

General Microbiology

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Introduction

- ✓ **Microbiology** is the study of living organisms of microscopic size, which include **bacteria, fungi**, the infectious agents at the border line of life that are called viruses and prions, infectious protein particles.
- It is derived from three Greek words (Micros = Small, Bios = life, Logos = study)
- It studies about their **structure, reproduction, physiology, metabolism and classification**

Subdivisions of microbiology

➤ It can be sub divided into: Virology (studies about viruses), Bacteriology (studies about bacteria), Mycology (studies about Fungi).

Viruses: The smallest infectious particles Ranging in diameter from 18 to 300 nm. They are consist of either DNA or RNA not both. They have living and non living characteristics.

- **Bacteria:** are prokaryotes, relatively simple in structure, reproduce by asexual division, covered by cell wall simple unicellular organisms without organelles like: Nuclear membrane, Mitochondria, Golgi bodies and Endoplasmic reticulum
- **Fungi:** are eukaryotes, that is why cellular structure is more complex and contain a well defined: Nucleus, Mitochondria, Golgi bodies, Endoplasmic reticulum,

- Fungi exist either in a unicellular form (Yeast) or in filamentous form (Mold)
- Veterinary microorganism deals with microbial agents affecting animals.

Prokaryotic and Eukaryotic cells

- Living cells, the smallest functional units of life capable of independent existence can be divided into two sharply differentiated groups: Prokaryotes and Eukaryotes

Prokaryotes

- Are primitive organisms that lack a clearly defined membrane-bound nucleus
- Their genetic information is contained in a single circular chromosome.

Eukaryotes

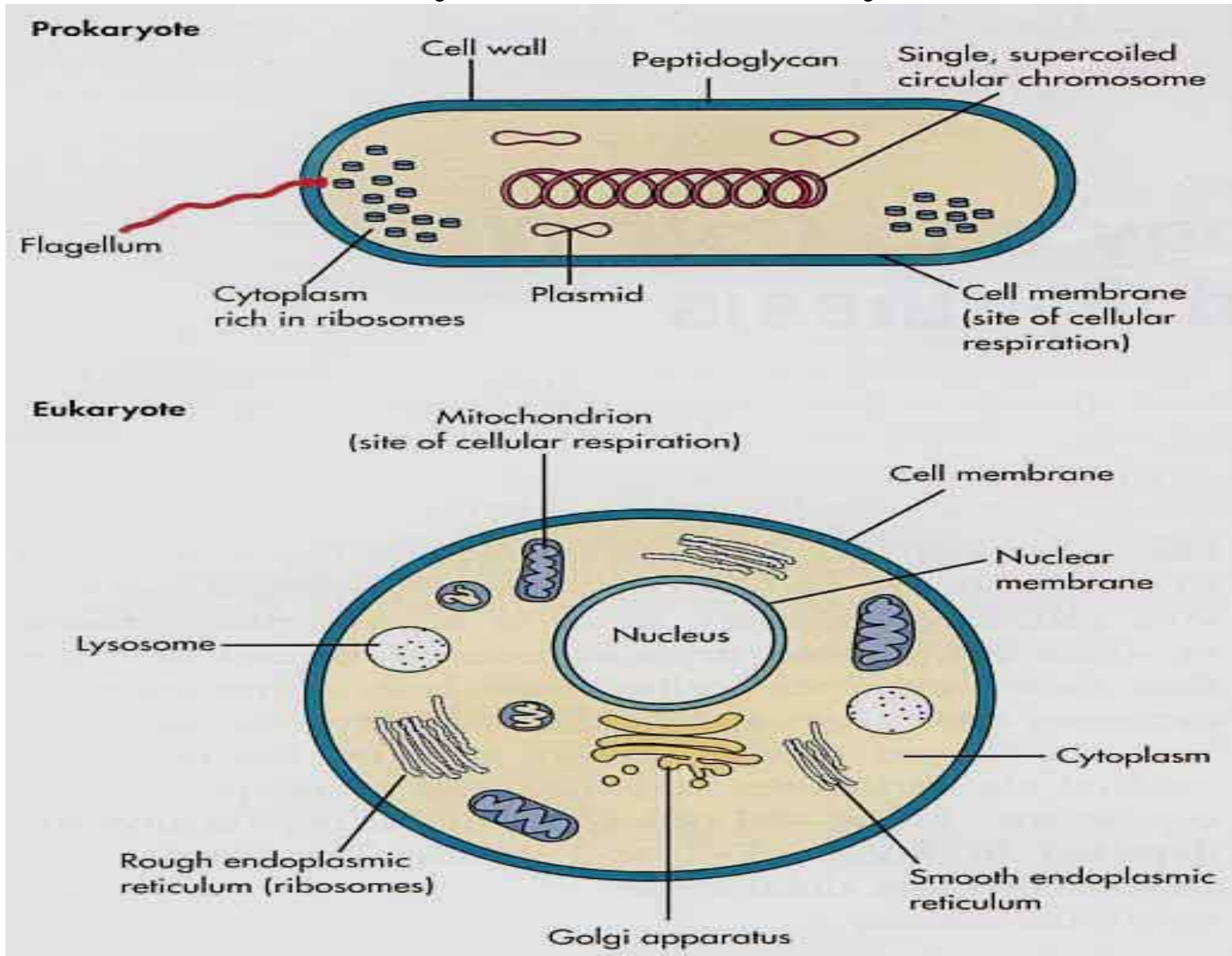
- Are more advanced life forms
- Possess a proper membrane bound nucleus (true nucleus), which contain chromosomes and contain organelles such as mitochondria, golgi apparatus, lysosomes and ribosomes
- Replicate by mitosis eg; Fungi, protozoa, plants and animals.

- Eukaryotes are grouped into the kingdom protista where as prokaryotes into kingdom monera
- Viruses fall into neither category

Comparison of eukaryotic and prokaryotic cells

Property	Prokaryotic	Eukaryotic
▪ True membrane bound nucleus	Absent	Present
▪ Number of chromosomes	One *	More than one
▪ Nucleolus	Absent	Present
▪ Replication	By binary fission	By mitosis
▪ Membrane bound organelles	Absent	Present
▪ Ribosomes	70S	80S
▪ Cell wall	Chemically complex with peptidoglycan	Chemically simple & lacking peptidoglycan
▪ Size	usually $\leq 5 \mu\text{m}$ in dm	Usually $\geq 100 \mu\text{m}$ in dm
▪ Differentiation	Rudimentary	Tissues & organs

Prokaryotes and Eukaryotes



Microscopy and microorganisms

- Microscope is an instrument most characteristics of the microbiology laboratory. It enables us to see microorganisms and their structures which are invisible to the naked eye. Depending upon the principle on which magnification is based, microscopes are of two categories
 - ✓ **Light or optical microscope**
 - ✓ **Electron microscope**

Light microscopy: magnification is obtained by a system of optical lenses using light waves.

- Two types namely Simple and Compound Microscope

Simple Microscope consists of a single lens.

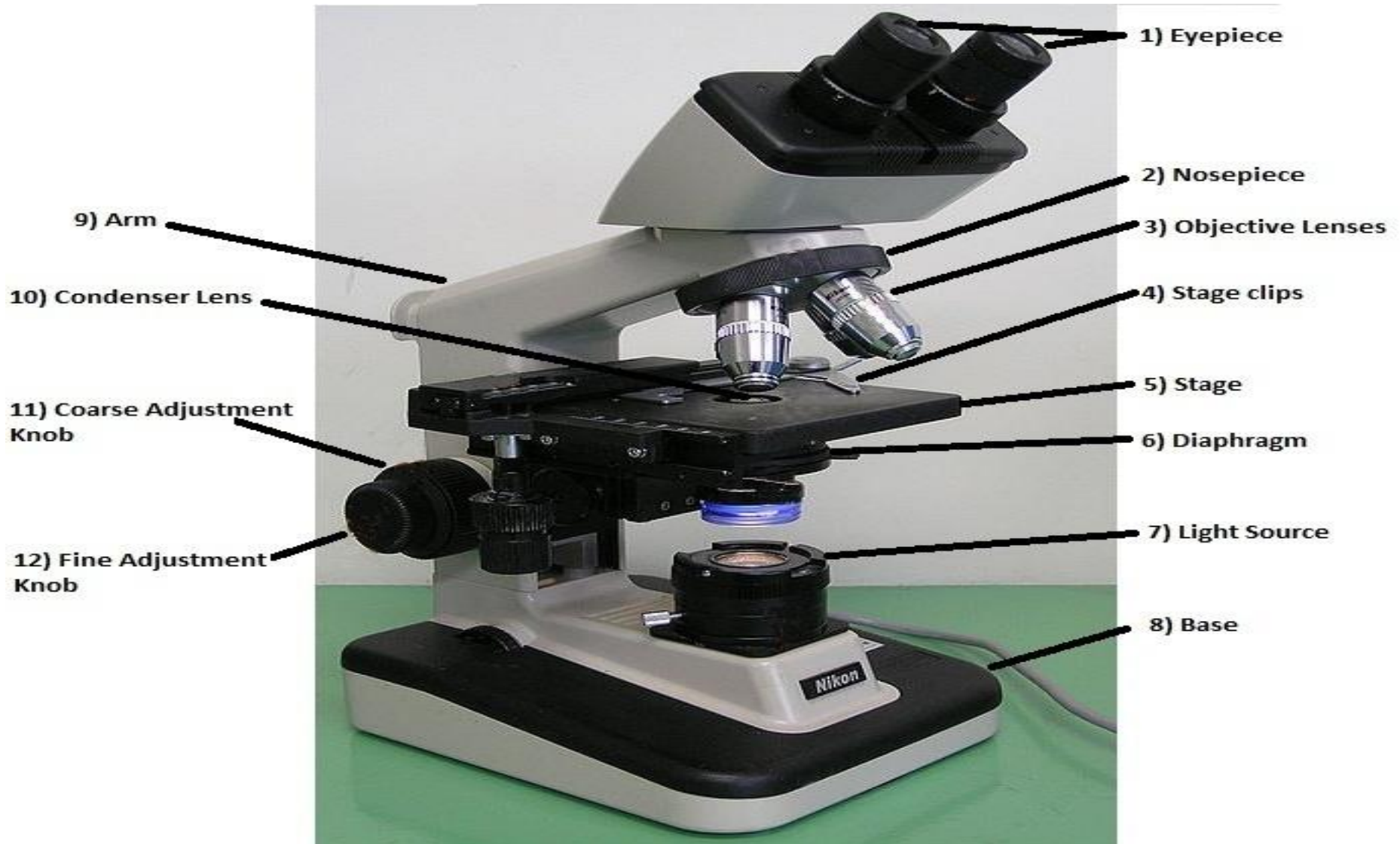
- A hand lens is an example of a simple Microscope.

Compound Microscope consists of two or more lenses in series. The image formed by the first lens is further magnified by another lens.

Simple microscope: consists of only a single lens. it is similar to a magnifying glass



Parts of a Compound Microscope



- Light microscopy, in which magnification is obtained by a system of optical lenses using light waves.

Types of light microscopes

Bright field microscope:

- ✓ Is the ordinary light microscope
- ✓ It is called a bright field because it forms a dark image against a brighter background

Dark- field microscope:

- The effect produce by the dark- field technique is that of a dark back ground against which objects are brilliantly illuminated

Phase-contrast microscope:

- ✓ Is valuable for studding living unstained cells and is widely used in applied and theoretical biological studies .

- ✓ It uses a conventional light microscope fitted with a phase contrast objective and a phase contrast condenser.

Fluorescence microscope:

- ✓ Exposes a specimen to ultraviolet, violet or blue light and forms an image of the object with the resulting fluorescent light.
- ✓ Usually the specimens have been stained with dye molecules, called fluorochromes

- To understand how a light microscope operates one must know the three principles of a light microscope; Magnification, Resolving power, Illumination

Magnification:

- The magnification of a compound microscope depends on ocular and objective lens systems.
- The total magnification of a microscope is equal to the product of the ocular lens and the objective lens magnifications.

Resolving power:

- The ability of a microscope to distinguish two adjacent points (particles) as distinct and separate

Resolving power can be increased either

- by reducing the wave length of the light or by increasing the numerical aperture.
- Since the spectrum of visible light is relatively narrow, the increase of the wave length of the light used is of limited value.

Illumination: The easily available source of illumination is ordinary day light but usually artificial light is used.

- The light from illumination source is refracted in to the sub stage condenser via the mirror located just below the condenser.

Electron microscope

- Uses a beam of electrons in place of light waves to produce the image. Specimens can be examined by either transmission or scanning EM
- EM has a practical resolution roughly 1,000 times better than the light microscope

- If electron illuminates the specimen, the microscope's resolution is enormously increased because the wavelength of the radiation is around 0.005 nm, approximately 100,000 times shorter than that of visible light.

Uses of different types of microscopes

Type of microscopy	Maximum useful magnification	Appearance of specimen	Useful application
Bright -field	1,000- 2,000	Specimens stained or unstained; bacteria generally stained and appear color of stain	For gross morphological features of bacteria,, yeasts, molds, algae and protozoa
Dark-field	1,000- 2,000	generally unstained; appear bright or “lighted” in an otherwise dark field	For microorganisms that exhibit some characteristic morphological feature in the living state and in fluid suspension eg. spirochetes
Fluorescence	1,000- 2,000	Bright and colored;; color of the fluorescent dye	Diagnostic techniques where fluorescent dye fixed to organism reveals the organism’s identity
Phase-contrast	1,000- 2,000	Varying degree of darkness	For examination of cellular structures in living cells of the larger microorganisms eg. Yeasts, algae, protozoa, and some bacteria
Electron	200,000- 400,000	Viewed on fluorescent screen	Examination of viruses and the ultrastructure of microbial cells

Morphology and Structure of Bacteria

- The word bacterium (plural, bacteria), is derived from the **Greek word** = *bakterion* = a small stick
- Bacteria vary in size as much as in shape (ranging in size from 0.2 μm -10 μm), **spiral organism** up to 100 μm , majority of the bacteria less than 5 μm , **Mycoplasma**=0.3 μm , **Nanobacteria** or **ultra microbacteria** = 0,2 to $\leq 0.05\mu\text{m}$ in dm. Some **spirochetes** occasionally reach 500 μm in length

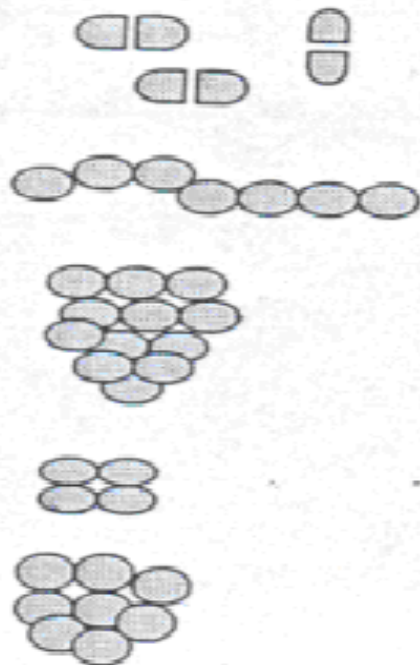
Generally, **bacterial cells** have three basic shapes

- **Round** (spherical): cocci/cocus
- **Rod-shaped** (bacilli/bacillus)
- **Spiral** with some varieties; spirilla/spirillum, spirochetes or curved rods

Cocci (Gr. Kokkos =berry) Cocci (coccus) are;

- Roughly spherical cells
- Can exist as individual cells
- Found in different arrangement depending upon their dividing planes

Cocci



Pairs (Diplococci) eg.
Neisseria

Streptococci

Staphylococci

Plates of four eg.
Gaffkya

Clubs of eight eg.
Sarcina

Rods or Bacillus (in Latin: bacillus =sticks)

- ✓ Bacilli differ considerably in their length-to-width ratio
- ✓ Coccobacilli being so short and wide that they resemble cocci
- ✓ The shape of the rod's varies between species and may be flat, rounded, cigar-shaped, or bifurcated

Rods



Coccobacilli eg. *Pasteurella*

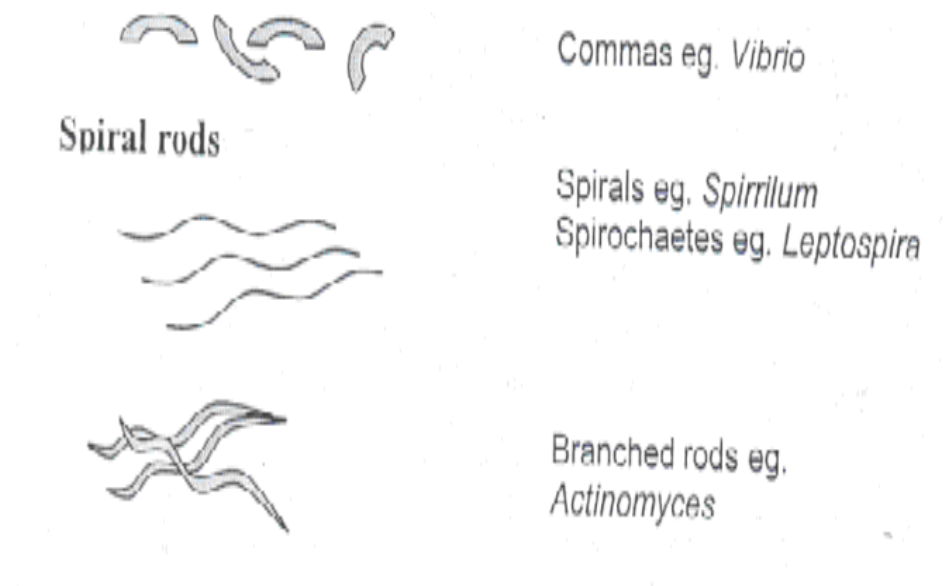


Chains eg. *Bacillus*

Spiral or curved:

Consists in the form of

- ✓ Vibrio
- ✓ Spirillum

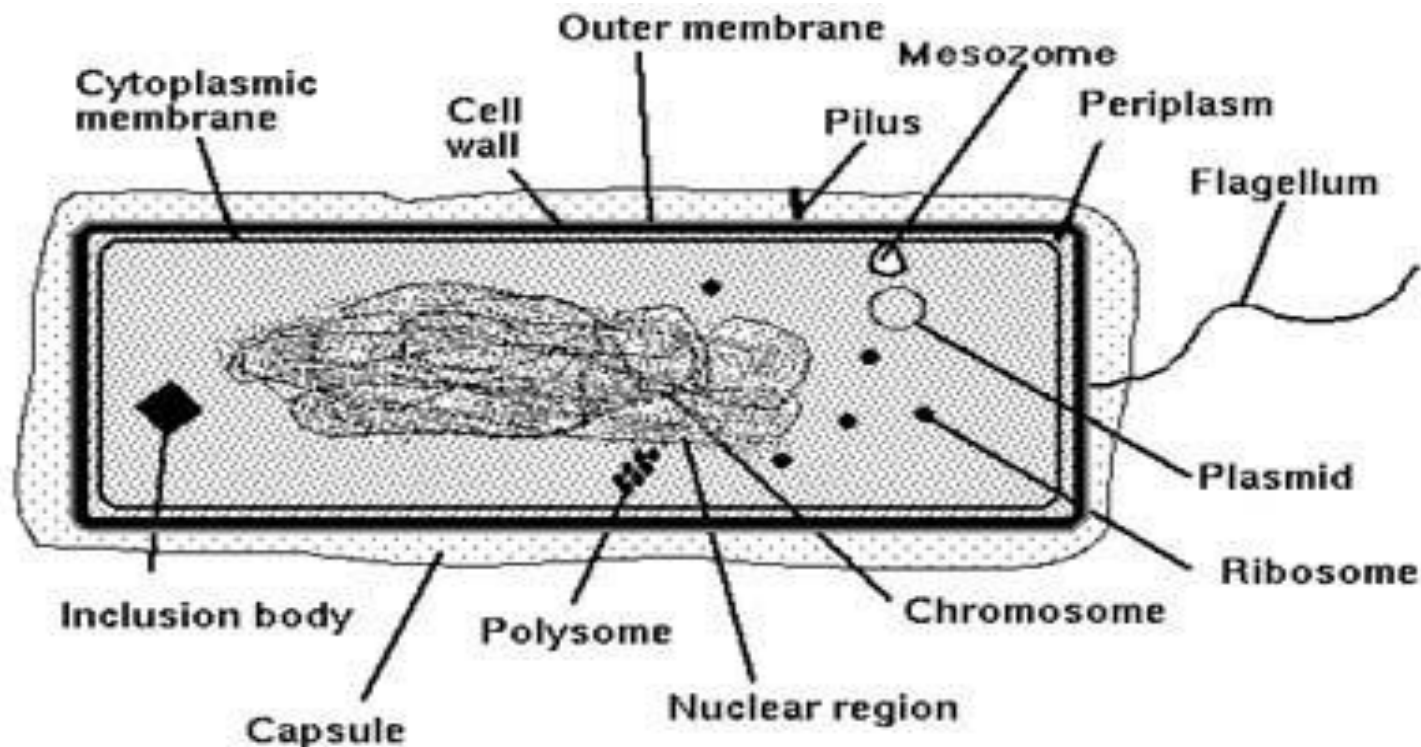


Pleomorphic bacteria

- ✓ Have variable shapes
 - ✓ Some bacteria show a degree of pleomorphism
 - ✓ example: *Mycoplasma*

Structure of the Bacterial cells

- Bacteria contain all the machinery required for growth and self- replication



Bacterial ultrastructures are:

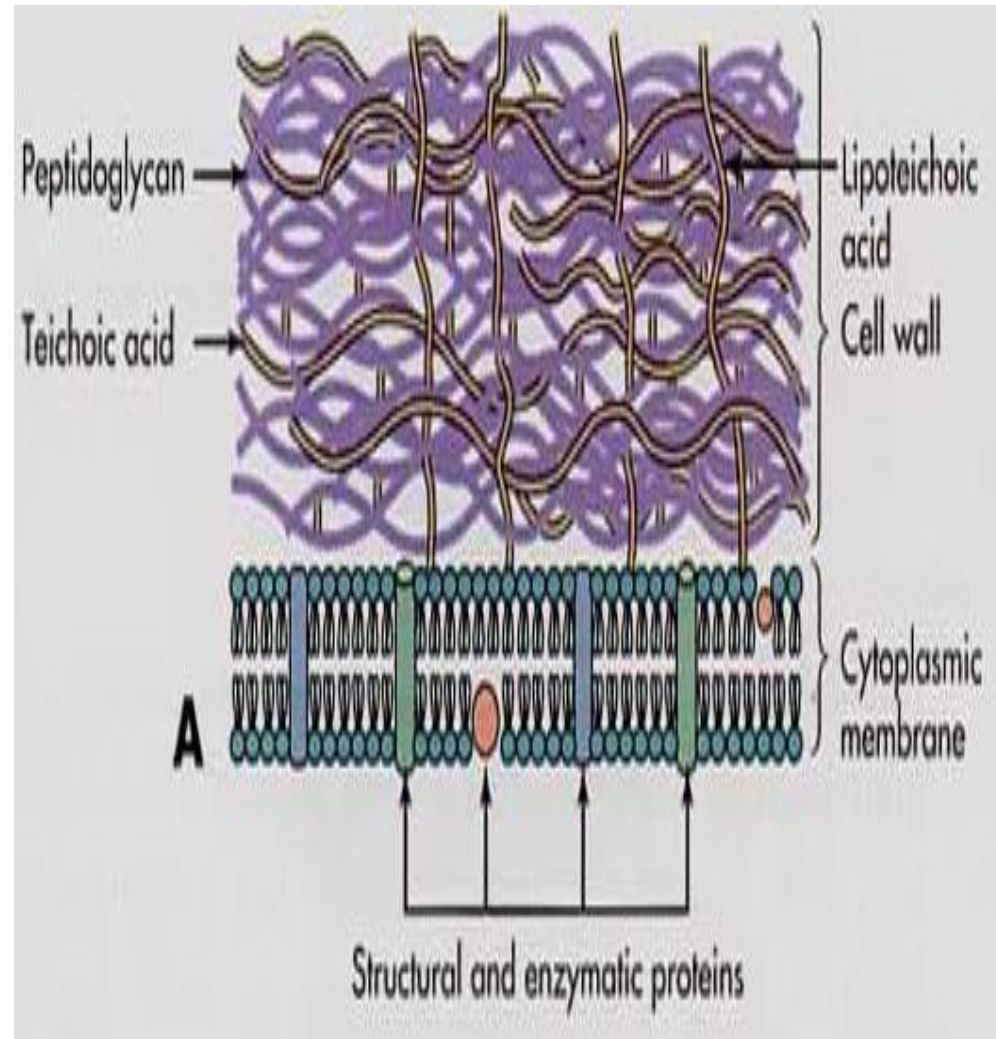
- ✓ Capsules
- ✓ Cell wall
- ✓ Cytoplasmic membrane
- ✓ Flagella
- ✓ Pili (Fimbriae)
- ✓ Spores
- ✓ Cytoplasm
- ✓ Ribosomes
- ✓ The nucleoid

Cell wall

- ✓ Protect the bacteria from chemical and physical action:
 - Determines the shape of the organisms
 - Bacteria are divided in to two major groups, on the basis of color when stained by Gram method:
 - ✓ Gram positive
 - ✓ Gram negative

Gram positive

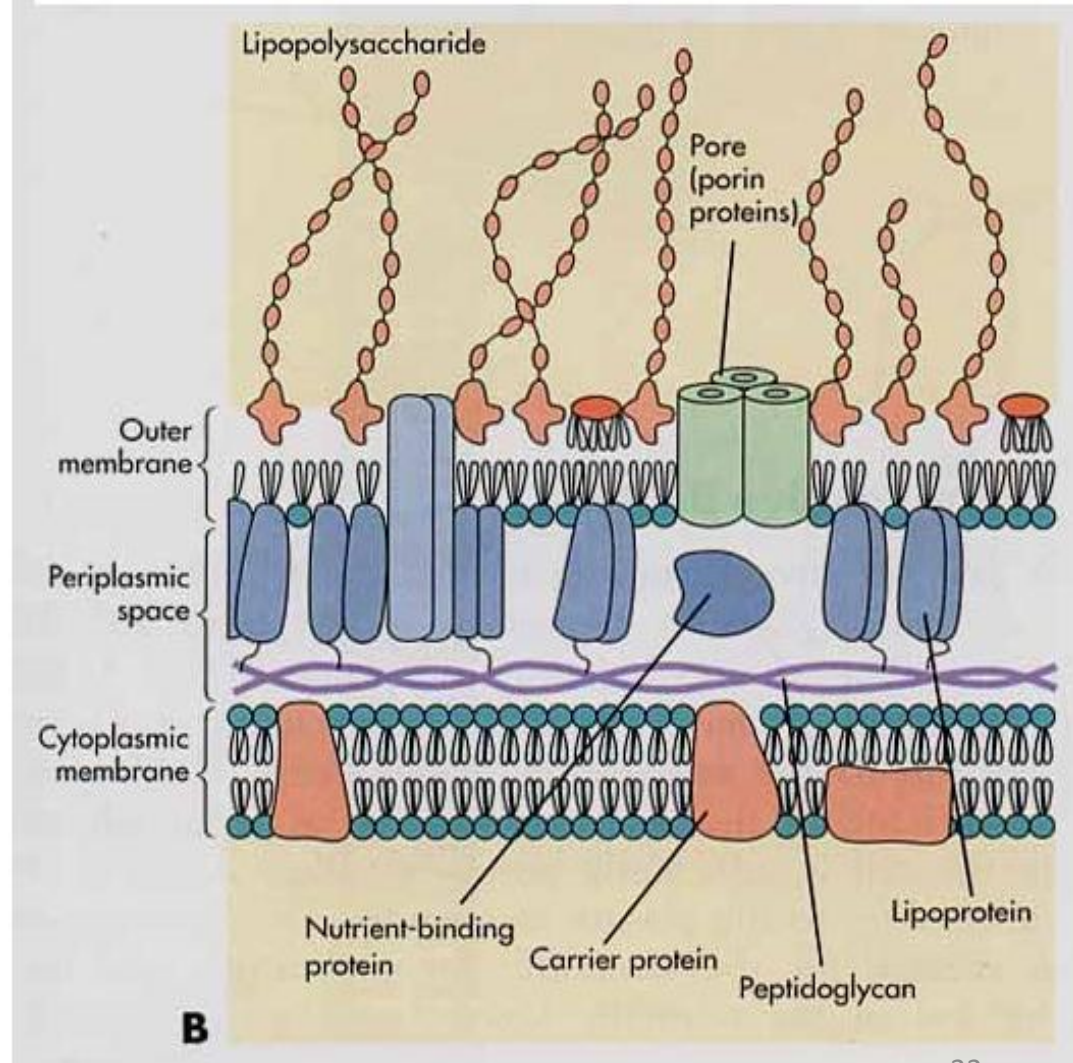
- Gram positive cell wall is relatively thick
- Composed mainly of peptidoglycan (Up to 40 layers)
- Include other components such as teichoic and lipoteichoic acid and polysaccharides



Gram -negative

Gram negative cell wall

- more complex
 - Contains two layers external to the cytoplasmic membrane
1. A thin peptidoglycan layer (only one or two layers)
 2. Outer membrane external to the peptidoglycan



Cytoplasmic membrane

- ✓ Composed of phospholipids and proteins
- ✓ It is permeable
- ✓ Located between the cell wall and the cytoplasm
 - Secret hydrolytic enzymes
 - Regulate cell division
 - Energy generation

Cytoplasm

- ✓ Aqueous fluid containing the nuclear material (DNA chromosomes ribosomes: protein synthesis)

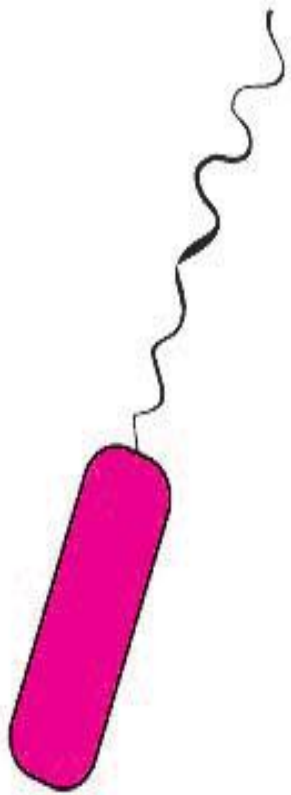
Capsule (Glycocalyx): mostly of water and 1-2% polysaccharide, polypeptide, or protein according to individual bacterial species. Its roles are

- ✓ Protection from adverse environmental condition
- ✓ Interfere with phagocytes in animal
- ✓ Facilitate the adherence of bacteria to animal tissue body

Flagella

- Attached to the bacterial cell wall
- Used for locomotion
- Composed of the protein flagellin
- Originate in the bacterial cytoplasm.
- Many Gram negative bacteria have flagella
- Flagella contain antigen (H antigen) which is used for identification and classification

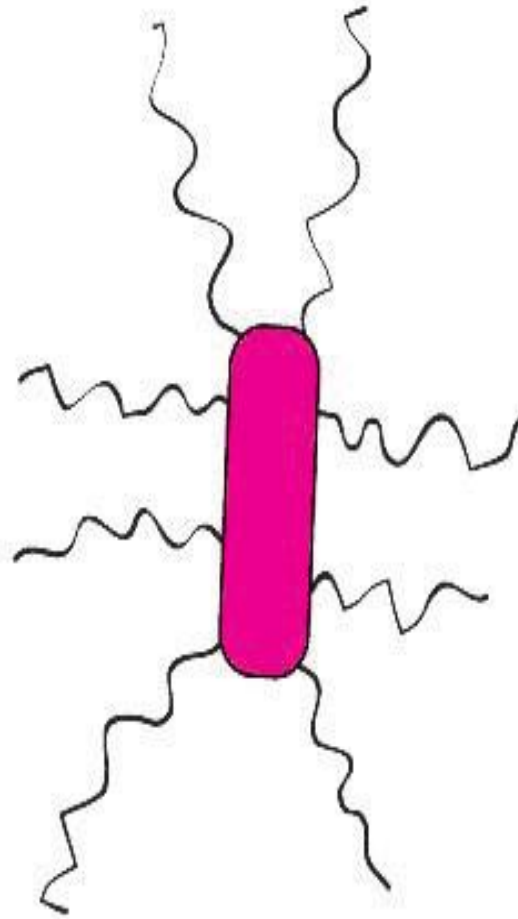
According to the position of flagella on the their surface, bacteria are divided in to 4



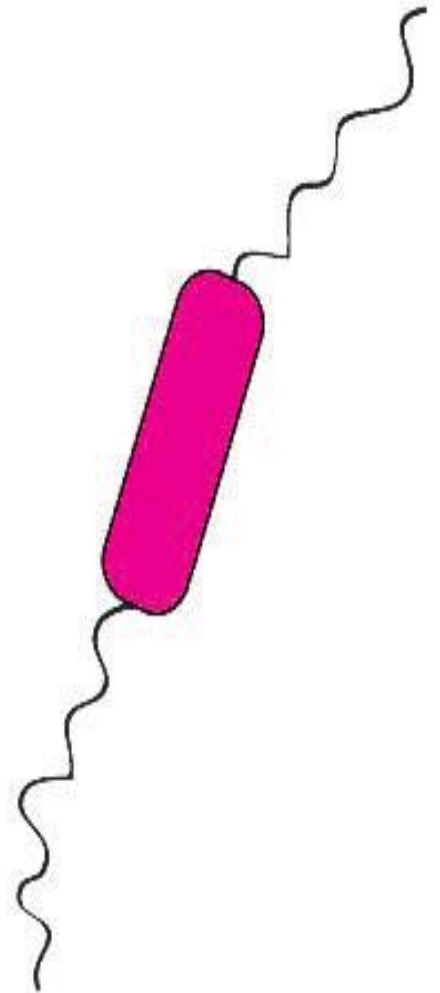
(a) Monotrichous
(polar)



(b) Lophotrichous



(c) Peritrichous



(d) Amphitrichous

Pili (Fimbriae):

- Hair like filaments appendages that extend from cell surface. They are composed of the protein pilin
- Found mainly on gram-negative organisms

Roles

- ✓ Promote the organism to adhere to mammalian cells
- ✓ Sex (conjugation) pilus promote the transfer of generic material (chromosomes) of bacterial during conjugation

Bacterial Spores

- Small, dehydrated metabolites produced during conditions that inhibit growth of cell division (nutritional depletion, in pH change, oxygen depletion)
- Formed inside the cell
- They are resistant to Heat, Dehydration, Radiation and Chemicals

Factors that play major role for the resistance

- ✓ Calcium dipicolinate in core
- ✓ Keratin spore coat
- ✓ New enzymes (dipicolinic acid synthetase, heat-resistant catalase)
- ✓ Increases or decreases in other enzymes.

Bacterial metabolism, growth and reproduction

Metabolic requirements

- Bacterial growth requires a source of energy and raw materials to build the protein structures and membranes that make up the structure and biochemical machines of the cell
- Bacteria must obtain or synthesize the amino acids, carbohydrates and lipids used as building block of the cell
- The minimum requirement for growth of bacteria need a source of Carbon and Nitrogen as energy source, Water and various ions are also necessary.

Bacteria can drive energy

- ✓ From the oxidation of metals ions
- ✓ By photosynthesis
- ✓ By metabolizing sugars, fats and proteins

Bacteria utilize energy for:

- ✓ The constriction of the physical parts of the cell (cell wall or membrane)
- ✓ Synthesis of enzymes, nucleic acid, polysaccharides, and other chemical component

Physico-chemical requirements for bacterial growth

- Bacteria grow if their environment is suitable. If the environment is not optimal; growth may occur at a lower rate or not at all or the bacteria may die.

Essential requirements for growth of bacteria include

- ✓ A Supply of suitable **nutrients**.
- ✓ A source of **energy** and **Water**
- ✓ An appropriate **temperature** and **pH**
- ✓ Appropriate levels (or the absence) of **oxygen**

Nutrients: Cell needs nutrients as raw materials for Growth, Maintenance and, Division.

- What ever the organism, cells need sources of, Carbon, Nitrogen, Phosphorus, Sulfur and other trace elements (materials) from which living mater is made

- Macro minerals are required in relatively large quantities
 - Carbon
 - Nitrogen
 - Hydrogen
 - Oxygen
 - Phosphorus
 - Sulfur
 - Calcium
 - Magnesium
 - Iron
- Micro minerals are required in relatively tiny quantities or trace amounts
 - Cobalt
 - Copper
 - Sodium
 - Manganese
 - Molybdenum
 - Zinc

Carbon:

- All microorganisms require carbon in, organic or inorganic forms
- Organic carbon forms the backbone of carbohydrates, lipids and proteins.

Bacteria can use

- Simple two carbon (acetate), Complex molecules (cellulose) or Monosaccharide (glucose)

Nitrogen

- It is the major component of proteins and nucleic acids
- Bacteria acquire Nitrogen from organic or inorganic sources

Inorganic sources of Nitrogen

- ✓ Ammonia, Nitrate and nitrite reduction ($\text{NO}_3^- / \text{NO}_2^- \rightarrow \text{NH}_3$)
- ✓ Gaseous N_2 (N_2 - fixation) $\text{N}_2 \rightarrow \text{NH}_3$

Organic sources of Nitrogen

- ✓ Proteins or their degradation products, In the process of N_2 assimilation: NH_3 formed from different N sources
- ✓ Glutamic acid + NH_3 = produce glutamine, which serves as a storehouse of N_2

Phosphorus:

- Sources of Phosphorus: inorganic phosphate (PO_4^-)
- Essential for the synthesis of important biomolecules like Nucleic acids and ATP (Adenosine triphosphate)

Sulfur:

Needed for the biosynthesis of the Amino acids like

- ✓ Cystine
- ✓ Methionine

Calcium;

- ✓ Acts as enzyme cofactor
- ✓ Contributes to heat resistance of spores

Potassium:

- Activates various enzymes

Magnesium:

- Serves a cofactor for many enzymes. It also stabilizes membranes and ribosomes and nucleic acids

Iron:

- It is important component of the cytochromes (electron carries in oxidation reduction reactions) system

Temperature requirements for bacteria

- ❖ For a given type of bacterium, growth occurs most rapidly at a particular temperature (optimum temperature). There are also maximum and minimum temperatures beyond which growth will not occur.
- Based on temperature requirements: bacteria grouped into
 - ✓ Psychrophiles
 - ✓ Mesophiles
 - ✓ Thermophiles

Psychrophiles (Cold- loving)

- Grow between -10°C and 20°C
- Psychrophilic bacteria and fungi are major factors in the spoilage of refrigerated foods
- Found in cold water, mud and soils such as in the polar region
- Examples: Pseudomonas, Flavobacterium
alcaligenes, Polaromonas vaculata

- **Mesophiles:** are microorganisms with growth temperature optima $20^{\circ}\text{C} - 45^{\circ}\text{C}$
 - Example: most human and veterinary pathogens
- **Thermophiles:** They can grow between $45^{\circ}\text{C} - 70^{\circ}\text{C}$
 - ✓ Possess heat stable enzymes and
 - ✓ Protein synthesis system able to function at high temperature

Hyperthermophilic bacteria

- Have growth optima between 80 –113 °C
- ✓ Do not involve in infectious diseases
- ✓ They are found in
 - Volcanic areas
 - Compost heaps
 - Hot springs

pH requirements of bacteria

- Based on pH requirements bacteria are classified in to
 1. **Neutrophiles** prefer the pH range of 5.5 to
 2. **Acidophiles** have their growth optimum between pH 0 and 5.5.
 3. **Alkalophiles** prefer the pH range of 8.5 to 11.5
- Most bacteria grow best at or near pH 7.0 (neutral)

Oxygen requirements of bacteria

- Some bacteria need oxygen for their growth.
 - Others do not need oxygen for their growth
 - others can grow regardless of the presence or absence of oxygen.
- ❖ Based on the requirements of O_2 bacteria can be grouped
- a. Aerobes**
 - b. Strict or obligate anaerobes**
 - c. Facultative anaerobes**
 - d. Microaerophilic bacteria**
 - e. Aerotolerant**

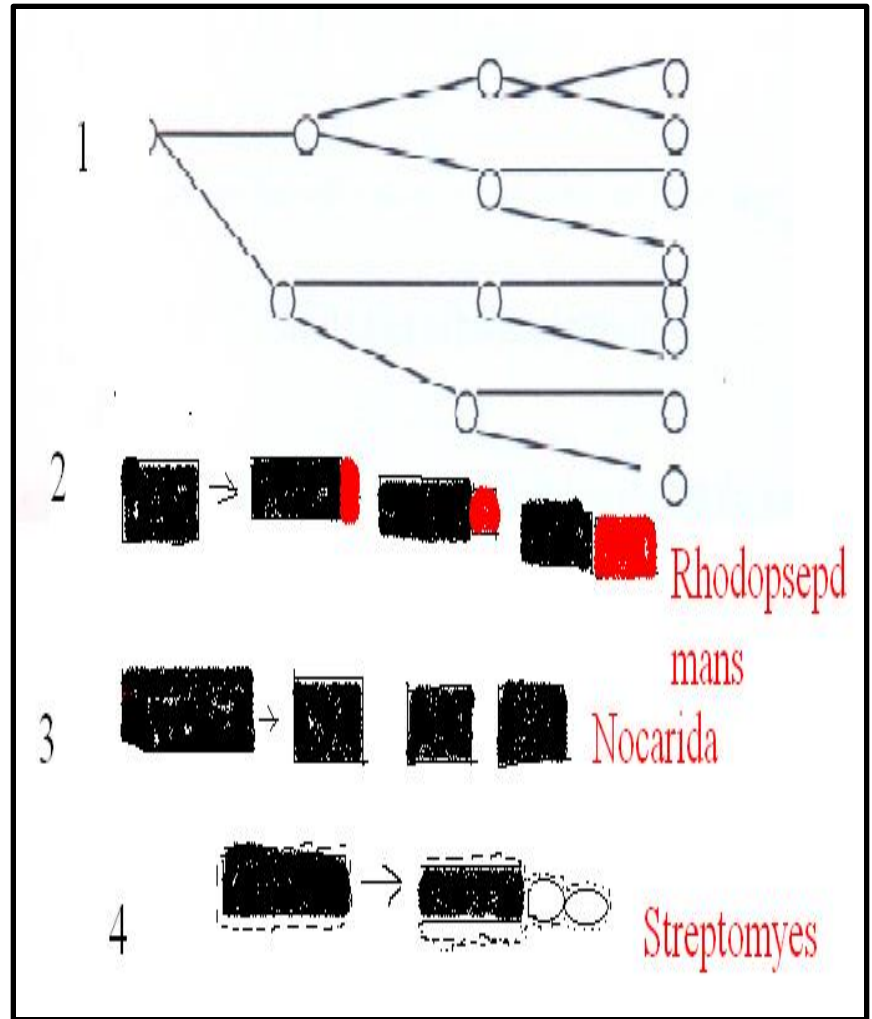
- **Aerobes:** organisms that grow in the presence of oxygen
- **Strict or obligate anaerobes:** grow only when oxygen is absent
- Bacteria which normally grow in the presence of oxygen but which can still grow under anaerobic conditions (ie. In the absence of oxygen) are called **facultative anaerobes**
- Similarly those which normally grow anaerobically but which can grow in the presence of oxygen are called **facultative aerobes**

- **Microaerophilic bacteria:** generally grow best when the concentration of oxygen is /usually much/ lower than the normal atmospheric level
- **Aerotolerant anaerobes:** Ignore oxygen and grow equally well whether it is present or not

Con't..

Group	Environment		O ₂ Effect
	Aerobic	Anaerobic	
Obligate Aerobe	Growth	No growth	Required (utilized for aerobic respiration) 1atm(21%)
Microaerophile	Growth if level not too high	No growth	Required but at levels below 0.2 atm
Obligate Anaerobe	No growth	Growth	Toxic
Facultative (An)aerobe	Growth	Growth	Not required for growth but utilized when available
Aerotolerant Anaerobe	Growth	Growth	Not required and not utilized

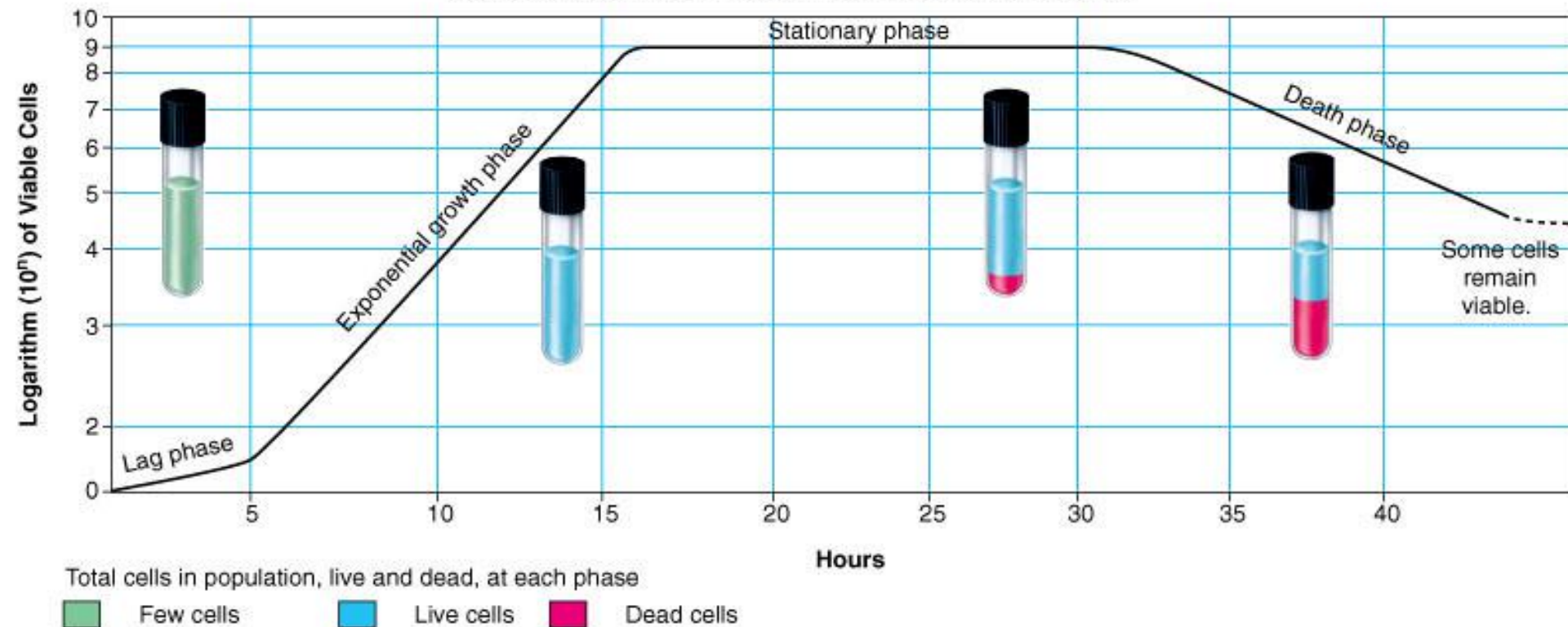
- Reproduction of bacteria take place by
 - ✓ binary fission: most common
- Alternative means
 - ✓ Budding
 - ✓ Conidiospores (filamentous bacteria)
 - ✓ Fragmentation



Bacterial growth phases (curves)

- The growth curves of bacteria are characterized by distinct stages from the time it is introduced into a medium until it ceases growth. These are **Lag, log (exponential), stationary, decline or death**.

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Nomenclature and classification of Bacteria

- Taxonomy is an area of biological science which comprises three distinct, but highly interrelated disciplines that include classification, nomenclature and identification.
- Applied to all-living entities, taxonomy provides a consistent means to classify, name and identify organisms. The common language that taxonomy provides minimizes the confusion about names of Mos.

- Classification is the organization of organisms that share similar morphologic, physiologic and genetic traits into specific groups or taxa.
- Nomenclature, the naming of microorganisms according to established rules and guidelines provide the accepted labels by which organisms are universally recognized.

- So the major aim of taxonomy is to set up classification of microorganisms
- ✓ This classification can then be used to identify individual bacterial species or strains (identification) and name them (nomenclature)

Rules of bacteriological nomenclature

- ✓ Bacterial nomenclature is the assignment of names to taxonomic groups according to international rules which are governed by 'Bacteriological code' including:
 - Particular species name has only one name understood by many scientists

- The name does not need to be descriptive one:- in fact descriptive names are prone to be misleading

For example: the name *Staphylococcus aureus* is descriptive of the golden *Staphylococcus*, however, it is now recognized that this organism may become non pigmented or white

- The first name (Genus name) of the organism should be a proper noun and always begins with a capital letter
- The second name begins with small letter and designates the species name
- The whole binomial name must be Latinized and italicized or underlined (Example: *Staphylococcus aureus*)

- ✓ The taxonomic categories into which the bacteria can be ranked from highest to lowest,

Kingdom, Phylum, Class, Order, Family, Genus, Species

- ✓ The names of each level ends with specific suffix. Eg.
Family- **aceae** (Enterobacteriaceae) Order- **ales**
(Enterobacterales), Class-**ia** (Gammaproteobacteria)

Genus: Escherichia

Species: *Escherichia coli*

Characteristics used in bacterial identification and classification

Bacteria are classified by using any of the characteristics under the following general heads

I. DNA test: comparison of DNA content and sequence between strain. These tests are the most definitive methods for separating organisms into different groups.

- DNA is analyzed by measuring the guanine- cytosine (G-C) content or the amount of DNA homology between strains.

II. Colony morphology: The shape, texture and color of colonies of microorganisms growing on solid agar plates are used for differentiation when ever practical. eg. *Staphylococcus aureus* is so named as colonies are of a yellow color; aureus comes from Latin for gold

III. Reaction to stains: Gram staining classified bacteria into Gram positive and Gram negative

- Stains may also be used to show other morphological features, which may used for classification, such as
 - Spores (Malachite green stain)
 - Unusual cell wall (acid fast stain)
 - Capsules (Indian ink stain)
 - Intracellular lipids (Sudan black)
 - Flagella (flagellar stain)

IV. Growth Characteristics:

- Relation to temperature (*Campylobacter jejuni*, grows well at 42° C; another, *Yersinia enterocolitica*, grows better than most other bacteria at 4° C)
- Relation to pH,
- Oxygen requirements of an isolates are useful tests for the identification (aerobically, anaerobically, facultatively (i.e., in either the presence or absence of oxygen))

V. Biochemical tests: Used to measure various aspects of bacterial metabolism such as ;-

- What Carbon and nitrogen sources the bacteria can use ...like sugars
- The end product of their metabolic process, such as acetoin, acids, gases
- What enzymes the bacteria produce,
- Resistance to antibiotics

VI. Antigenecity:

- Cellular components such as the O-side chains of lipopolysaccharide (LPS) or capsule are frequently used to distinguish between strains of one species
- The antigenic relationships of bacteria are detected by the use of serological tests agglutination and precipitation

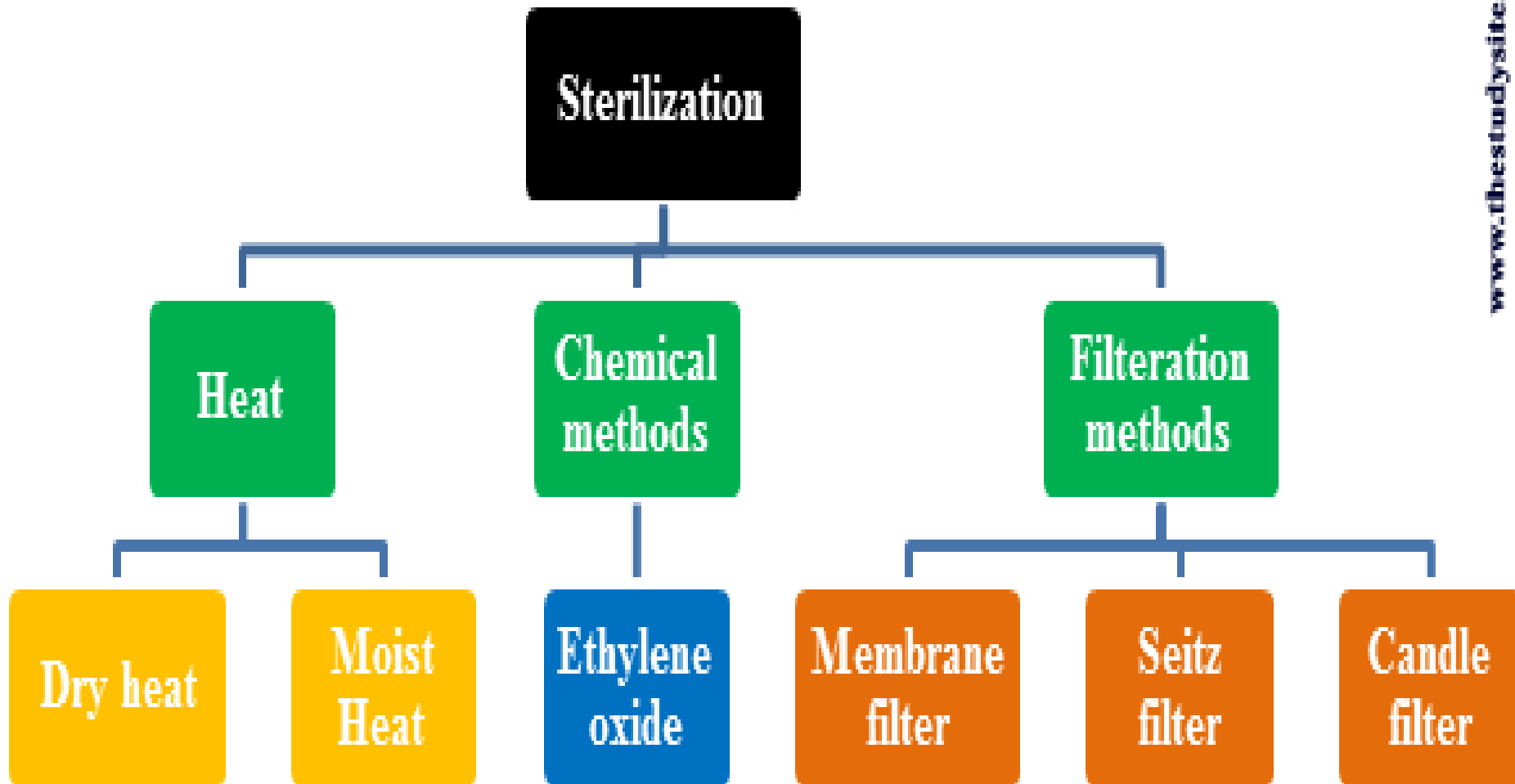
Sterilization and disinfection

- Bacteria are present on the surface of all laboratory apparatus, in the dust, upon the hands and are generally found every where.
- They are the source of contamination, infection, and decay. Hence it is necessary to remove them from materials and areas.

Sterilization

- ✓ Means total destruction of all microbes including the more resilient forms such as bacterial spores, mycobacterium, non-enveloped viruses and fungi
- ✓ Sterilization can be achieved by
 - Heat
 - Chemical
 - Filtration methods

Methods of sterilization



Heat sterilization

- Most common method of sterilization.
- The heat kills the microbes in the substance.
- The amount of heat and duration of heating are the factors that affect extent of sterilization. two types based on the type of heat used
 - ✓ Moist heat method
 - ✓ Dry heat method

Moist heat methods of sterilization:

- ✓ Boiling
- ✓ Pasteurization
- ✓ Use of pressurized steam (Autoclaving)

- Boiling is preferred for metallic devices like surgical scissors, scalpels, needles etc.



B. Pasteurization is a process of heating the milk at a temperature of 63 °C or 72°C for 30 min and 15 sec, respectively

C. Steam (autoclaving): Substances are subjected to sterilization in an autoclave.

- Three major factors required for effective autoclave
 - ✓ Pressure
 - ✓ Temperature
 - ✓ Time

Pressure	Temperature	Time (Min.)
15 psi	121°C	15
20 psi	126°C	10
20 psi	134°C	3

Dry-heat sterilization

- Requires a higher temperature than moist heat and a longer exposure time.
- More convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam (strong glasses like petridish and tubes)

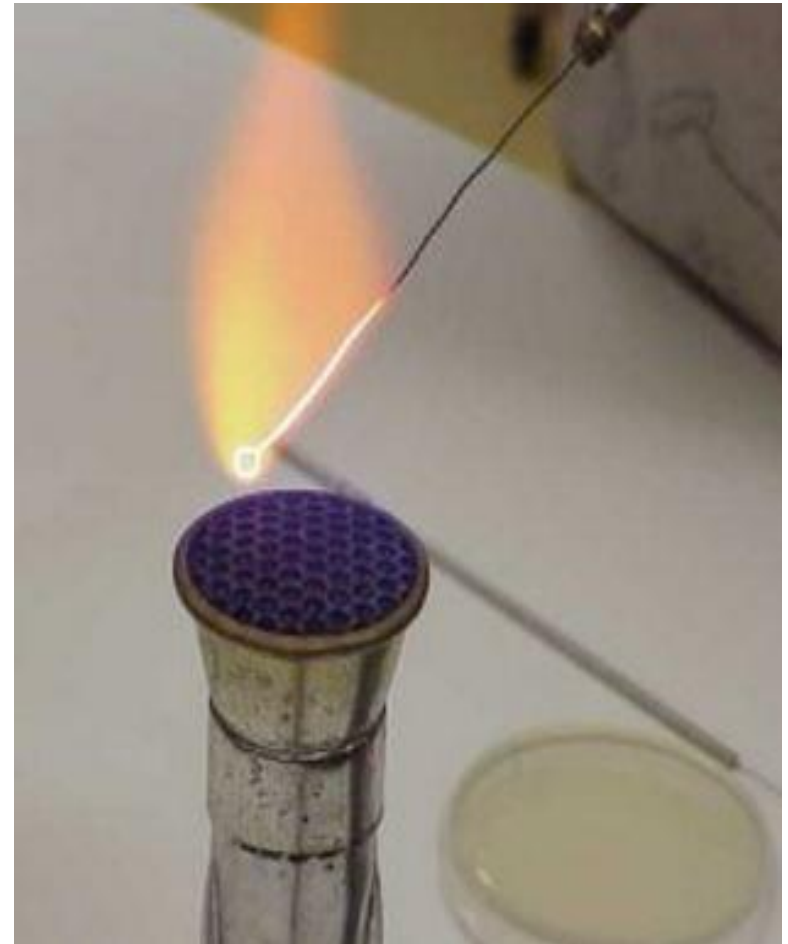
<u>Temperature °C</u>	<u>time in min</u>
• 160	180
• 170	60
• 180	30

This method includes techniques like

- ✓ Red heat
- ✓ Flaming
- ✓ Incineration
- ✓ Hot air oven
- ✓ Radiation sterilization

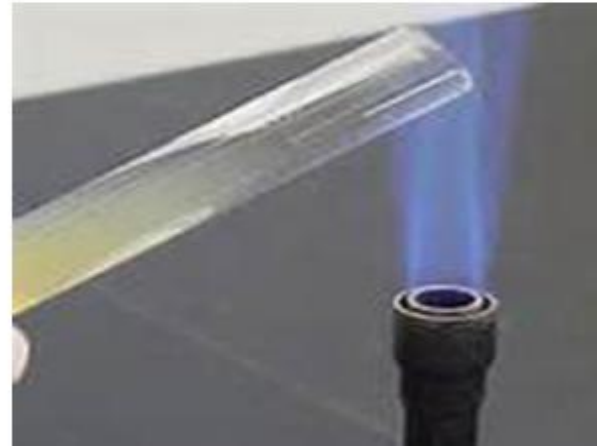
Red heat

- It is used to sterilize metallic objects (needle, scalpels, scissors etc) by holding them in flame and heated red hot. The fire burns the microbes and other dust on the instrument



Flaming

- The material is passed over flame without allowing it to become red hot. used for sterilizing scalpel, mouth of culture tubes, glass slides etc.



- **Incineration:** for items like as bandages, paper dishes, and sputum caps
- **Hot air oven:** (oven baking) suitable for drying materials like powder, metal devices, glassware



Radiation method:

- Involves exposing the packed materials to radiation for sterilization
- ✓ Two types of radiation
- Non ionic radiations: Safe to the operator , like UV radiation
- Ionizing radiations: powerful radiation, harmful the operator needs protect himself from exposure by dressing special cloth. Eg. X-rays, gama-rays. Etc

Chemical methods of sterilization

- Articles are subjected to sterilization by using toxic gases
- Gas penetrates quickly into the material
- But the chances of explosion and cost factors are to be considered
- The commonly used gas is ethylene oxide with combination of CO_2 . CO_2 is added to minimize the chances of an explosion

Filtration method of sterilization:

- Liquids are filtered through bacterial filters to remove any microbes present
- Useful to sterilize heat sensitive objects.

Disinfection: is a process, which involves use of physical procedures or chemical agents (disinfectants) to destroy most microbial forms

Disinfectants are subdivided into

- ✓ High Level
- ✓ Intermediate level
- ✓ Low-level agents
- ✓ applied on inanimate objects

Con't..

- Examples of disinfectants include Glutaraldehyde, hydrogen peroxide, peracetic acid, chlorine dioxide, and other chlorine compounds

Antisepsis

- Is the use of chemical agents on skin or other living tissues to inhibit or eliminate microbes; no sporicidal action is implied. Antiseptics include
 - ✓ Alcohols
 - ✓ Iodophors .
 - ✓ Chlorhexidine
 - ✓ Triclosan etc

Culture media

- Media (Medium S.): is a substance used to provide nutrients for the growth and multiplication of microorganisms. It gives artificial environment, simulating natural condition necessary for growth of bacteria
- Used for
 - ✓ Isolation
 - ✓ Identification
 - ✓ antibiotic sensitivity

Culture media should contain

- ✓ Energy source
- ✓ Carbon source
- ✓ Nitrogen source
- ✓ Salts
- ✓ Satisfactory PH
- ✓ Adequate oxidation-reduction potential and
- ✓ Growth factor

Based on their consistency culture media are classified into:

- ✓ Fluid laboratory media
- ✓ Semisolid laboratory media
- ✓ Solid laboratory media

Liquid media

- ✓ Used as enrichment media before plating on solid media
- Bacteria grow very well in fluid media in 3 to 4 hours
- These are not suitable to study colony types

Types :- (Broth, Peptone , Yeast extract)

Solid

Liquid

semi-solid



Culture Media

Solid Media

- Used to study colonies of individual bacteria.
- Essential for isolation of organism in pure form

Types

- ✓ **Agar:** It is complex polysaccharide obtained from sea weeds (Algae geledium species)
- ✓ **Gelatin:** protein prepared by hydrolysis of collagen with boiling water

Based on the ingredients that lab media contain
classified : Defined or synthetic media and Complex
Media

Defined or synthetic laboratory medium

- ✓ The exact amount of each component is known
- ✓ Used widely in research, as it is often desirable to know what the experimental microorganism is metabolizing

Complex Media

- ✓ Contain some ingredient of unknown chemical composition.
- ✓ Examples: Meat extract, yeast extract, MacConkey agar

Based on the purpose in which laboratory media are used can be divided in to the following categories

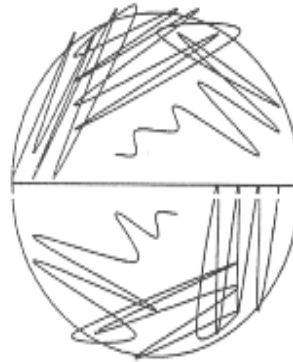
- **General purpose media:** support the growth of many MOs e.g. Tryptic soya broth, nutrient agar
- **Enriched media:** support the grow of fastidious bacteria (require many growth factors) blood ,serum, etc
- **Selective media:** made selective for the growth of a particular bacterium. They contain substance that prevents the growth of unwanted bacterial species. Eg. MacConkey agar, Endo agar , Eosin methylene blue agar

- **Indicator media:** Designed to give a presumptive identification of bacterial colonies and contain fermentable sugars plus a pH indicator that gives a color change in the media. Example MacConkey media
- **Differential media:** distinguish between different groups of bacteria based on their biological characteristics. e.g. Blood agar

Inoculation of Culture Media:

- The processes of introducing a tiny sample into a container of medium.
- There are different types of inoculation (planting) methods on to petridish these are:
 - A. Quadrant plating method: using the whole plate
 - B. Half plating: using half of the plate
 - C. Quarter plating: using quarter r of the plate

Different bacterial inoculation methods



Con't..

There are also different techniques of inoculation some of them are

1. Streak culture: -

- The objective of plate Streaking is to obtain isolated bacterial colonies.

2. Spread Plate: is used to separate microorganisms contained within a small sample volume, which is spread over the surface of agar plate,

3. Pour plate culture

- ✓ Appropriate dilution of inoculums mixed with melted agar is poured or dispense in to Petri-dish. gives viable bacteria count in a suspension.

Measurement of bacterial growth: can be done by direct or indirect methods

- ✓ **Cell mass:** Directly weighing or by measurement of cell nitrogen or indirectly by turbidity
- ✓ **Cell activity:** indirectly by relating the degree of biochemical activity to the magnitude of the bacterial population
- ✓ **Cell count:** directly by microscopy or using an electric particle counter. Bacteria can be counted easily and accurately with the Petroff-Hausser counting chamber
- ✓ **Colony count:** The most frequently used method of counting bacteria is plate count method

Staining of bacteria

- ✓ Living microorganisms can be directly examined with the light microscope.
- ✓ They often must be fixed and stained to increase visibility of specific morphological features, and preserve them for future study.
- ✓ Before we can begin the staining procedure, the cells have to be mounted (smear) and fixed onto a glass slide.

Staining techniques

✓ Simple and differential staining techniques

Simple: using only one dyes,

- Cover the fixed smear with anyone of the following dyes: (Gentian violet ,Crystal violet, Safranin, Methylene blue, Basic fuchSION)
- After 30-60 seconds the slide is washed under the water tap and the smear is gently blotted dry.
- Size, shape and arrangement of bacteria are appreciated

Differential Staining Techniques

- In microbiology, differential staining techniques are used more often than simple stains.
- Require more than one stain and several steps, are referred to as such because they permit the differentiation of cell types or cell structures.
- The most important of these is the Gram stain. Other differential staining methods include the endospore stain (to identify endospore-forming bacteria) and the acid-fast stain

Gram staining procedure

Fixation

- Make a thin film of the material on a clean glass slide
Air dry, fix the slide by passing it several times through a flame (the slide should not become too hot to touch)



Spread culture in thin film over slide



Dry in air



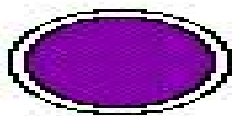
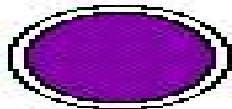
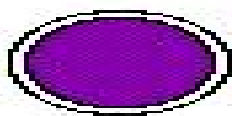
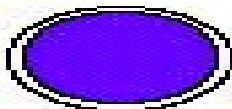
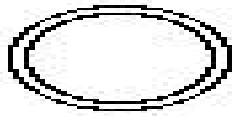
Pass slide through flame to fix

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- Flood the fixed smear with crystal violet solution and allow to remain for 1 minute
- Wash off the crystal violet with tap water.
- Flood the slide with iodine solution for one minute.
- Wash off the iodine solution with tap water.
- Flood the slide with decolorizer (alcohol) for one to 5
- Wash off the decolorizer with tap water
- Flood the slide with safranin for 30 seconds.
- Rinse off the safranin with tap water.
- Dry the slide on bibulous paper and place in an upright position
- Examine the slide for bacterial organisms under a 100X objective microscope. G+= violet / blue, G- = pink /red

Cond'

GRAM +



Fixation



Crystal
Violet



Iodine
treatment

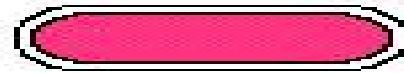
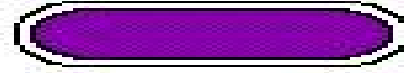
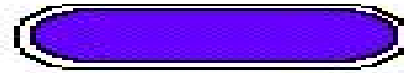


Decolorization



Counter stain
(safranin)

GRAM -



- **Acid-Fast stain:**(also called the Ziehl-Neelsen stain)
it is used to stain , those in the genus mycobacterium do not bind simple stains readily and must be stained by a harsher treatment. This is due to the high lipid content of acid-fast cell walls; in particular high content of mycolic acid

Staining specific structures

Negative staining: this technique reveals the presence of the diffused capsules surrounding many bacteria.

Spore staining

By Schaeffer Fulton procedure

- ✓ Endospores are first stained by heating bacteria with malachite green
- ✓ After malachite green treatment, the rest of the cell is washed free of dye with water
- ✓ Then counter stained with safranin.
- ✓ Result: will be a green endospore resting in a pink to red cell.

THANK YOU!!!