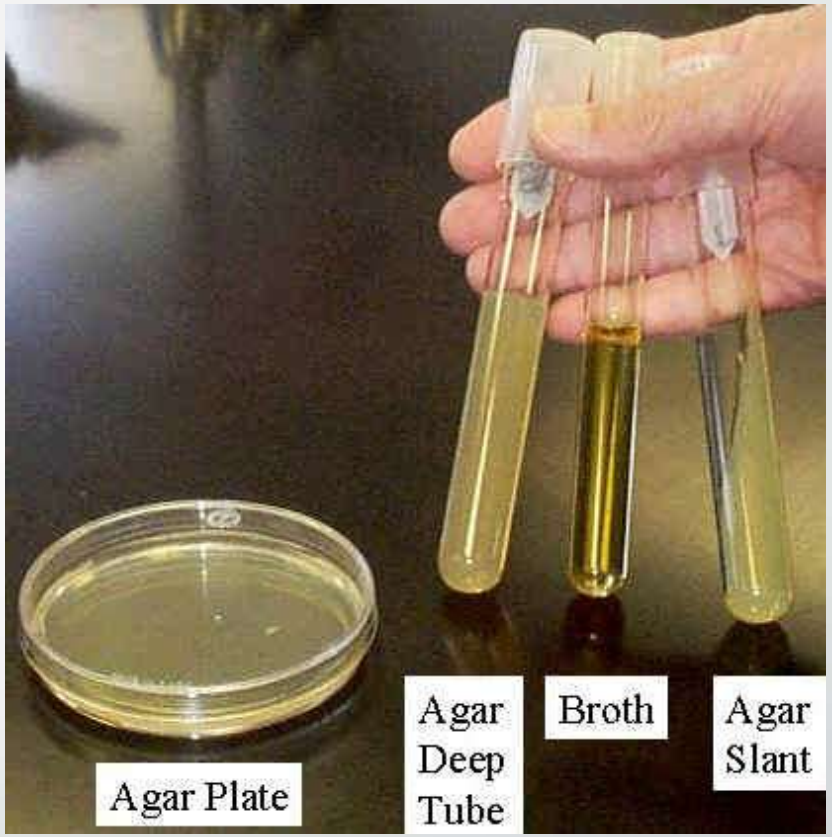
What is a light microscope?

- A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects, and enlarging them.
- They use lenses to focus light on the specimen, magnifying it thus producing an image. The specimen is normally placed close to the microscopic lens.
- The functioning of the light microscope is based on its ability to focus a beam of light through a specimen, which is very small and transparent, to produce an image. The image is then passed through one or two lenses for magnification for viewing. The transparency of the specimen allows easy and quick penetration of light. Specimens can vary from bacterial to cells and other microbial particles.



Petri plates

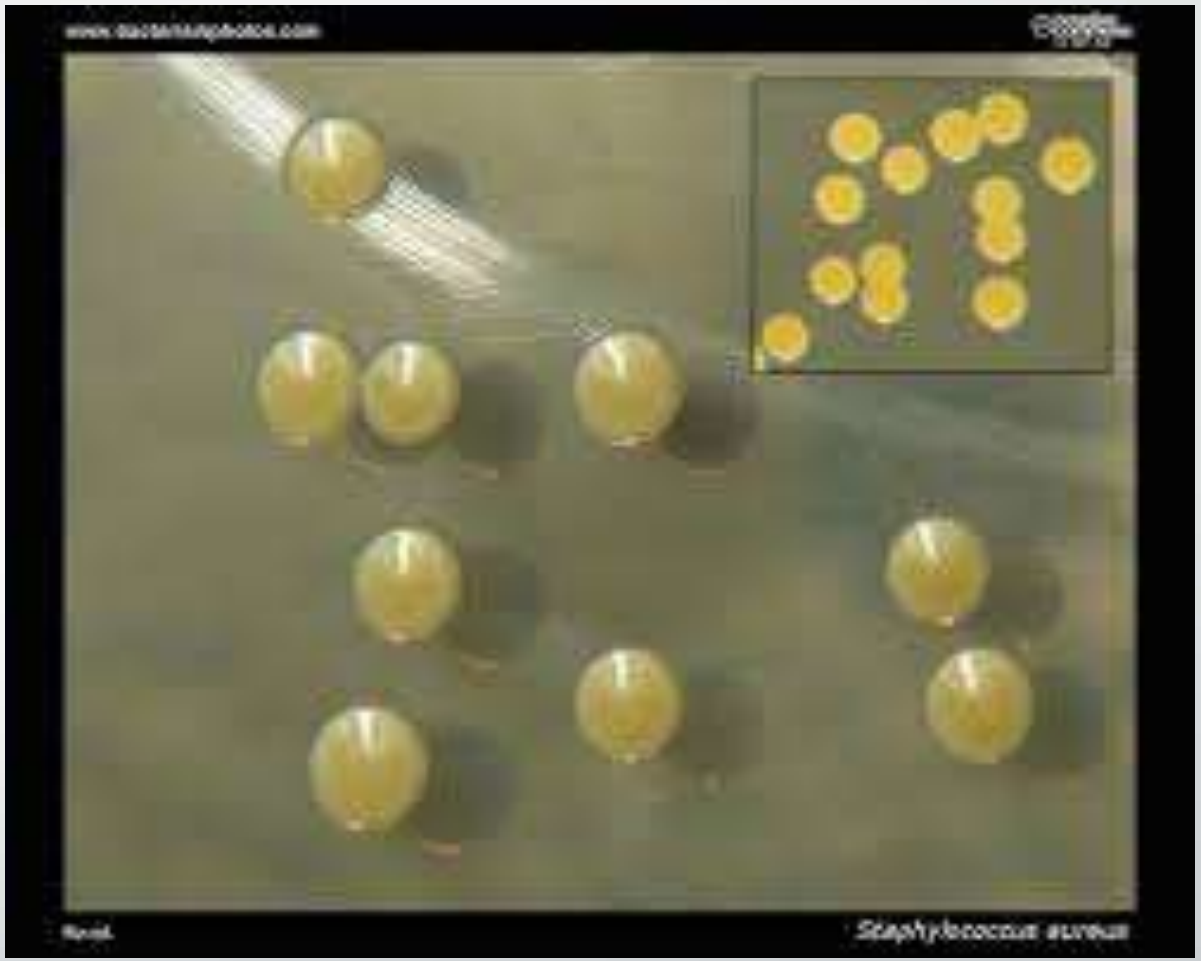
Agar media plates (e.g. Nutrient agar)



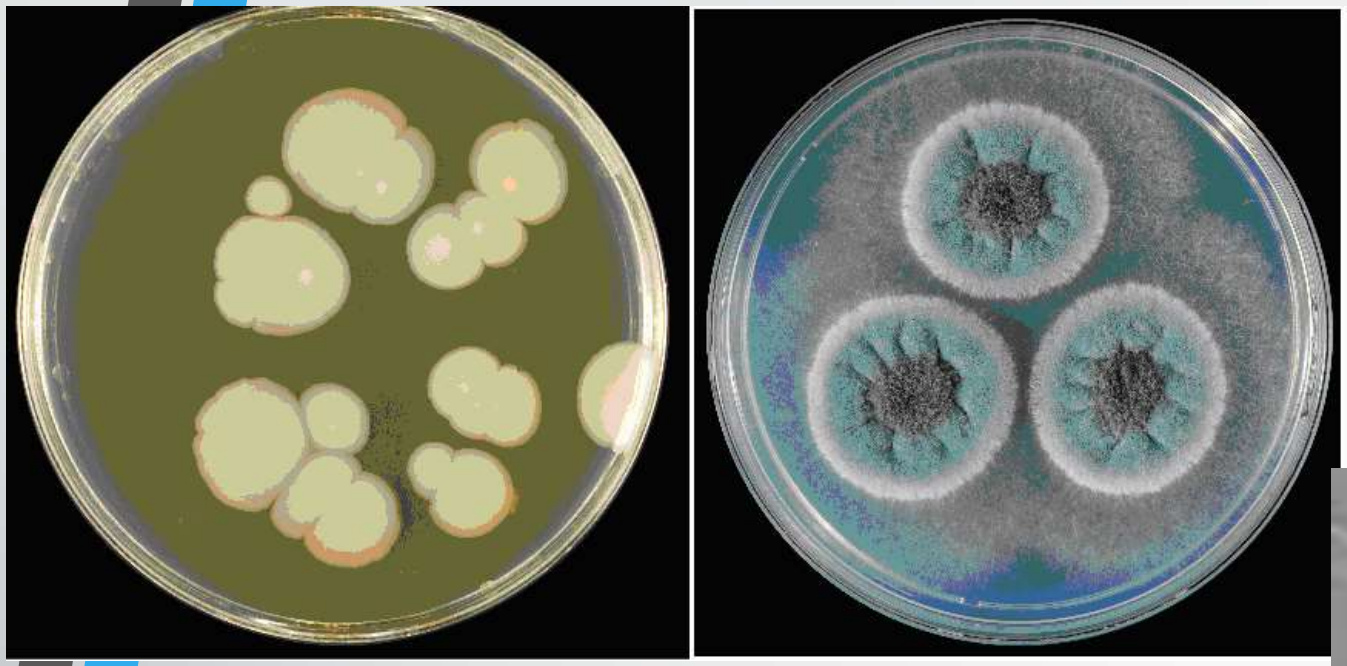
Nichrome wire loop

Freshly prepared media plates

Streaked plates/ culture

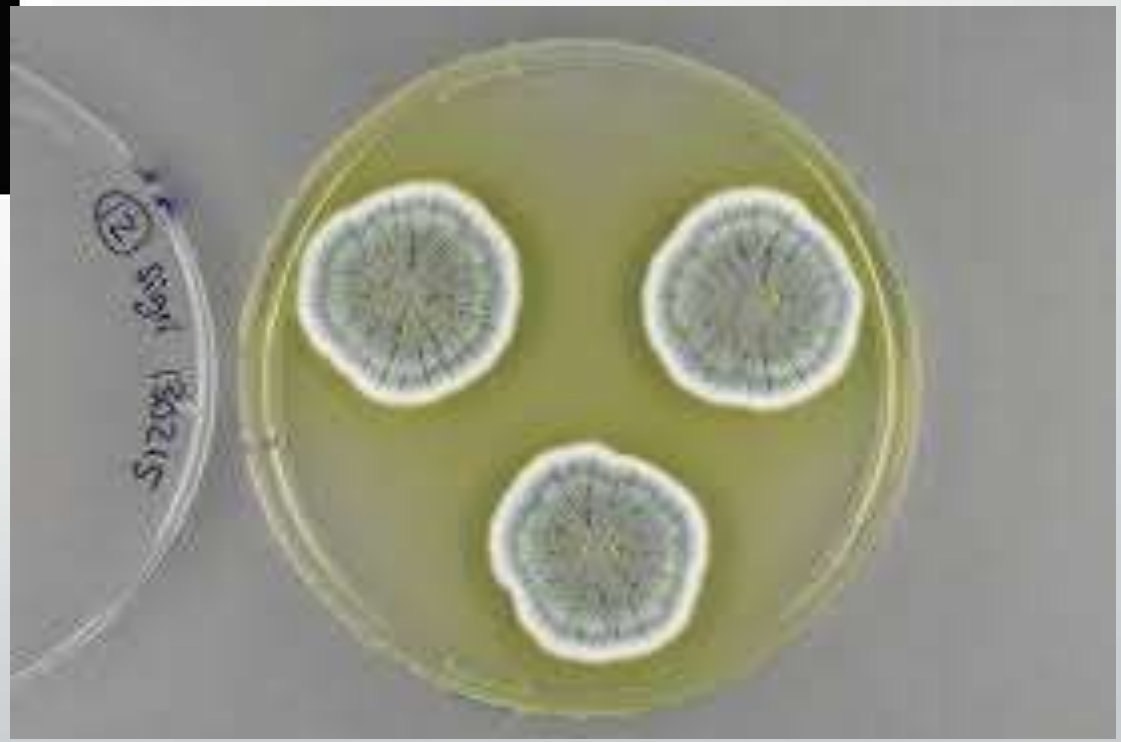


Bacterial colonies on agar plates

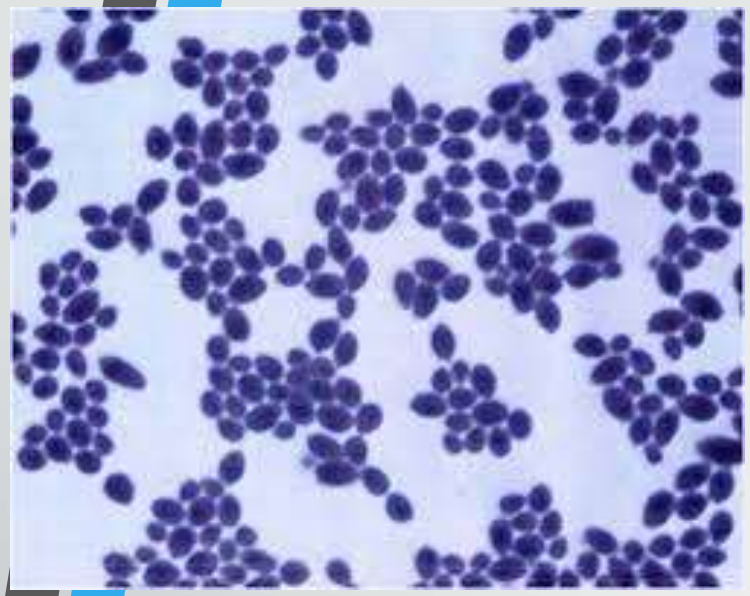


Yeast and mold

Fungal colonies on agar plates



Penicillium species

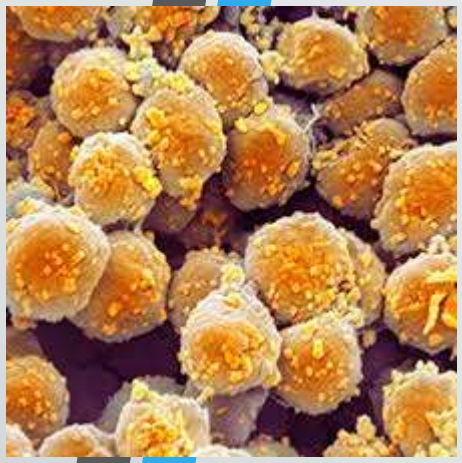


Gram positive violet and Gram negative pink

Budding yeast cells (under microscope)

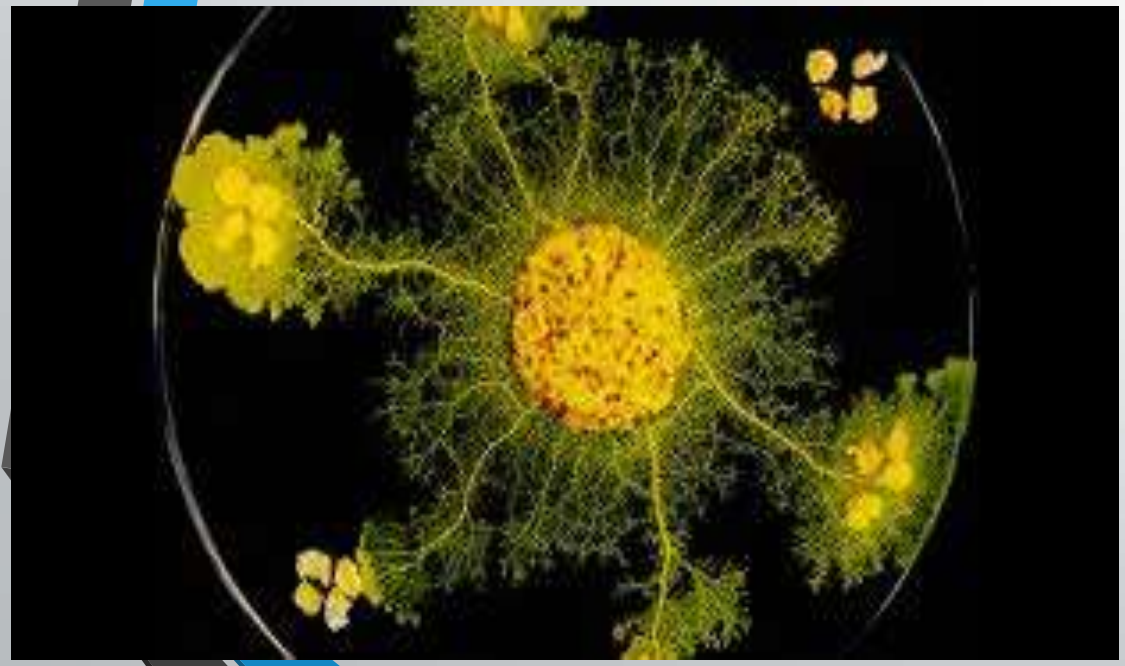
Oral thrush caused by yeast named as *Candida albicans*

Viruses attached to the surface of the host cells



Achaea

Protozoa



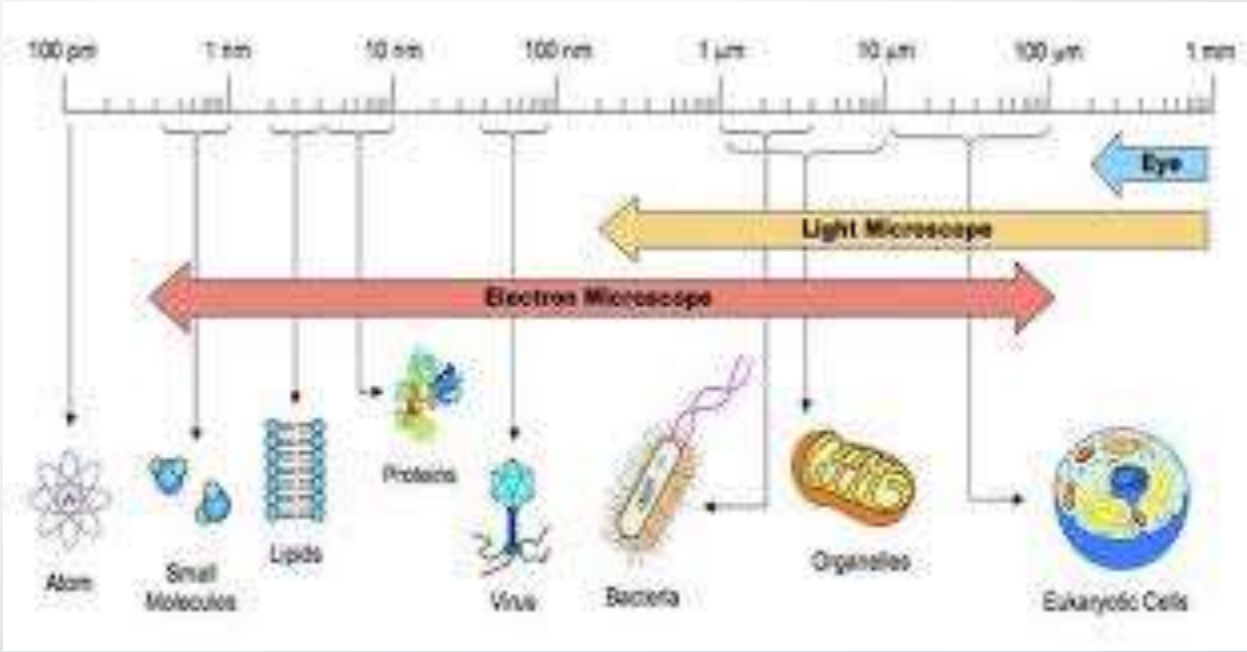
Mold

Fungi

Mold spores fungus grown on bread

Morphology and cytology of bacteria

The word morphos consists of two words with different meaning: 'morphos' means external characters and 'logus' means systematic study. Thus, systematic study of external characters of micro organisms is called as morphology. Morphology includes the study of bacteria with espect to size, shape and arrangements.



Size: Size is the most important and most variable character of bacteria. Some species of bacteria are too small as cant be observed even with a compound microscope while others are so large that one can observe with naked eye! The largest known bacteria was isolated from the sediments of coast of Namibia, named as Thiomargarita namibiensis which is almost 1 mm in diameter (1988)

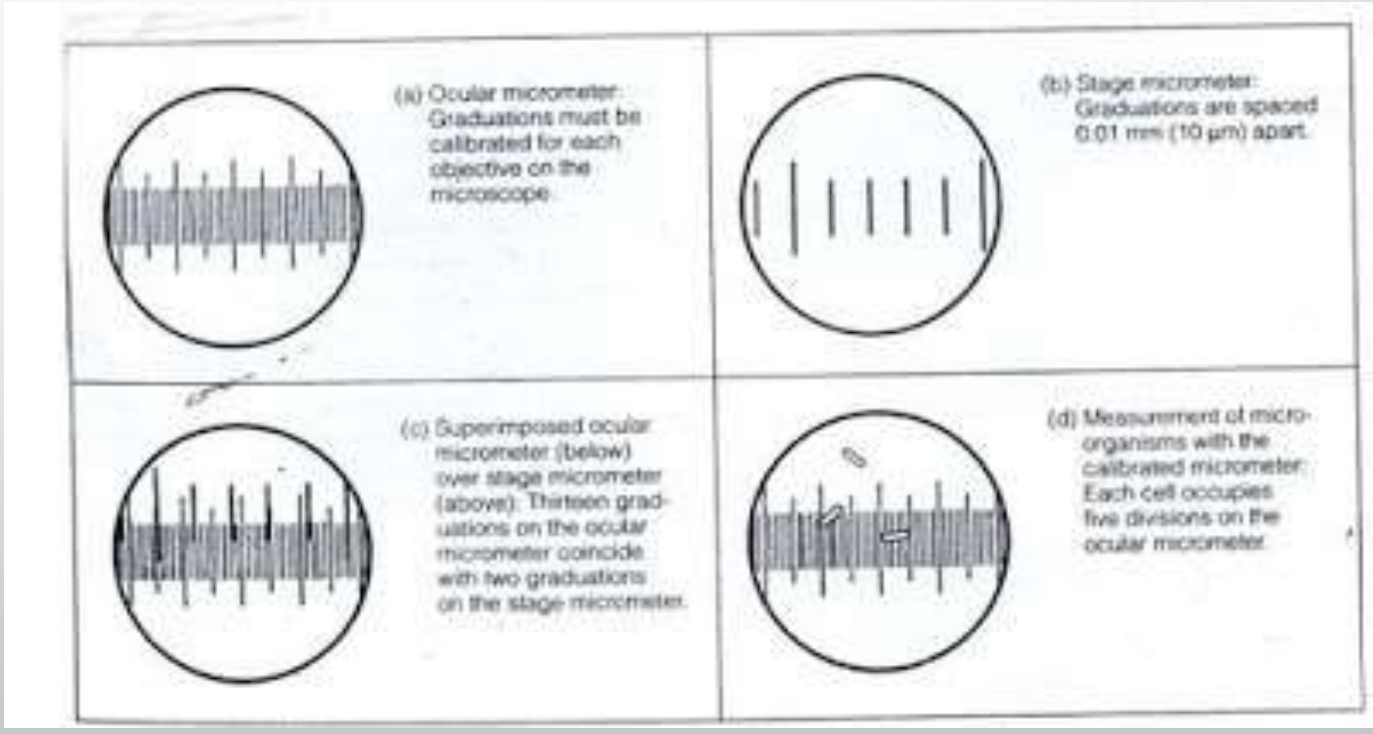
- Calibrated slides and ocular of compound microscope is needed for the measurement of the bacterial cells. The method by which the size is measured is called 'micrometry'. The size of bacteria can be measured by photographic micrometry or by electron microscopic micrometry.
- The unit of measurement used in bacteriology is

1) Micron (µ) or micrometer (µm)
2) Nanometer (nm)
3) Angstorm (A°)

Size of Bacteria

- Unit of measurement in bacteriology is the micron (micrometre, µm)
- 1 micrometre (10^{-6})= 1/1000 mm = 1/10000 cm = 1/100000 metre
- 1 nanometer (10^{-9})= 1/1000 micrometer = 1/100000000 meter
- Bacteria of medical importance
  - 0.2 – 1.5 µm in diameter
  - 3 – 5 µm in length

Dr. Ashish Jawarkar 3

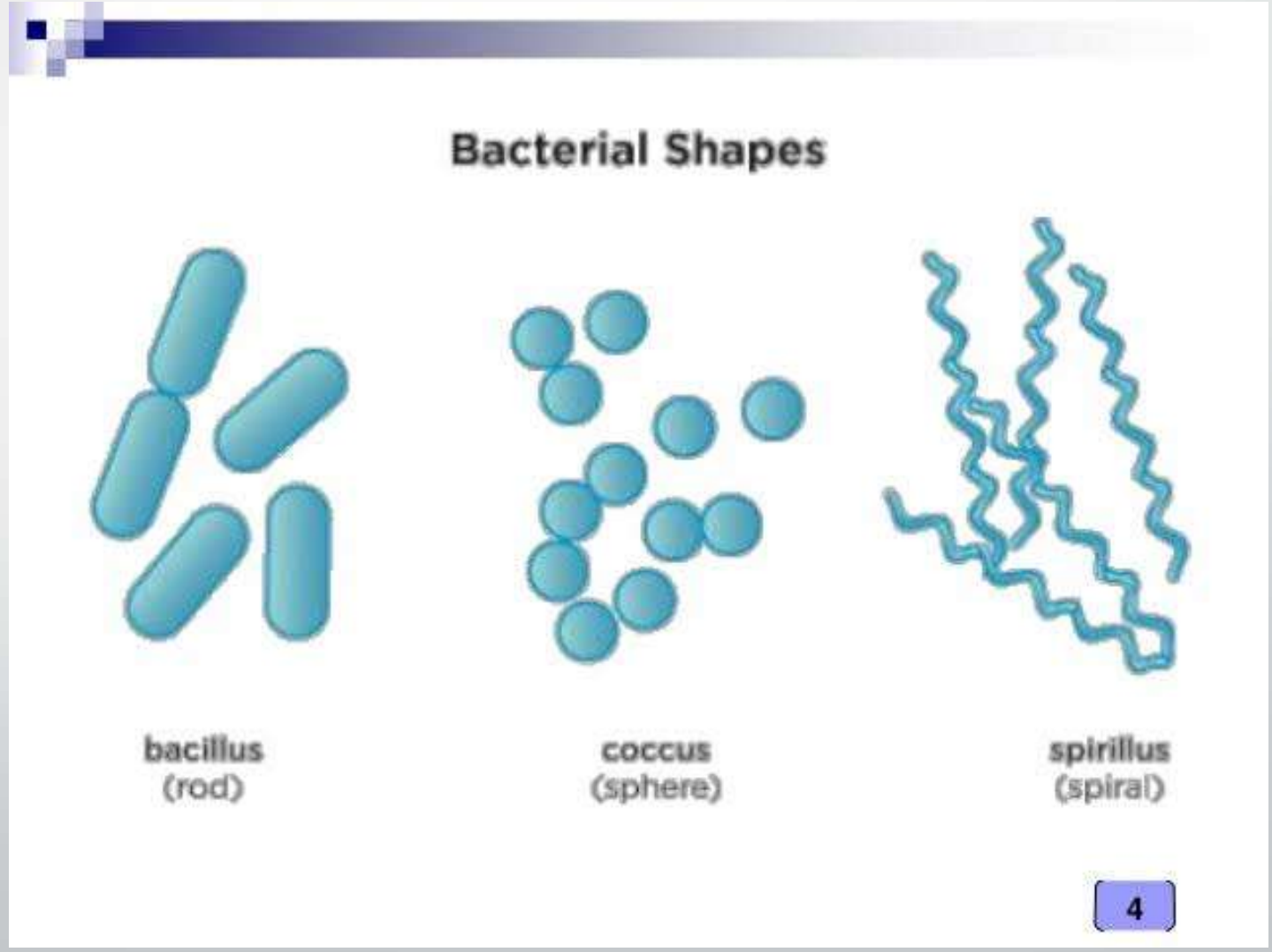


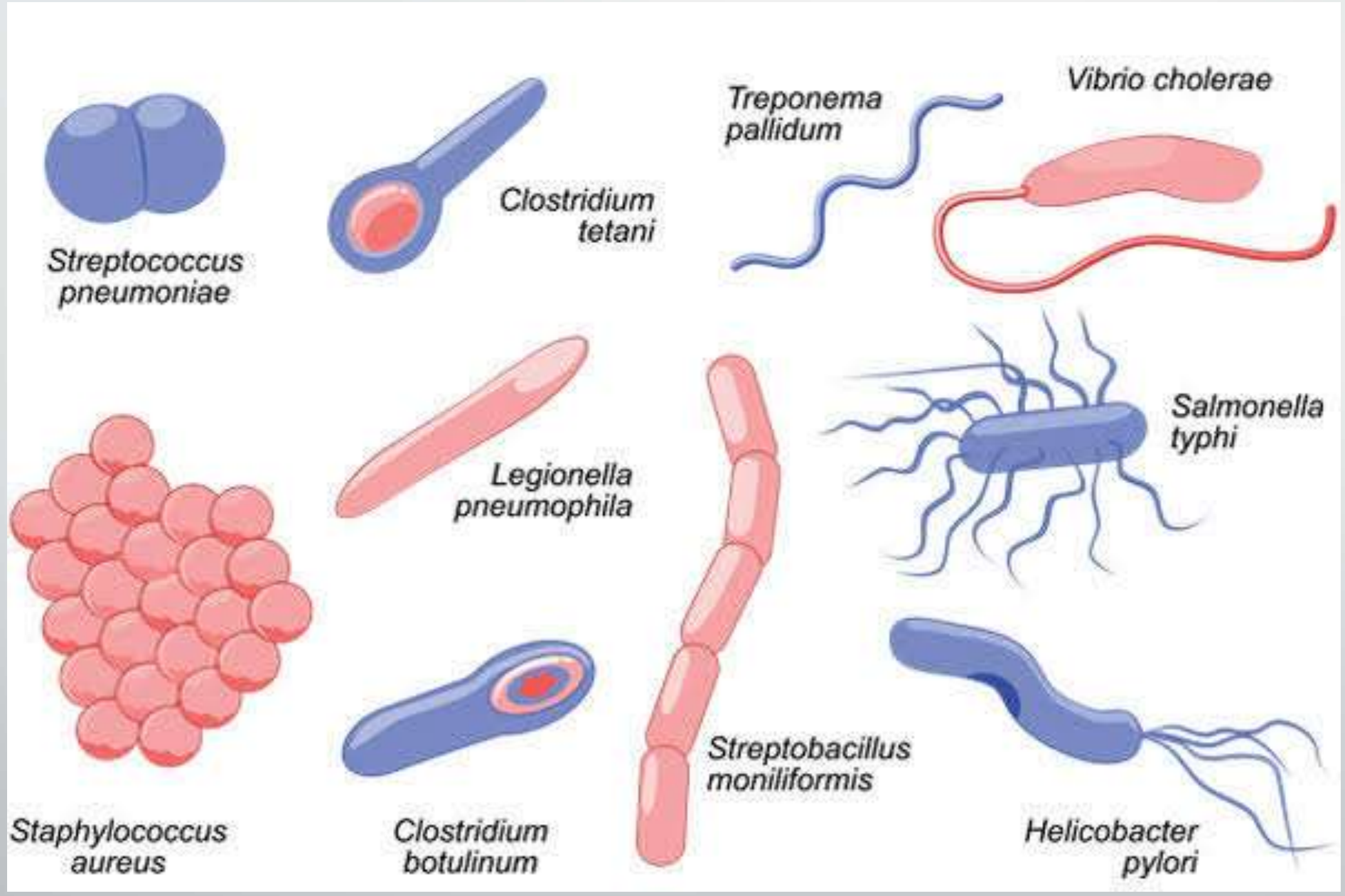
Shape: Bacterias are mainly of three different shapes:

a) Cylindrical / spherical

b) Spherical / ellipsoidal

c) Spiral / helical




Streptococcus pneumoniae
Clostridium tetani
Treponema pallidum
Vibrio cholerae
Legionella pneumophila
Salmonella typhi
Staphylococcus aureus
Clostridium botulinum
Streptobacillus moniliformis
Helicobacter pylori

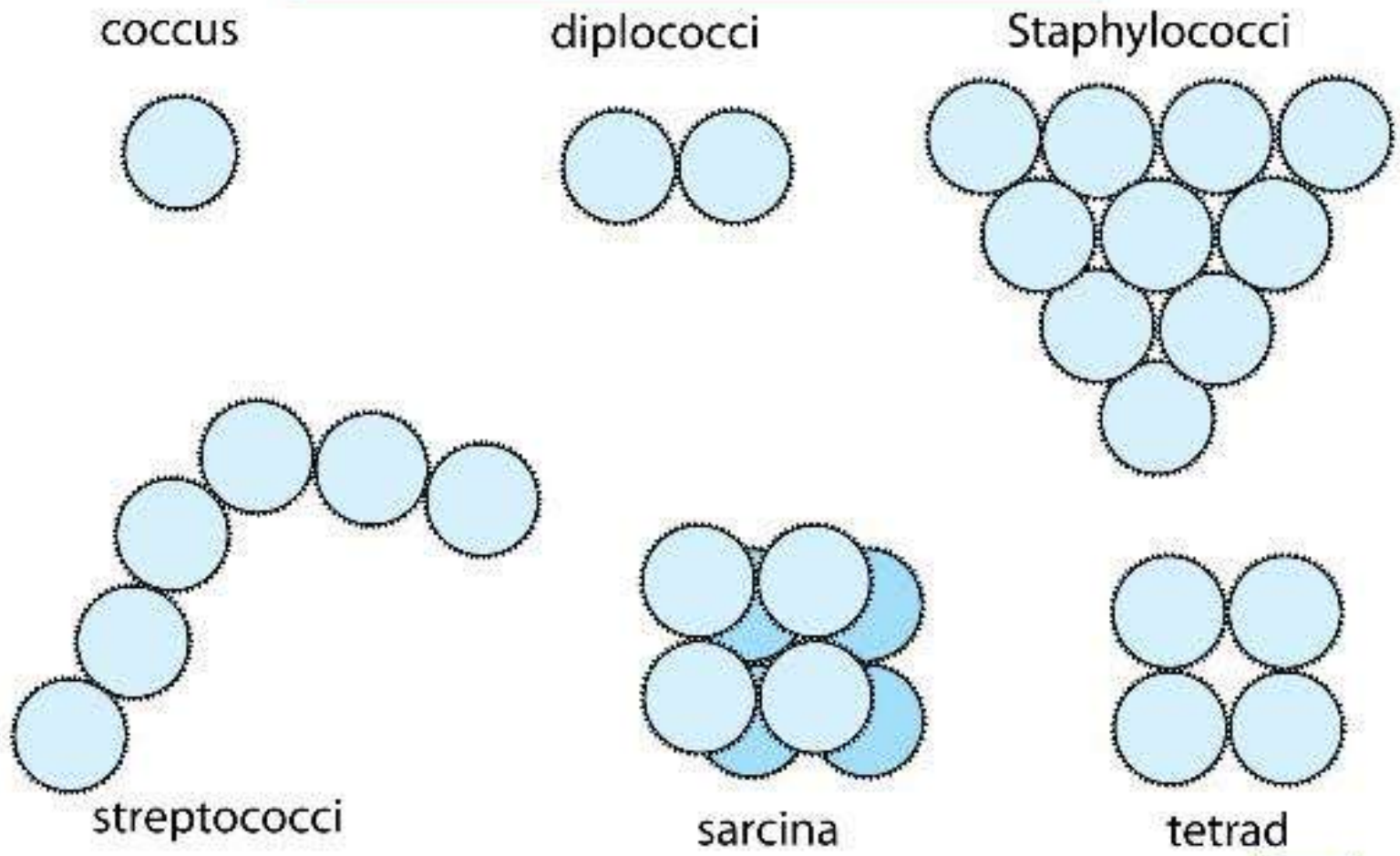
- Arrangement : Bacteria function as an independent single unicellular organisms. Some bacteria have a characteristic pattern of attachment or style or grouping. It is called as the arrangement of bacteria. Each arrangement is typical for that species and it becomes useful for identification and taxonomic (classification) purposes.

1. Arrangement of cocci: Greatest variety of micro organisms show this type of arrangement.

Different kinds of patterns are:

Single- Organims live singly or as an individual organisms.
Diplococci- When a coccus divides in one plane and remain predominantly in pairs.
Streptococci-When cocci divide in one plane and remain attached to each other several cycles of division to form along chain cocci.
Tetrads- If a single a coccus divides in one plane to form a pair, then the pair divides simultaneously in the plane which is perpendicular to previous one, forming a group of 4 cells.
Sarcina- Cell divides in three planes in a regular pattern producing eight cocci arranged in cuboidal manner**.**
Staphylococci- Cell divides in three planes but in an irregular pattern form a bunch of cells called as Staphylococci (Staphyle-Greek word- meaning a bunch of grapes)

Arrangements of Cocci

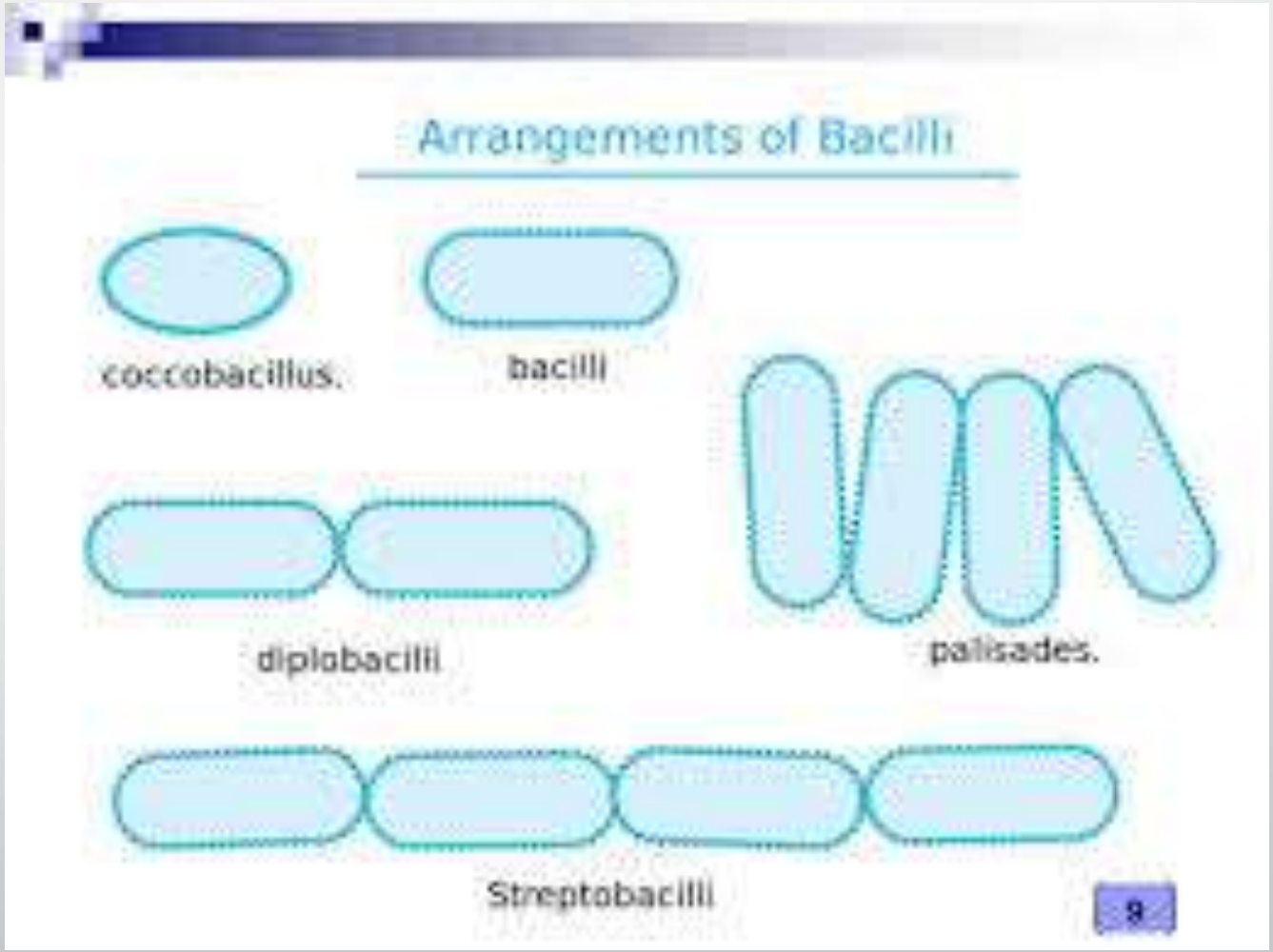


- Arrangement of Bacilli:

Bacilli are less varied in arrangement because they can divide in only one plane i.e. transverse plane. (perpendicular to axis)

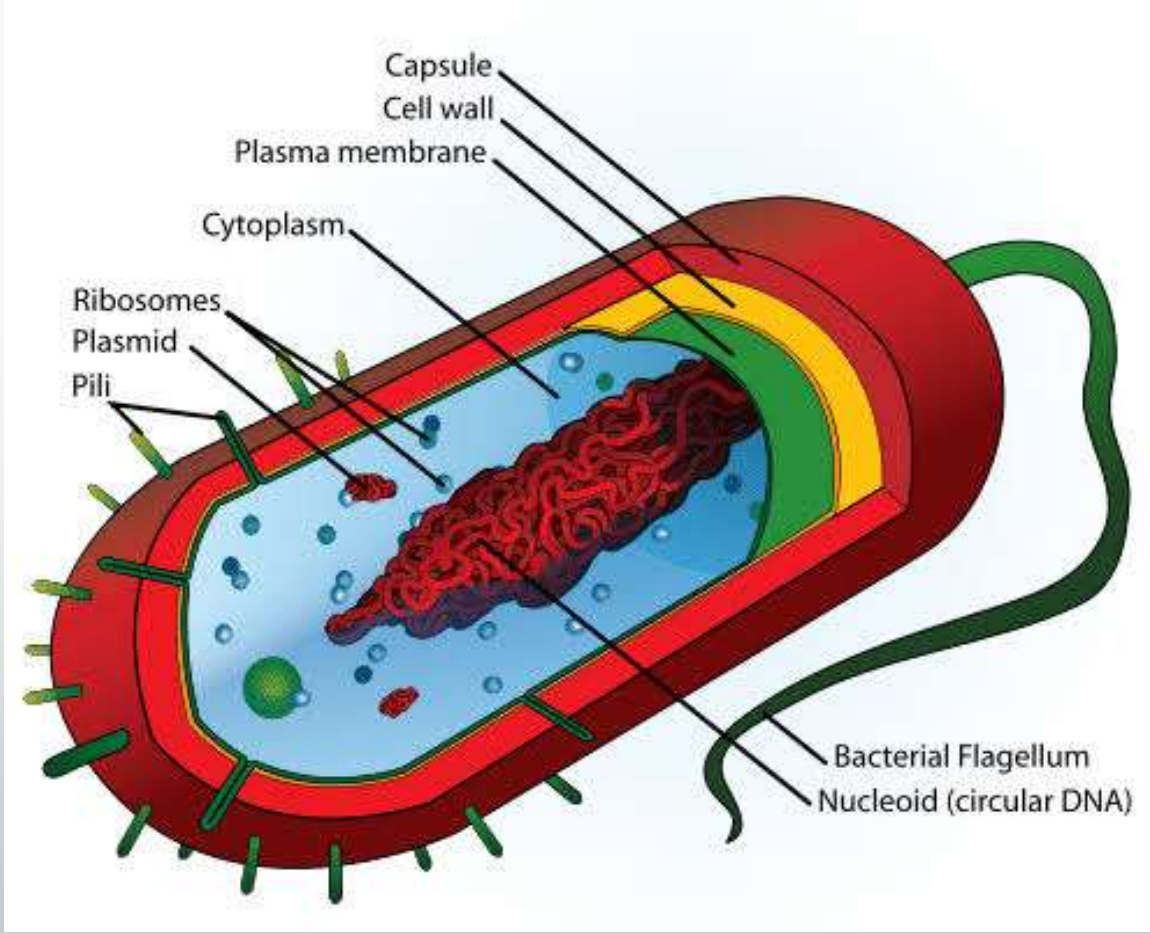
Bacilli can occur either as:

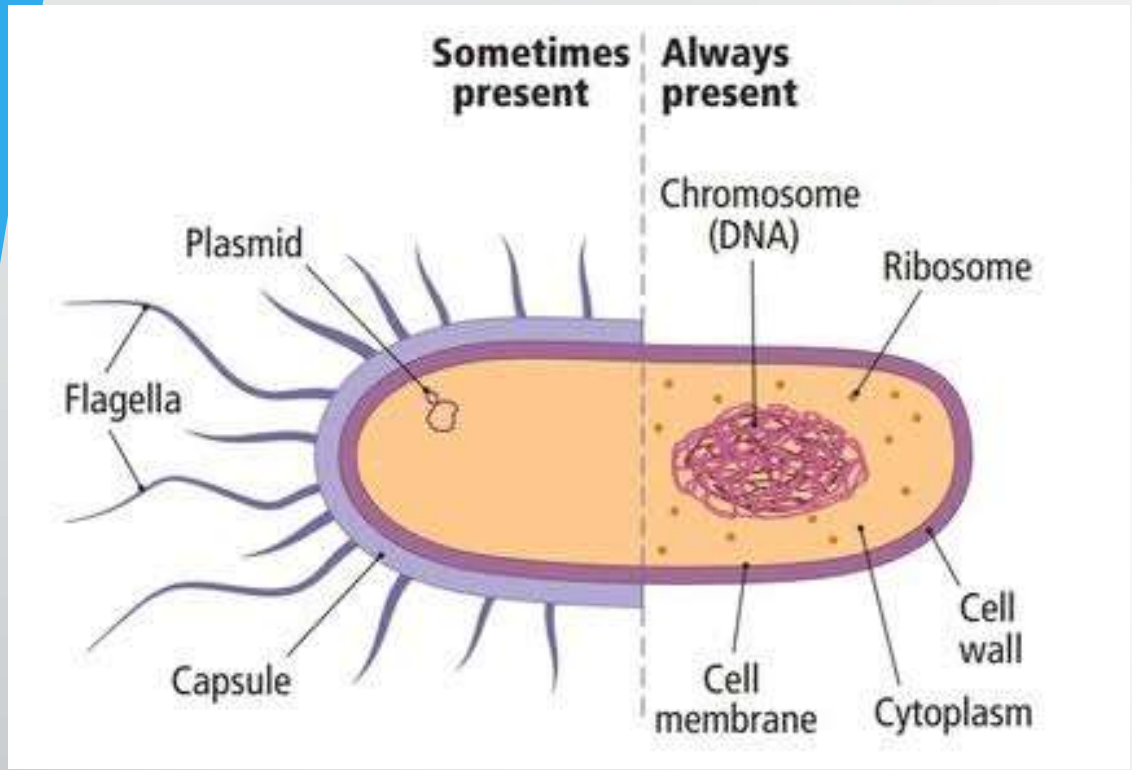
1) Single cells
2) Diplobacilli
3) Streptobacilli

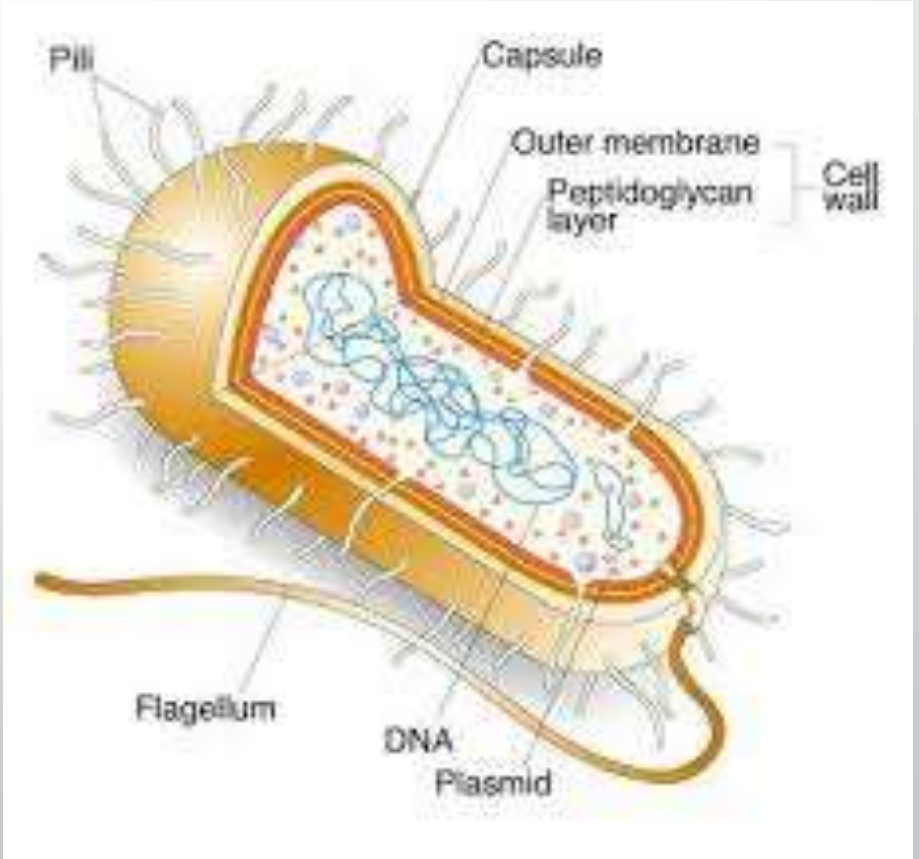


Cytology of a bacteria

- Structure of a typical bacterial cell: Bacterial cells show various anatomical parts; some of these parts are external to rigid cell wall while others are internal to the cell wall. The external parts include flagella, pili, while internal parts include cell membrane, cytoplasm, nuclear region, ribosomes, reserve food materials, vacuoles, etc. Some structures are common for all species or they are characteristic of that species.




Sometimes present
Always present
Plasmid
Chromosome (DNA)
Ribosome
Flagella
Capsule
Cell membrane
Cytoplasm
Cell wall


Pili
Capsule
Outer membrane
Peptidoglycan layer
Cell wall
Flagellum
DNA
Plasmid

- Structure and functions of different parts:

1) Cell wall- Cell wall is the most important and semi rigid part that lie beneath the capsule and cell membrane. All bacteria possess a cell wall except Mycoplasma, Ureoplasma and L forms.

DEFINITION: It is the outermost rigid covering which gives shape, rigidity and protection to the bacterial cell.

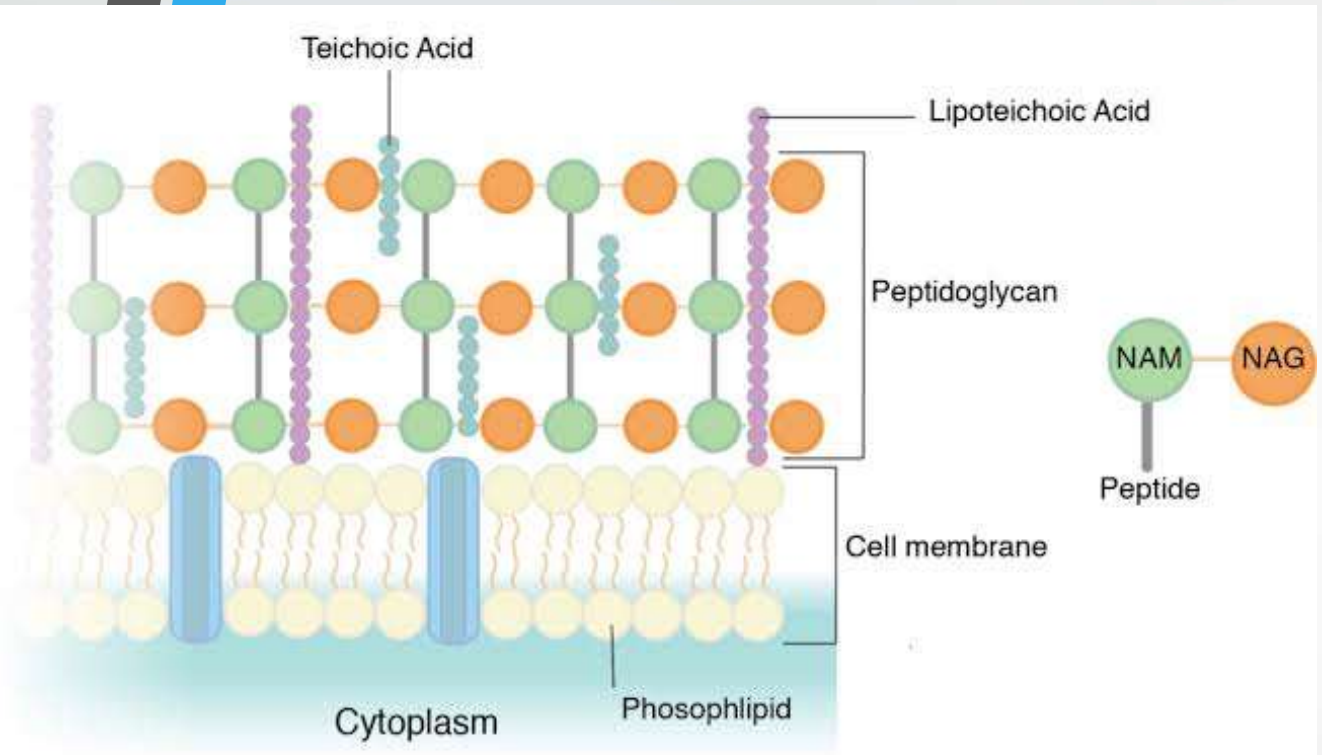
Depending upon the structure and chemical composition of cell wall, bacteriasare calssified into two categories as

A) Gram positive bacteria

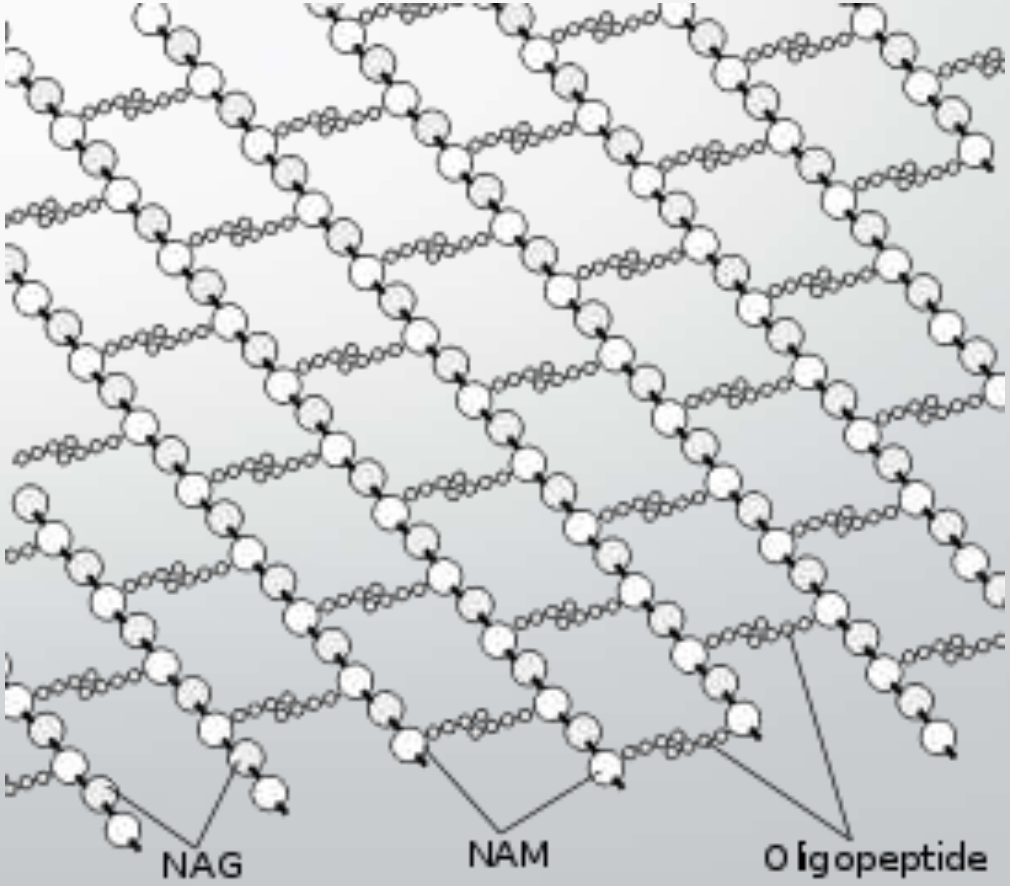
B) Gram negative bacteria

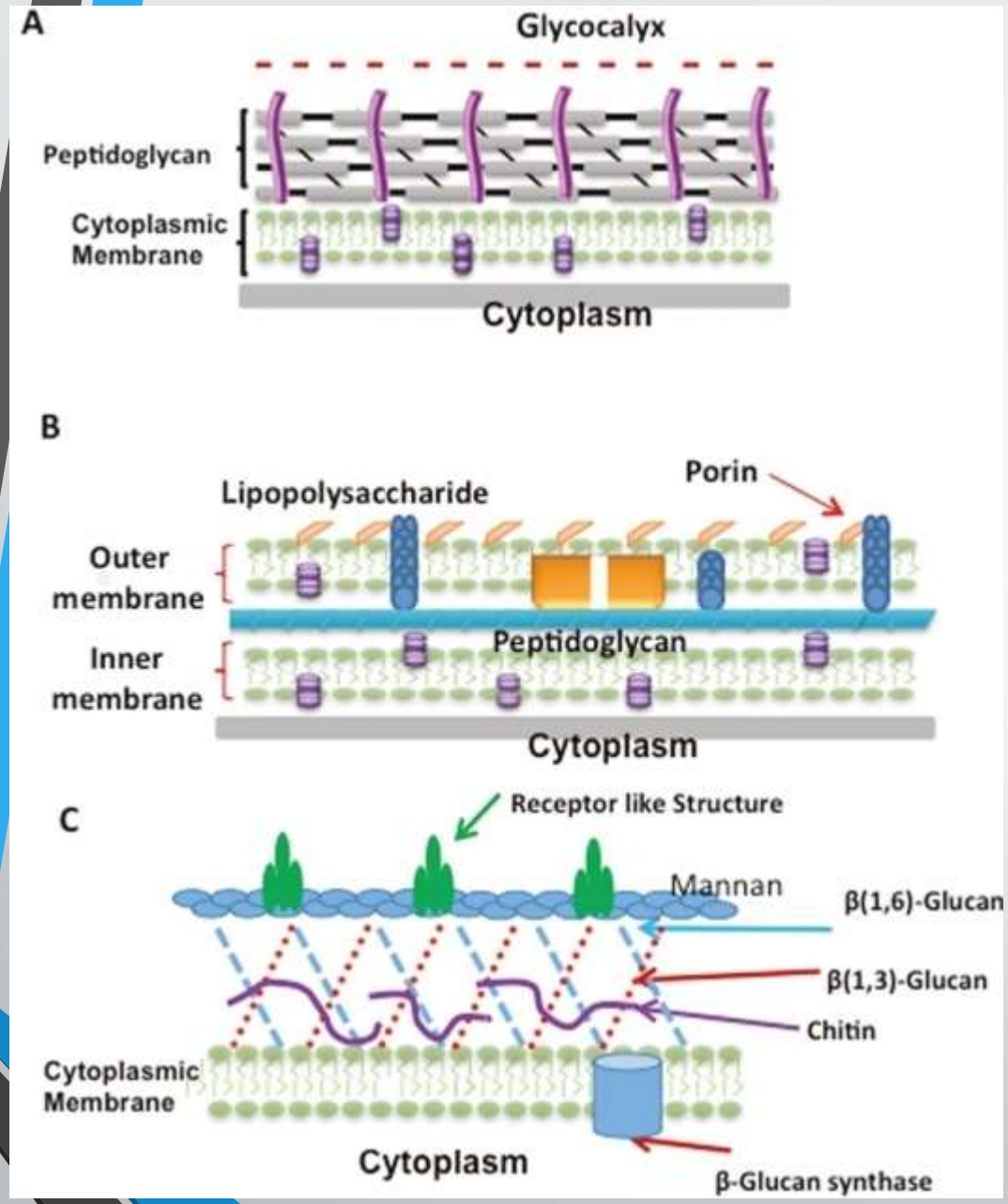
A) Cell wall of Gram positive bacteria:

- Monolayered, thick and homogenous
- Thicker than Gram negative bacteria
- Accounts for 10-50% of dry weight of the cell
- Made of two major components i.e. peptidoglycan and teichoic acid (small amount of lipids, proteins are present)



Gram Positive Bacteria Cell Wall

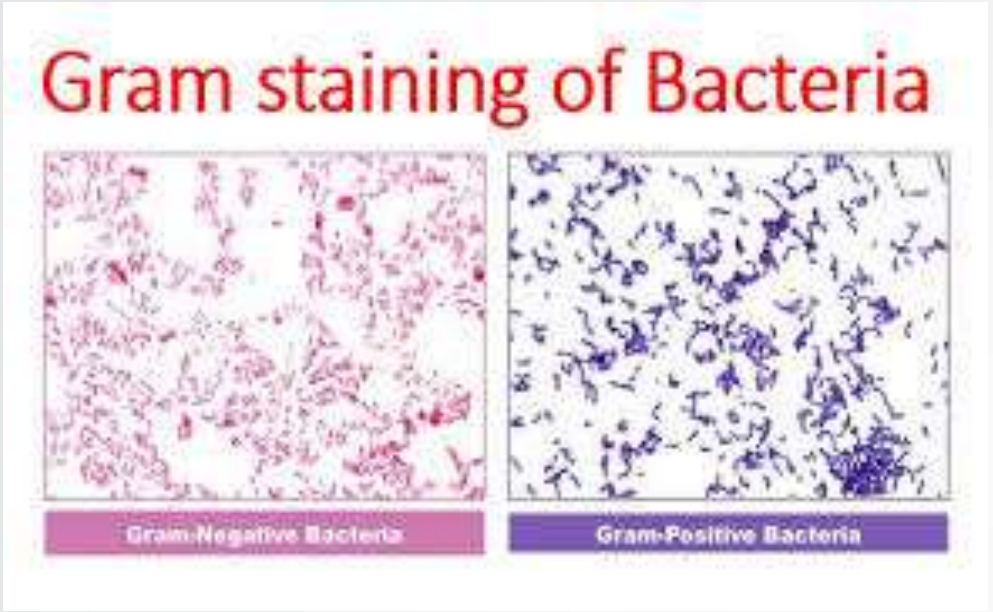


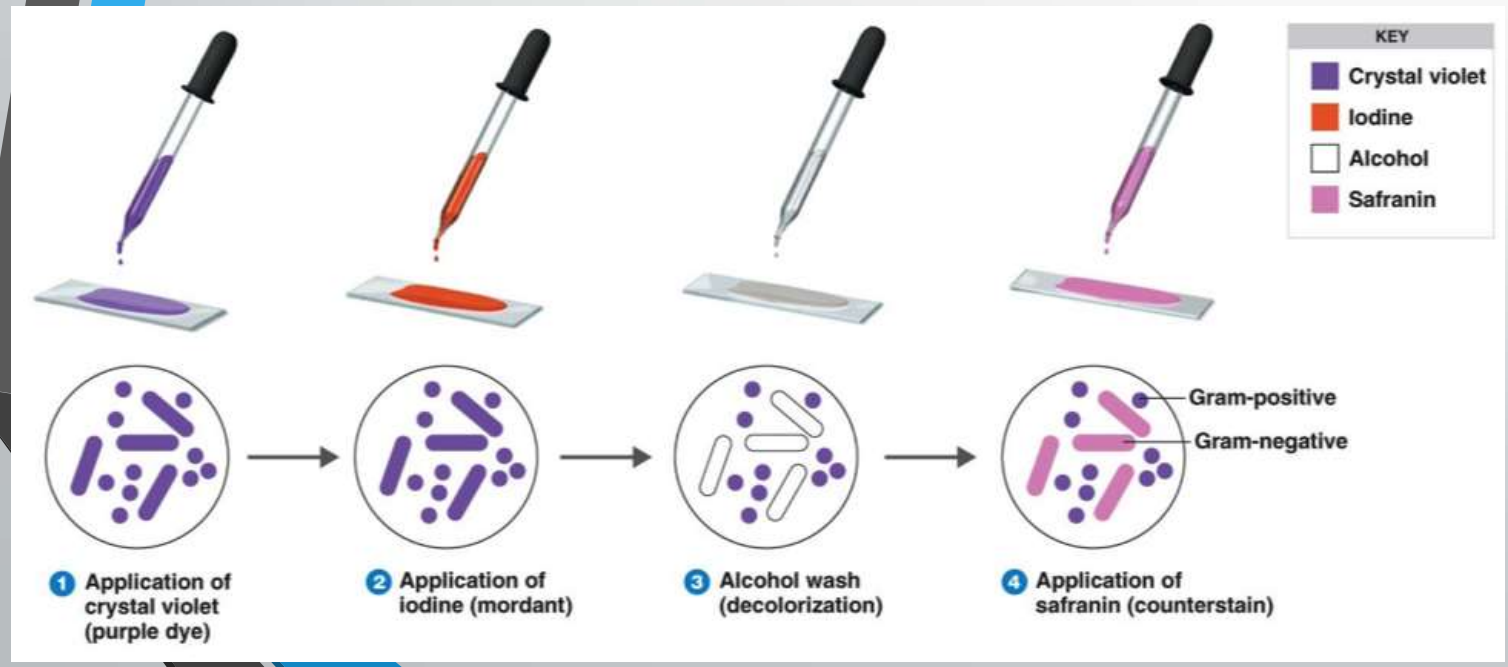


B) Cell wall of a Gram negative bacteria:

-The cell wall of a Gram negative bacteria is more complex than Gram positive.

- Cell wall is bilayered
- Thinner than cell wall of Gram positive bacteria (thickness 8-11 mm)
- Accounts for 10-20% of dry weight of cell wall.
- Made of two layers i.e. inner or peptidoglycan layer and outer wall layer





The two major groups of bacteria can be divided into gram-positive and gram-negative. The Gram stain technique is based on the differential structure of the cellular membranes and cell walls of the two groups.

Gram-positive organisms contain a highly cross-linked layer of peptidoglycan that retains the primary dye, crystal violet (CV), following the application of the mordant, iodine (I). The iodine and crystal violet form a complex within the peptidoglycan. When decolorizer is applied to the cells, the CV-I complex remains within the cell, making it appear dark purple to blue.

The gram-negative organisms do not contain a thick cross-linked layer of peptidoglycan. The peptidoglycan is loosely distributed between the inner cell and the outer cell membranes. Following the application of the crystal violet and iodine, the CV-I complexes are not trapped within the peptidoglycan. Application of the acid-alcohol decolorizer dehydrates the outer cellular membrane, leaving holes in the membrane and effectively washing or removing the CV-I complex from the cells. The cells appear colorless. To make the colorless cells visible, a secondary stain, safranin, is applied, leaving the gram-negative cells pink.

Functions of cell wall:
-It gives specific size and shape to the bacterial cell.
- The high salt and other molecules are packed in small cell interiors, exerting powerful pressure outward. But because of strong cell wall the cell is protected from explosion.
- Protection against osmotic pressure.
-It is an essential component for growth and division.
-Teichoic acid serves as the major surface antigen of Gram positive bacteria.
-O side chain of lipopolysaccharides of outer wall layer serves as an antigenic determinant of Gram negative bacteria.

2) Cell membrane: All vitally important components of bacterial cell like nuclear material, ribosomes, enzymes are present in the cytoplasm or protoplasm. Just beneath the cell wall, there is a thin membrane which covers or surrounds such a cytoplasm. The membrane is called as plasma membrane, cytoplasmic memnrane or protoplasmic membrane.

If the cell membrane is observed through an electron microscope by taking a thin section then it is observed that cell membrane is a bilayered structure with 7-8 nm. The structure of cell membrane of a bacteria cell has considerable resemblance with the plasma membrane of all living cells., therefore it is called as 'unit membrane' or 'elementary membrane'

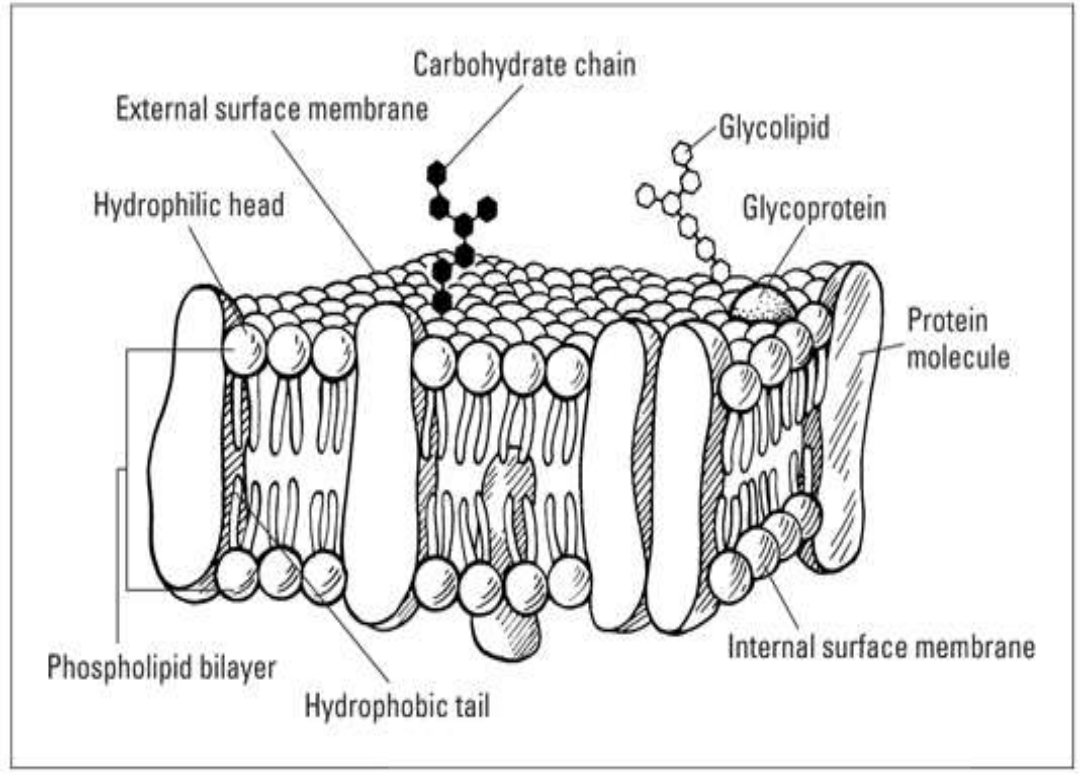
The cell membrane is made up of two components as;

1. Phospholipids (20-30% of membrane mass)
2. Proteins (60-70% of membrane mass)

Major exceptions are; cell membrane of *Mycoplasma* and *Archaebacteria*

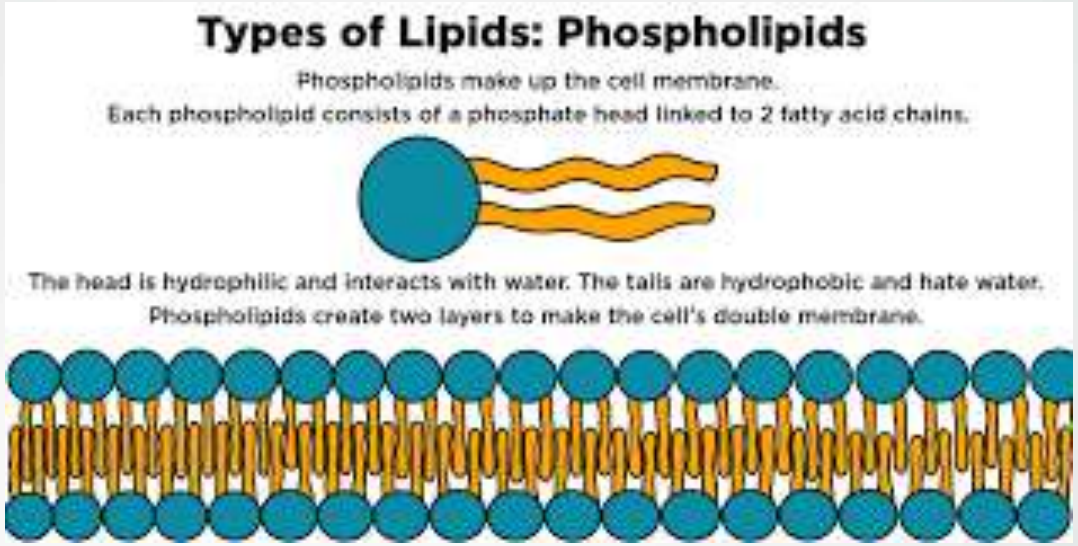
STRUCTURE:

To explain the structure of cell membrane various models are proposed but the most accepted model is the 'Fluid Mosaic Model'. It is proposed by S.J.Singer and G.L.Nicholsan in 1972.



According to them, the cell membrane is a phospholipid bilayer in which proteins are embedded. The phospholipids of the cell membrane are in fluid form at the normal temperature, while the proteins embedded are in fluid form at the normal temperature, while the proteins embeded give mosaic pattern, therefore the name is given as Fluid Mosaic Model.

The phospholipids of the cell membrane are glycerophospholipid bilayer in which proteins are embedded. They are in fluid form because of having glycerol 3-phosphate as a basic unit. It consists of two parts as head and tail with knob like structure as shown.



Head is hydrophilic and tail is hydrophobic. The phospholipids always form a bilayer where hydrophilic heads face outwards on both sides, protecting the hydrophobic tail projecting towards the center. Due to bilayered structure the hydrophilic heads are allowd to come in contact with watery cytoplasm or outer environment., side by side, avoiding the contact of hydrophobic tails with water. Because of it, structural stability is achieved.

The membrane protein are globular proteins and they are of two types:

1) Peripheral : The peripheral or extrinsic proteins are located only on the outer surface of the membrane.

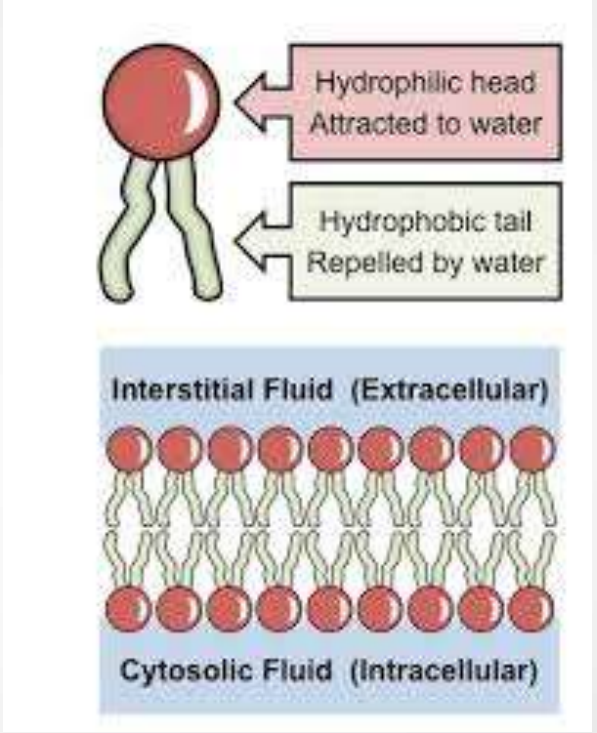
They are soluble and can easily dissociate from membrane.

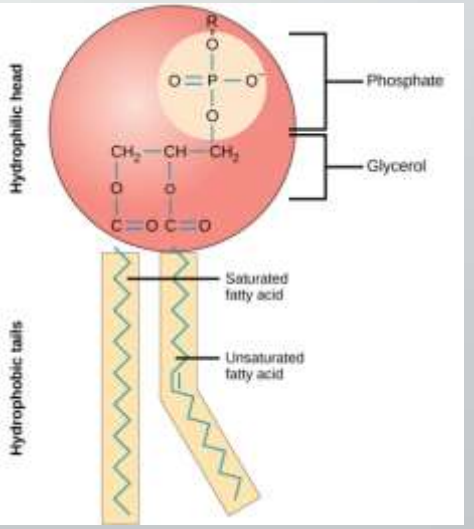
2) Integral : Integral or intrinsic proteins are embedded throughout the bilayer and are insoluble.

The cell membrane is stabilized by hydrophobic forces between fatty acids and electrostatic forces between hydrophilic heads exposed to the exterior surfaces.

FUNCTIONS :

1) It is an ultimate organelle of the bacterial cell. A cell cannot survive without a cell membrane. Integrity of the cell is lost by causing leakage of cell components.
2) Serves as a selective permeable barrier.
3) Acts as an osmotic barrier.
4) Attachment sites for chromosome and plasmid while replication.
5) Acts as a center for biosynthesis of certain constituents, especially cell wall, capsule, flagella, etc.
6) Site for ATP production
7) It plays an important role in cell division and sporulation during septum formation.
8) It also involves in secretion or discharge of metabolic products like toxins, enzymes, outside the cell.
9) It also provides anchorage site for flagella.





3) Mesosomes:

Mesosome is a Greek word. Mesos means middle and somas means body.

Philip Fitz James first examined the mesosomes under electron microscope. Mesosomes are prominent in Gram positive bacterias but are difficult to see in Gram negative bacterias maybe because of their relatively small size.

Mesomes are complex localized infoldings or invasions of cell membrane filled with the clusters of vesicles and tubules of membranous whorls or both.

As mesosomes are the invasions of the cell membrane chemically. They are similar to cell membrane i.e. made up of phospholipid bilayer in which proteins are embedded. The phospholipids are exactly smilar to the cell membrane but the proteins are different.

Depending upon the location mesosomes are of two types:

- Central (when it is present at the center of the cell)
- Peripheral (when it is located at the periphery of the cell)

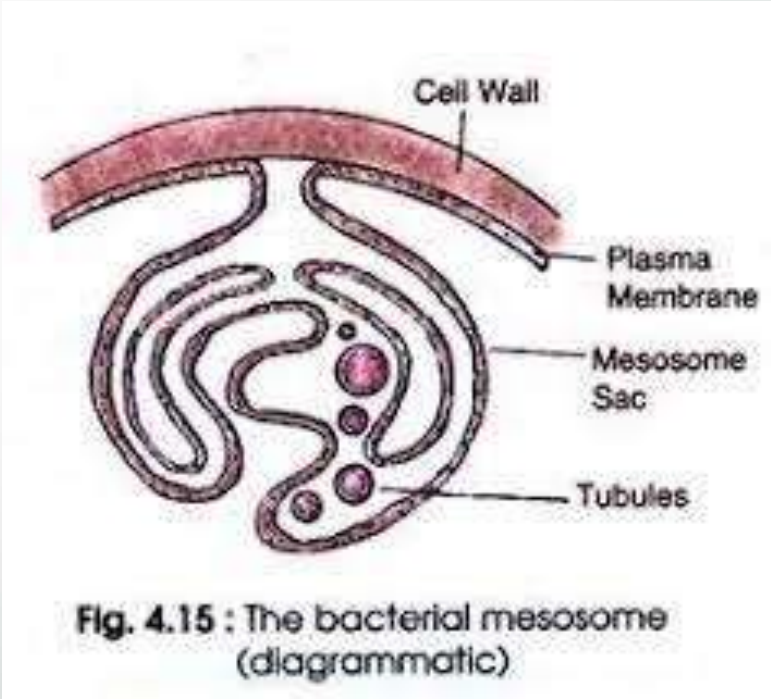
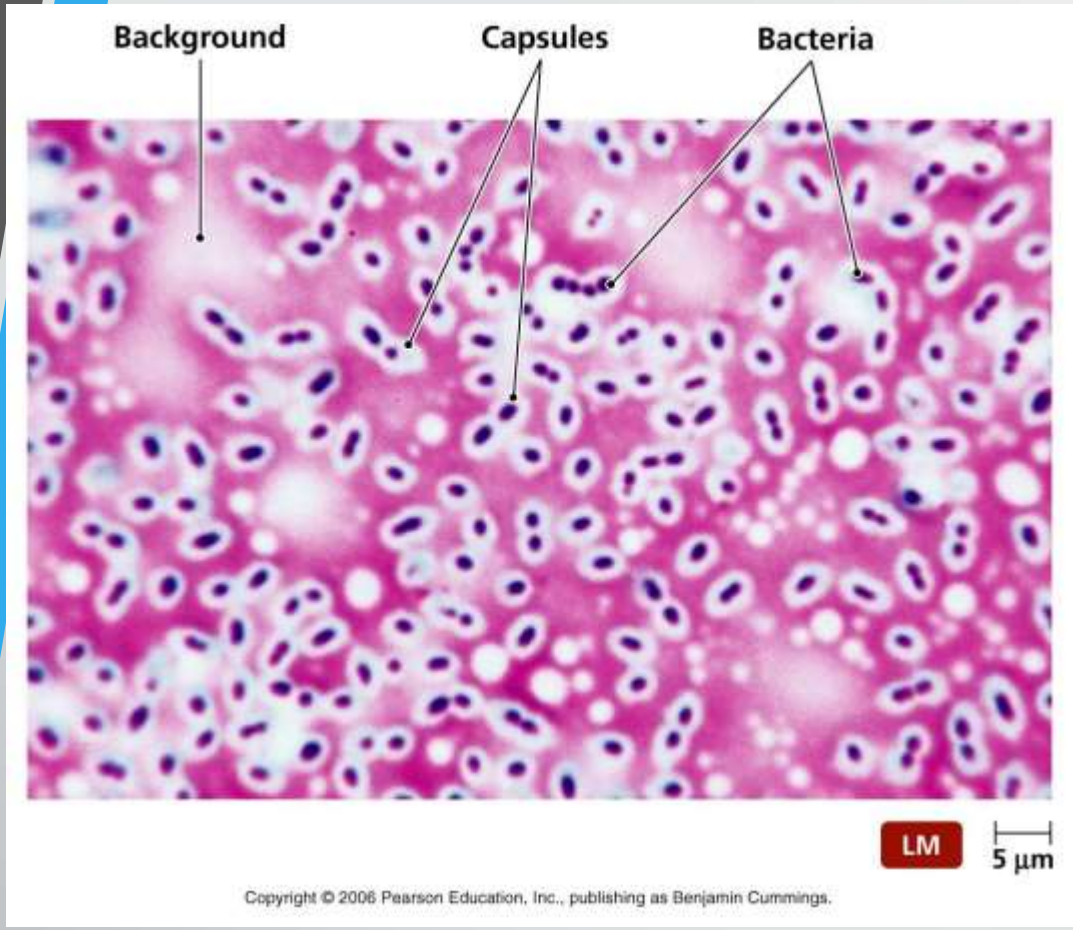
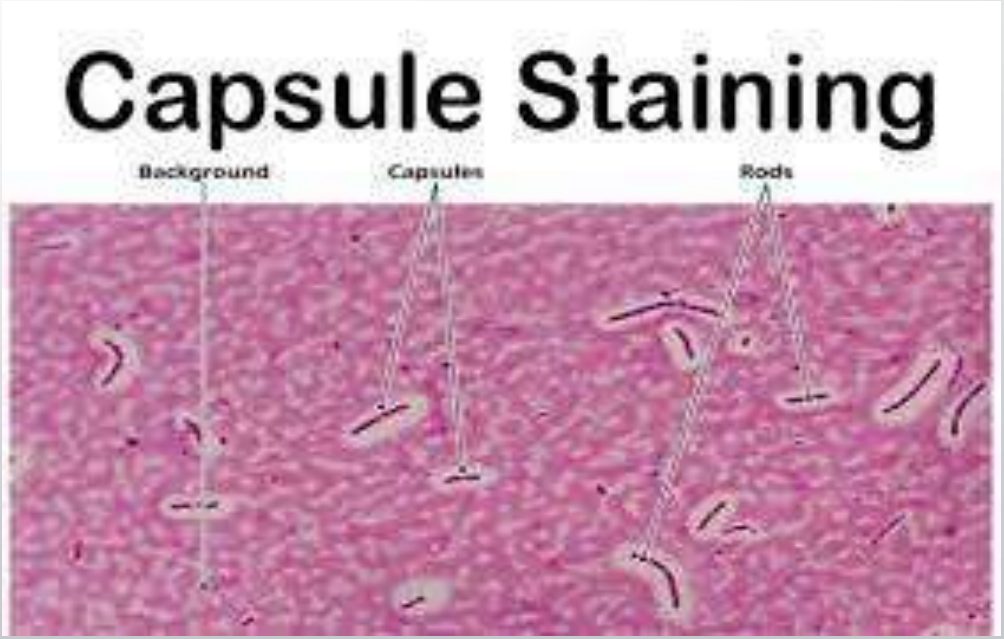


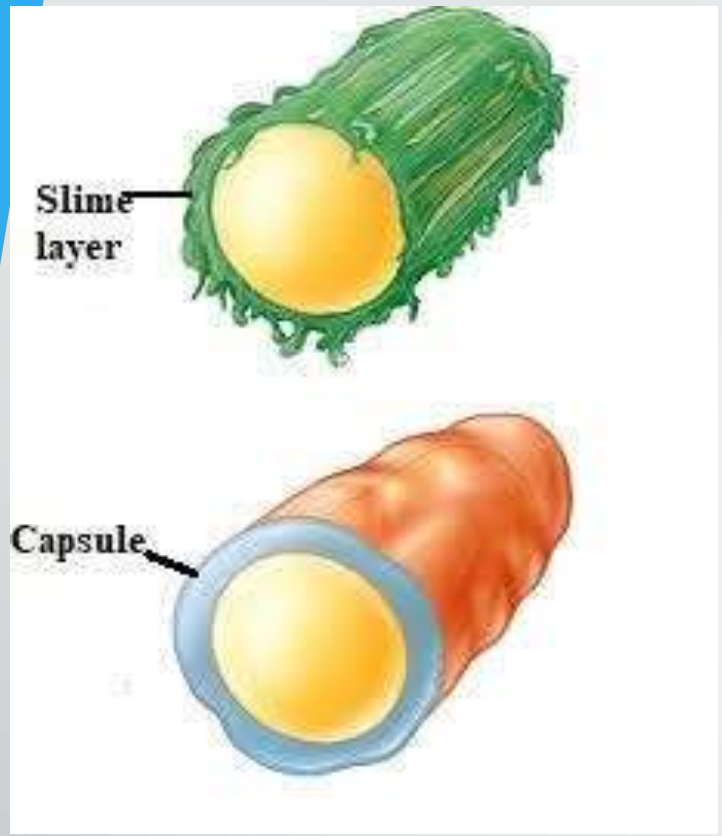
Fig. 4.15 : The bacterial mesosome (diagrammatic)

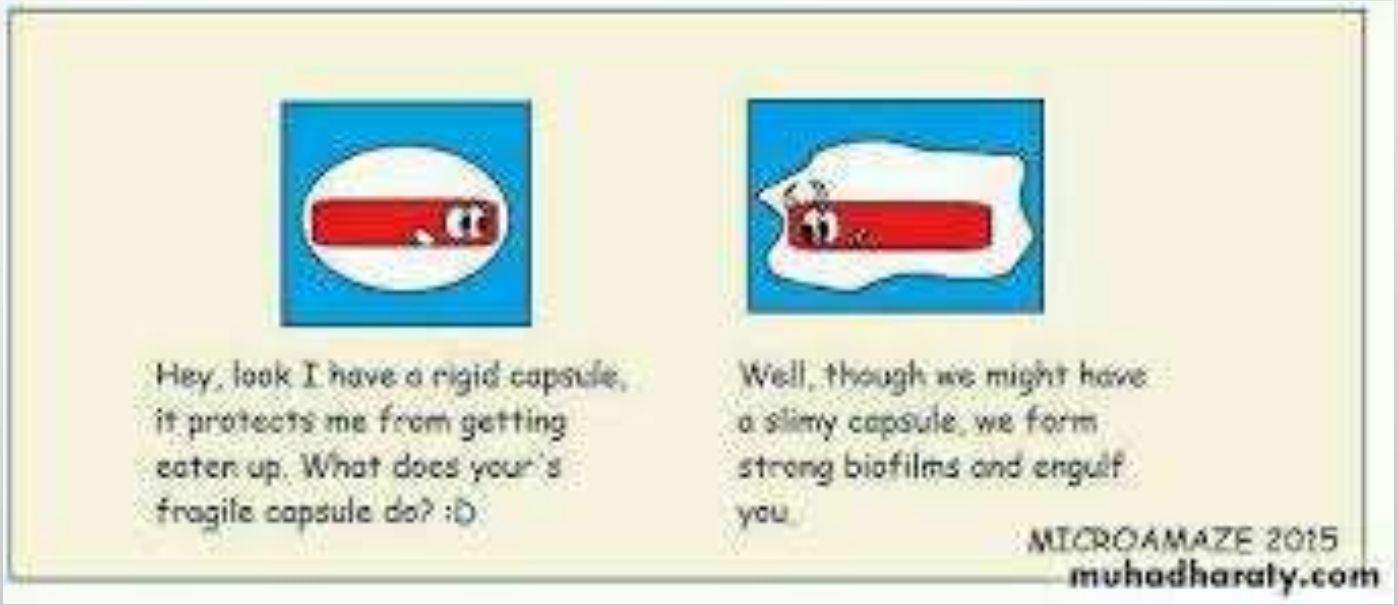
4) Capsule or slime layer: Many micro organisms are suurounded by a viscous substance forming an extra covering around the bacterial cell it is called as a glycocalyx or exoplolymer.
The glycocalyx is of two types – capsule or slime layer.

Capsule	Slime layer
1. It is a part of the cell	1. It is only the secretion of the cell
2. Has a definite shape	2. It is amorphous and thus having a less definite shape
3. It has more or less same density throughout the width of the capsule	3. Its density decreases with the increase in the distance from the cell surface
4. Ability to produce a capsule is under genetic control of the bacterial cell.	4. Production of slimelayer is not under genetic control but it is influenced environmentally
5. Capsular material does not diffuse in surrpunding material	5. Slime diffuses in the medium and medium becomes sticky
6. Capsule has great immunological significance	6. It has no immunological significance
7. Eg. Streptococcus, azotobacter, Rhizobium	7. Eg. E.coli, Salmonella


Background
Capsules
Bacteria
LM
5 µm
Copyright © 2006 Pearson Education, Inc., publishing as Benjamin Cummings.

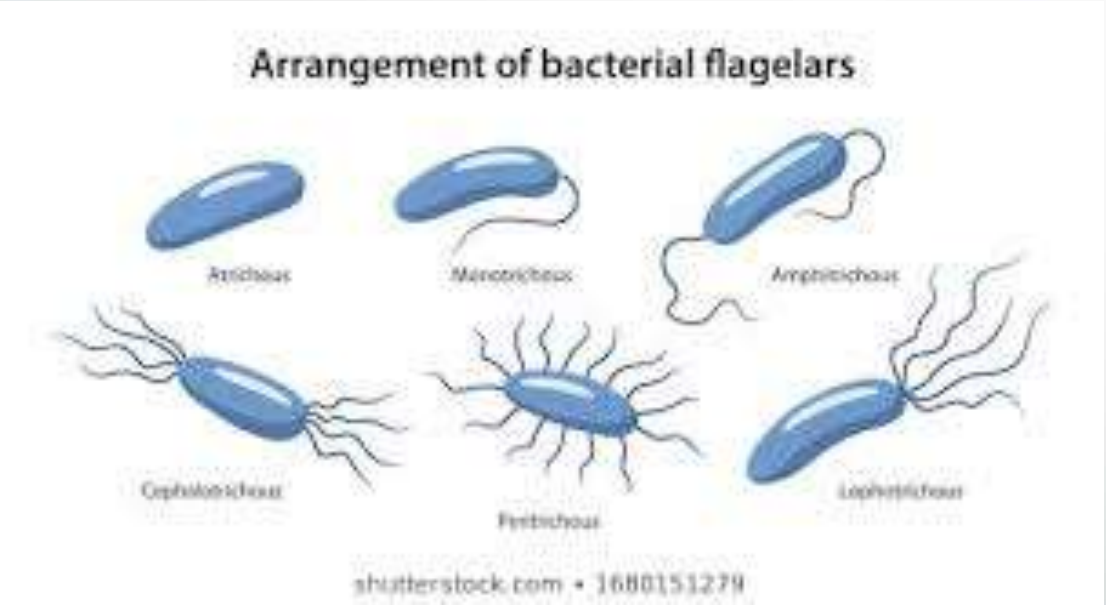

Capsule Staining
Background
Capsules
Rods

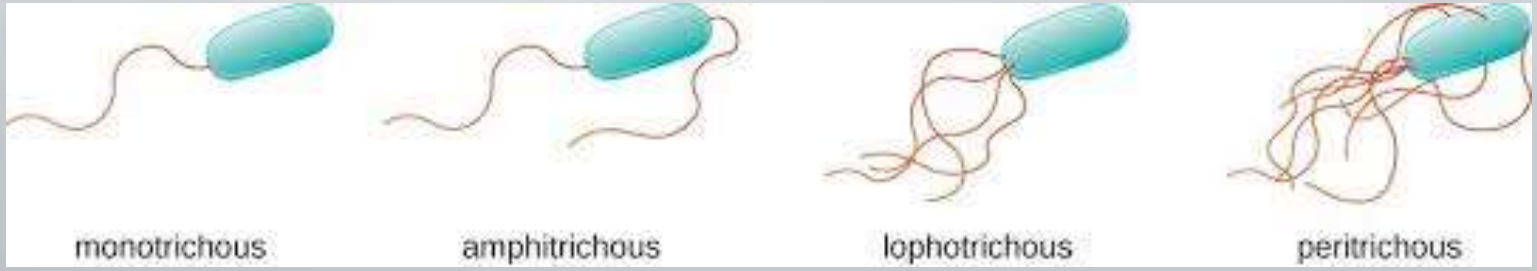

Slime layer
Capsule


Hey, look I have a rigid capsule, it protects me from getting eaten up. What does your's fragile capsule do? :D
Well, though we might have a slimy capsule, we form strong biofilms and engulf you.
MICROAMAZE 2015
muhadharaty.com

5. Flagella: Many but not all groups of micro organisms process possess appendages (means any external projection) on the cell surfaces. The appendages of bacteria are of two types

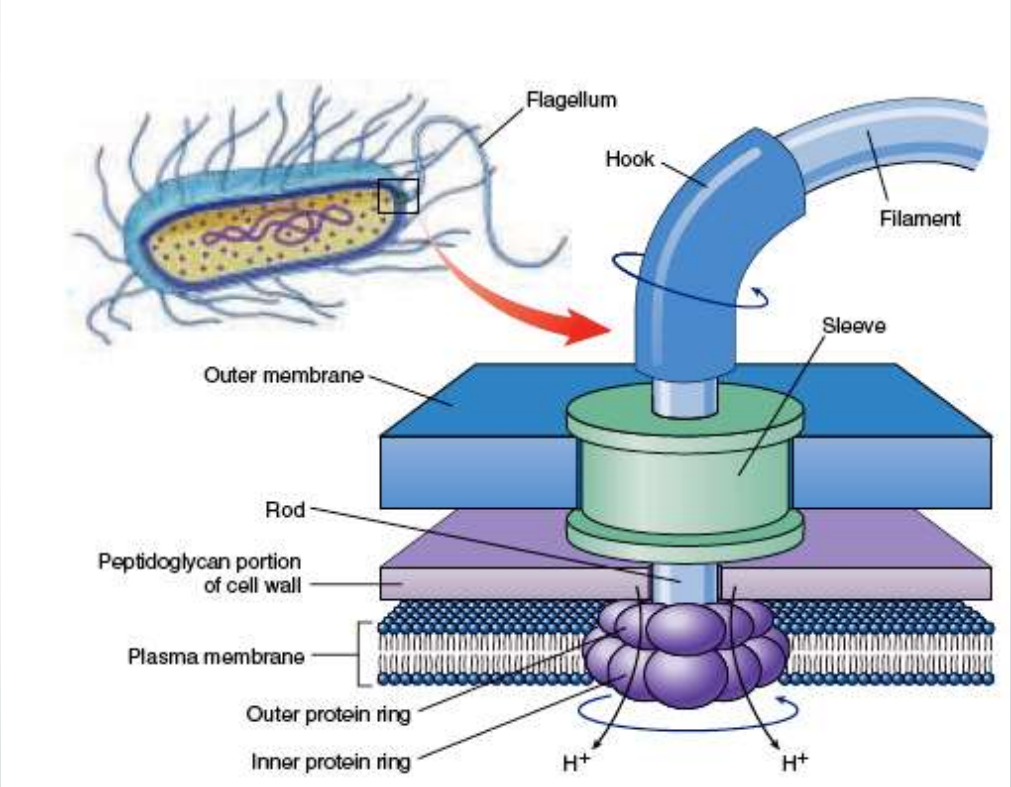
- Flagella: those that provide motility
- Pili : those that provide attachment





Structure of a flagellum:
It is made up of three parts
a) Basal boy b) Hook c) filament

Functions:
- Swimming motility of bacteria
- Serves as a receptor site for bacteriophages
- Acts an an antigenic determinant
- Move freely in response to chemicals, oxygen and light
- It can help the pathogen penetrate certain host defense barrier in the human body



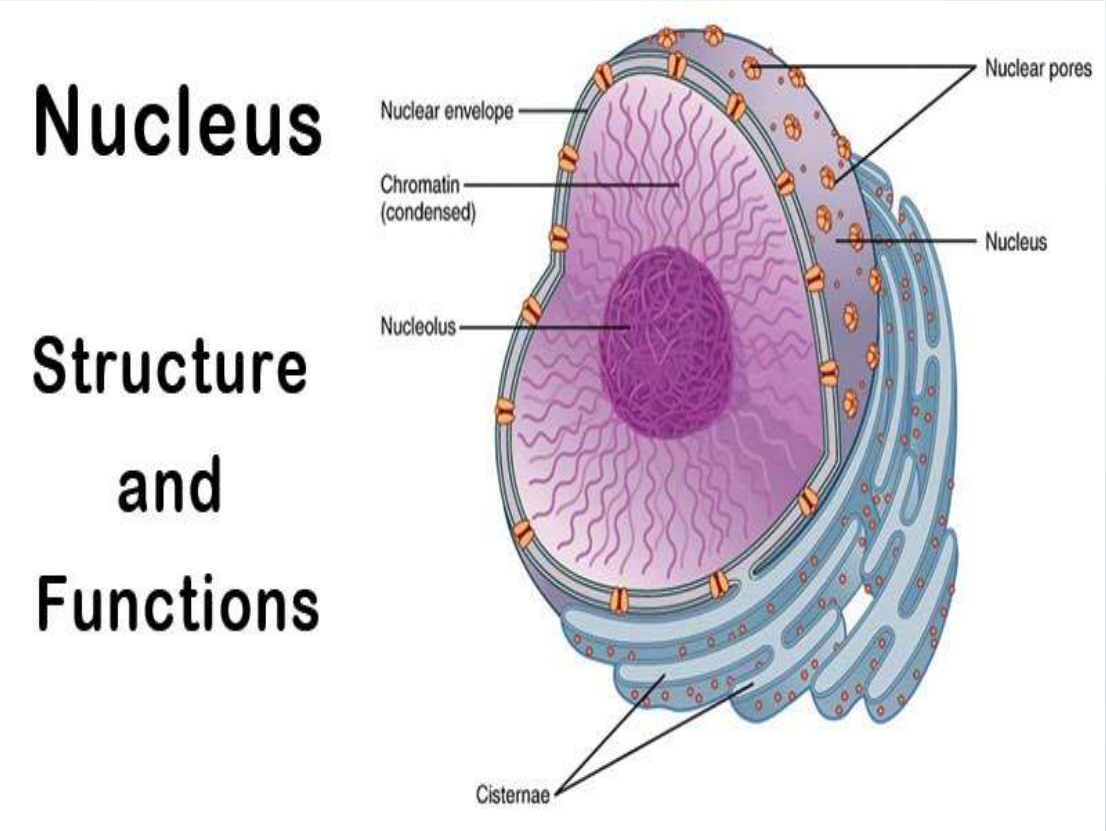
7. Nuclear material:

The cell nucleus is a membrane-bound structure that contains the cell's hereditary information and controls the cell's growth and reproduction. It is the command center of a eukaryotic cell and is commonly the most prominent organelle in a cell accounting for about 10 percent of the cell's volume.

In general, a eukaryotic cell has only one nucleus. However, some eukaryotic cells are enucleated cells (without a nucleus), for example, red blood cells (RBCs); whereas, some are multinucleate (consists of two or more nuclei), for example, slime molds.

The nucleus is separated from the rest of the cell or the cytoplasm by a nuclear membrane.

As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell.



The structure of a nucleus encompasses the nuclear membrane, nucleoplasm, chromosomes, and nucleolus.

Nuclear Membrane

The nuclear membrane is a double-layered structure that encloses the contents of the nucleus. The outer layer of the membrane is connected to the endoplasmic reticulum.

Nucleoplasm

Nucleoplasm is the gelatinous substance within the nuclear envelope.

Also called karyoplasm, this semi-aqueous material is similar to the cytoplasm and is composed mainly of water with dissolved salts, enzymes, and organic molecules suspended within.

Nucleolus

Contained within the nucleus is a dense, membrane-less structure composed of RNA and proteins called the nucleolus.

Some of the eukaryotic organisms have a nucleus that contains up to four nucleoli.

Chromosome

The nucleus is the organelle that houses chromosomes.

Chromosomes consist of DNA, which contains heredity information and instructions for cell growth, development, and reproduction.

Chromosomes are present in the form of strings of DNA and histones (protein molecules) called chromatin.

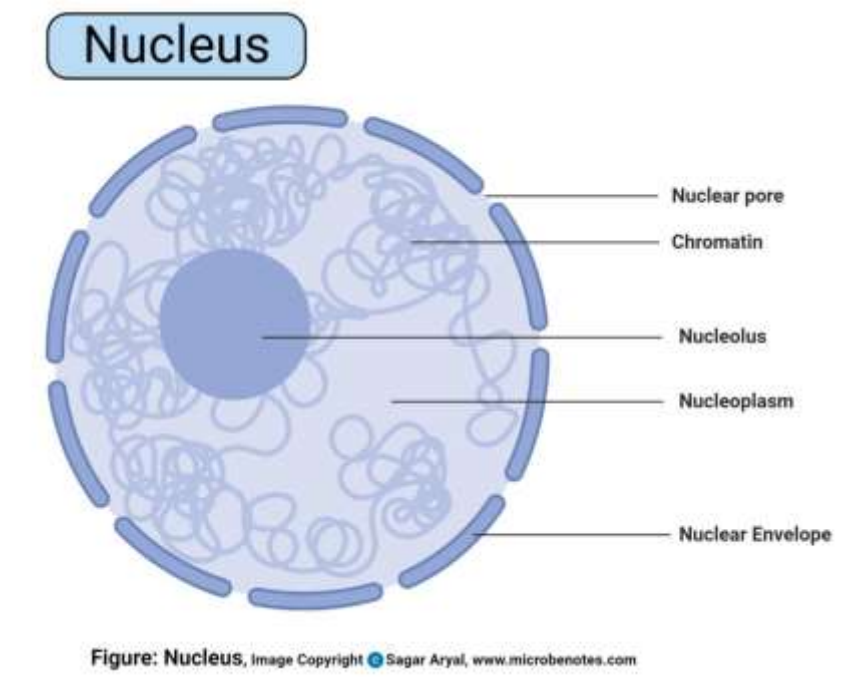


Figure: Nucleus, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Nucleus

The nucleus provides a site for genetic transcription that is segregated from the location of translation in the cytoplasm, allowing levels of gene regulation that are not available to prokaryotes. The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle.

It controls the hereditary characteristics of an organism.

The organelle is also responsible for protein synthesis, cell division, growth, and differentiation.

Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatin.

Storage of proteins and RNA (ribonucleic acid) in the nucleolus.

The nucleus is a site for transcription in which messenger RNA (mRNA) are produced for protein synthesis.

During the cell division, chromatins are arranged into chromosomes in the nucleus.

Production of ribosomes (protein factories) in the nucleolus.

Selective transportation of regulatory factors and energy molecules through nuclear pores.

8. Ribosome:

The ribosome word is derived – 'ribo' from ribonucleic acid and 'somes' from the Greek word 'soma' which means 'body'.

Ribosomes are tiny spheroidal dense particles (of 150 to 200 A^0 diameters) that are primarily found in most prokaryotic and eukaryotic.

They are sites of **protein synthesis**.

They are structures containing approximately equal amounts of RNA and proteins and serve as a scaffold for the ordered interaction of the numerous molecules involved in protein synthesis.

The ribosomes occur in cells, both prokaryotic and eukaryotic cells.

In prokaryotic cells, the ribosomes often occur freely in the cytoplasm.

In eukaryotic cells, the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of the endoplasmic reticulum.

The location of the ribosomes in a cell determines what kind of protein it makes.

If the ribosomes are floating freely throughout the cell, it will make proteins that will be utilized within the cell itself.

When ribosomes are attached to the endoplasmic reticulum, it is referred to as rough endoplasmic reticulum or rough ER.

Proteins made on the rough ER are used for usage inside the cell or outside the cell.

The number of ribosomes in a cell depends on the activity of the cell.

On average in a mammalian cell, there can be about 10 million ribosomes.

Each ribosome is divided into two subunits:
A smaller subunit which binds to a larger subunit and the mRNA pattern, and
A larger subunit which binds to the tRNA, the amino acids, and the smaller subunit.
Prokaryotes have 70S ribosomes respectively subunits comprising the little subunit of 30S and the bigger subunit of 50S.
Eukaryotes have 80S ribosomes respectively comprising of little (40S) and substantial (60S) subunits.

Functions of Ribosomes

The ribosome is a complex molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation).
Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules.
Ribosomes act as catalysts in two extremely important biological processes called peptidyl transfer and peptidyl hydrolysis.