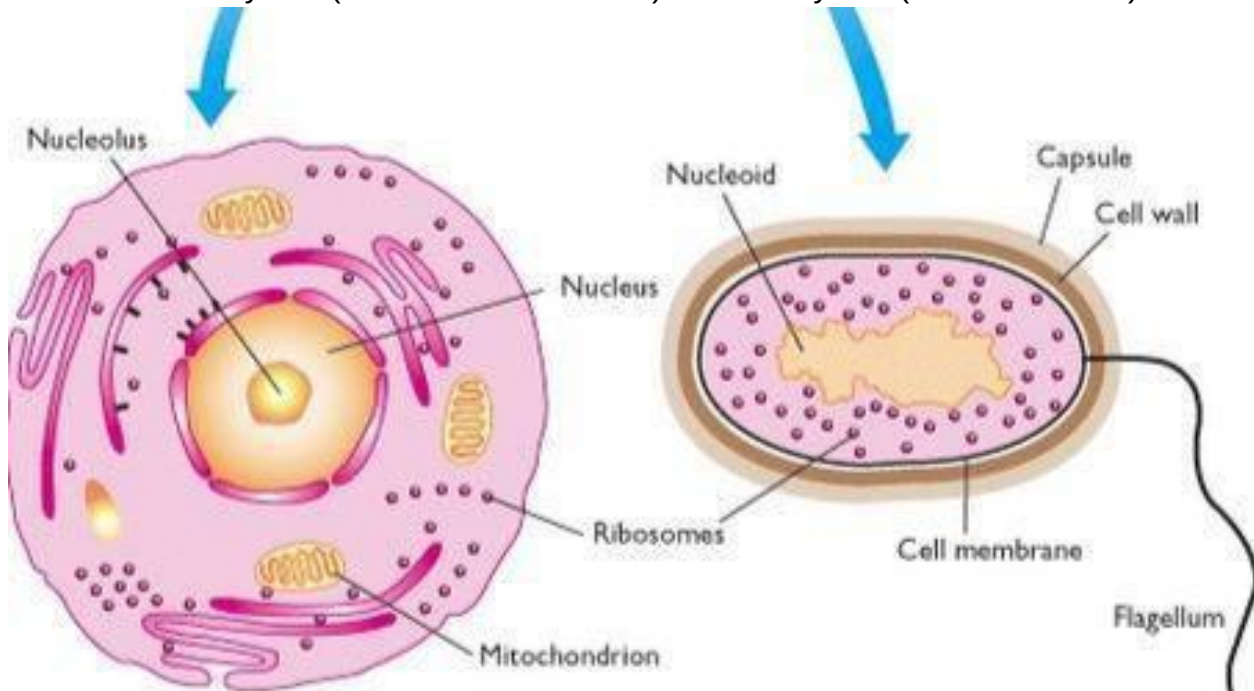


Topic-001 Microbes and Microbiology

What are Microbes?

- Also called germs or microorganisms
- Too small to be seen with the unaided eye
- A microscope is needed to see them
- Studying microbes is microbiology
- Microbes are simple.
- Diverse
- Classification has been a challenge.
- Prokaryotes (no nuclear membrane) and eukaryotes (distinct nucleus)



Topic-002 Why do we Classify Organisms?

- Studying them becomes convenient
- Easy Identification
- Easy to establish relationship between organisms
- Evolutionary relationships can be established

Bases of Classification

Similarities among them

- Morphology used to be the main feature prior to the advent of molecular biology
- Biochemical similarities
- Genetic similarities
- Evolutionary relationships

Five Kingdom Classification of Organisms

- Monera: Bacteria and Archea
- Protista
- Fungi
- Plantae
- Animalia
- Microbes placed in the first 3 kingdoms

Reasons for Changes in the Classification

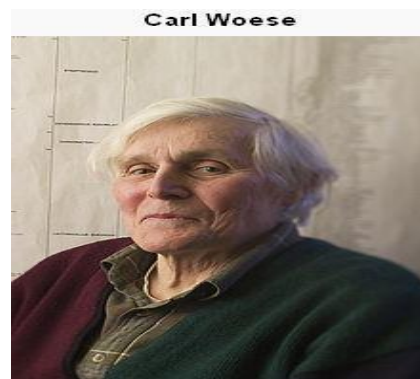
- Electron microscope
- Biochemical and physiological characteristics
- Nucleic acid and protein sequencing
 - Ribosomal RNA (rRNA)
- Bacteria and Archea two different groups



Carl Linnaeus, 1735

Three Domain Classification (1977)

- =
- Bacteria: True or eubacteria
- Archea:
- Eucarya
 - Protists (slime molds, protozoa, algae)
 - Fungi
 - Plants
 - Animals

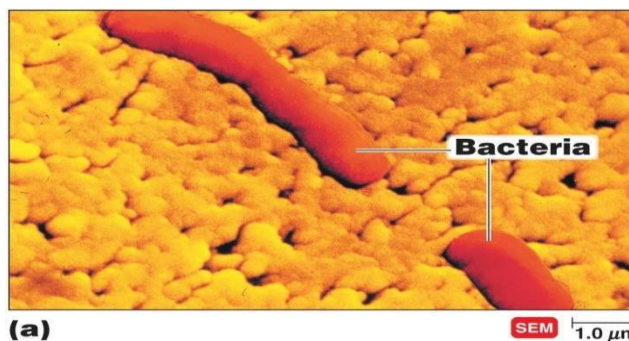


Domains & Kingdoms

- Domain: Bacteria, Kingdom Bacteria
- Domain: Archea, Kingdom Archea
- Domain: Eucarya Kingdom Protista Kingdom Fungi Kingdom Plantae Kingdom Animalia

Bacteria

- Procaryotes
- Usually single-celled organisms
- Cell wall made up of peptidoglycan



Archea

- Procaryotes
- Cell wall has no peptidoglycan, but has special lipids.
- Vary in rRNA sequencing
- Live in extreme conditions – Thermophiles
 - Halophiles

Eucarya

1. Protists
2. Fungi
3. Animals
4. plants

Protists

- **Algae:** Cellulose cell wall, Photosynthetic Produce (O₂)
- **Protozoa:** motile, unicellular organisms
- **Slime mold:** Protozoa like at one stage and fungus like in another

Fungi

- Unicellular form is called yeast.
- Multicellular is known as mold or mushroom

Other Microbes

- Viruses: Acellular organisms
 - Proteins and nucleic acids
- **Viroids:** composed only of circular ssRNA (potato spindle tuber viroid)
- **Virusoids:** Contain circular ssRNA, need helper viruses for replication and encapsidation; also called satellite viruses
- **Prions:** Infectious proteins

Naming Organisms: Binomial Nomenclature

- **Linnaeus** (in 1735) established the system of scientific nomenclature
- Each organism has two names: the **genus** and **specific epithet** (descriptive word)
 - *Escherichia coli*

Scientific Names

- Are italicized or underlined. The genus is capitalized, and the specific epithet is lowercase.
- Are —Latinized and used worldwide.
- May be descriptive or honor a scientist.

Escherichia coli

- Honors the discoverer, Theodor Escherich
- Describes the bacterium's habitat—the large intestine, or colon

Staphylococcus aureus

- Describes the clustered (*staphylo-*) spherical (*cocci*) cells
- Describes the gold-colored (*aureus*) colonies

Scientific Names

- After the first use, scientific names may be abbreviated with the first letter of the genus and the specific epithet:
 - *Escherichia coli* and *Staphylococcus aureus* are found in the human body. *E. coli* is found in the large intestine, and *S. aureus* is on skin.

Bacterium Vs Bacteria

- E.coli as a species is a bacterium.
- One E.coli cell is a bacterium.
- Many E.coli cells are bacteria.
- **Medium Vs Media**

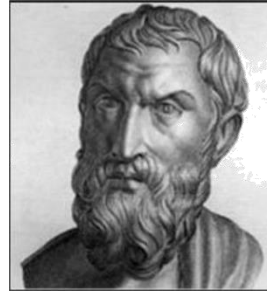
Topic-003 History of Microbiology

Learning Objectives

- First microscopic observation of microorganism

Discovery of Microbes

- Investigators suspected existence of microbes.



Lucretius



Fracastoro

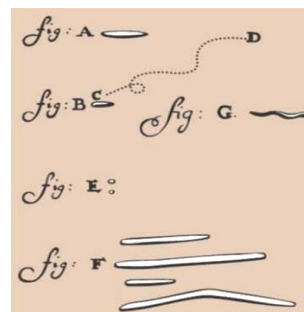
Robert Hooke

- First published drawing of microbes in 1665.
- Man behind the cell theory.



Antony van Leeuwenhoek

- First to publish extensive and accurate observations of microbes
- He called them —animalculesll.



Topic 004 History of Microbiology

Learning Objectives

- What was spontaneous generation?
- How was spontaneous generation issue solved?

Spontaneous Generation

- Aristotle: Simpler invertebrate could arise by spontaneous generation

Francesco Redi challenged



Leeuwenhoek

- His observations of microbes renewed the controversy.

John Needham (1748)

- Boiled extracts of hay or meat can give rise to microorganisms
- He boiled mutton broth and then tightly stoppered the bottles.
- Conclusion: organic matter has vital force.

Spallanzani

- Sealed bottles before heating
- No growth was seen
- Proposed that air carries the germs
- Also commented that air was necessary for vital force.



Louis Pasteur (1861)

- Microbes are present in the air.
- They can contaminate sterile solutions
- Pasteur's work ended the spontaneous generation controversy.



Topic-005 The Golden Age of Microbiology

Learning Objectives

- What discoveries were made in the golden age of microbiology?

1857-1914

- A lots of discoveries in microbiology with Pasteur's work
- In about 60 years, Microbiology became a real science.
- Spearheaded by Pasteur and Koch
- Disease agents identified
- Role of Immunity established
- Chemical activities of microbes studied
- Improved culturing techniques introduced
- Vaccines
- Surgical techniques developed

Fermentation and Pasteurization

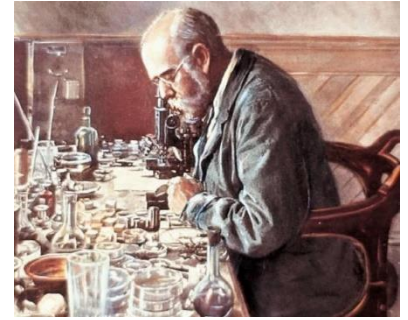
- Souring of beer and wine
- Pasteur found the yeast that fermented sugar to alcohol
- In the presence of O₂, bacteria grow and change alcohol to acetic acid

Germ Theory of Disease

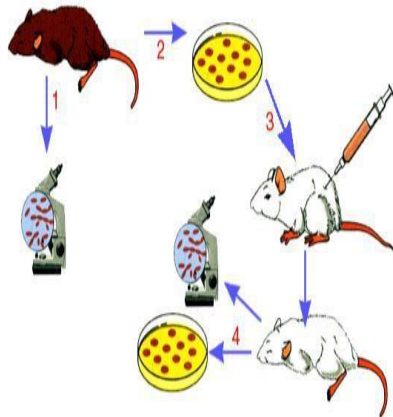
- Treatments for many diseases were discovered before Pasteur.
- Causes of the disease were unknown.
- Yeast fermentation by Pasteur was a clue
- 1860, Joseph Lister, applied phenol as disinfectants in treating surgical wounds and hand sanitizing.
- In 1865, Pasteur found that silkworm disease caused by a protozoan.

Robert Koch

- 1876: *Bacillus anthracis* established as a cause of anthrax, first proof
- Isolation of bacteria made possible by agar and Petri dish



Koch's Postulates



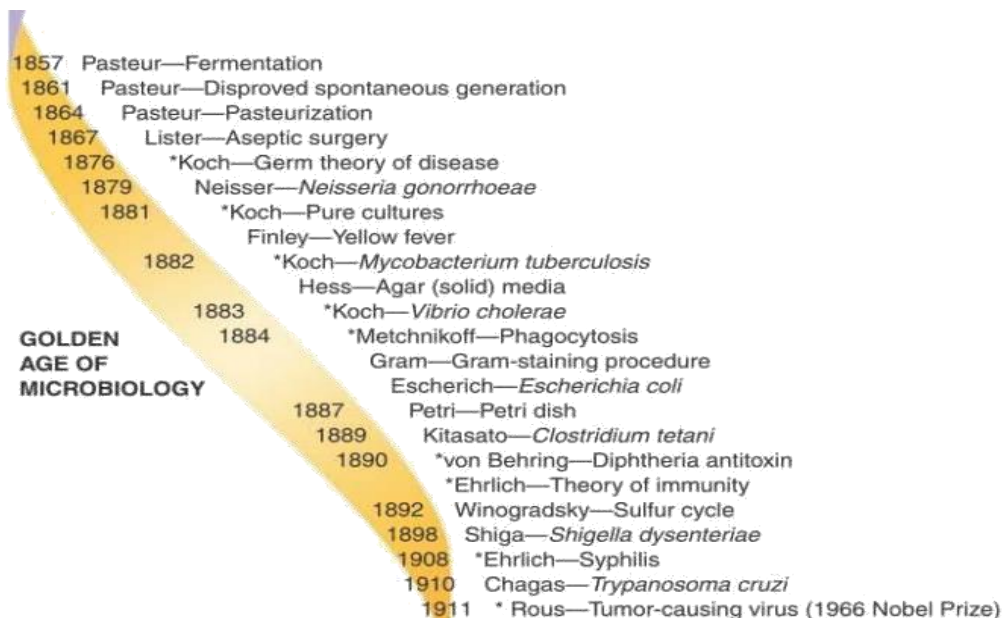
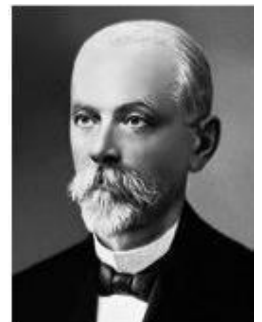
Vaccines

- Edward Jenner in 1796 and small pox
- Pasteur in 1888, discovered why vaccination works
- Avirulent microbes as vaccine
- The word —vaccinell

Ivanowsky

Viral pathogens

- Tobacco mosaic virus



Topic 006 Branches of Microbiology

Learning Objectives

- What are various branches of Microbiology?
- We live in the age of bacteria.
- First living organisms on our planet.
- They created the atmosphere that we live in today.
- Biosphere depends upon their activities.

Bacteriology

- Study of bacteria

- Deals with:
 - Isolation
 - Identification
 - Characterization
 - Classification

Mycology

- Study of fungi
- Medical mycology
- Agricultural mycology
- Ecological mycology
 - Decomposers of woods, leaves, feces and other organic matters

Basic & Applied Microbiology

- Basic: Biology of microbes
- Applied: Practical problems (diseases, wastewater Rx, food spoilage etc)

Medical Microbiology

- Deals with diseases of humans and animals
- Identifications of disease causing agents
- Control and elimination of microbes
- Tracking down new pathogens

Epidemiology

- Monitoring diseases in a population
- Detection of outbreaks and developing epidemics
- Implementation of appropriate control measures
- Surveillance studies

Immunology

- Study of the immune system
- How it works
- Allergies
- Vaccines
- Antibodies for the treatment of diseases and cancers

Food Microbiology

- Microorganisms that either create or contaminate foods
- Cheese
- Bread
- Yogurt
- Wine

Industrial Microbiology

- Use of microbes or their enzymes for large scale production of biomolecules

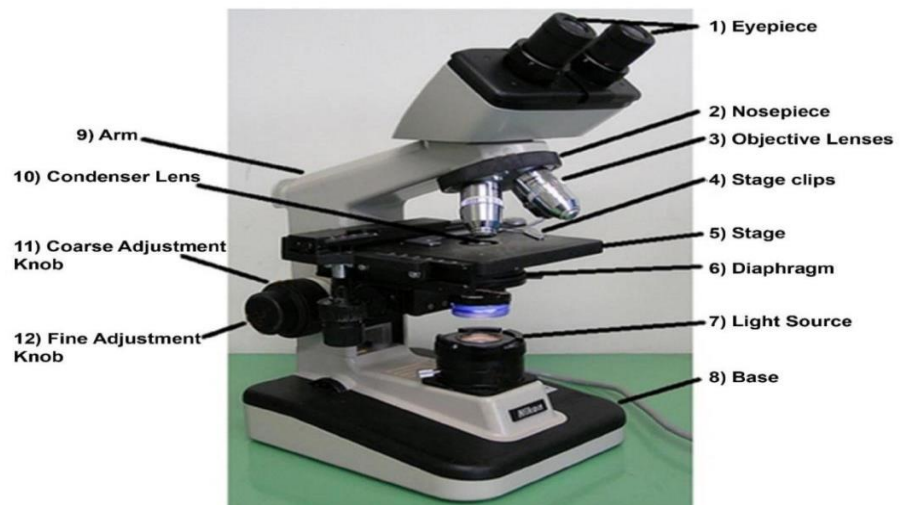
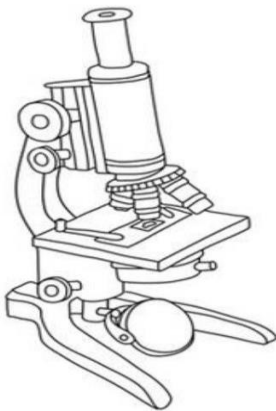
Microbial Genetics & Mol. Biology

- How genes regulates the development and function of cells and organisms.
- *E. coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, T4 and lambda phages as study models

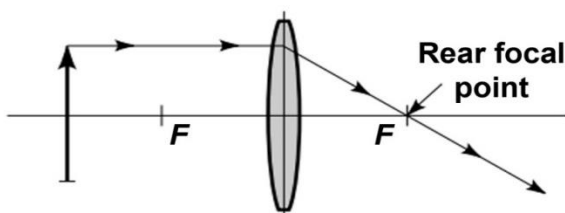
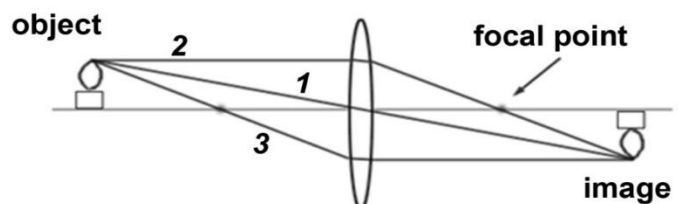
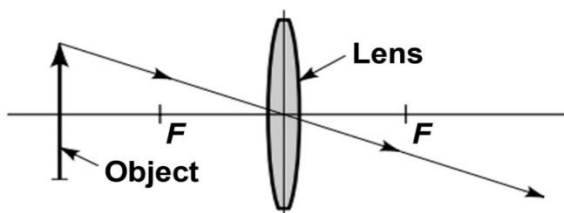
Topic-007 Microscopy

- What is light microscopy.
- How a light microscope works.
- What are various parts of light microscope.
- What is total magnification.
- What is resolution and refractive index .

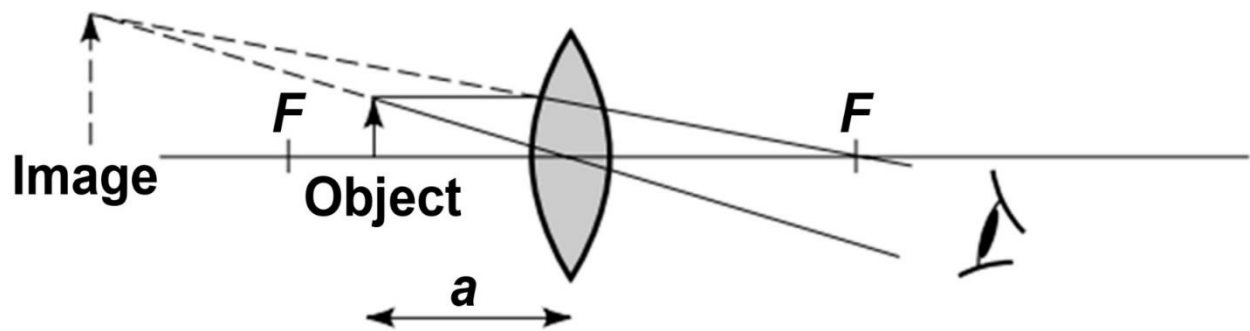
Uses visible light to observe the specimen



How a microscope works (Real Image)



How a microscope works (Virtual Image)



How a microscope works

Parts of Compound Light Microscope

- **Illuminator:** a light source
- **Condenser:** Directs the light through the specimen
- **Objective Lenses:** Close to the specimen
- **Ocular Lens (Eyepiece):** Close to the eye

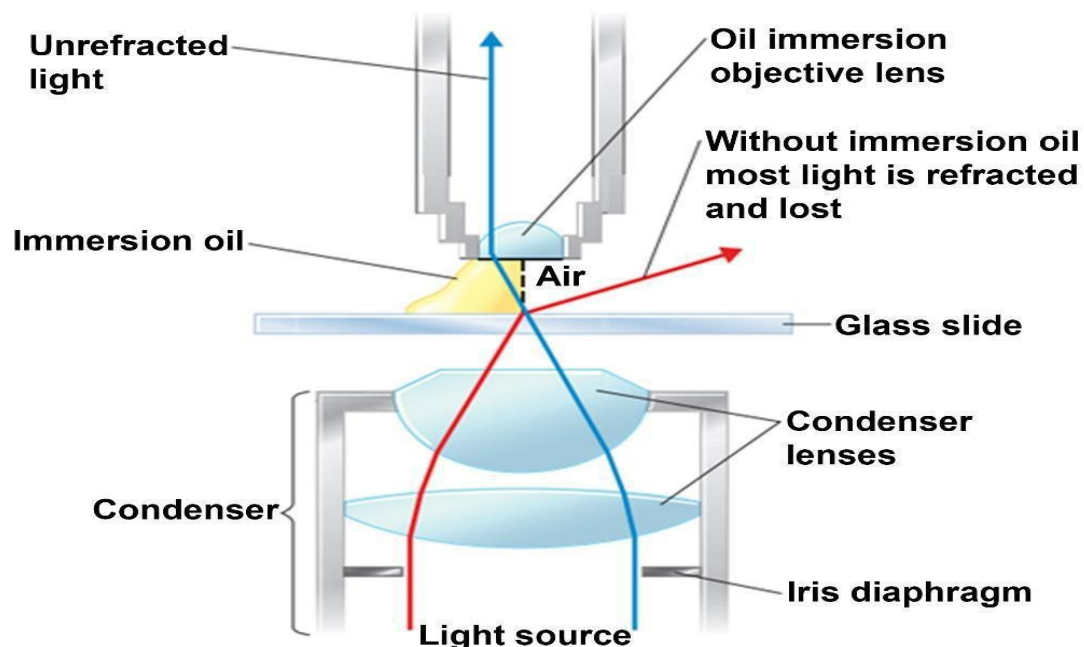


What is total magnification?

- Magnification of objective lens x magnification of ocular lens
- Objective lenses:
 - Low: 10 X
 - High: 40 X
 - Oil immersion: 100 X
- Ocular lens: 10 X
- Low magnification power: $10 \times 10 = 100$
- Oil immersion power: $100 \times 10 = 1000$

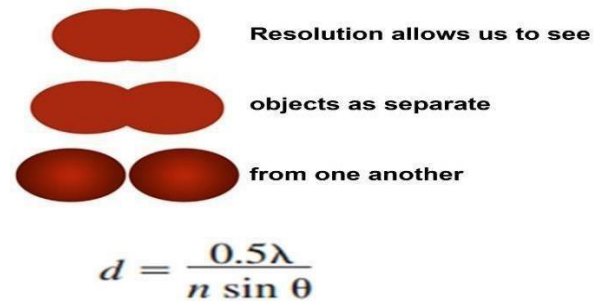
Refractive Index and Image

- **Refractive Index:** Ability of a medium to bend the light
- Light rays move in a straight line through a single medium
- To increase contrast, we stain the specimen



What is resolution?

- Ability of the lenses to distinguish between two closely lying objects as separate
- Light microscope resolving power is 0.2 μm .
- n = refractive index, 1.5 for immersion oil



Summary

- Ordinary light microscope
- Stained images are used
- Can resolve 200 nm objects

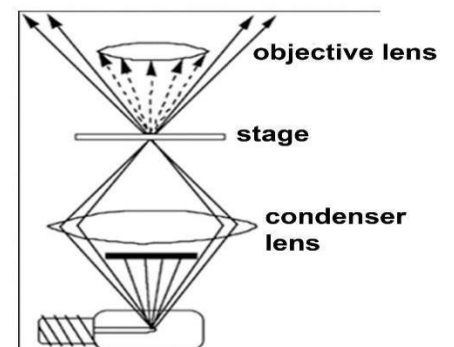
Topic-008 Types of Microscopy

Learning Objectives

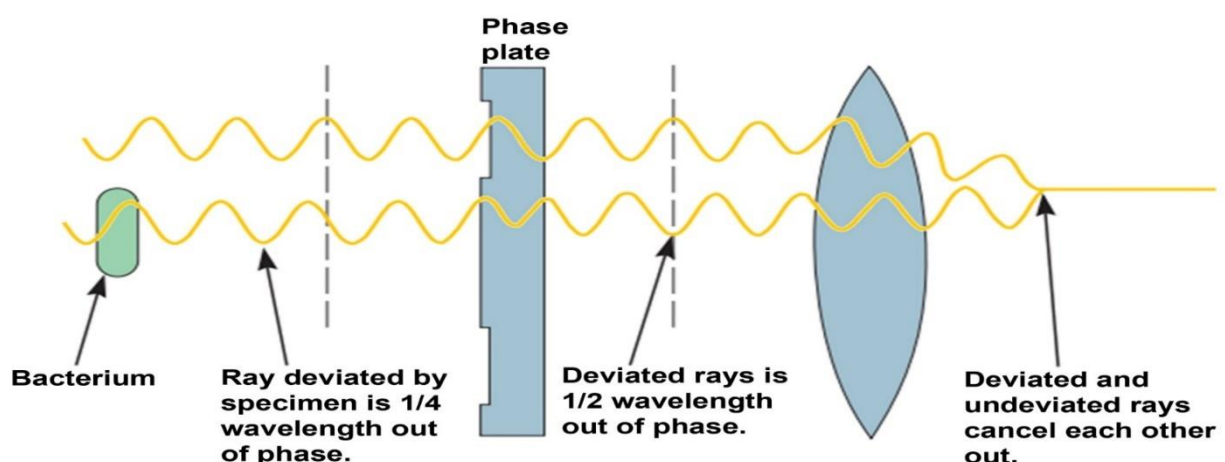
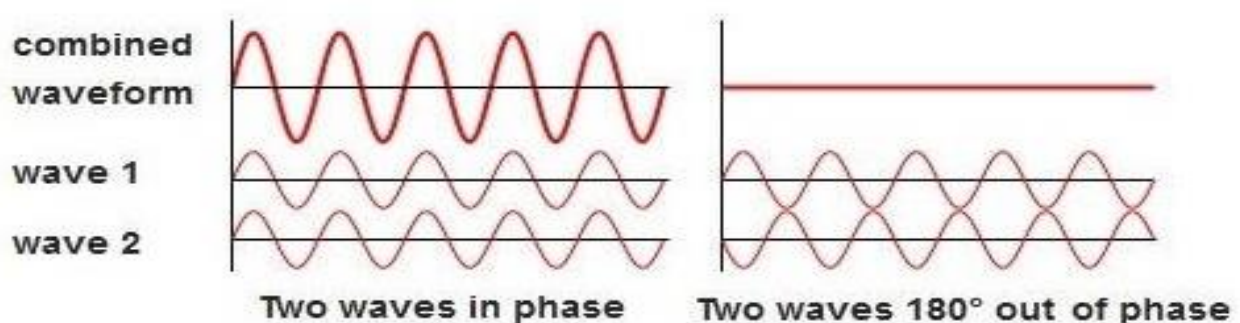
- Darkfield microscopy
- Phase contrast microscopy
- Fluorescent microscopy
- Transmission electron microscopy
- Scanning electron microscopy

Darkfield Microscopy

- Uses a darkfield condenser having an opaque disk
- Used for unstained specimens suspended in liquids
- Light rays can be in phase or
 - out of phase



Phase Contrast Microscopy



Fluorescent Microscopy

- Uses fluorochromes and UV light as illumination source
- Fluorochromes: substances that absorb short wavelength of light and emit longer wavelength (visible)

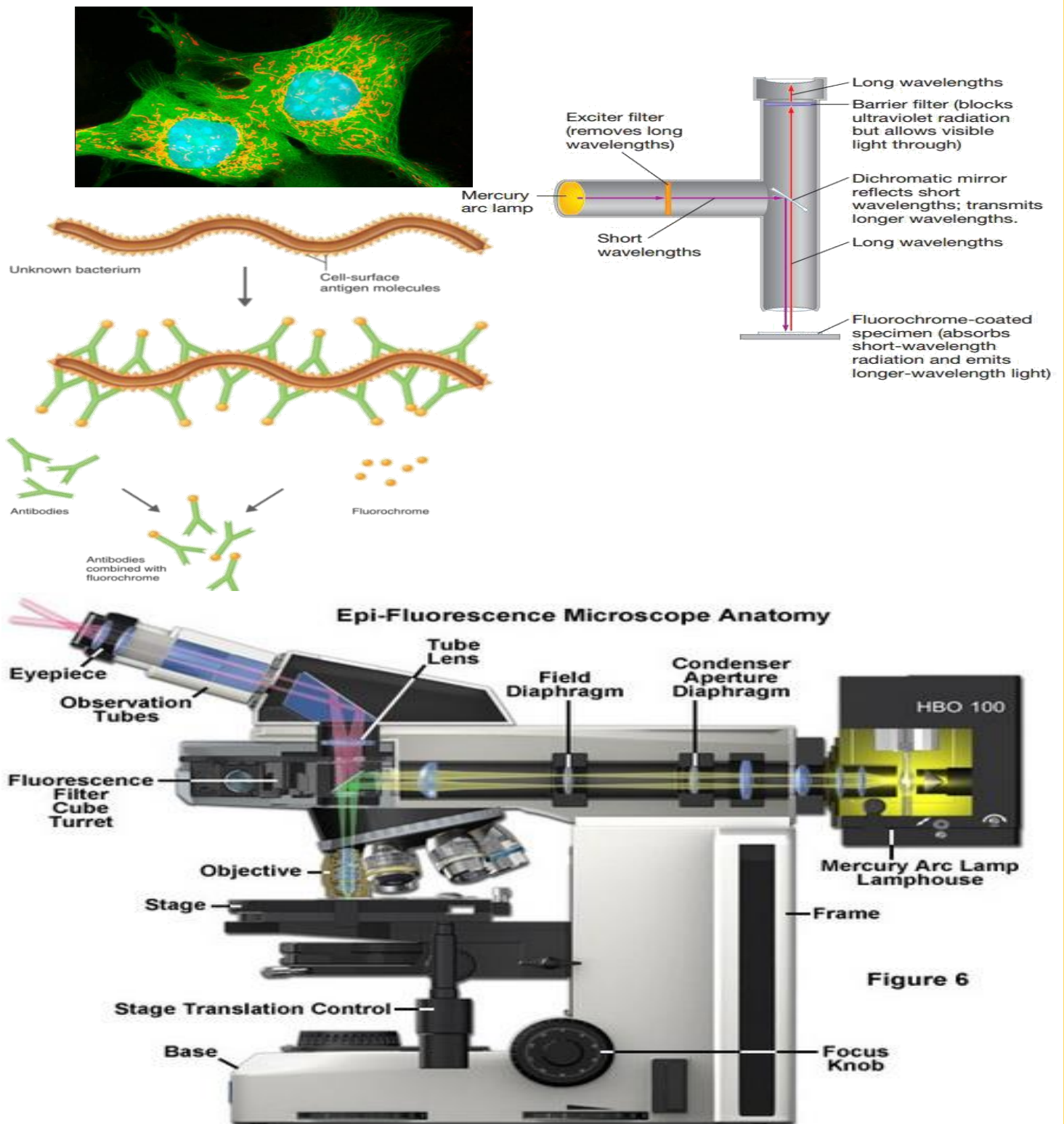
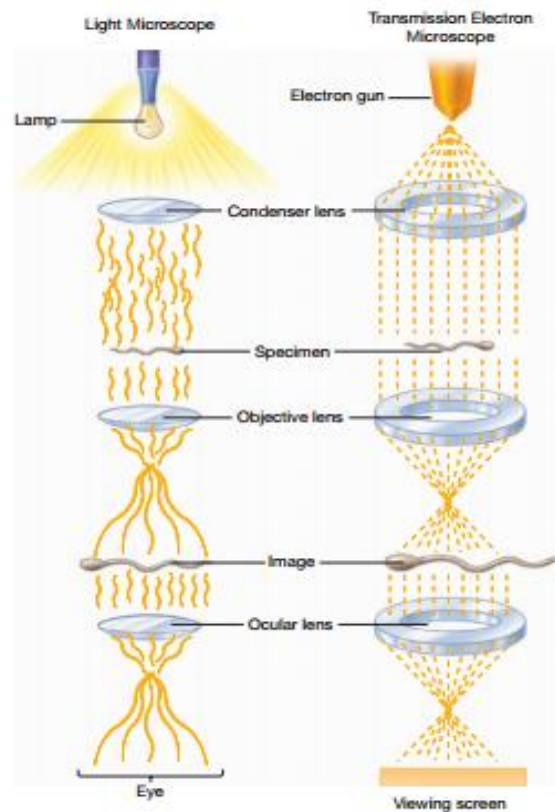
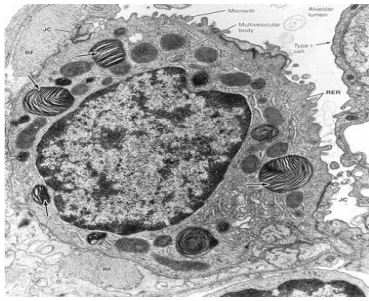


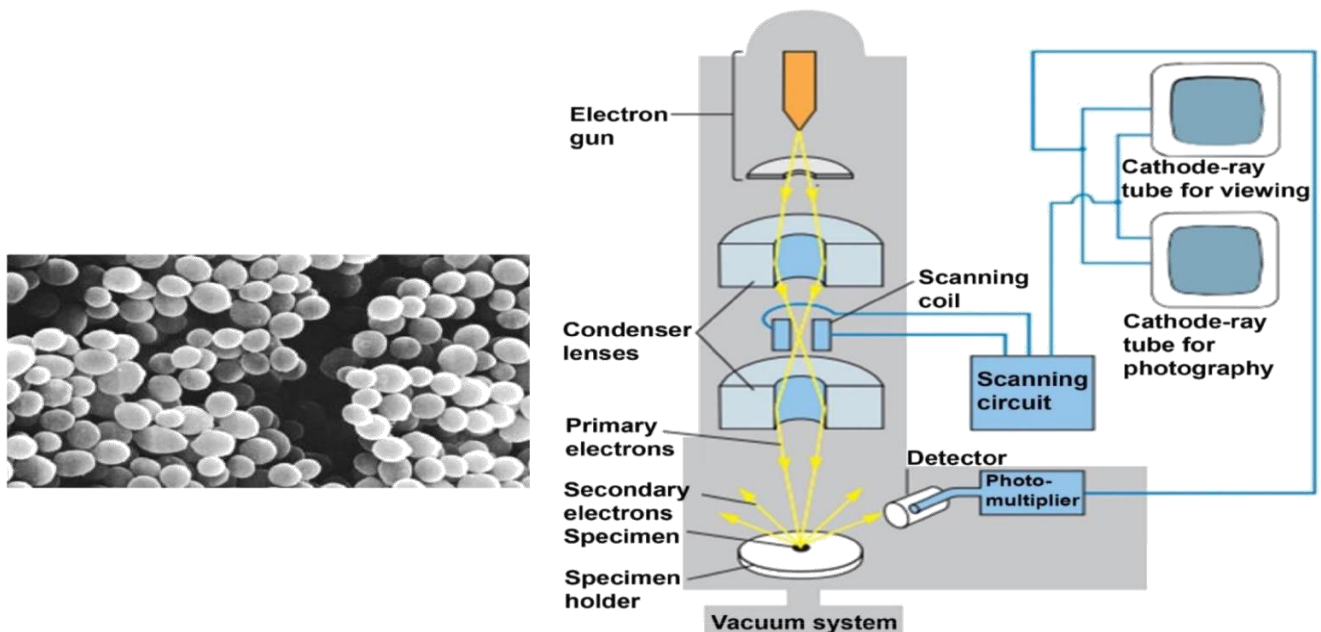
Figure 6

Transmission Electron Microscopy

$$d = \frac{0.5\lambda}{n \sin \theta}$$



Scanning Electron Microscopy



Summary

- A host of microscopes can be used to observe the microbes for their morphology with or without staining.

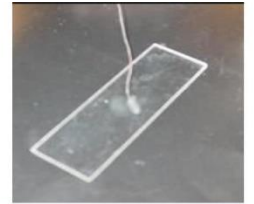
Topic009 Staining Microbes

Learning Objectives

- Why we stain microbes.
- What is a basic or an acidic stain?
- What is a simple stain?
- What is a negative or a positive staining?

Why we stain microbe

- Most organisms appear colorless when seen under a microscope.
- Staining emphasizes certain structures of the organisms.
- Staining is just coloring with a dye.



Advantages of Staining

- Increases visibility of microbes
- Shape, size and arrangements
- Purity or contamination of a culture
- Differentiation and classification of microbes
- Various parts of bacteria can be detected with staining

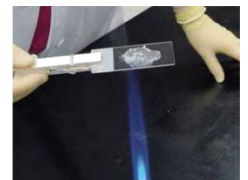
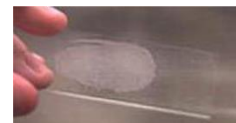
How to make a smear?

- Using a platinum loop, a colony or a drop of broth culture can be smeared into a thin film on a glass slide for making a smear.



Fixing the smear to the slide

- The specimen is spread into a thin film (smear).
- Smear is air-dried.
- Smear is fixed (attached) to the slide before staining.
- Heating the slide is one way of fixing the smear.
- Methyl alcohol can be used.



Fixing a smear

- Physically attach the specimen onto the slide
- To preserve internal and external structures in place
- Physical method: Heat
- Chemical Methods: Ethanol or formaldehyde

Staining a smear

- Stain is applied and then washed off with water.
- The slide is air dried or blotted.

What is a stain?

- Stains are salts composed of a negative and a positive ion.
- Chromophore: A colored ion.
- Basic dye: The color is in the positive ion.

- Acidic dye: The color is in the negative ion.
- Bacteria are negatively charged at pH 7.
- Basic dyes are most commonly used for bacteria.

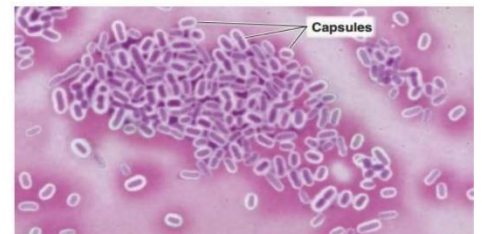
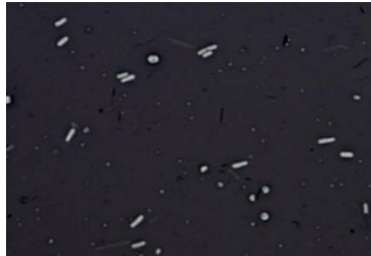
Basic Dyes: Positive Staining

- Crystal violet
- Methylene blue
- Malachite green
- Safranin



Acidic Dyes: Negative Staining

- Eosin
- Acid fuchsin



Topic 10 Types of Staining 1

Objectives

- What is simple and differential staining?
- What are advantages of differential staining?

Simple: only one stain is used.

Methylene Blue staining

Differential: A couple of stains used.

Gram Staining

Special:

Capsular staining

Endospore staining

Simple Staining

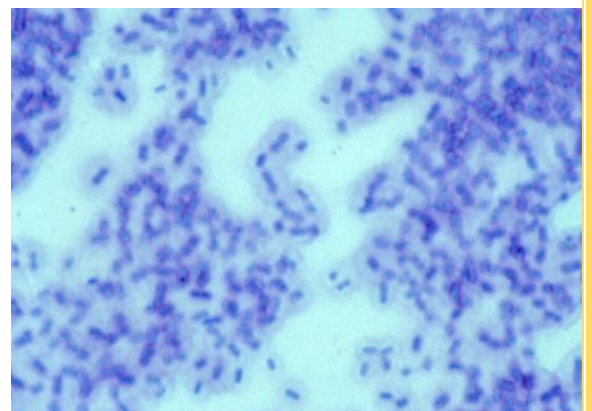
- A single stain is used.
- Stain is washed off.
- Microbes seen under the microscope.

Methylene blue

Carbolfuchsin Crystal

violet, and safranin

- A mordant is added, sometimes.

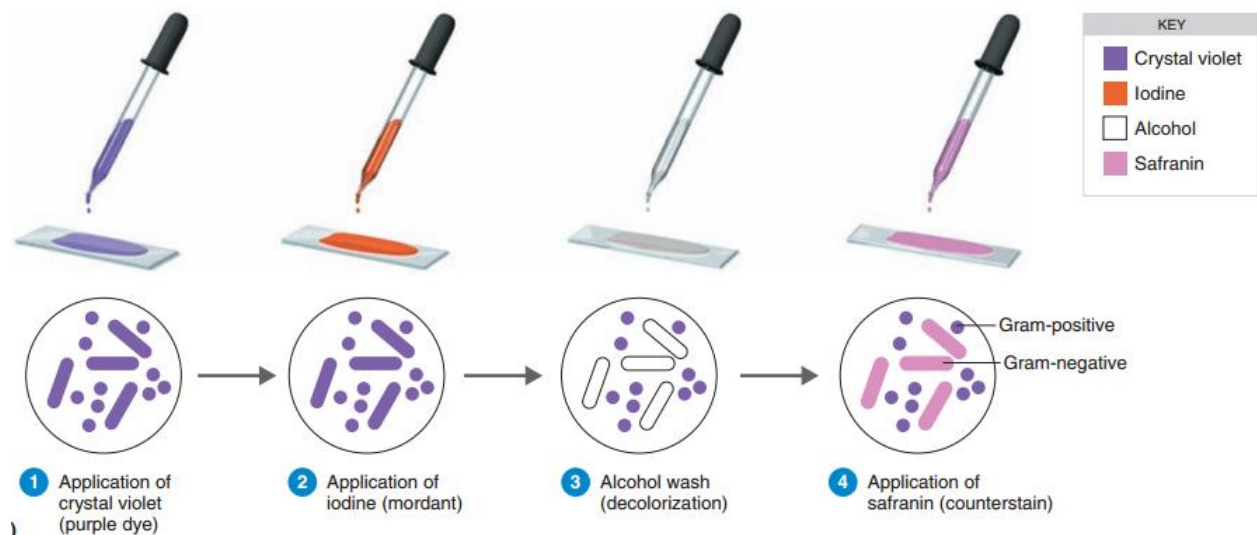


Differential Staining: Gram Staining

- Differential stains react differently with different kinds of bacteria.
- Most common is Gram staining.

– 1884, by Hans Christian Gram

Gram Staining

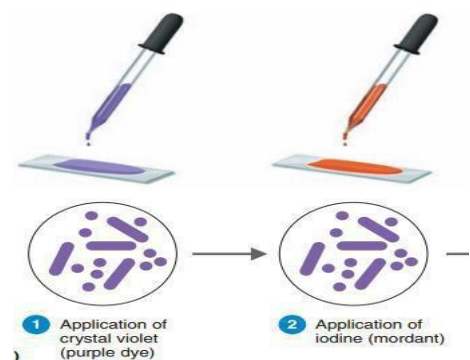


Gram Staining

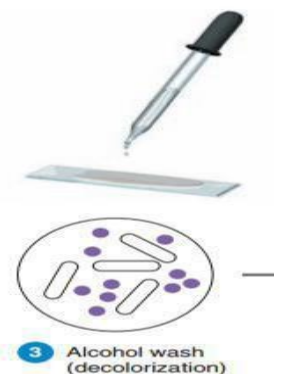
Crystal violet is added.

– Primary stain

- Crystal violet is washed off.
- Iodine is added to enhance binding.
 - Mordant

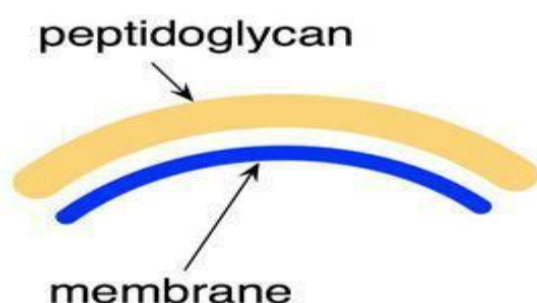


- The slide is washed off with alcohol.
 - Decolorizing agent
- Gram positive bacteria retain crystal violet
- Gram negative bacteria appear colorless.

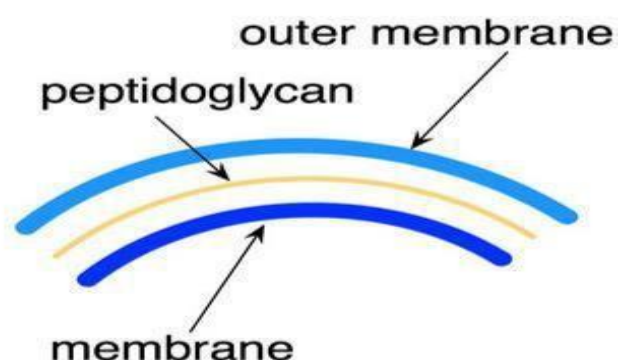


- Crystal violet and iodine make a complex in the cytoplasm
- Peptidoglycan layer is thicker in Gram positive bacteria and CV-I is retained.

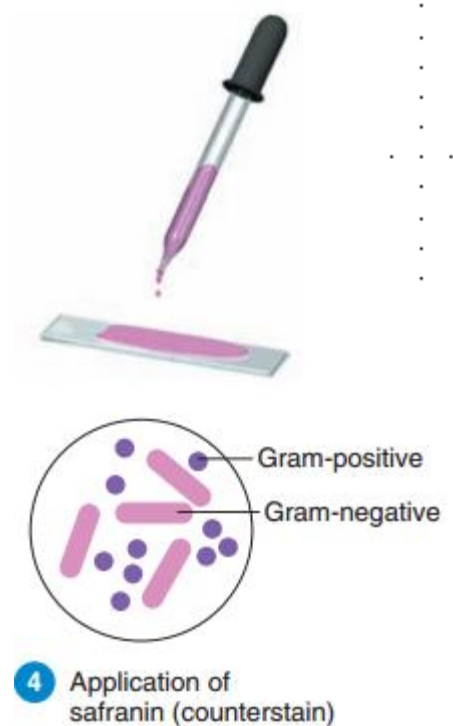
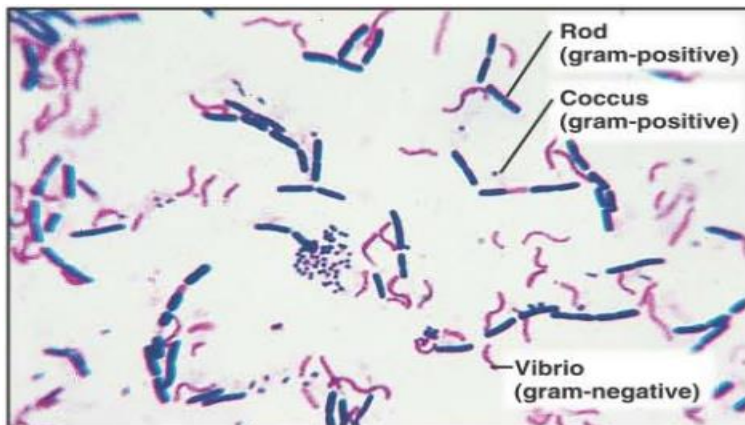
GRAM-POSITIVE



GRAM-NEGATIVE



- Alcohol is rinsed off and stained with safranin.
 - Counterstain
- The smear is washed again.
- Blotted dry and examined microscopically.



Topic 11 Types of Staining 2

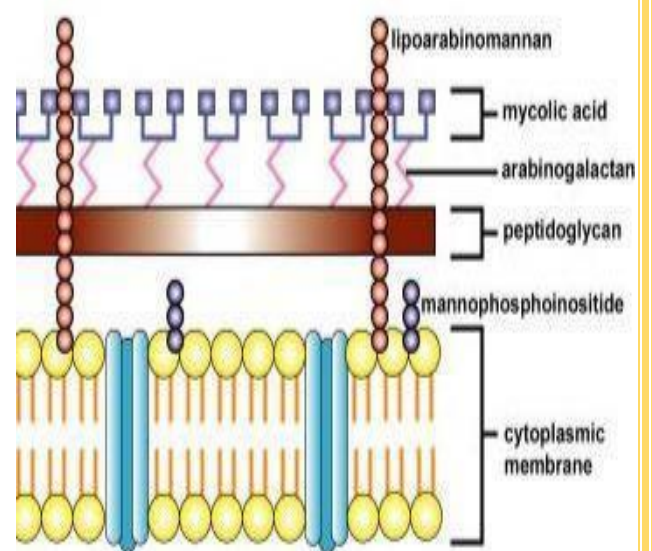
Objectives • Acid fast

staining

- Special staining:

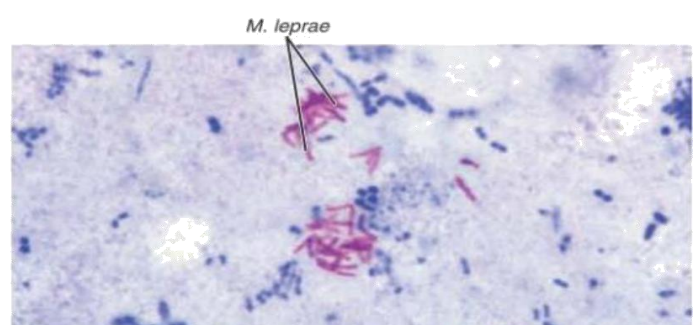
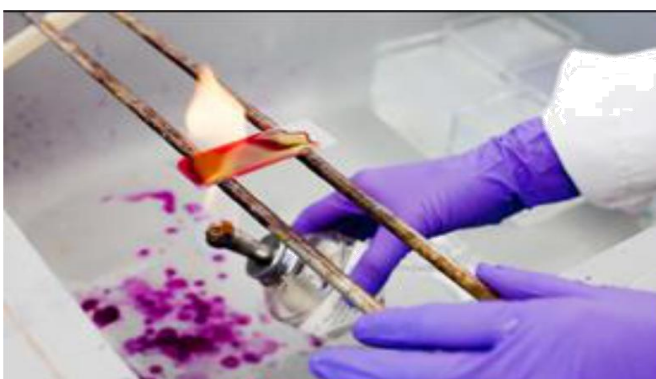
Acid-Fast Staining

- Some bacteria such as Mycobacterium has a waxy material in their cell wall.
 - Mycolic acid
- M. tuberculosis: tuberculosis
- M. leprae: leprosy



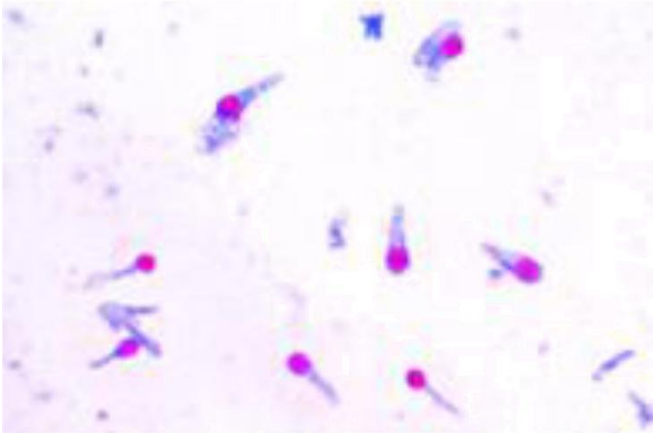
Acid-Fast Staining

- Mycobacteria are lipophilic, not easy to stain
- Once stained, are resistant to counterstain
- Carbol fuchsin: primary stain
- Heat is used to enhance penetration of the stain.
- Acid alcohol is decolorizer.
- Methylene blue is counterstain.



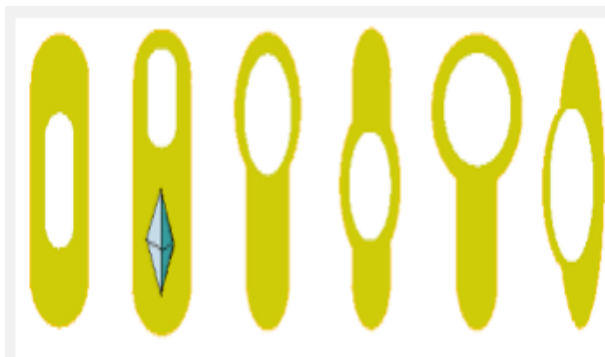
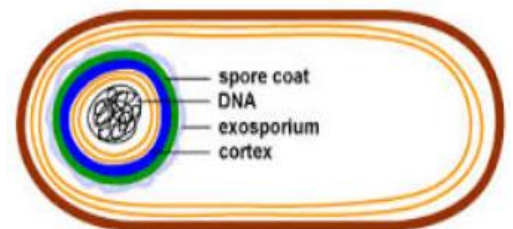
Special Staining

- Used for coloring specific parts of an organism.
 - Endospore
 - Flagella
 - Capsule

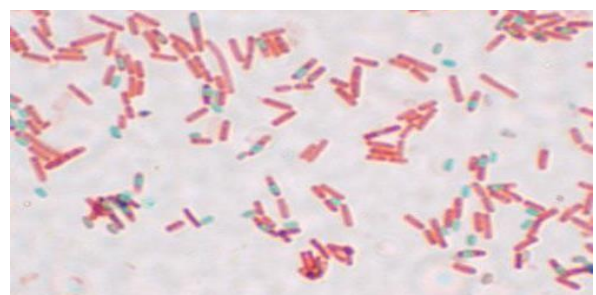


Endospore Staining

- Endospore: A resistant dormant structure within a cell
- Position of the endospore varies within the cell
- Appear as a clear halo in Gram stained smears



- Heated to steaming for 5 min.
- Washed with water.
- Counterstained with safranin.



Summary

- Various options for staining of bacteria are available.
- Positive/Negative staining
- Differential staining
- Special staining

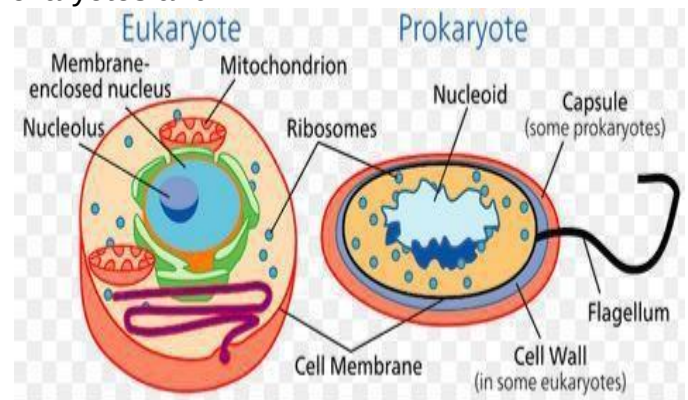
Topic-012 Anatomy of Prokaryotes I

Learning Objectives

- Overall differences in cell structures of prokaryotes and eukaryotes

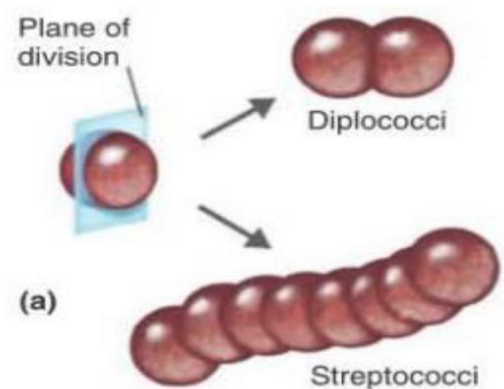
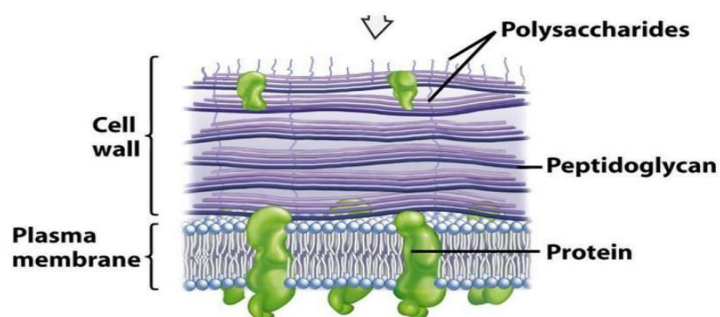
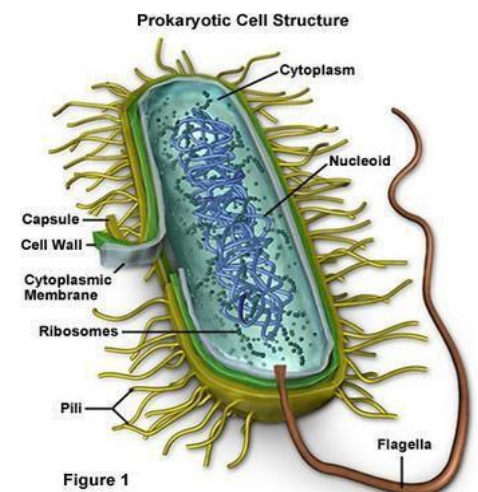
Prokaryotes and Eukaryotes

- Prokaryotes and eukaryotes are chemically similar.
 - All made up of nucleic acids, protein, lipids, and carbohydrates.
 - All metabolically similar.



Prokaryotes

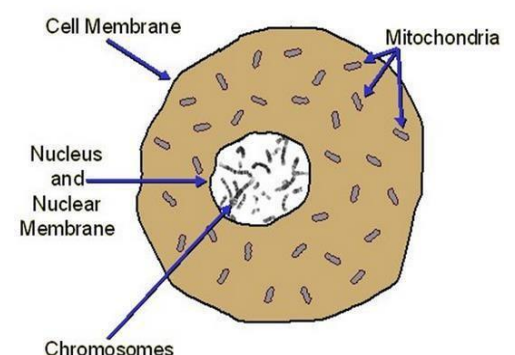
- DNA is not enclosed in a nuclear membrane.
- Chromosome: mostly Single, circular
 - DNA not associated with histones
- No membrane enclosed organelles
- Cell wall has peptidoglycan, a complex carbohydrate.
- Divide by binary fission.



Eukaryotes

- DNA is enclosed in a membrane-bound nucleus.
- DNA is found in multiple chromosomes.
- Chromosomes are linear, thread-like structures.

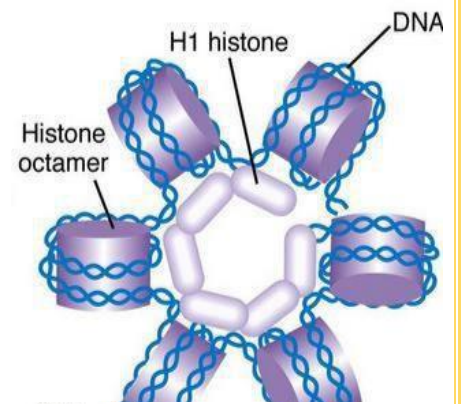
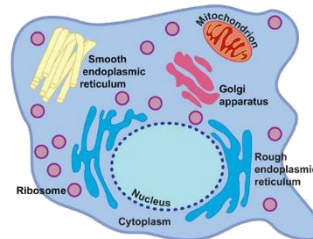
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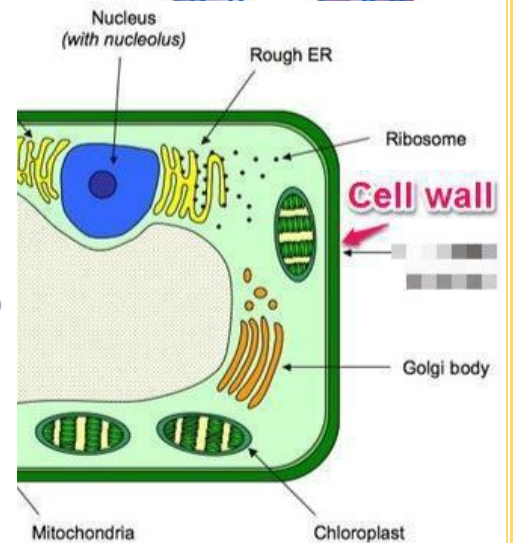
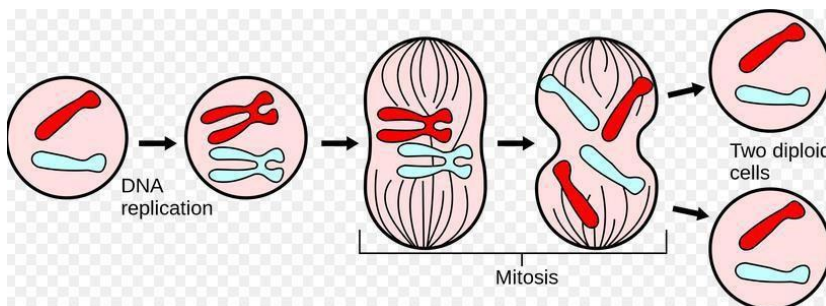
-
-
- **DNA is associated with histones.**

Cell wall, if present, is chemically simple.

- Cellulose
- Chitin
- **They have membrane enclosed organelles.**
 - Mitochondria or chloroplasts
 - Endoplasmic reticulum
 - Golgi apparatus
 - Lysosomes



- **Cell division involves mitosis.**



Summary

- Cell wall
- Nucleus
- Chromosomes
- Organelles
- Cell division

Topic-013 Anatomy of Prokaryotes II

Learning Objectives

- Shapes and arrangements of prokaryote

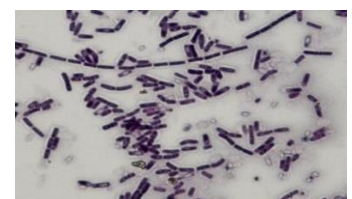
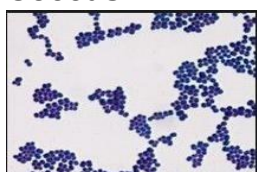
Prokaryotes

- Unicellular organisms – Bacteria
 - Archea
- Although, look similar, their chemical composition is different.
- Majority of prokaryotes are bacteria.

Bacillus

3 Basic Shapes of bacteria

Coccus



Shape of bacteria

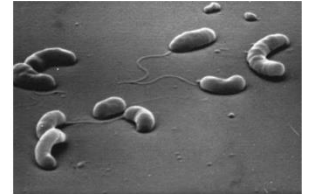
Spiral

- Vibrio: curved rods
- Spirillum: Helical but rigid
- Spirochete: Helical but flexible Move by axial filaments



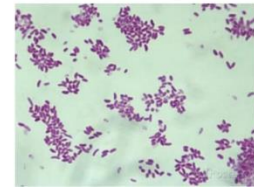
- **Spiral**

- Vibrio: curved rods



- **Spiral**

- Spirillum: Helical but rigid
- *Spirillum minus*: causes rat bite fever – 0.2 μm wide and 3 -5 μm long.



- **Spiral**

- Spirochete: Helical but flexible
- Move by axial filaments
- Size: 5 and 250 μm long
- 0.1–0.6 μm wide

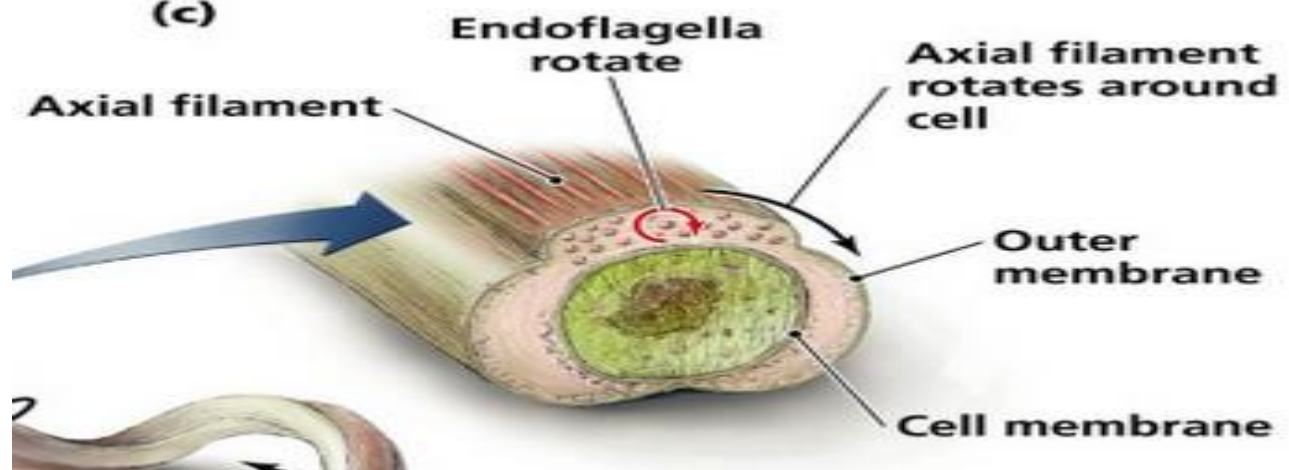
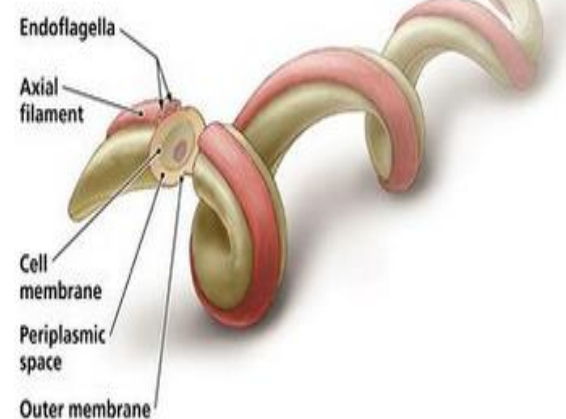


Spirochete

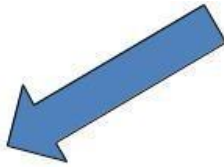
- Cause diseases



(c)



Diseases by Spirochetes



Leptospira spp: Leptospirosis

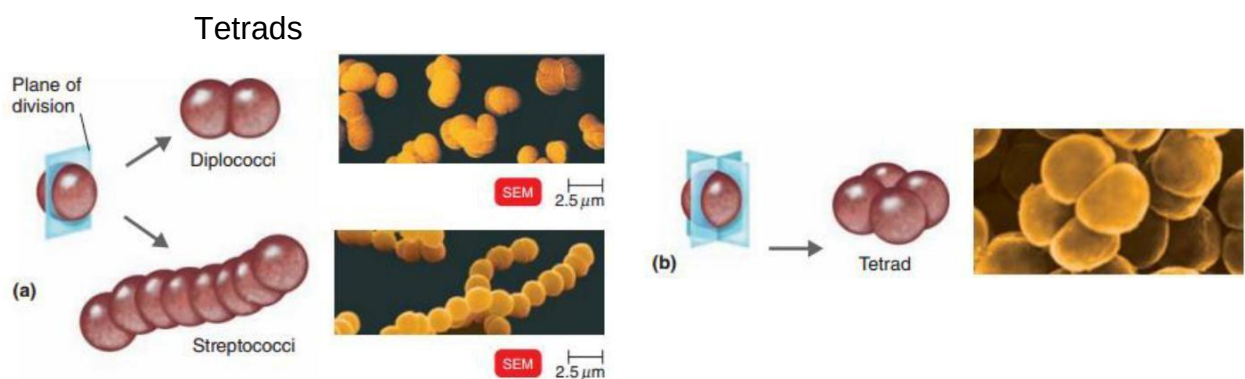
- *Borrelia burgdorferi*: Lyme disease
- *Treponema pallidum*: Syphilis

Cocci

Division in one plane

Diplococci and streptococci (chain)

- Division in two planes



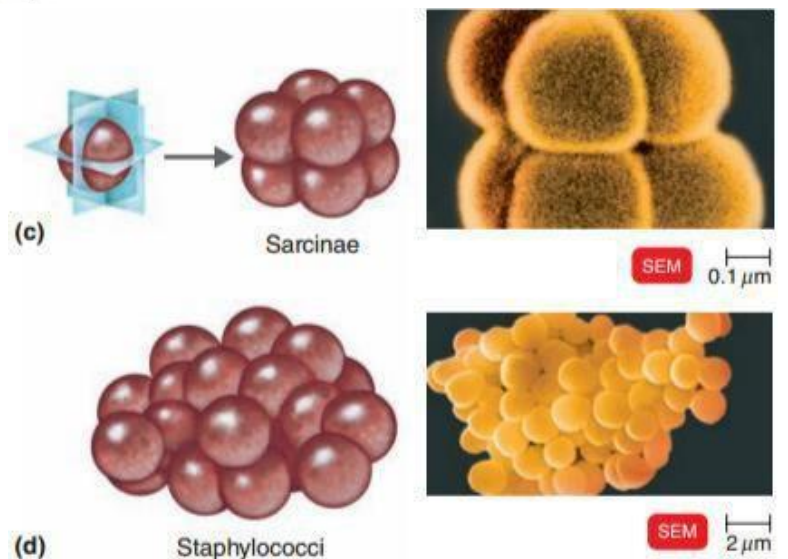
Arrangement of Cocci

- Division in 3 planes –

Sarcinae

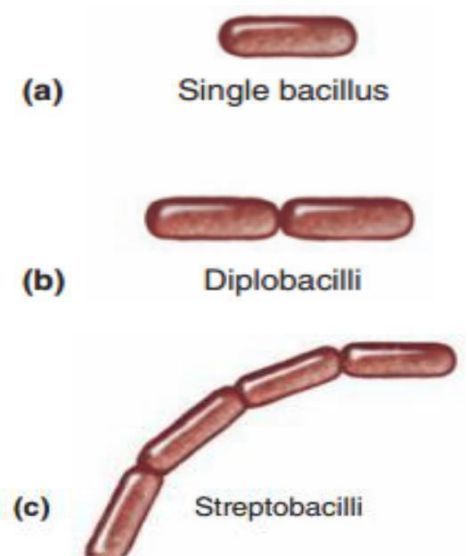
- Division in multiple planes

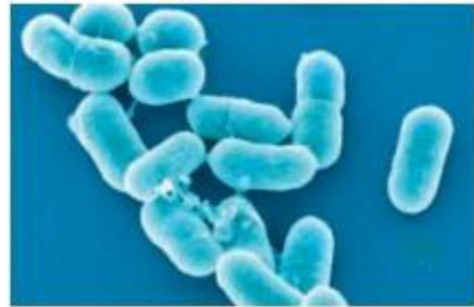
- Staphylococci



Arrangement of Bacilli

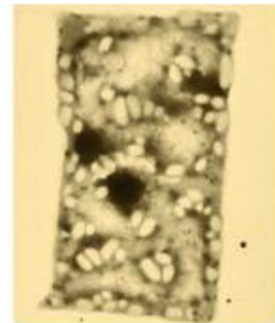
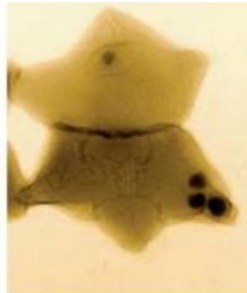
- Bacillus
- Diplobacilli
- Streptobacilli
- Coccobacillus
- Spiral: become single after division





Shapes of Archea

- Star shaped:
 - genus *Stella*
- Rectangular flat:
 - genus *Haloarcula*
- Triangular:



Variation in Shape

- Usu monomorphic (maintain a single shape)
- Could be pleomorphic (many shapes) – Naturally
 - Acquired (environment)

Summary

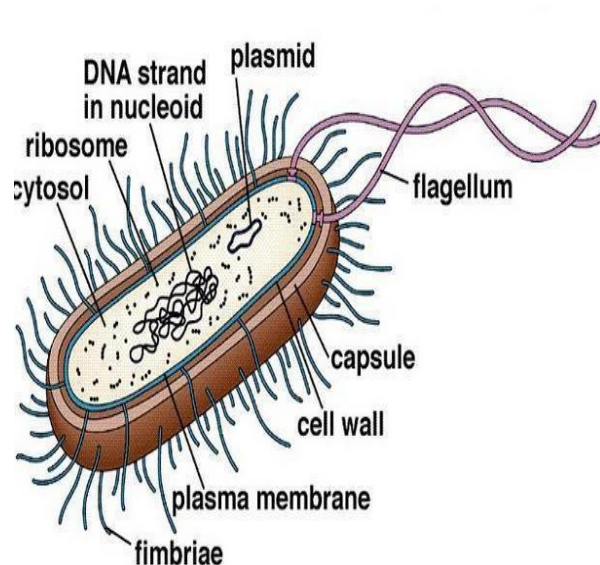
- 3 Basic shapes of bacteria
 - Cocci
 - Bacilli
 - Spirals
- 3 Shapes of Archea – Star-shaped
 - Falt rectangular
 - Triangular
- Arrangements
 - Single
 - Two: diplococci or diplobacilli
 - Chains: Streptococci or streptobacilli
 - Coccobacilli

Topic-014 Anatomy of Prokaryotes III

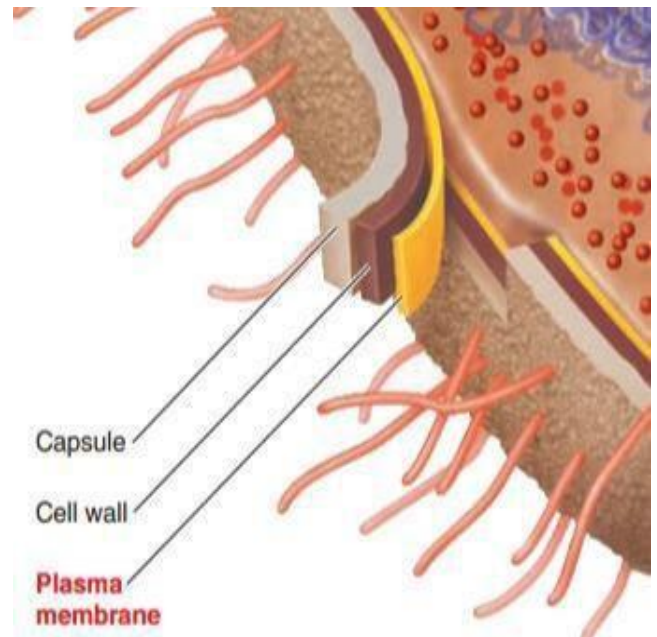
Learning Objectives

- Brief introduction to various structures of prokaryotes and their functions
- Structures external to the cell wall
- What is glycocalyx and its importance for bacteria

A Prokaryote



Cell Wall as a land mark for discussion

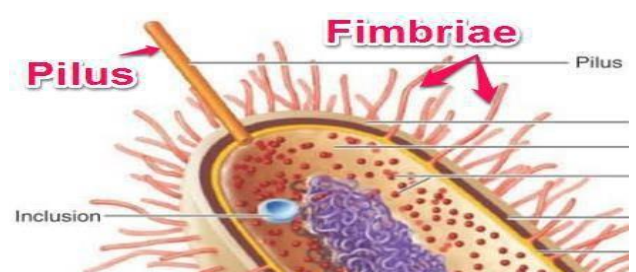


Structures and their functions

Plasma Membrane	Selective permeable barrier, nutrient transport, respiration, photosynthesis,
Gas vacuoles	Buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusion bodies	Storage of carbon, phosphate, and other substances
Nucleoid	DNA or genetic material location
Periplasmic space	Contains hydrolytic enzymes and binding proteins for nutrient processing and uptake
Cell wall	Shape and protection from osmotic stress
Capsule and slime	Resistance to phagocytosis, attachment to surfaces
Fimbriae and Pili	Attachment and bacterial mating
Flagella	Locomotion
Endospore	Survival under harsh environmental conditions

Structures external to the cell wall

- Glycocalyx
- Flagella
- Axial filaments
- Fimbriae
- Pili



Glycocalyx

- A viscous and gelatinous secretion that surrounds the cells
 - Polysaccharide and polypeptide or both
- Capsule: Organized and firmly attached
 - Virulence
 - Vaccine

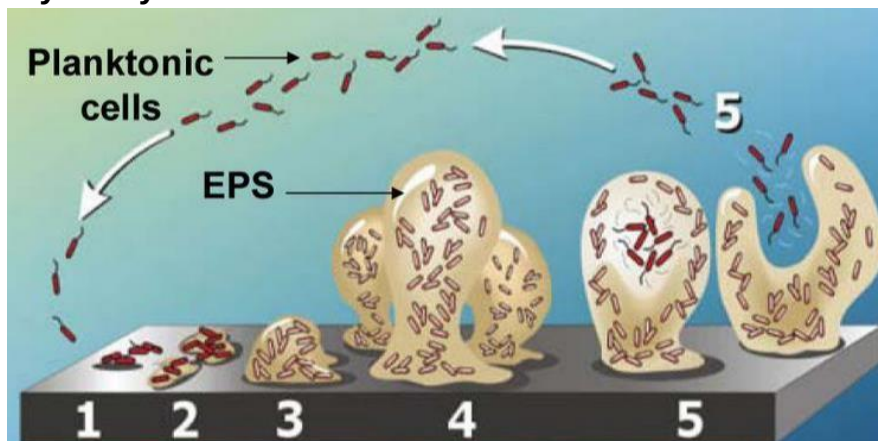
Capsule and Virulence

- Bacillus anthracis
- Streptococcus pneumoniae
- Klebsiella

Glycocalyx as biofilm

- Bacteria secrete extracellular polymeric substance (EPS)
- Protects cells within it.
- Facilitates communication amongst cells
- Enables the cells to attach to various surfaces for survival

Glycocalyx as biofilm

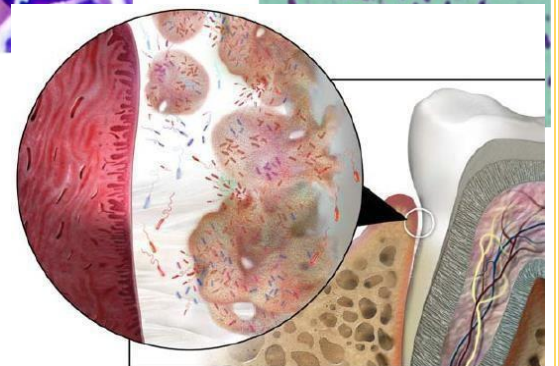
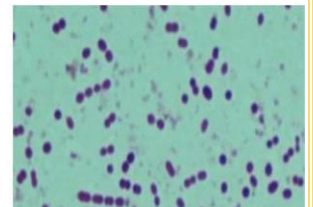
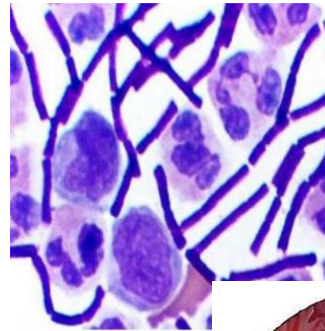
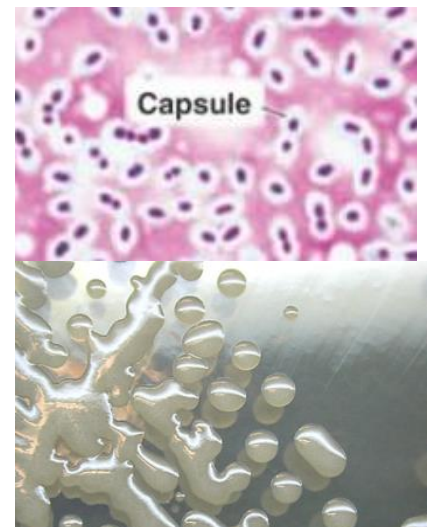
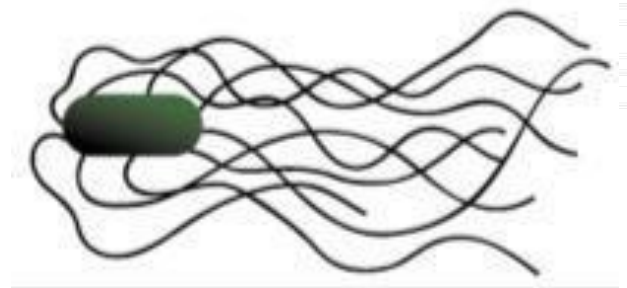


Flagella

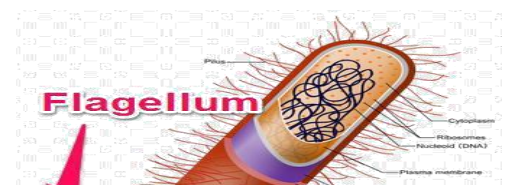
- Flagellum: Long filamentous structure that propels bacteria

Classification of bacteria based on flagella

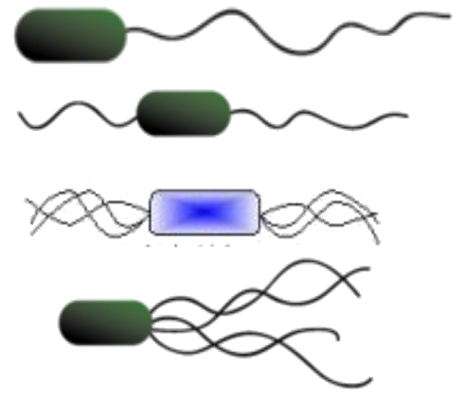
- Atrichous: No flagellum •
- Peritrichous: Distributed over the entire cell



Biofil o



- **Polar:**
 - Monotrichous
 - Amphitrichous
 - Lophotrichous



Summary

- Glycocalyx
- Flagella
- Axial Filaments
- Fimbriae
- Pili

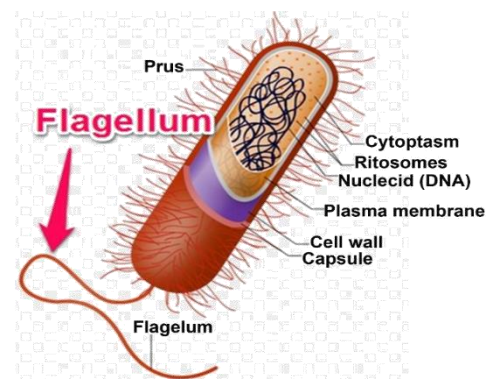
Topic-015 Anatomy of Prokaryotes IV

Learning Objectives

- Structure and function of flagella
- Motility of bacteria by flagella

Flagella

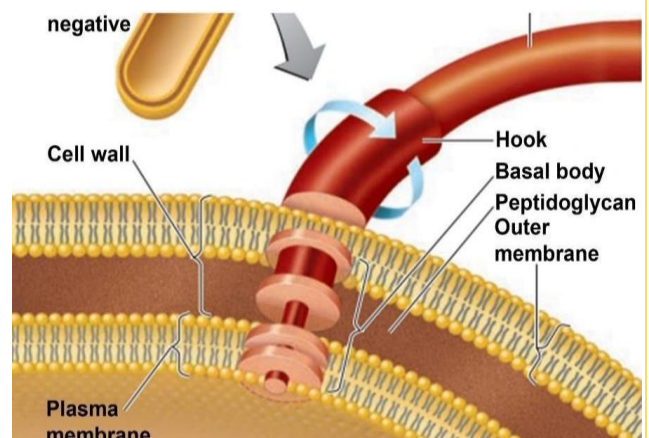
- Flagellum: Long filamentous structure that propels bacteria



Anatomy of flagella

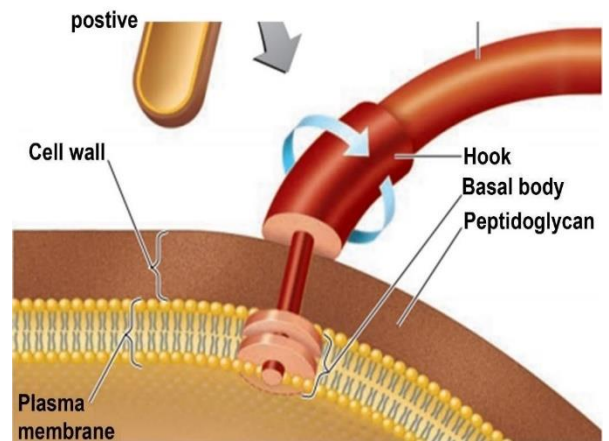
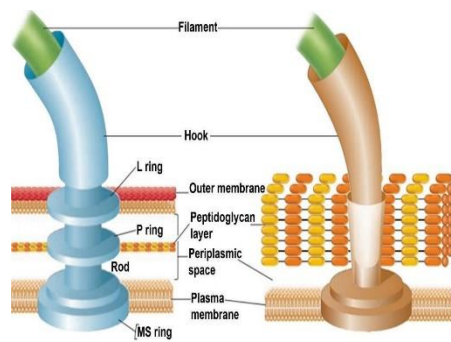
- Filament: Flagellin
- Hook: A different protein
- Basal Body: The most complex

Gram Negative Flagellum



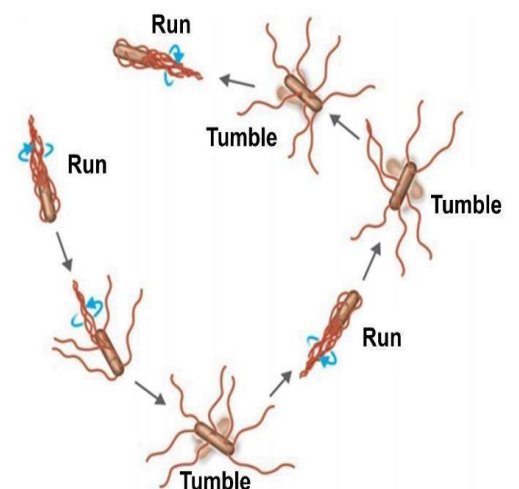
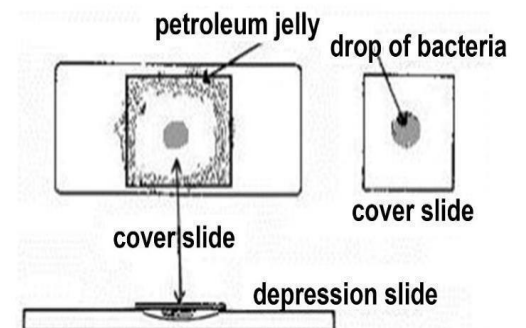
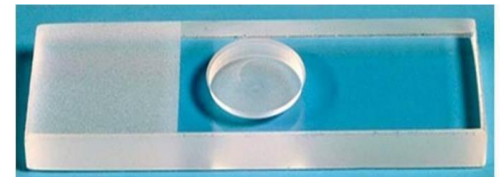
Gram Positive Flagellum

Flagellum



Flagella

- Used for bacterial movement – E. coli: 270 rms
- Taxis: movement
 - Chemotaxis
 - Phototaxis
- H antigen for serovar identification in G-neg bacteria
- Flagella move counterclockwise for forward thrust (run or swim)
- Clockwise when tumbling



Videos for Flagellar Motility



Videos for Flagellar Motility



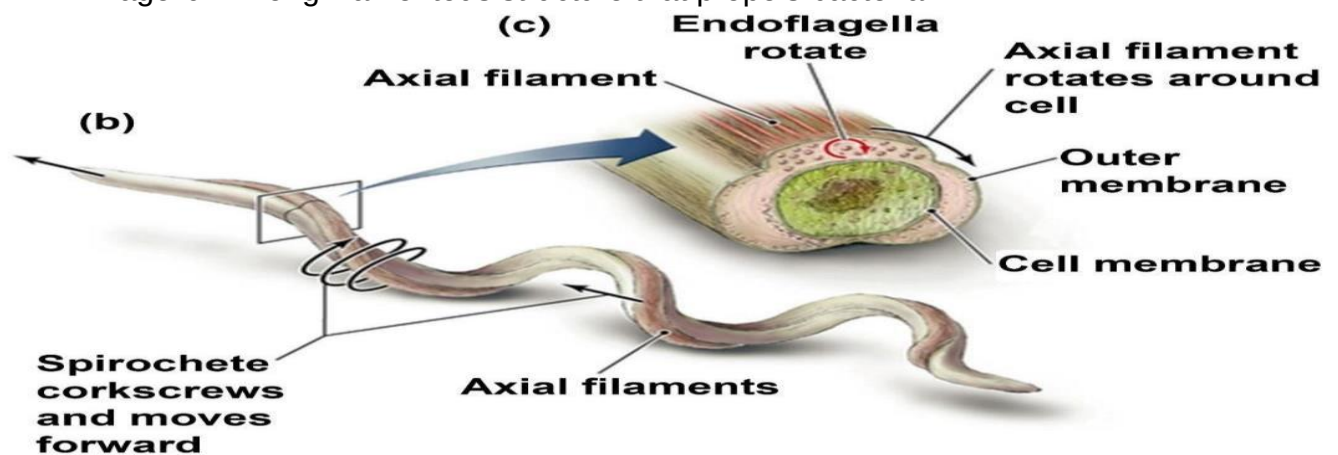
Topic-016 Anatomy of Prokaryotes V

Learning Objectives

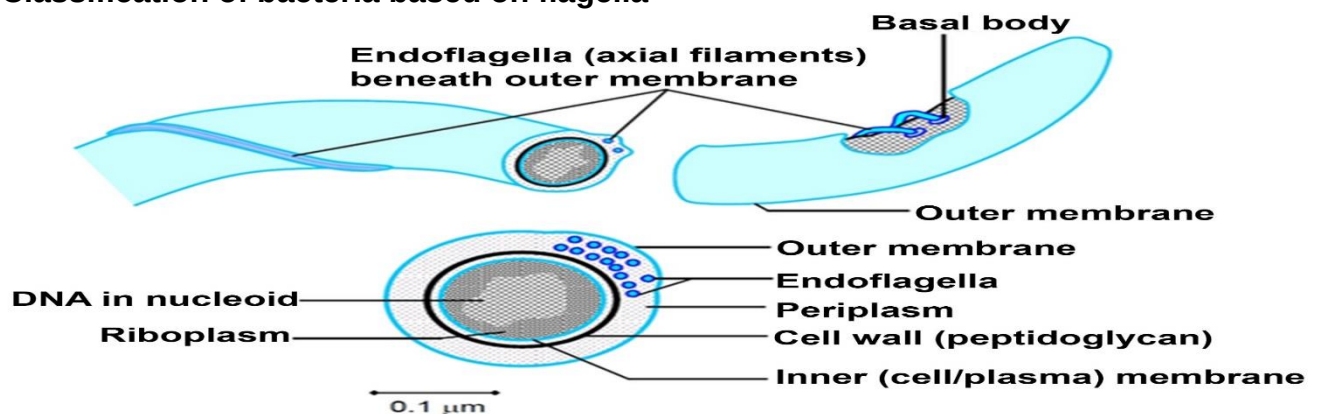
- Structure and function of axial filament
- Motility of bacteria by axial filament
- Fimbriae and their role
- Pili and their role

Axial Filament

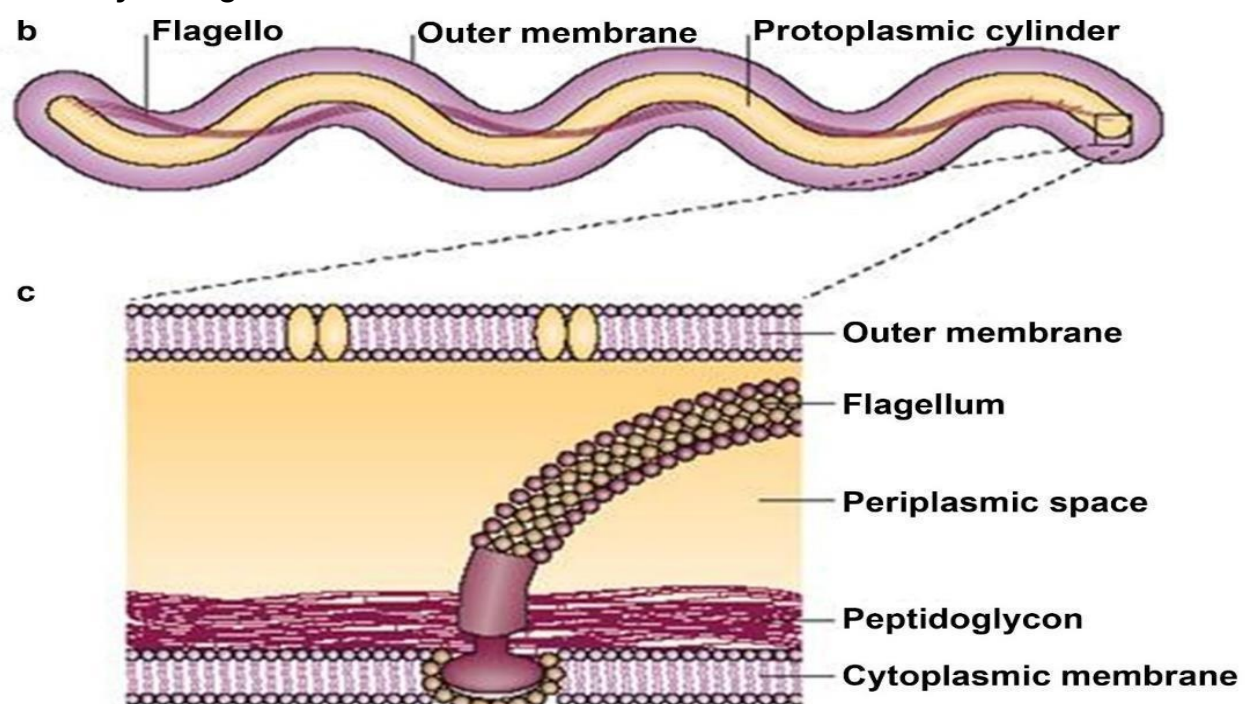
- Flagellum: Long filamentous structure that propels bacteria



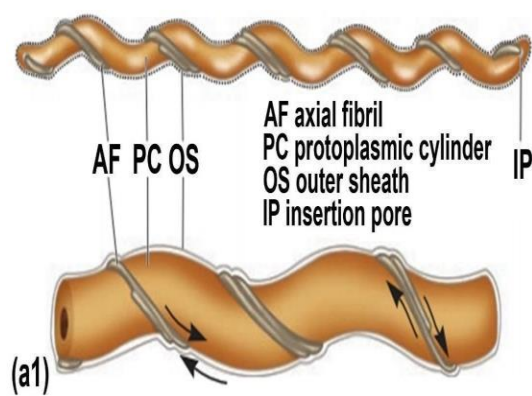
Classification of bacteria based on flagella



Anatomy of flagella



Axial Filament

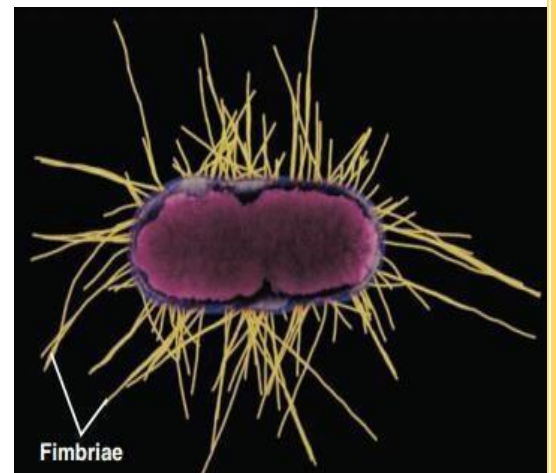


Axial Filament Motility (movie)

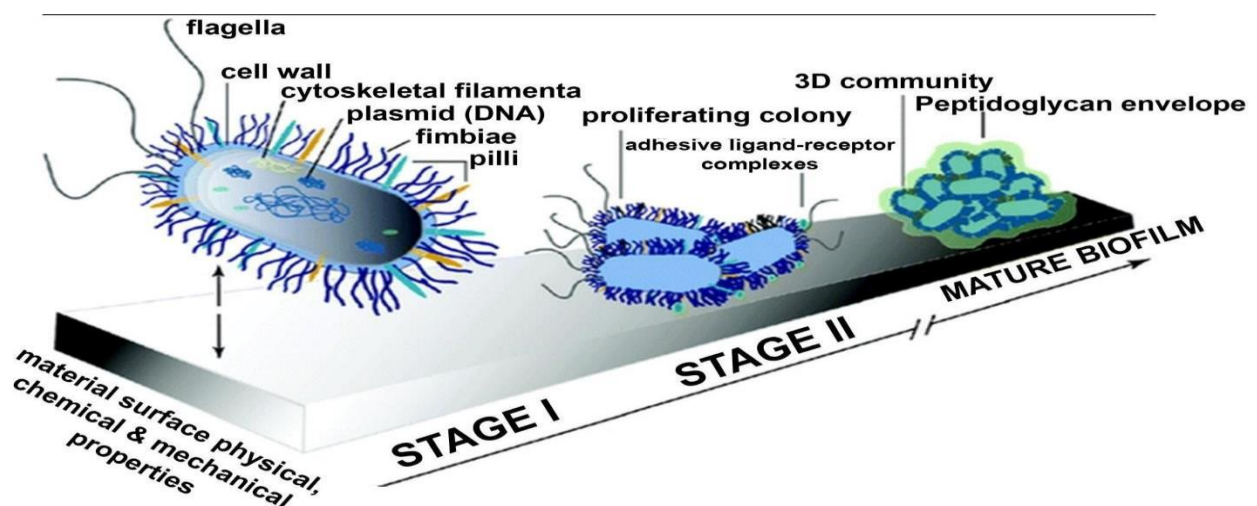


Fimbriae

- Hair-like small appendages on G-neg cells
- Composed of pilin
- Can be at one pole or around the entire cell
- Used for attachment to surfaces or epithelial cells
- Fimbria: Singular

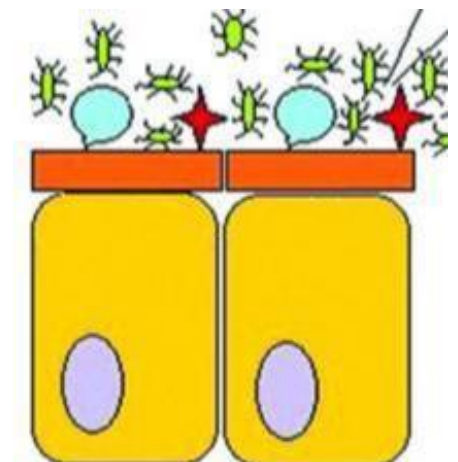


Fimbriae attachment in biofilm formation



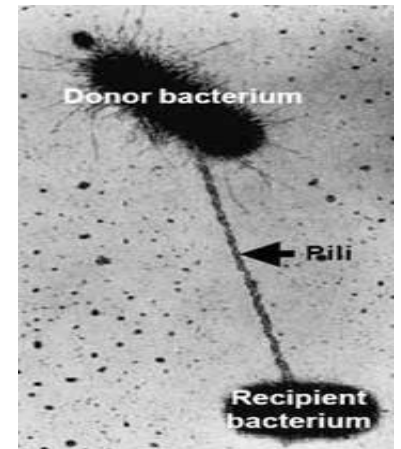
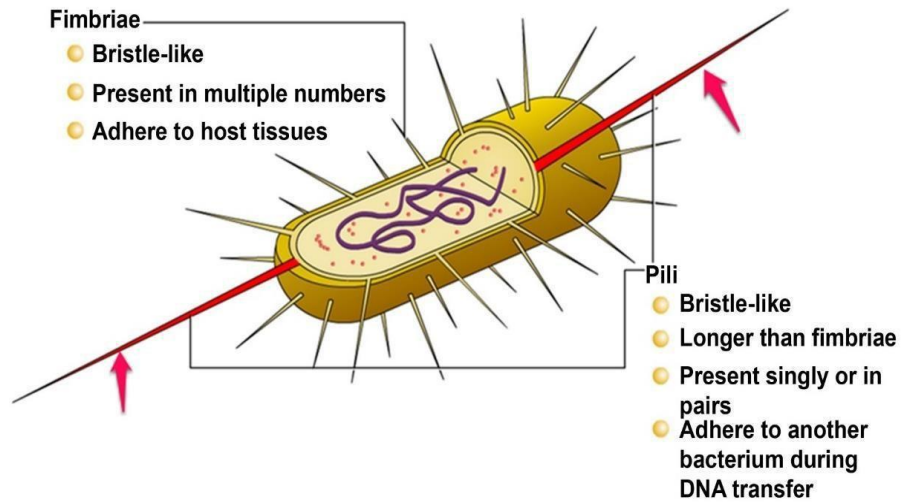
Fimbriae

- Attachment to epithelial cells – *Neisseria gonorrhoeae*
- *E.coli* O157: Diarrhea



Pili

- Longer than fimbriae
- One or two per cell
- Used for attachment:
 - Host cells
 - Bacteria
- **Used for attachment:**
 - DNA transfer: Conjugation (Sex pili)
 - Twitching Motility
 - Gliding Motility



Summary

- Axial filament
- Fimbriae • Pili
 - Attachment
 - Motility

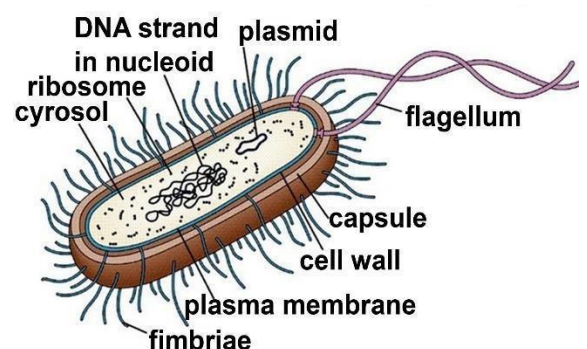
Topic 17 Anatomy of Prokaryotes 6

Learning Objectives

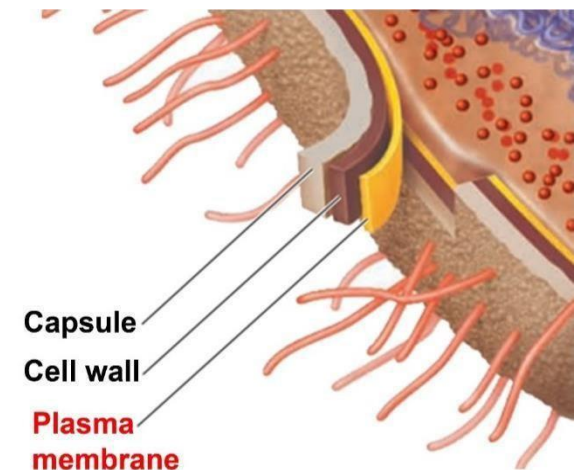
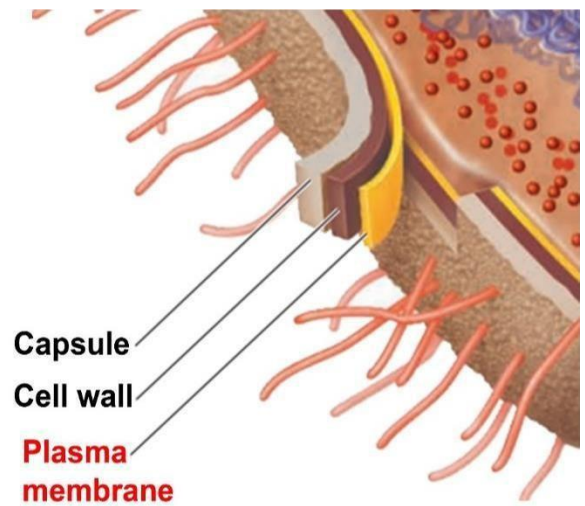
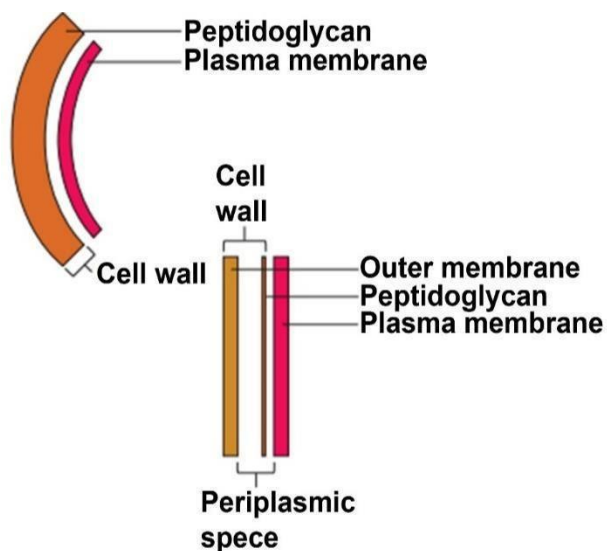
- **Structure and functions of cell wall**

Cell Wall

- Rigid layer just outside the plasma membrane
- Most important structure in prokaryotes –
 - Shape
 - Protection from osmotic lysis
 - Pathogenicity

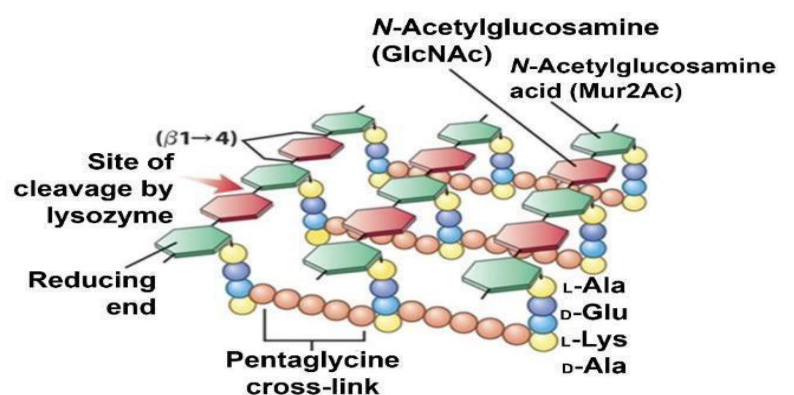


- Most important structure in prokaryotes –
Several antibiotics act on it.
 - 1988: Gram staining
 - Gram –ive and +ive
 - TEM revealed true differences
- Peptidoglycan thick in G +ive cells
- Thin in G –ive cells



Peptidoglycan Structure

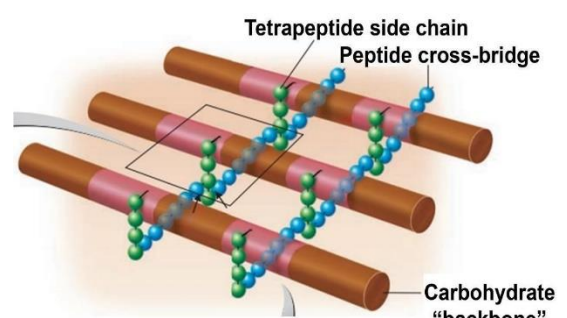
- Meshlike polymer
 - N-acetylglucosamine
 - N-acetylmuramic acid
- Meshlike polymer
 - Amino acids
 - D-amino acids (some)
 - Not degraded by body proteases
 - L-isomers found in the body

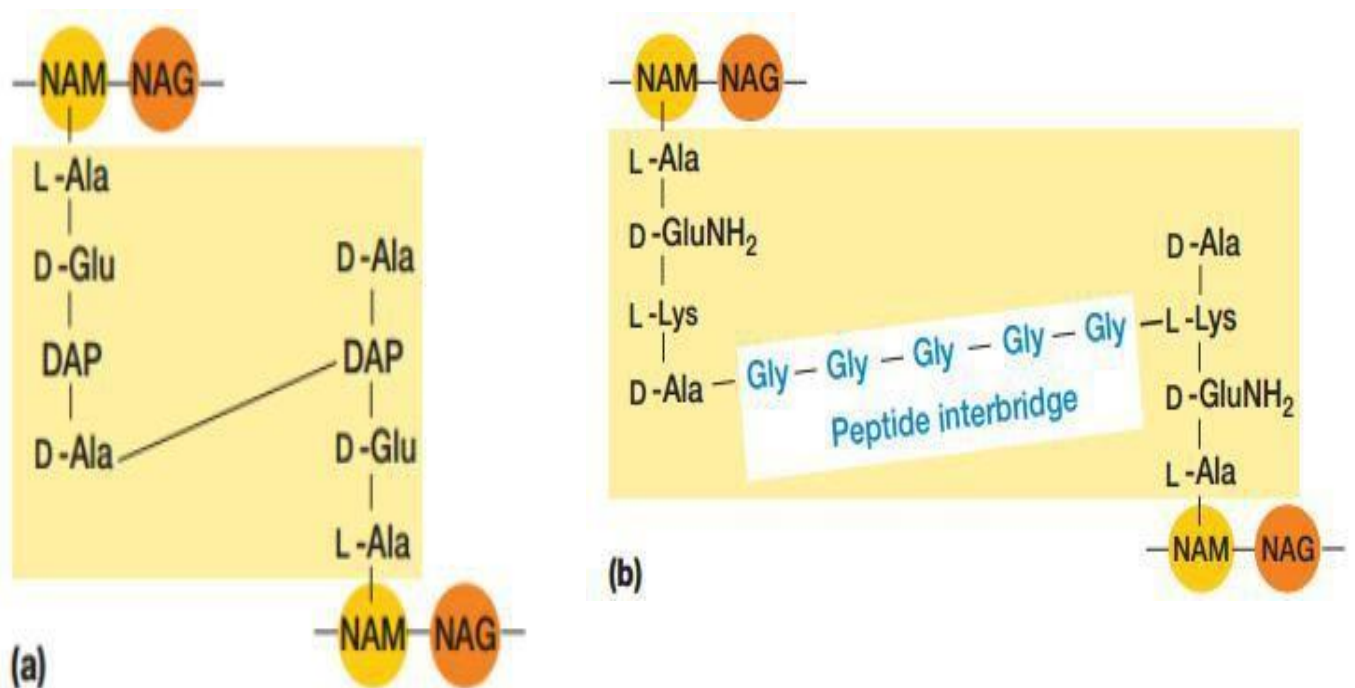


Peptidoglycan Structure

Peptidoglycan Structure

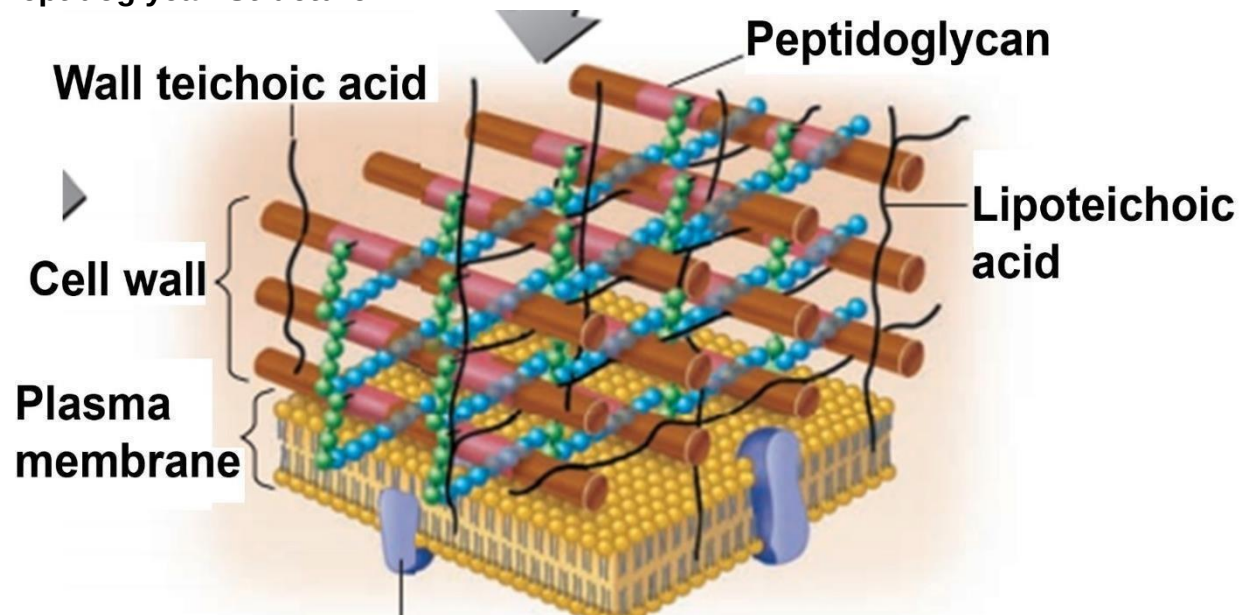
- Cross links



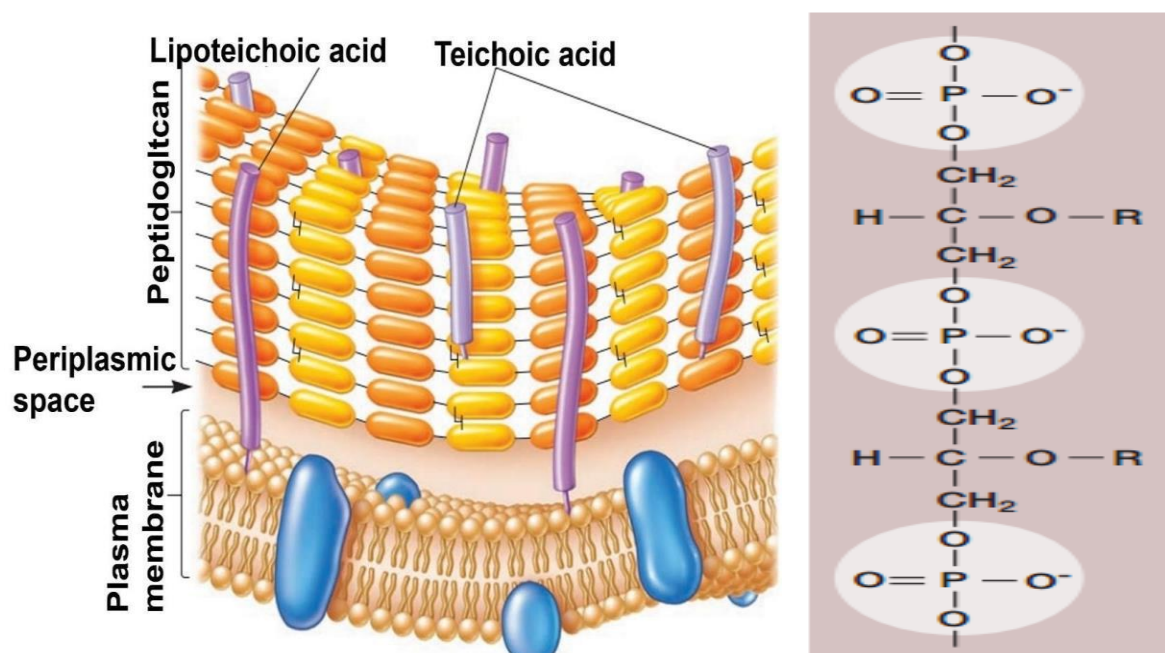


- Peptide interbridges or cross bridges
- Penicillin interferes with cross bridges

Peptidoglycan Structure



Teichoic Acid (glycerol or ribitol + Phosphate)

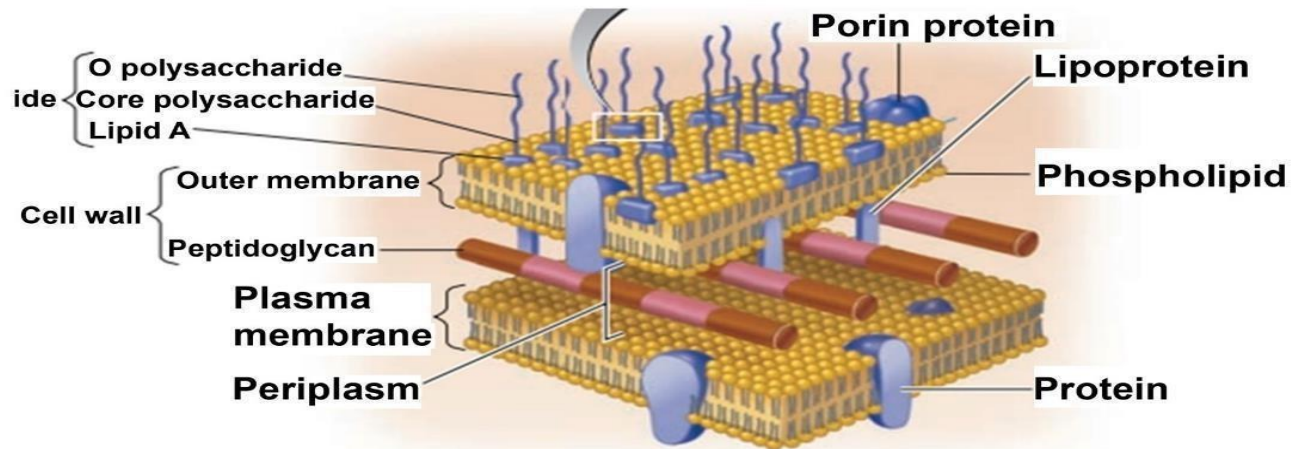


Topic-018 Anatomy of Prokaryotes VI-b

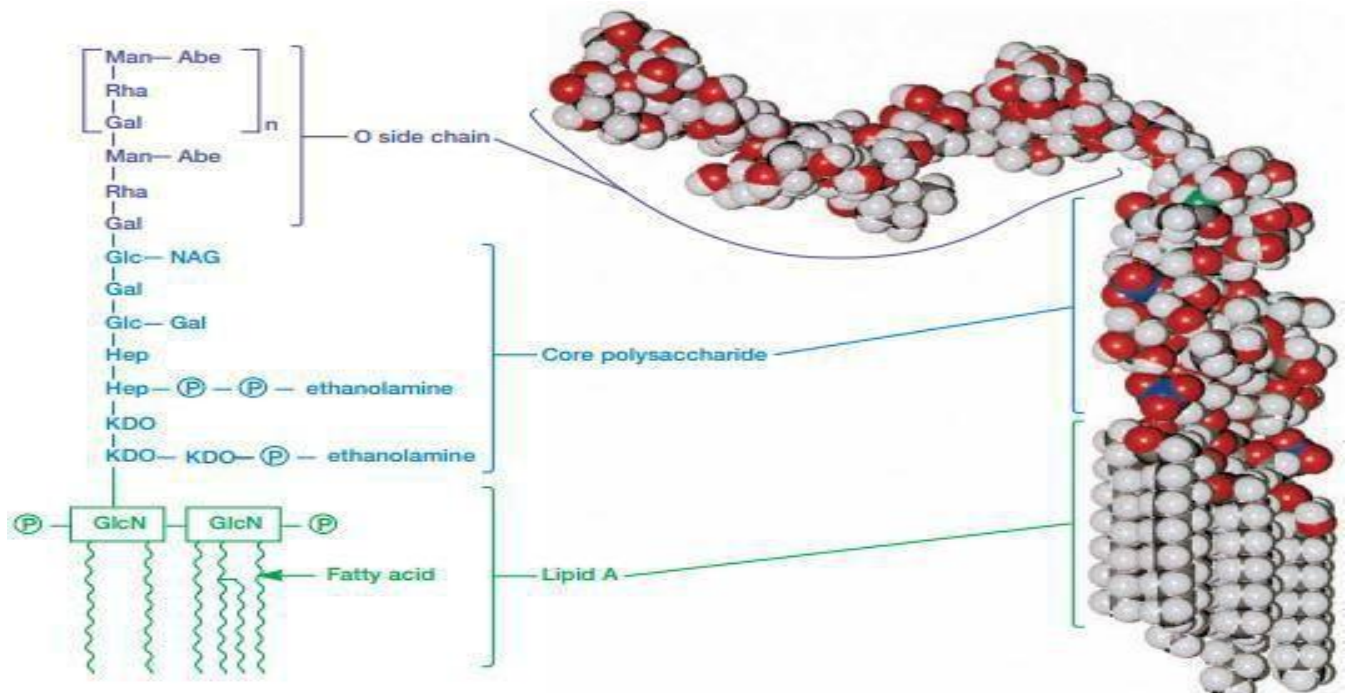
Learning Objectives

- Structure and functions of G-negative cell wall
- Comparison of G-positive and G-negative cell walls

Gram Negative Cell Wall



Lipopolysaccharide (LPS)



LPS

- Lipid A: endotoxin
 - Fever, vasodilation and shock
- Core polysaccharide
 - Structural support
- O side chain

- Antigenic like teichoic acid in gram positive bacteria
- Comparison of G-ive and G+ive Bacteria**

Characteristics	Gram Positive	Gram Negative
Gram reaction	Retain CV dye	Decolorized
Peptidoglycan layer	Thick	Thin
Teichoic Acid	Present	Absent
Periplasmic Space	Absent (generally)	Present
Outer membrane	Absent	Present
LPS	Virtually none	High
Lipid and lipoprotein contents	Low	High
Flagellar structure	2 rings	4 rings
Toxin Produced	Exotoxins	Exo and endotoxins
Susceptibility to penicillin	High	Low
Overall resistance	High	Low

Summary

- Gram positive cell wall
 - Thick and contains teichoic acid
- Gram negative cell wall
 - Thin, outer membrane
 - LPS, Porins
- Gram staining differences

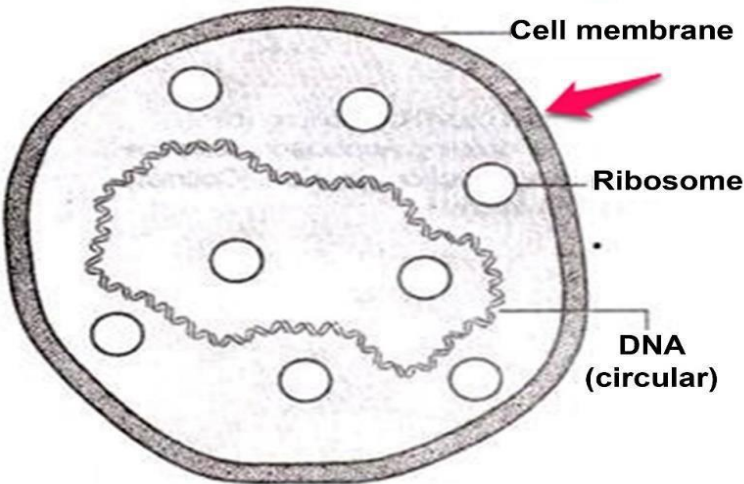
Topic-019 Anatomy of Prokaryotes VII

Learning Objectives

- Structure and functions of atypical cell wall

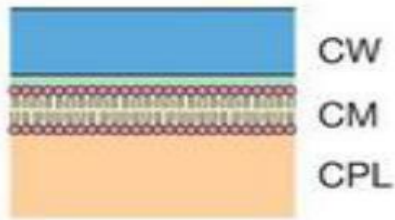
Atypical Cell Wall

- Some bacteria have no cell wall or have very little
 - Mycoplasmas
- Smallest bacteria
- Plasma membrane has sterols



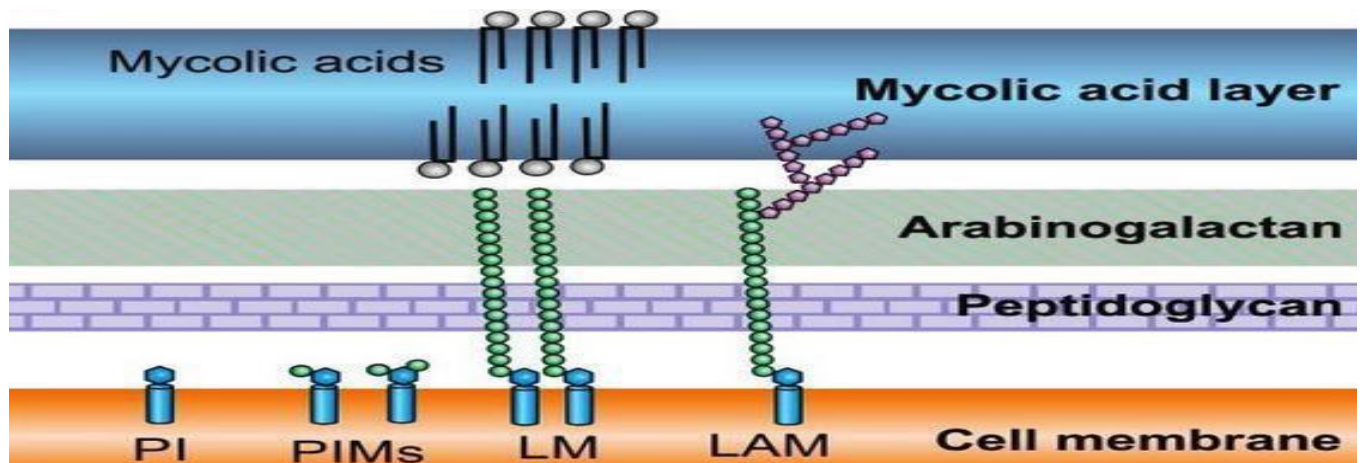
Archea: Cell Wall

- No cell wall or if they do, it is peptidoglycan-like
 - Pseudomurein
- No peptidoglycan, so behave like G –ive bacteria



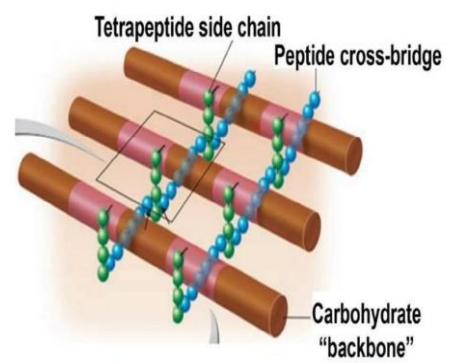
Acid fast cell wall

- Mycobacterium and Nocordia
 - Contain mycolic acid: waxy lipid
- Mycolic acid and peptidoglycan layers held by carbohydrate – Carbolfuchsin stains them.



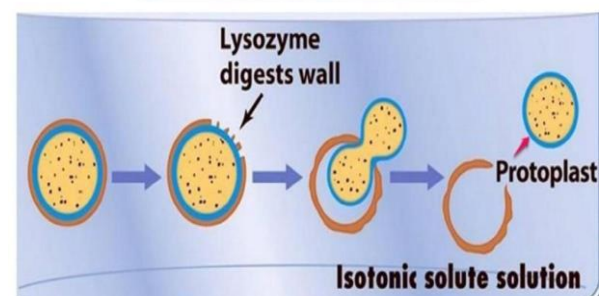
Damage to Cell Wall

- Lysozyme: breaks the sugar-derived backbone
- Protoplast: G +ve cells
- L-form bacteria: Loose their cell wall



Summary

- Some bacteria lack cell wall
- Archaea: Pseudomurein
- Mycobacterium: Mycolic acid
- Digestion with lysozyme – Protoplast



Topic-020 Anatomy of Prokaryotes VIII

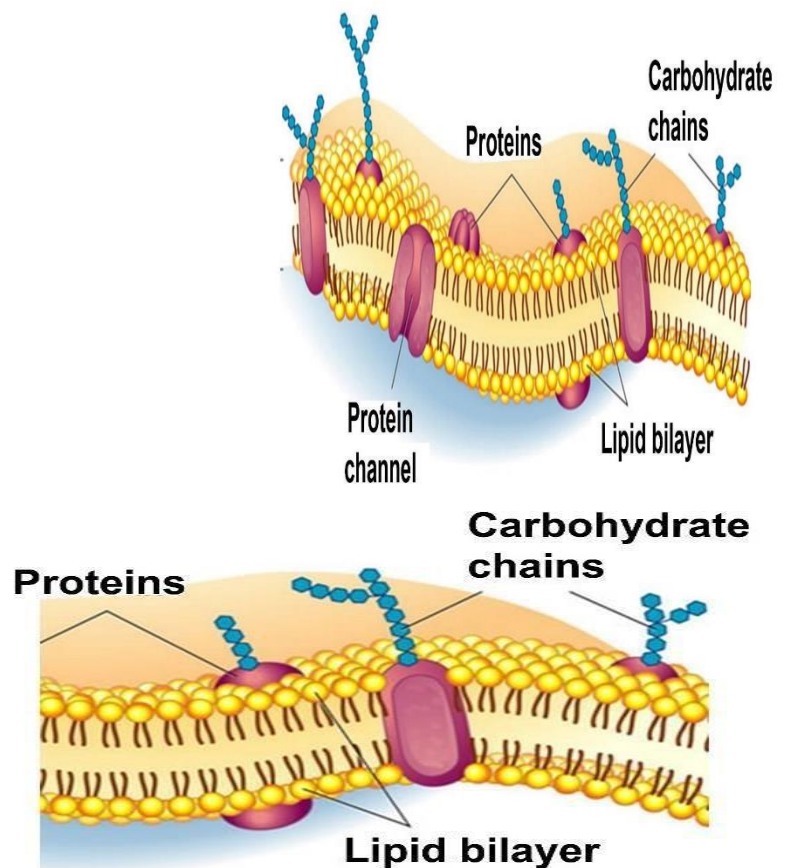
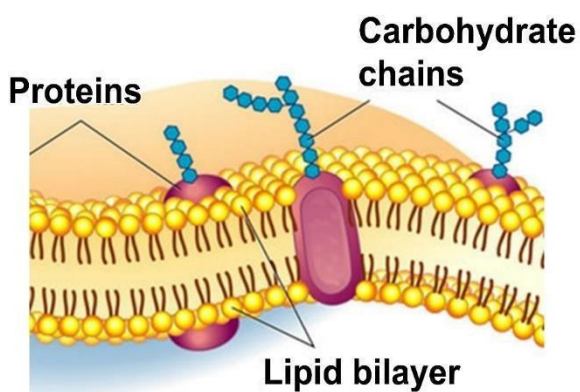
Learning Objectives

- Structure and functions of cell membrane

Cell Membrane

Functions of Cell Membrane

- Selective permeable barrier
- Passive and Active Transport
- Respiration
- Photosynthesis
- Lipid synthesis
- Cell wall parts
- Receptors



Phospholipid Bilayer

- Polar heads
- Nonpolar tails
- Amphipathic molecules

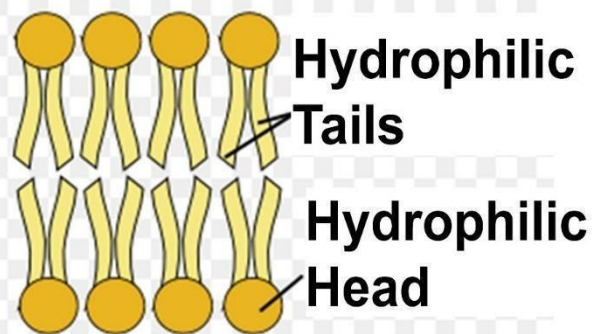
Types of Membrane Proteins

- Peripheral Proteins
 - Loosely connected to the membrane
 - Soluble in water
- Integral proteins
 - Imbedded in the membrane
 - Insoluble in water
 - Amphipathic in nature
- Some act as receptors

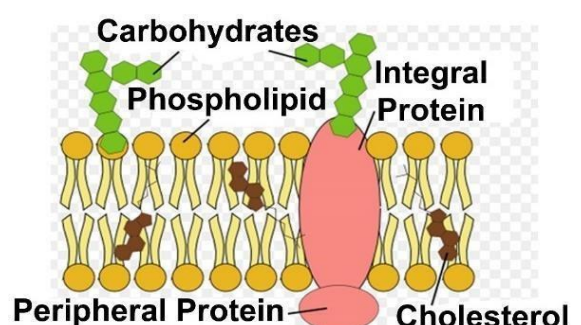
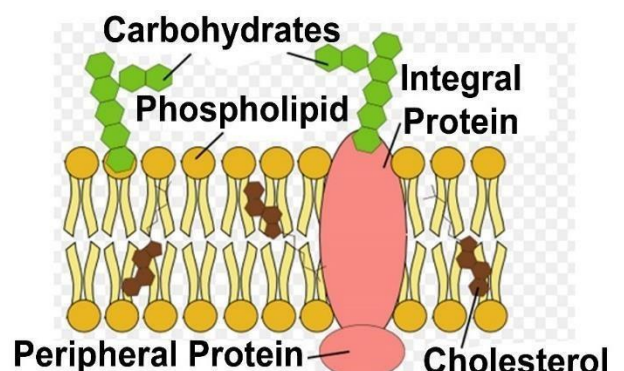
Fluid Mosaic Model of Cell Membranes

- Integral proteins are not static in position

Extracellular Fluid



Intracellular Fluid



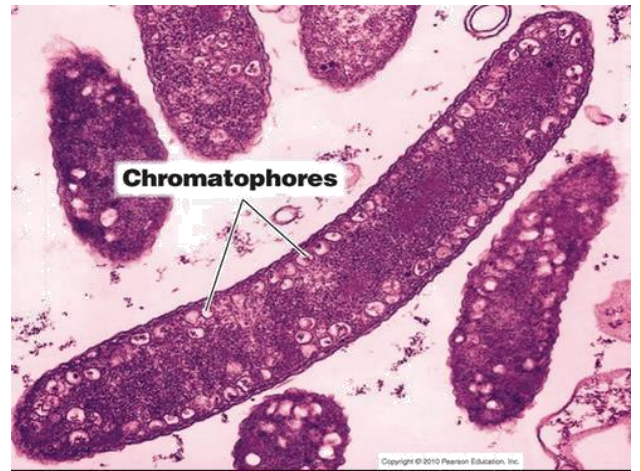
- Can diffuse laterally and change position in the membrane

Photosynthesis by Cell Membrane

- Chromatophores or thalakoids

Destruction of Cell Membrane

- Alcohol
- Quaternary ammonium compounds
- Polymixins



Summary

- Phospholipid bilayer
- Selectively permeable
- Integral proteins
- Peripheral proteins
- Performs many functions

Topic-021 Anatomy of Prokaryotes IX

Learning Objectives

- How do various materials move across cell membrane?
- What is passive movement?
- What is active movement?
- What is osmosis?

Passive Movement

- Movement with the concentration gradient
- No energy expenditure
- Substances move from area of high concentration to low concentration.

Active Movement

- Movement against the concentration gradient
- From low concentration to high concentration
- Requires energy (ATP)



Passive Movement

- **Simple diffusion:**

- Area of high conc. to low conc.
- Until ions or molecules evenly distributed

Equilibrium

- O₂ and CO₂

- **Facilitated diffusion:**

- Integral proteins as channels or carriers
- Called transporters or permeases

- No energy is required

Facilitated diffusion:

- Nonspecific transporters
 - Ions
- specific transporters
 - Change in shape
 - Sugars, vitamins

- **Facilitated diffusion:**

- Large molecules degraded by extracellular enzymes
- Smaller molecules then bind transporters

- Water molecules can pass through lipid bilayer by *simple diffusion* or through water channels, *aquaporins*

- **Osmosis**

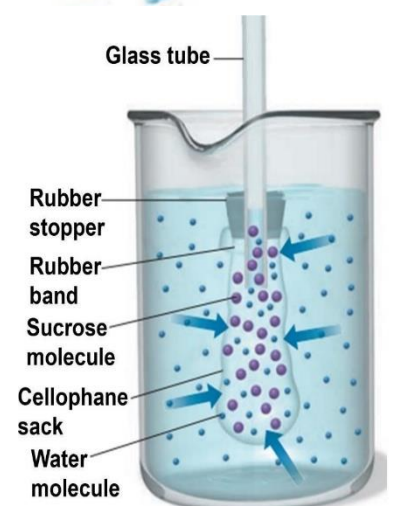
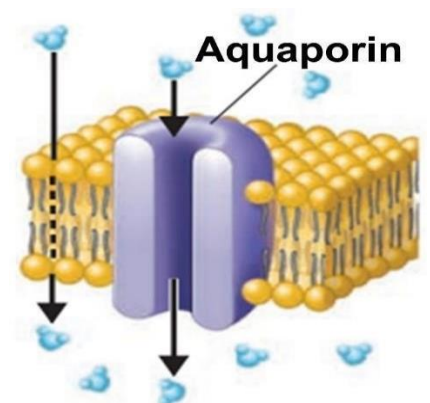
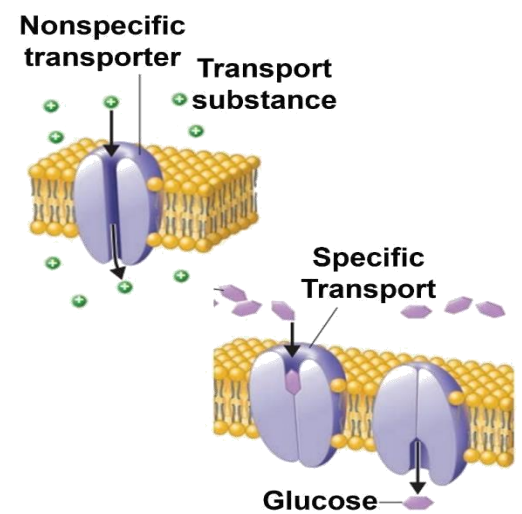
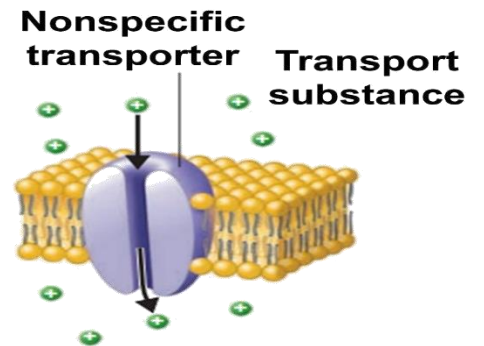
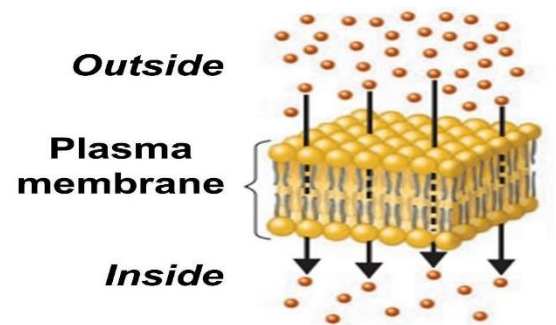
- Net movement of solvent
 - From high conc of solvent to low conc
 - OR low conc of solute to higher conc of solute

- **Osmosis**

- Solvent will move b/c of semipermeable membrane

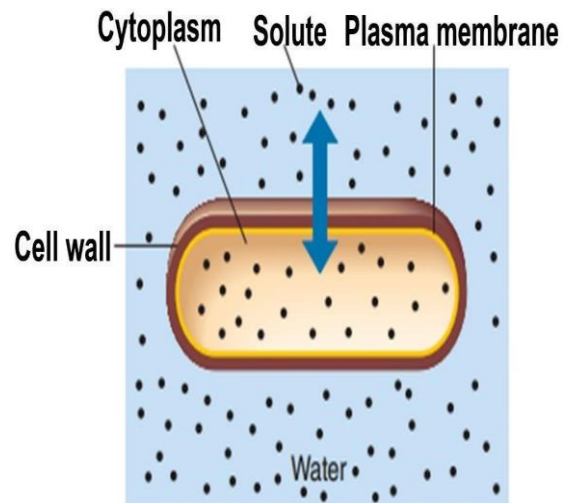
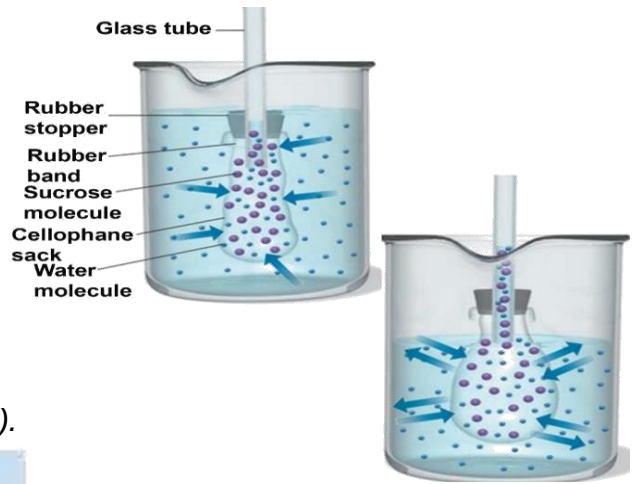
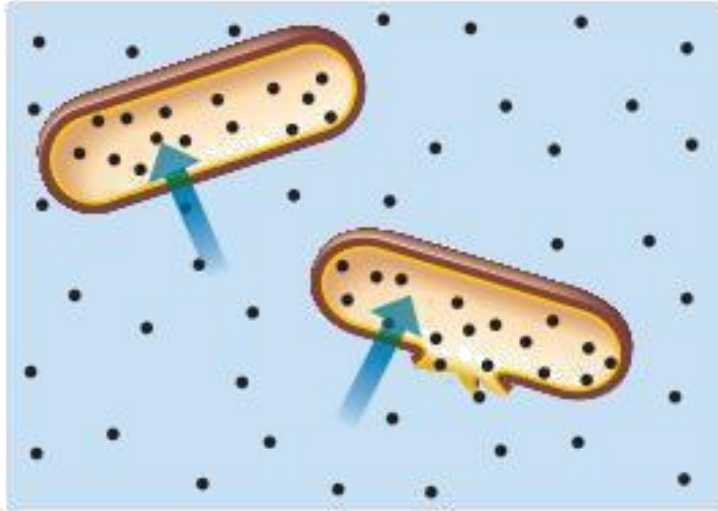
- **Osmotic Pressure**

- Pressure required to prevent movement of water into a solution containing some solute.

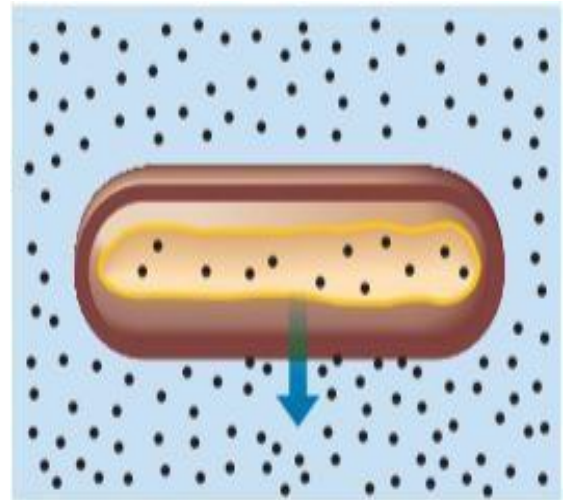


Solutions Vs Bacteria

- Isotonic Solution
 - No net movement of water
- Hypotonic Solution
 - Water moves into the cell (*Osmotic lysis*).



- Hypertonic Solution
 - Water moves out of the cell (*Plasmolysis*).
- Requires ATP
- Nutrients move from lower conc to higher conc
 - Na^+ , K^+ , H^+ , Ca^{2+} , and Cl^-
 - Amino acids
 - Simple sugars
- *Note: Can use passive diffusion too!*
- Active Transport
 - Substances are not altered during transport
- Group Translocation
 - Chemically altered during transport
 - Retained in the cell
 - *Glucose is an example.*



Summary

- Passive diffusion
 - No ATP used

- Conc gradient dependent
 - Simple diffusion
 - Facilitated diffusion
- **Active diffusion**
 - ATP used
 - Go against concentration gradient Active transport
 - Group translocation

Topic-022 Anatomy of Prokaryotes 10

Learning Objectives

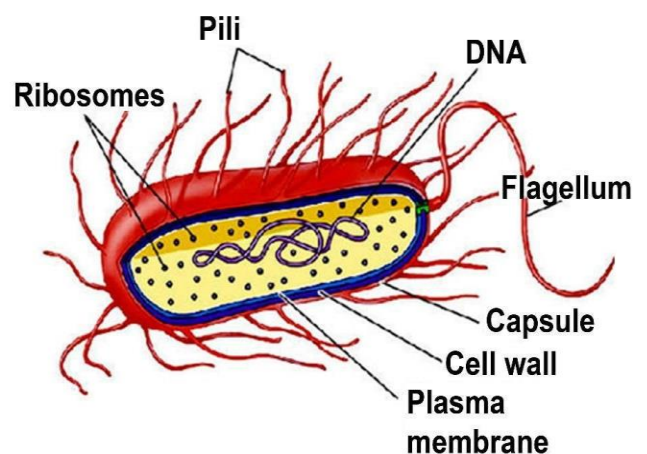
- What lies in the cytoplasm
- Nucleoid
- Ribosomes
- Inclusions

Prokaryotic Cytoplasm

- Substances of cells inside the plasma membrane
- 80% is water.
- Nucleoid
- Ribosomes
- Inclusions
- No cytoskeleton and streaming

The Nucleoid

- Circular dsDNA (bacterial chromosome)
- No nuclear membrane
- No histones
- Chromosome attached to plasma membrane
- Plasmid: extrachromosomal DNA
 - Not needed but advantageous



Ribosomes

- Composed of proteins + ribosomal RNA

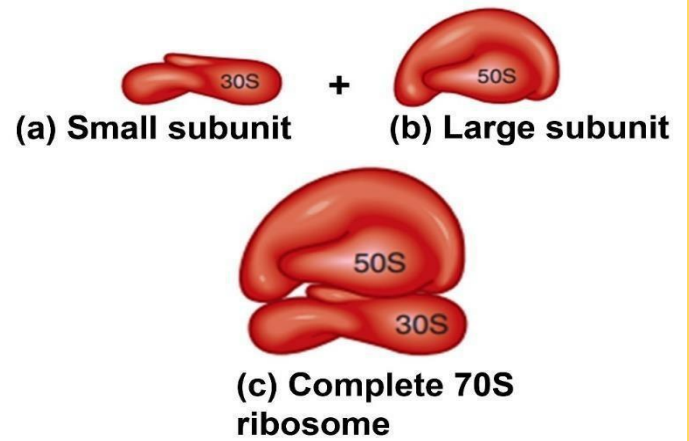
- 2 subunits

- 70S ribosomes

50S: one rRNA

30S: two rRNA

- Several antibiotics act on ribosomes and inhibit protein synthesis
 - Streptomycin attach to 30S

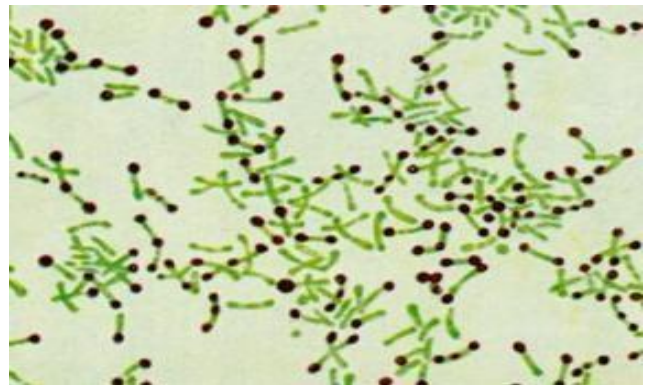


Inclusions

- Reserved deposits of nutrients
- Avoid the increase in osmotic pressure – Metachromatic granules
 - Polysaccharide granules
 - Lipid inclusions
 - Sulfur granules etc.

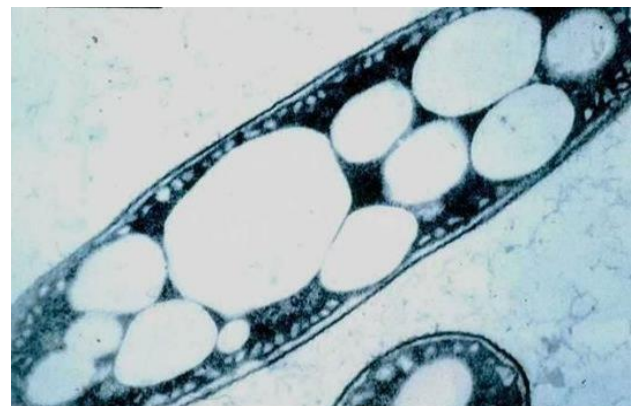
Metachromatic Granules

- Also called volutin
- Large inclusions
- Stained red with methylene blue
- Contain inorganic phosphates
- Characteristics of *Corynebacterium diphtheriae*



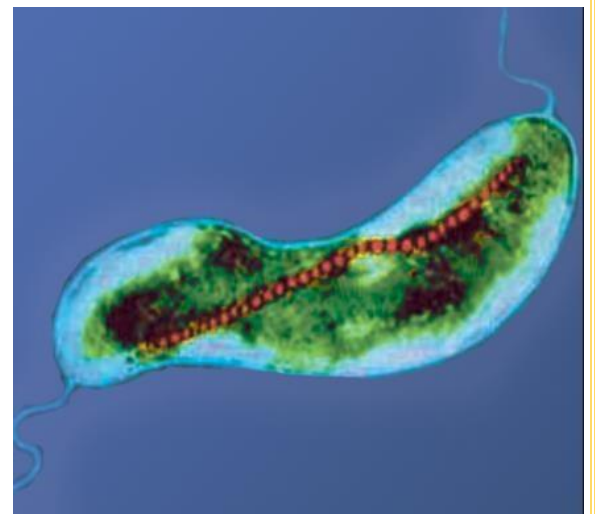
Gas Vacuoles

- Aquatic bacteria
- Maintain buoyancy for obtaining:
 - Nutrients
 - O₂
 - Light



Magnetosomes

- Inclusions of iron oxide
- Surrounded by invaginations of plasma membrane
- Present in G negative bacteria – Act like magnet
 - Decompose H₂O₂



Summary

- Cytoplasm
- Bacterial chromosome
- Plasmid
- 70S Ribosomes
- Inclusion bodies Metachromatic granules
 - Gas vacuoles
- Magnetosomes

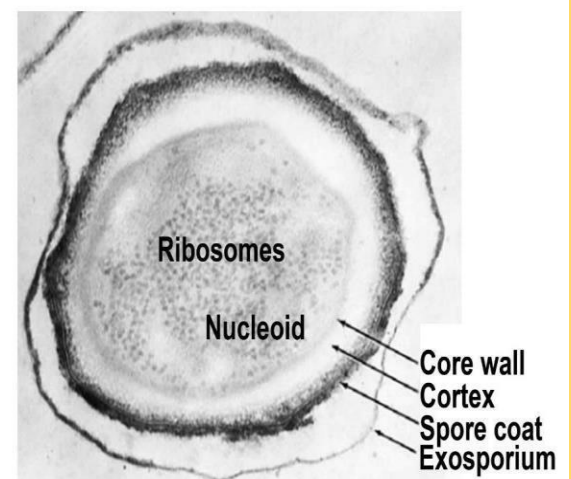
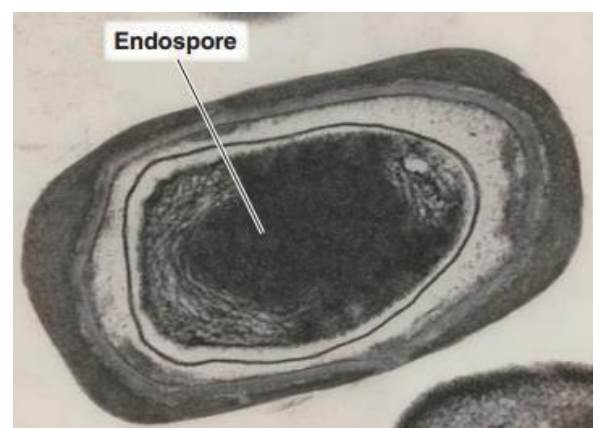
Topic-023 Anatomy of Prokaryotes XI

Learning Objectives

- What are endospores and how they form?
- What are the advantages of having an endospore?

Endospores

- Specialized resting cells
 - Highly durable, dehydrated forms of bacteria
 - Formed inside a bacterium
 - Survive extremes of temperature
- Lack of water
 - Radiation
 - Toxic environment
- Mostly formed by Gram positive bacteria
 - Clostridium
 - Bacillus
 - One gram negative: *Coxiella burnetii* also has it.
 - Formation of spores is called sporulation or sporogenesis.
 - Exosporium: A thin delicate covering



- Spore coat: Thick, composed of several protein layers
 - Resistant to chemicals
 - Contains enzymes germination

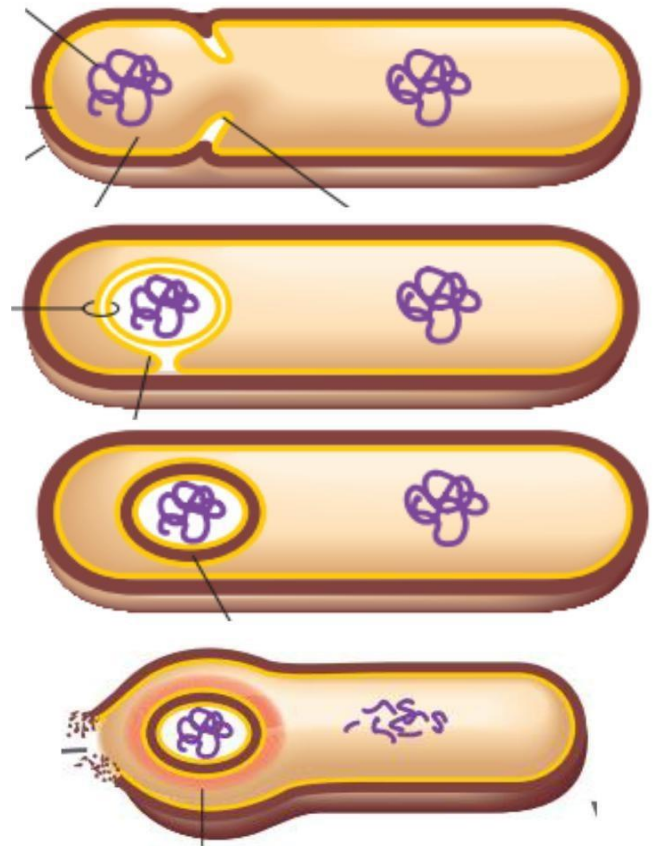
Cortex: Has peptidoglycan

Spore cell wall or core wall: Surrounds the protoplast or spore core

Spore core: Contains nucleoid and ribosomes

Sporogenesis

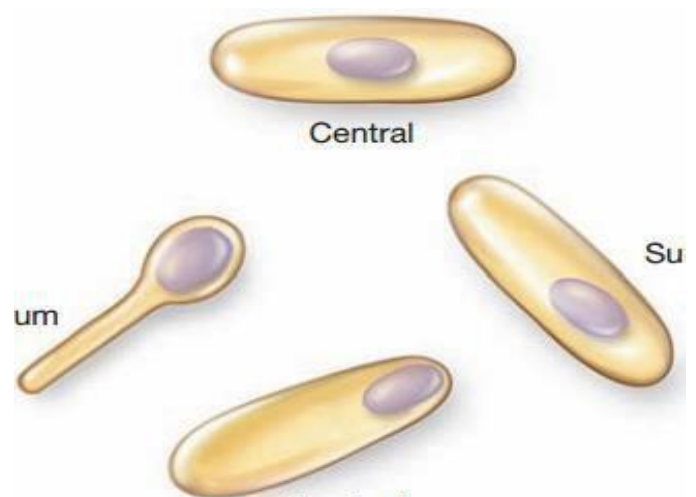
- In-growth of plasma membrane (spore septum)
- Spore septum becomes double layered •
Peptidoglycan is laid down between the two membranes
- A thick spore coat is formed.
- Core has dipicolinic acid complexed with calcium
 - Thought to stabilize DNA
- Small acid-soluble acid binding proteins
 - Protect DNA from heat, desiccation and chemicals



Location and Size

- Bulging or non-bulging
- Central
- Terminal
- Subterminal
- Used for identification
 - *Clostridium tetani*

Drum-stick



Germination of Spore

- Activation: Prepares spore for germination.
 - Heating can initiate it.
- Germination: Spore starts swelling and losing its coats etc.

- Metabolically active
- Outgrowth: New components are made.

Spores and Sterilization

- Autoclaving:
 - 121 °C at 15 psi for 20 min
 - Sugars get damaged at this temp.

Tyndallization:

- Boil on day 1
- Incubate at 37 °C
- Boil on day 3

Summary

- Spore is a specialized cell
- Forms when nutrition gets depleted
- Resistant to extreme conditions
- Forms within a cell
- Spore germinates under favorable conditions and returns to vegetative form.

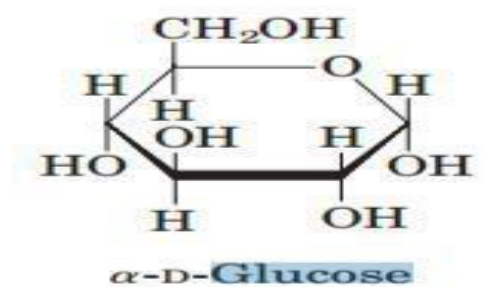
Topic-024 Microbial Metabolism I

Learning Objectives

- How is energy captured from various nutrient molecules to generate ATP?

Energy in Nutrients

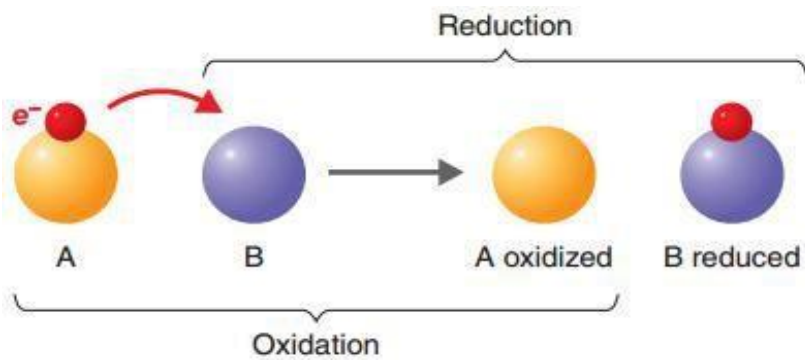
- Energy is associated with electrons that form bonds between their atoms.
- Nutrients are rich in hydrogen.
- Hydrogen atoms move from the substrates.



This energy is ultimately concentrated into bonds of ATP.

Oxidation Reduction (Redox) Reactions

- Oxidation: Removal of electrons from an atom •Reduction: Gain of electron
- Oxidation and reduction reactions are coupled.



Oxidation Reduction (Redox) Reactions

- In many cellular oxidations, electrons and protons (hydrogen ions, H^+) are removed at the same time.
 - This is equivalent to removal of hydrogen atoms. (Hydrogen has one electron and one proton)
- Most biological oxidations involve the loss of hydrogen atoms, they are also called dehydrogenation reactions.

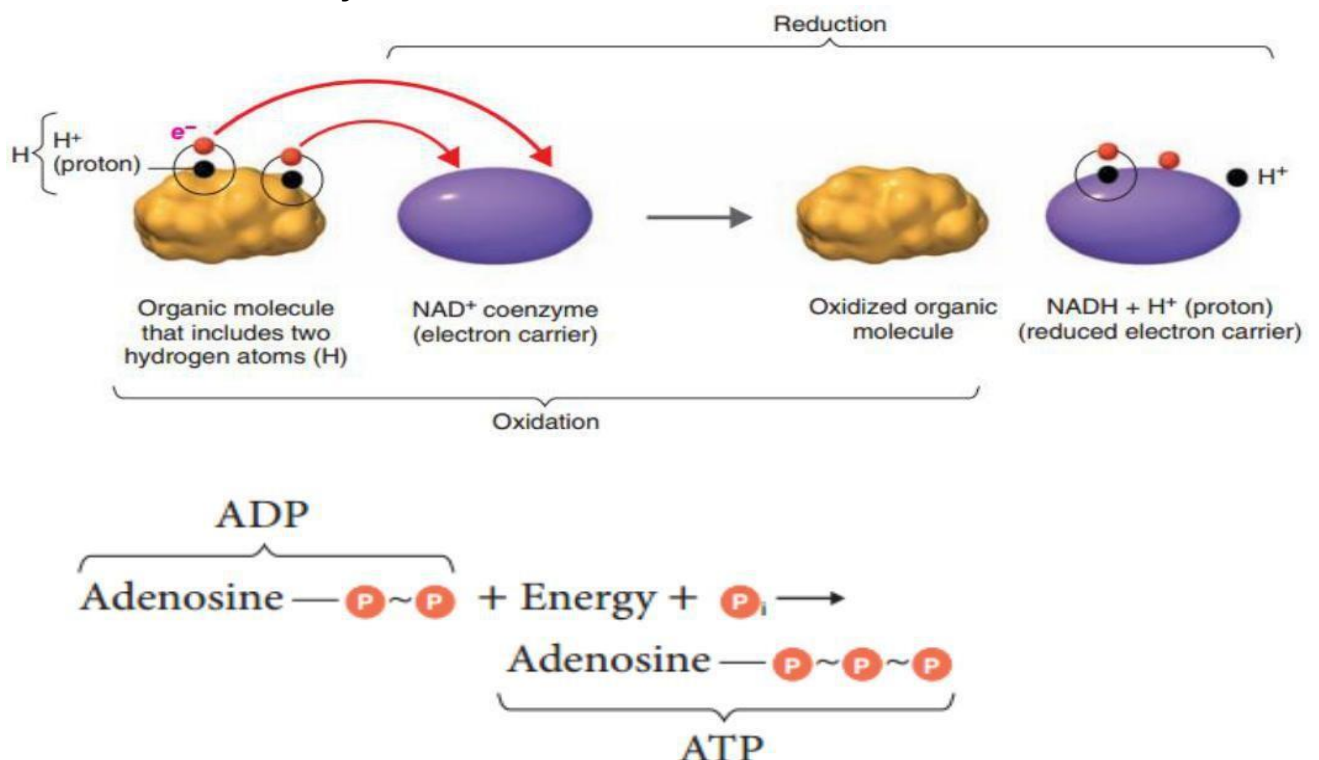
Coenzymes

- Act as electron carriers
- Receive electrons from nutrients
- Donate electrons to other molecules in subsequent reactions

Examples of coenzymes:

- NAD^+
- $NADP^+$
- FMN and FAD

Redox Pair and ATP Synthesis

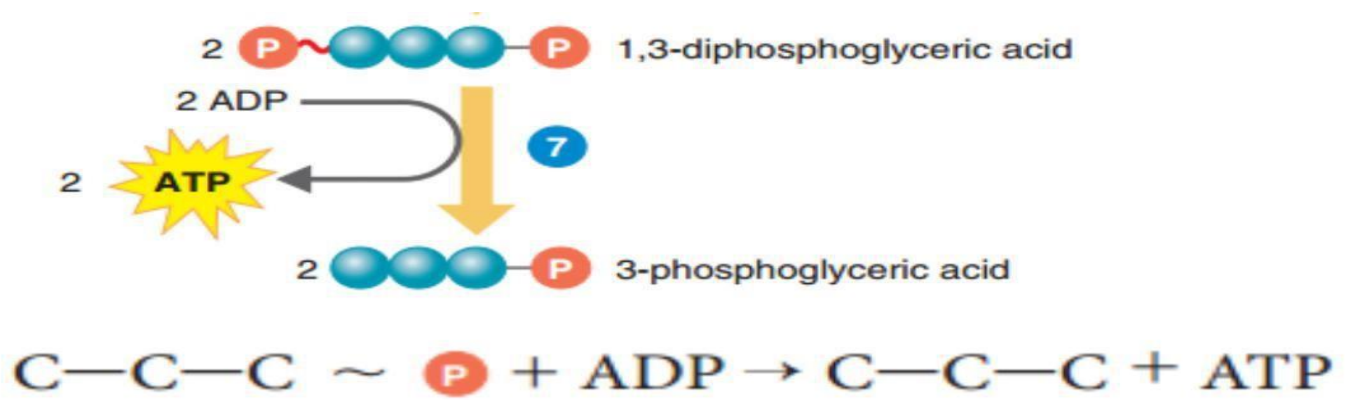


Phosphorylation and ATP Production

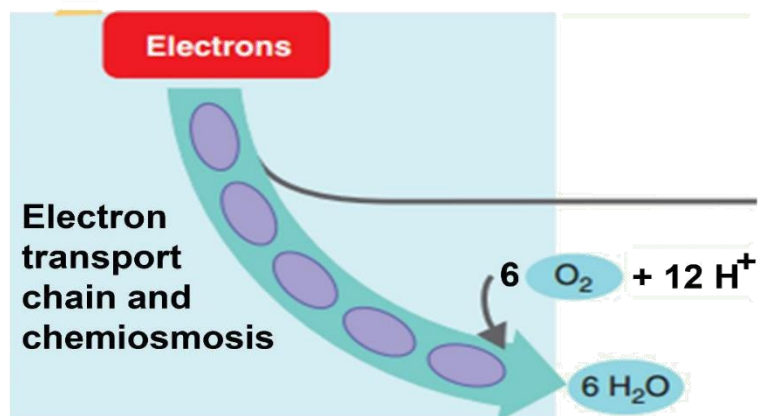
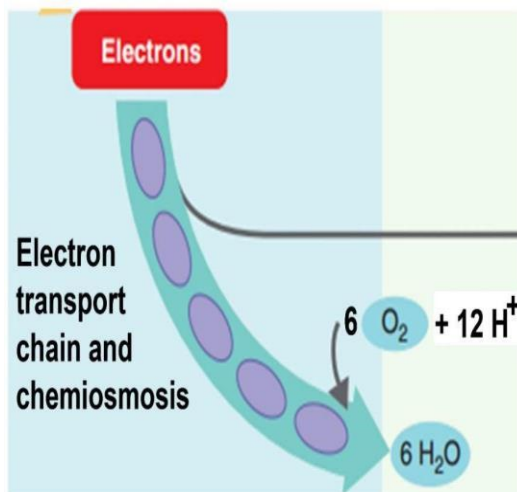
- The addition of P to a chemical compound is called phosphorylation.

Substrate level phosphorylation

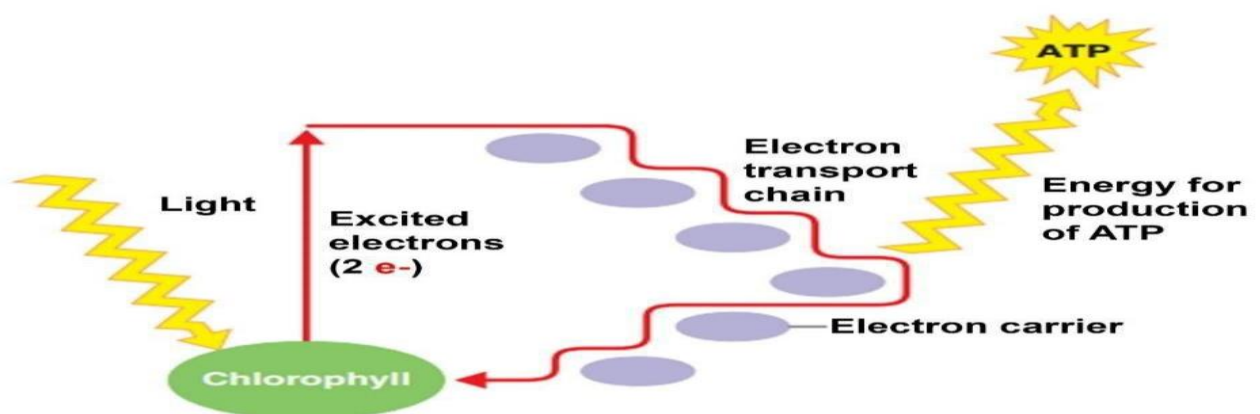
- Direct transfer of P to ADP



- Oxidative Phosphorylation
- Electrons transferred to NAD^+ or FAD
- Electron passed through a series of electron carriers to molecules of O_2
 - *Electron transport chain or system*
 - ATP generated by chemiosmosis



- Photophosphorylation
- Occurs in photosynthetic cells
- Light energy is converted into ATP.



Summary

- Energy associated with electrons in foods.
- These electrons are extracted by metabolic pathways.

- Electrons are used to make ATP.
- ATP used for various activities in the organism.

Topic-025 Microbial Metabolism II

Learning Objectives

- How are carbohydrates catabolized for ATP production.
- Cellular Respiration
- Fermentation

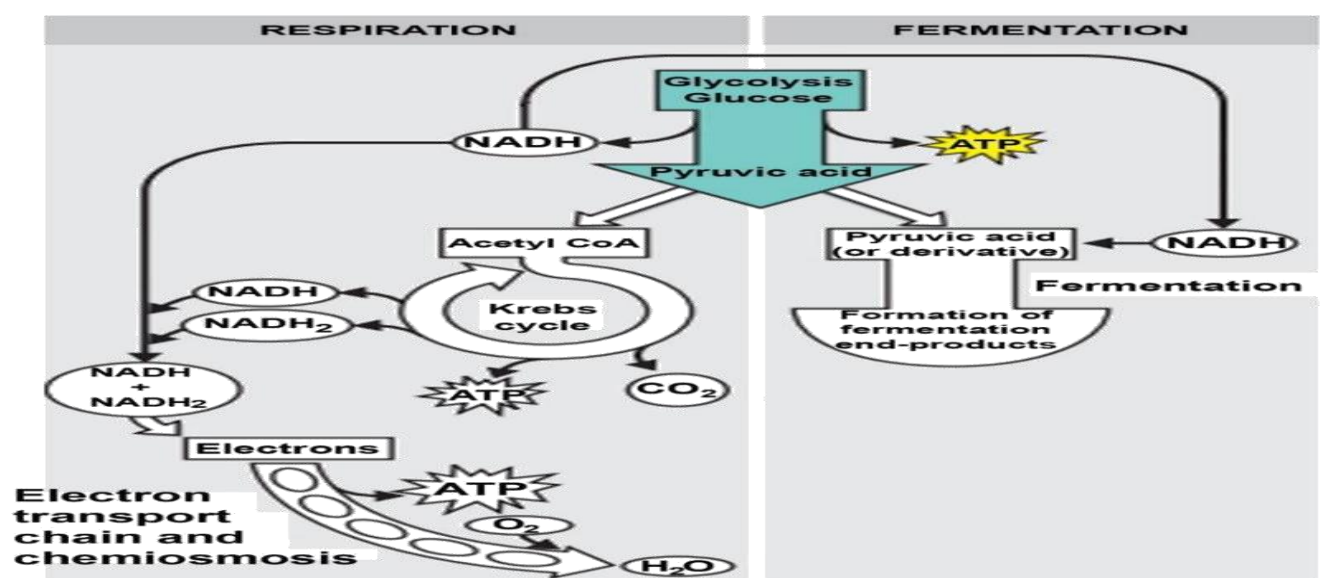
Carb Catabolism

- Primary source of cellular energy for most microbes
- Glucose is the most common carb energy source
- Lipids and proteins can also be used as energy sources
- Glucose is used by two processes:

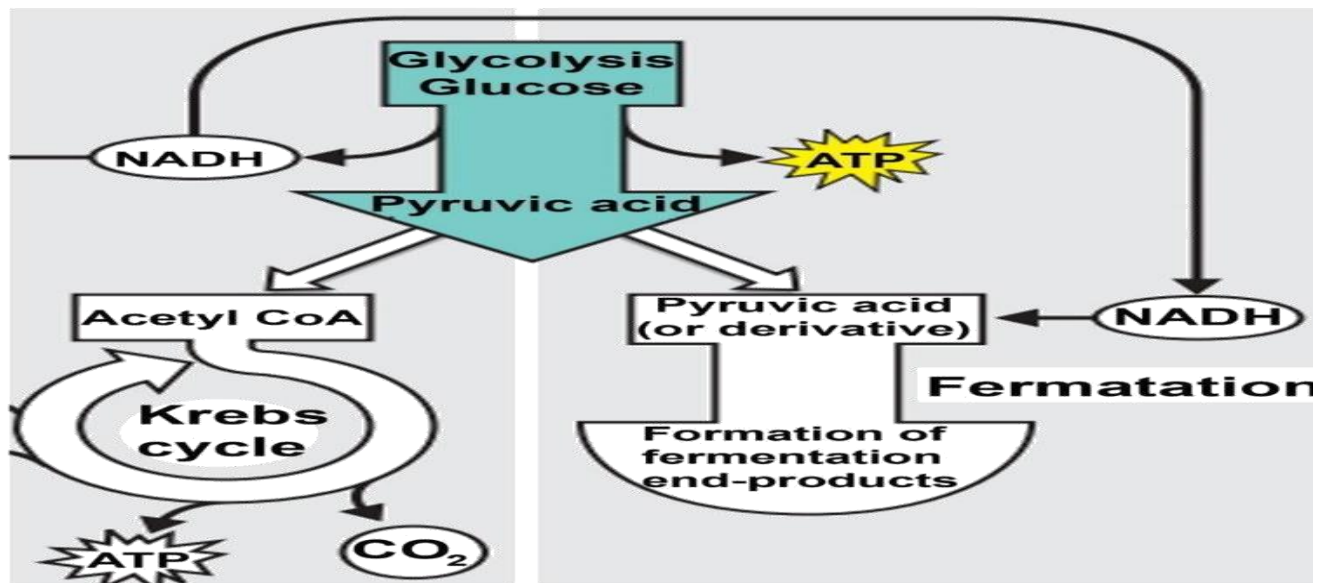
Cellular respiration (it is not breathing!)

Fermentation

Both usually start with glycolysis as the first step, but split after pyruvic acid formation.



Aerobic Respiration And Fermentation

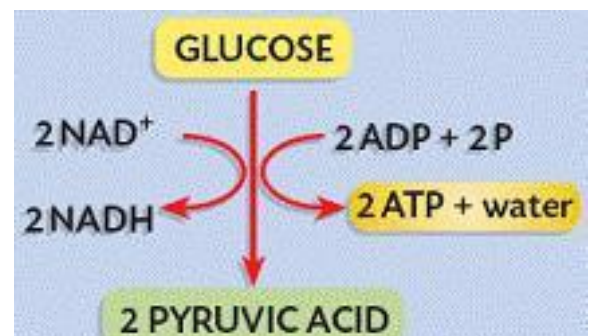


Respiration of Glucose

- Occurs in three stages:
 - Glycolysis
 - The Krebs cycle
 - Electron transport chain
- Most of ATP is generated in the last stage.

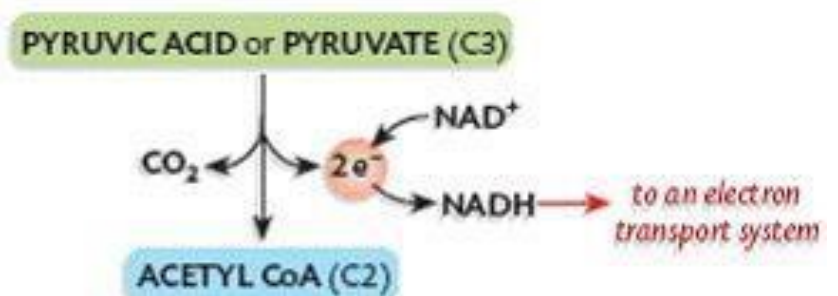
Glycolysis

- Glycolysis is the oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH.

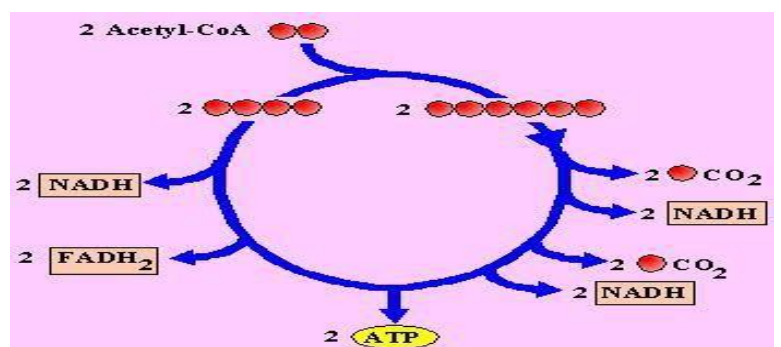


The Krebs Cycle

- Oxidation of acetyl CoA to carbon dioxide, with the production of some ATP and energy-containing NADH and FADH₂

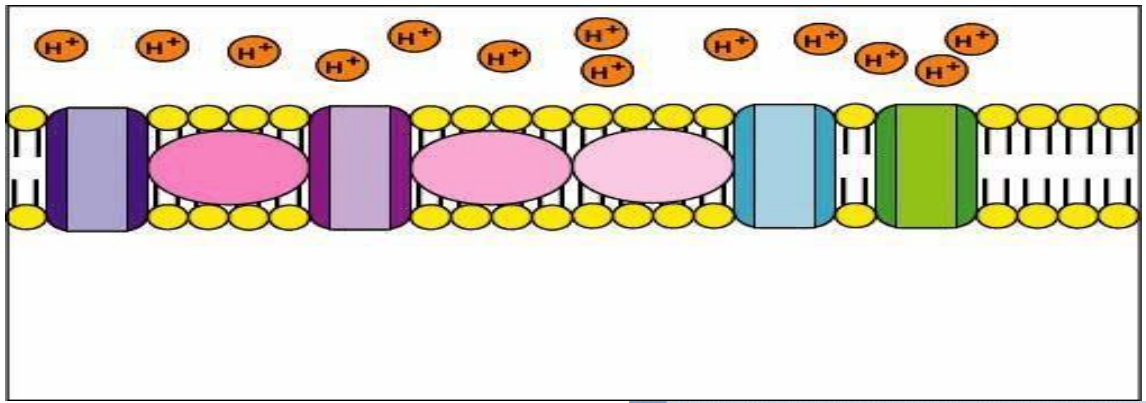


The Krebs Cycle



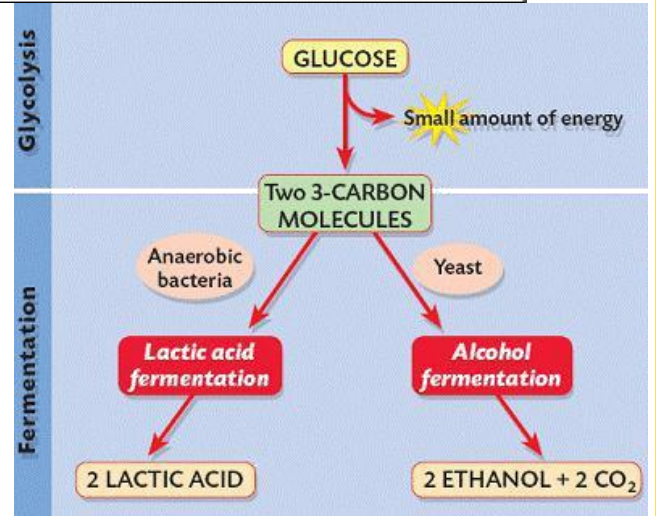
Electron Transport Chain

- NADH and FADH₂ are oxidized through a series of electron carriers to produce ATP



Fermentation

- Starts with pyruvic acid and has no Krebs cycle or electron transport chain for production of ATP.
 - ATP yield is low because energy only comes from glycolysis step.
- Does not require or use O₂.



Summary

- Glucose: Primary energy source for microbes
- Aerobic respiration:
 - Glycolysis
 - Krebs cycle
 - Electron Transport Chain
- Fermentation
 - No O₂ needed

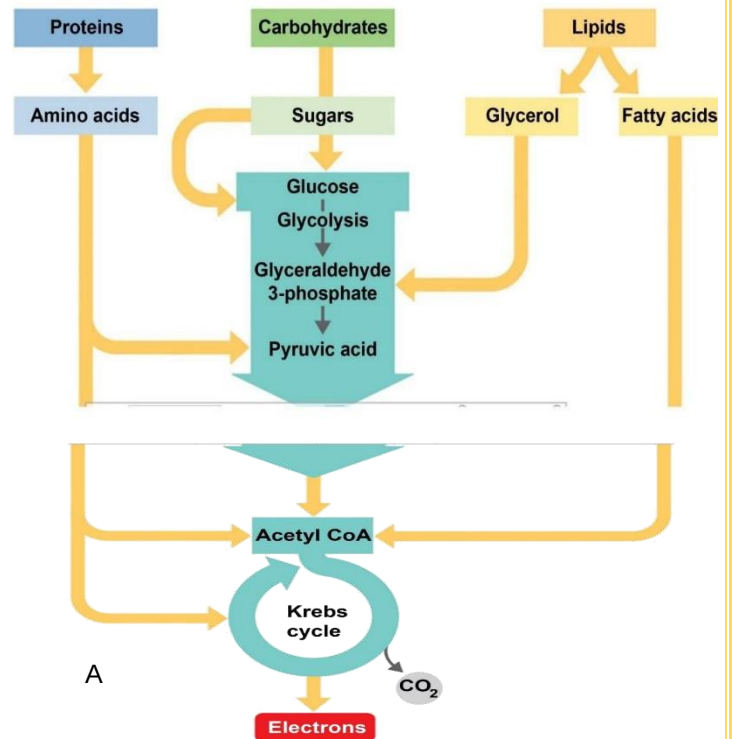
Topic 26 Microbial Metabolism 3

Learning Objectives

- Anaerobic Respiration
- How lipids and proteins are metabolized
- Biochemical testing for microbial identification
- Nutritional classification of bacteria

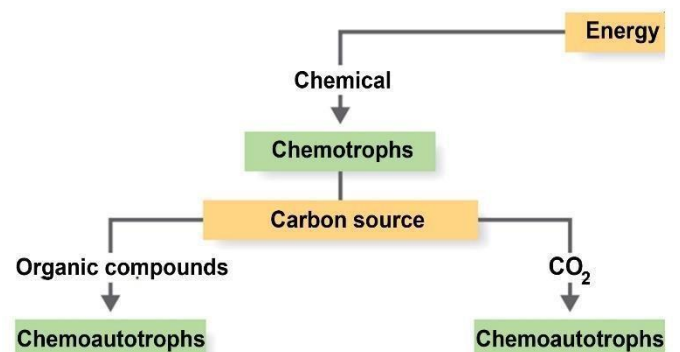
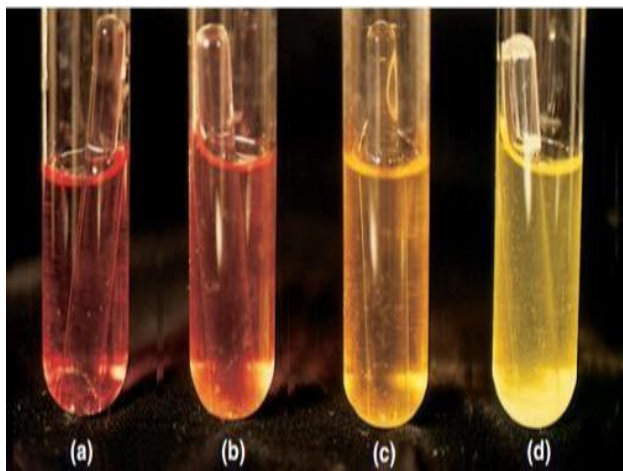
Anaerobic Respiration

- Final electron acceptor is inorganic substance other than O₂.
 - Nitrate ion
Nitrogen fixing bact
 - Sulfate ion
H₂S forming bact
 - Carbonate ion CH₄ forming bact



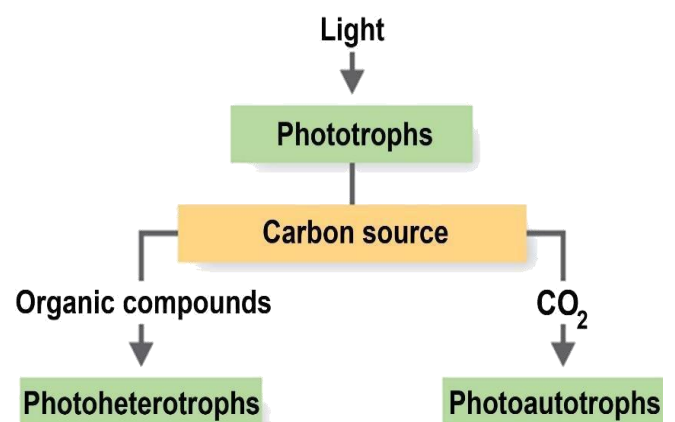
Fermentation Test for Bacterial Identification

Nutritional Classification of Bacteria



Photoautotrophs

- Use light as a source of energy
- CO₂ as a source of Carbon
- Hydrogen atoms of water are used to reduce CO₂



Photoautotrophs

Oxygen is given off (Oxygenic)

- Photosynthetic bacteria
 - Algae
 - Green plants
- ✓ Green bacteria: Non-oxygenic
 - ✓ Do not use hydrogen from water
 - ✓ Use hydrogen from H₂S or H₂ gas to reduce CO₂
 - ✓ Need total anaerobic conditions
 - ✓ Use light as a source of energy

- ✓ Use organic compounds as Carbon source

- Alcohol
 - Fatty acids
 - Carbohydrates etc.
- They are anoxygenic.
 - *Rhodopseudomonas*

Chemoautotrophs

- Use electrons from inorganic compounds as energy source
 1. H₂S
 2. Sulfur
 3. Ammonia
 4. H₂
- CO₂ as carbon source
 - *Thiobacillus*
- Energy source and carbon source are usually the same organic compound
 - Glucose
- Typically, use hydrogen atoms of organic compounds as electron source
 - Disease causing microbes

Summary

- Anaerobic respiration
 - O₂ is not electron acceptor
- Lipids and proteins can enter at various points in the glycolysis and Krebs
- cycle Microbes classified on the basis of nutritional requirements

Topic 27 Microbial Growth 1

Learning Objectives

- Requirements for bacterial growth
 - List of requirements
 - Classification of bacteria based on temperature
 - Food preservation Temp

Growth Requirements

Physical:

1. Temperature

2. pH
3. Osmotic Pressure

Chemical:

1. Source of C
2. N, S, P, O,
3. Trace elements
4. Growth factors

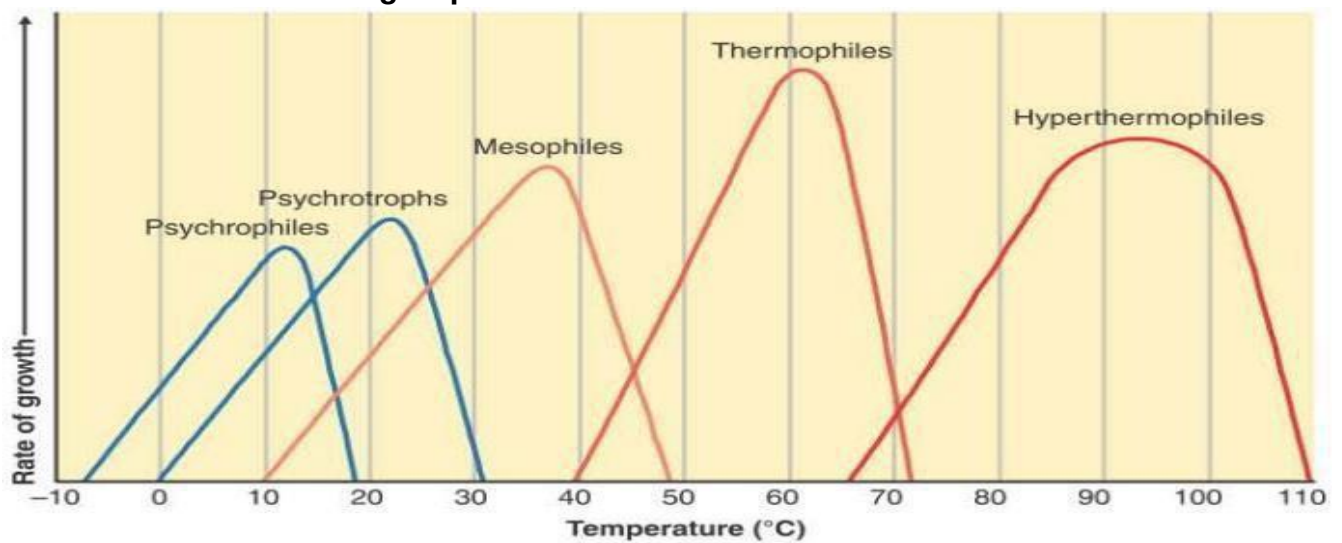
Classification of Bacteria

- Psychrophiles:
 - Cold loving: 15 °C
- Mesophiles: 25 – 40 °C
 - Moderate temp loving
 - Optimum: 37 °C
- **Thermophiles: 50 – 60 °C**
 - Heat loving
 - Important in organic compost piles

Temperature

- Every group has:
- Minimum growth temp
- Optimum G. T.
- Maximum G. T.
- Min and max temp: 30°C apart

Growth Rates of various groups of bacteria



Psychrotrophs

- Grow best at 20 – 30 °C
- Can grow at refrigeration temp
- Spoil foods

Hyperthermophiles

- Optimum temp: 80 °C
- Archaea in nature
- Live in hot springs

Summary

- Physical requirements
- Chemical requirements
- Classification based on temperature
- Food spoilage occurs between 5 to 60 °C

Topic-028 Microbial Growth II

Learning Objectives

- pH requirements for growth
- Osmotic pressure and microbial growth

pH (-log of H⁺)

- Acidity: < 7
 - Alkalinity: > 7
- Enzymes need certain pH for optimal function.
- Most grow at pH between 6.5 and 7.5

Effect of pH

- Very few grow below pH 4
 - Pickles and
 - Many Cheeses not spoiled
 - Acid is produced by bacteria during fermentation
 - **Acidophiles**
 - Molds and yeast grow
- At pH of 5 to 6
- **Acidophiles**
 - Alkalinity also inhibits microbial growth
 - Rarely used for preservation

Buffers in the culture

- Bacteria grown in lab produce acids
- Growth inhibited by acids

- Buffers are used to maintain desired pH

Peptones and amino acids

PBS

Osmotic Pressure

- Isotonic solutions
- Hypertonic solutions
 - Plasmolysis
 - Food preservation
- Hypotonic solutions
 - Lysis of cells
- **Extreme halophiles**
 - Require high salt concentrations for growth
 - Obligate halophiles
 - 30 % salt
- **Extreme halophiles**
 - Require high salt concentrations for growth
 - Facultative halophiles
- 2% Salt

Summary

- Most bacteria grow at 6.5 to 7.5 pH.
- Buffers are used to maintain pH in medium.
- Osmotic pressure is necessary for integrity of microbes.

Topic 29 Microbial Growth 3

Learning Objectives

- Various elements needed for microbial growth

Carbon

- Besides water, C is the most important requirement
- Structural backbone for living matter
- All organic compounds have it.
- Half the dry wt of microbes is C.
- Chemoheterotrophs Use C from energy sources

Proteins, carbs, and lipids

- Chemoautotrophs and photoautotrophs

Use CO₂

N, S, and P

- Nitrogen and Sulfur needed for protein synthesis
- Nitrogen and phosphorus needed for DNA and RNA synthesis and ATP

How bacteria get N

- Decomposing proteins
- NH₄⁺ ions
- Nitrates
- Gaseous N₂
- Nitrogen fixation

Sulfur

- Sulfate ions
- Hydrogen sulfide
- Sulfur containing amino acids

Phosphorus & other

- Phosphate ions
- Trace elements:
 - Usually not added in the medium
 - Assumed to be present in the ingredients used in the medium

Summary

- Carbon is the most abundantly needed requirement
- Nitrogen needed for protein and nucleic acids
- Sulfur and phosphorus needed for nucleic acids
- Trace elements too

Topic 30 Microbial Growth 4

Learning Objectives

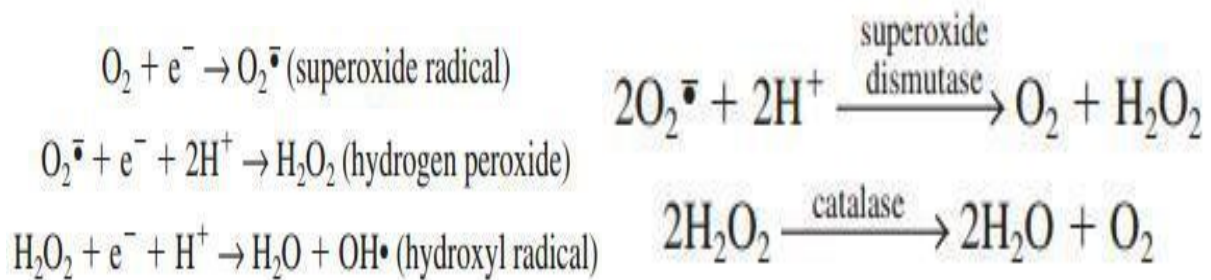
- Oxygen, a requirement or not a requirement
- Microbial classification on the basis of oxygen requirement for growth
- How microbes deal with O₂ radicals

Use of Oxygen

- Needed for aerobic respiration
- Use of O₂ means more ATP
- NADH and FADH₂ during Krebs cycle oxidized via ETC and generate 36 or 38 ATP molecules
- Obligate Aerobes
- Need O₂ for growth
- Mycobacterium tuberculosis
- Facultative Anaerobes:
- E. coli

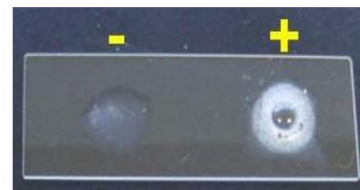


O₂ is harmful



Catalase Test

- A drop of H₂O₂ mixed with the culture



Use of Oxygen

- **Obligate Anaerobes:**

- Do not grow in the presence of O₂

- **Aerotolerant aerobes:**

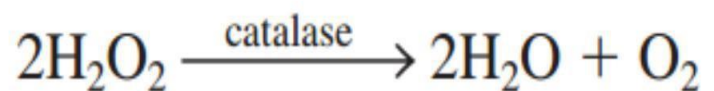
- Do not use O₂ but can tolerate it fairly well.
- O₂ has no effect.
- Growth evenly throughout

Helicobacter pylori

- **Microaerophiles:**

- Aerobes but O₂ is required in low amounts.
- Produce O₂ toxic compounds if exposed to more O₂

Listeria, Vibrio



Summary

- Oxygen usage provides more energy (ATP)
- Anaerobes do not have SOD, so cannot grow in the presence of O₂
- Organisms classified on the basis of O₂ requirement.

Topic 31 Microbial Growth 5

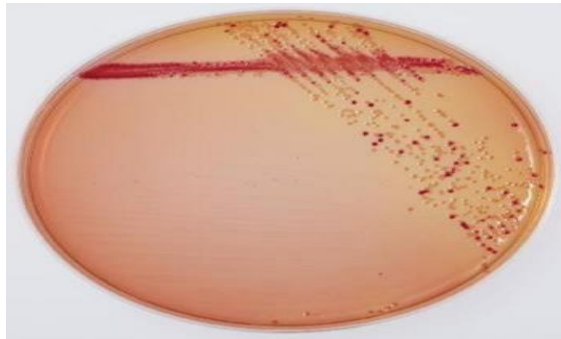
Learning Objectives

- What is a culture and culture medium
- What is needed to grow bacteria in lab?
- Uses of solid medium
- Colony characters

Culture Media

- Culture medium: A nutrient material for growth
- Some grow easily, other do not grow at all.
- Inoculum: Microbes introduced into a culture medium that initiate growth

- Culture: Microbes that grow or multiply in a culture medium

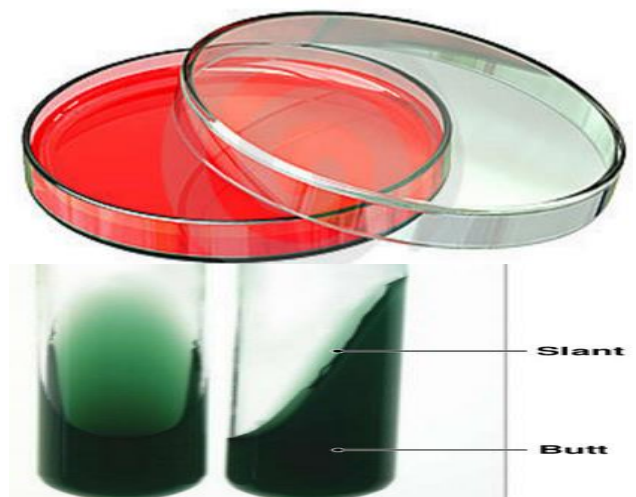


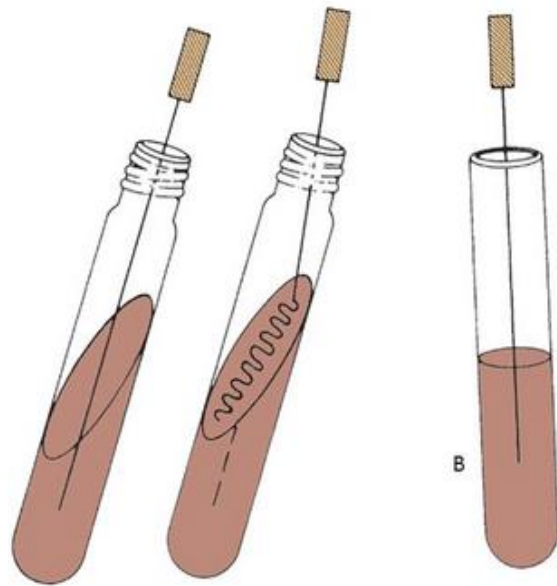
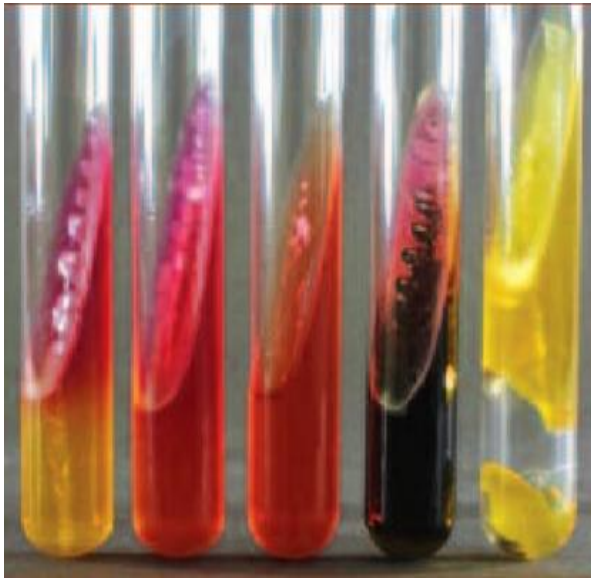
What is needed to grow an organism in culture?

- Right nutrients for the type of microbe
 - Carbohydrates, proteins, minerals, vitamins, water and oxygen or absence of oxygen etc.
- Incubation at right temperature, pH, and osmotic pressure
- Medium may be fluid or solid in nature.
- Sterility of the medium is important.

Agar for solidification

- Derived from a sea weed
- Complex carb, not used by bacteria
- Liquefies at 100 °C and solidifies at 40 °C
 - Petri plates
 - Slants



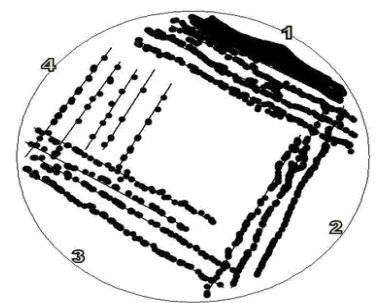


Butt

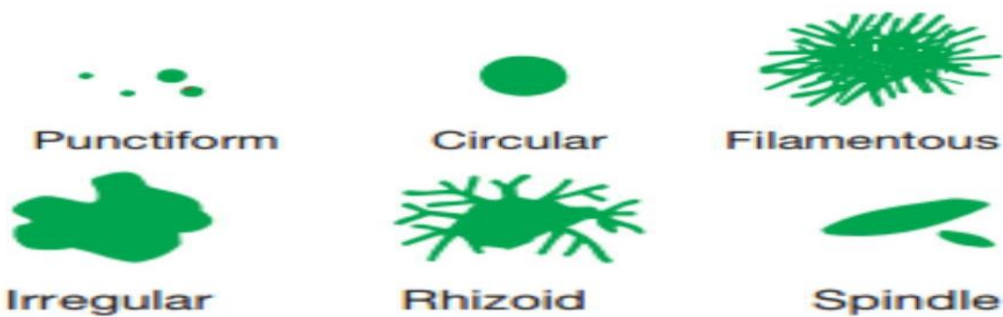
deep

Why a solid medium?

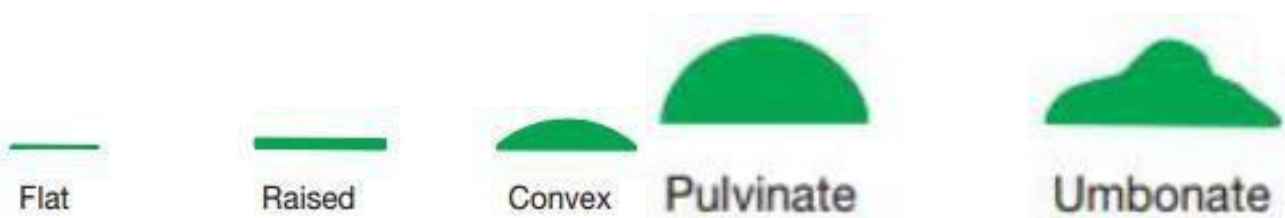
- Pure culture is easy to obtain by streaking the organisms
- Colony characters can be studied



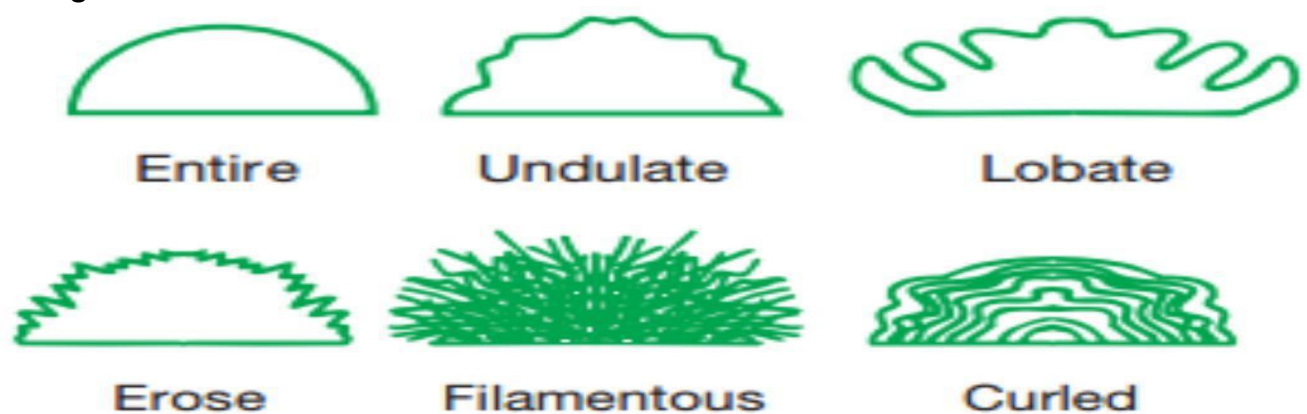
Forms of Bacterial Colonies



Elevations of Bacterial Colonies



Margins of Bacterial Colonies



Summary

- Inoculum
- Culture
- Culture medium
- Colony characters – Form
 - Elevation
 - Margins

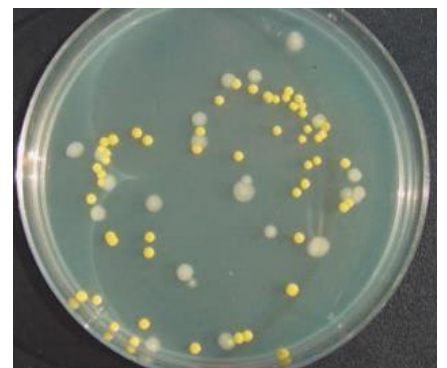
Topic 32 Microbial Growth 6

Learning Objectives

- Classification of culture media
- Pure culture
- Pure culturing techniques

Classification of Media

- **Chemical composition**
 - Defined (synthetic)
If we know the organism
 - Complex
When organism is not known or is fastidious
- **Physical Nature**
 - Solid
 - Liquid
- **Function**
 - General purpose
 - Selective
 - Enrichment
 - Differential



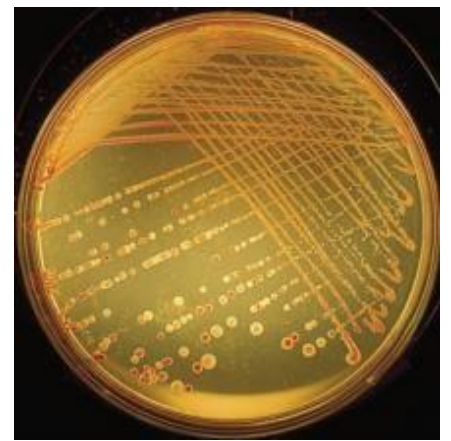
Blood Agar

- Nutrient agar + blood
- Enriched and differential
- Used for fastidious organisms
- Differential: hemolysin
 - β -hemolysis: clear zone
 - α -hemolysis: greenish halo



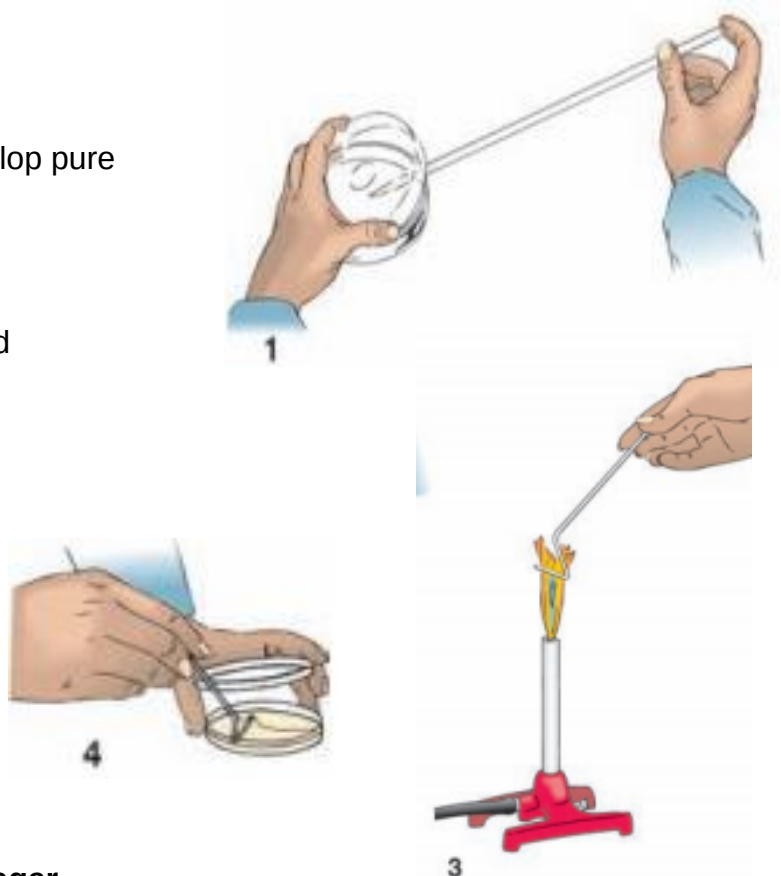
MacConkey Agar

- Nutrient agar + Bile salt + CV
- Selective and differential
- Used for gut bacteria
- Differential: lactose
 - Neutral red, a pH indicator
 - Lactose fermenter as red: E. coli



Pure Culture

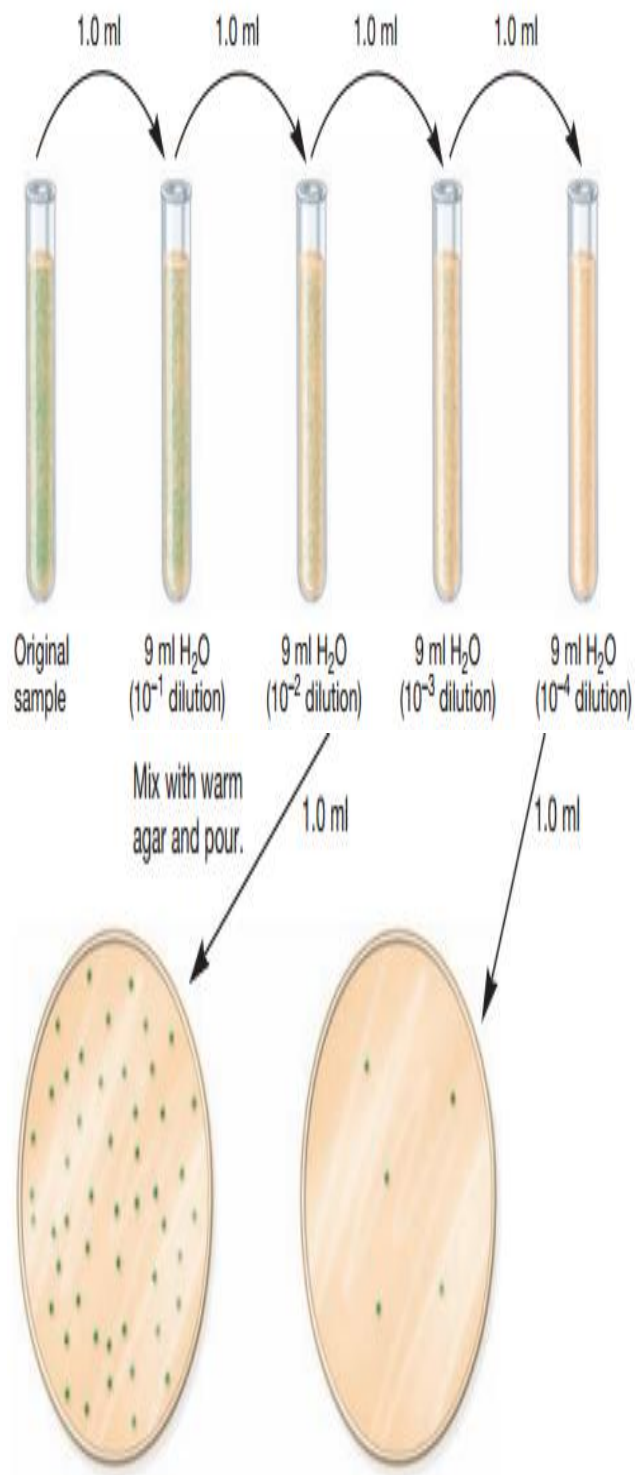
- A progeny of single cell
- Robert Koch was the first to develop pure culturing technique.
- Streak Plate method
 - Essentially a dilution method
- Spread plate and pour plate
- Sample is diluted before spreading
- Spread Plate method
 - On the surface



Pour Plate Method

- Embeds the cells in the agar

30 to 300 colonies



Summary

- Defined and complex media
- General purpose media
- Enriched media
- Selective media
- Differential media
- Culture purifying techniques

Topic-033 Microbial Growth VII

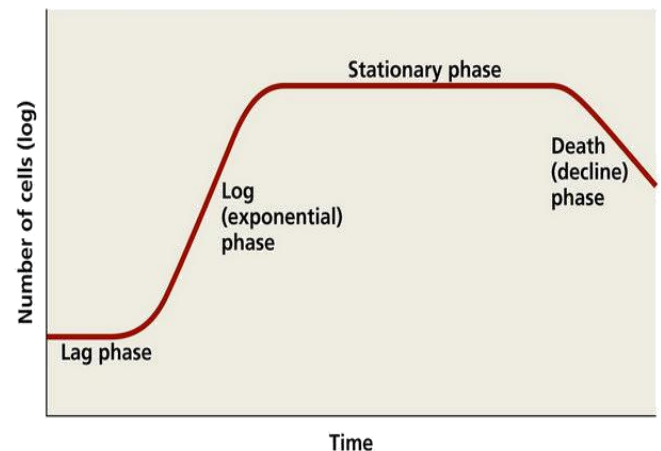
Learning Objectives

- What is growth curve
- Batch culture
- Uses of growth curve
- Generation time



Growth Curve

- Batch Culture
 - Liquid medium in a closed vessel
 - No fresh medium
- Nutrients depleted
- Waste products increased



- Generation time
- Doubling time
- Can be calculated from the exponential phase of growth

$$k = \frac{\log 10^9 - \log 10^3}{(0.301)(10 \text{ hr})} = \frac{9 - 3}{3.01 \text{ hr}} = 2.0 \text{ generations/hr}$$

$$g = \frac{1}{2.0 \text{ gen. hr}} = 0.5 \text{ hr/gen. or } 30 \text{ min/gen.}$$

Uses of Growth Curve

- Growth dynamic of any bacteria can be studied
- Exponential phase is ensured for mass production of microbes
 - Vaccine production
 - Seed for bacteria
 - Culture preservation

Summary

- Batch culture
- Growth curve
- Uses of growth curve
- Generation time

Topic 34 Microbial Growth 8

Learning Objectives

- How to estimate or enumerate microbial number or growth

Microbial Growth

- Measurement of Microbial Growth

– Direct counts

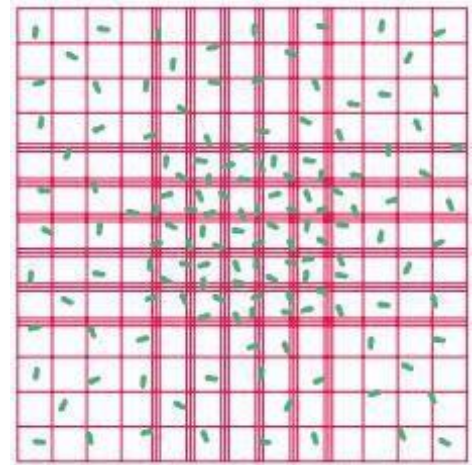
Counting chamber

Coulter Counter

Flow cytometer

Membrane filter technique for water samples

– Fluorescent dye



Microbial Growth

- Membrane filter technique

- Viable counting method

– Spread plate

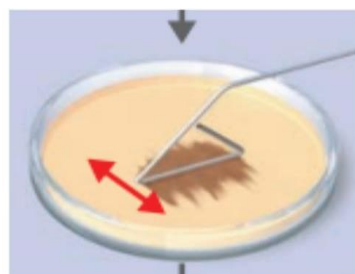
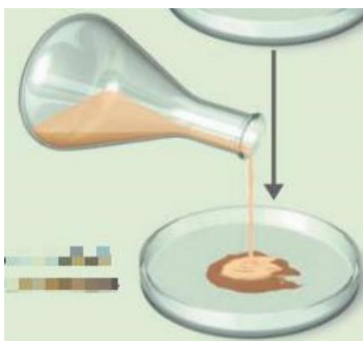
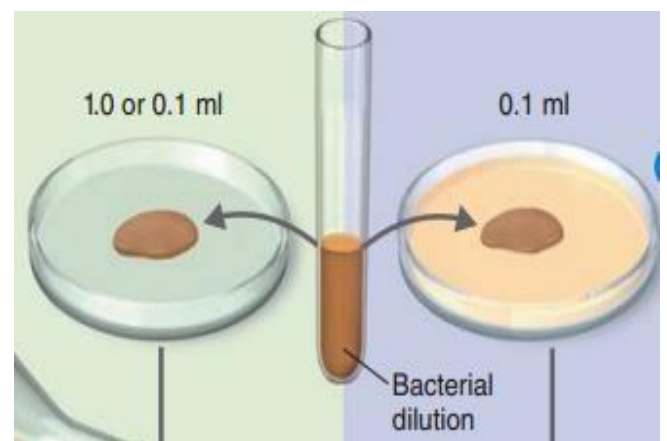
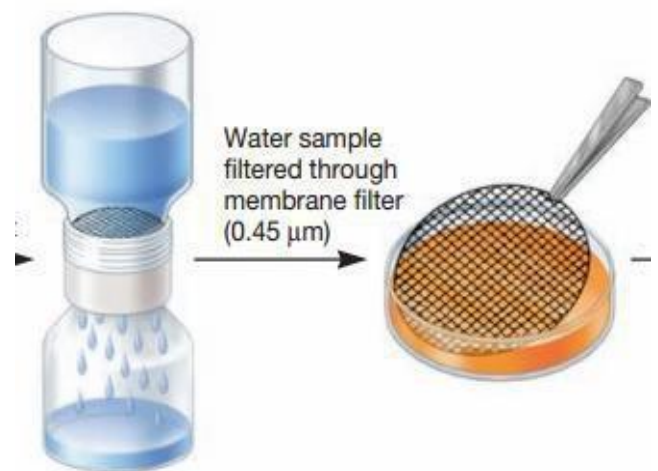
– Pour plate

- Pour plate and spread methods

- Pour plate and spread methods

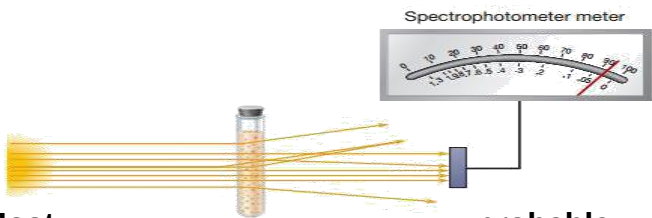
- Viable counting method

– Spread plate with serial dilutions



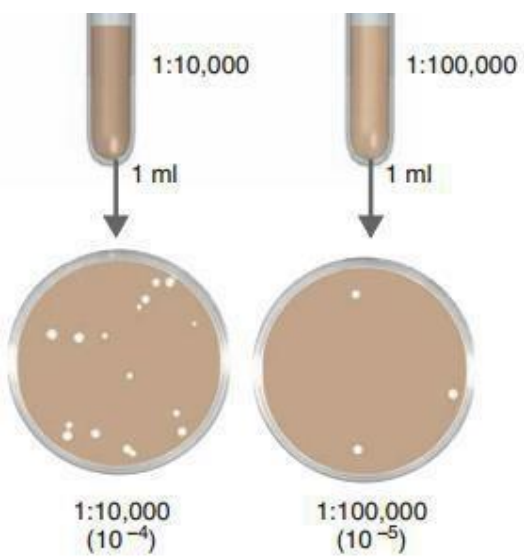
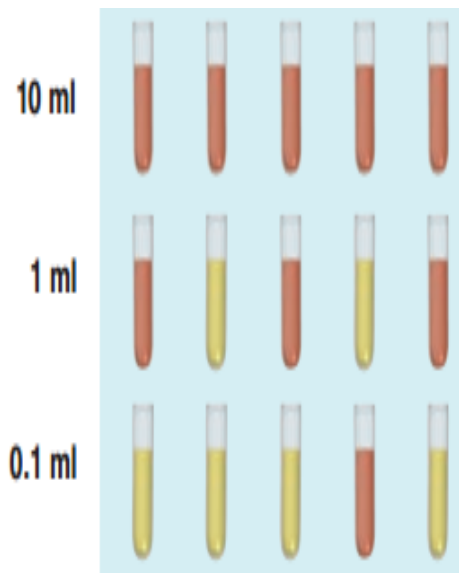
• **Measurement of cell mass – Dry weight**

– Spectrophotometry



• **Most probable number method**

– Statistical estimating technique



Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

Summary

- Direct methods for microbial count
- Indirect methods – Dry mass
 - Spectrophotometer
 - Most probable number

Topic-035 Microbial Control I

Learning Objectives

- Terms used for microbial controls and their meaning

Terminology

- Sepsis: Microbial contamination
- Asepsis: Absence of significant contamination
- Aseptic surgery techniques prevent microbial contamination of wounds
- Sterilization: Removing all microbial life including spores
- Disinfection: Removing pathogens significantly
- Antisepsis: Removing pathogens from living tissue
- Degerming: Removing microbes from a limited area (injection site)
- Sanitization: Lowering microbial counts on eating utensils
- Biocide/germicide: Kills microbes
- Bacteriostasis: Inhibiting, not killing, microbes
- Chemotherapy: killing microbes within the host tissue

Summary

- **Sterilization**
- **Disinfection**

Topic-036 Microbial Growth II

Learning Objectives

- pH requirements for growth
- Osmotic pressure and microbial growth

pH (-log of H⁺)

- Acidity:

< 7

- Alkalinity:
 > 7
- Enzymes need certain pH for optimal function.
- Most grow at pH between 6.5 and 7.5

Effect of pH

- Very few grow below pH 4
 - Pickles and
 - Many Cheeses not spoiled

Acid is produced by bacteria during fermentation

- **Acidophiles**
 - Molds and yeast grow
 At pH of 5 to 6
- **Acidophiles**
- Alkalinity also inhibits microbial growth
 - Rarely used for preservation

Buffers in the culture

- Bacteria grown in lab produce acids
- Growth inhibited by acids
- Buffers are used to maintain desired pH
 - Peptones and amino acids
 - PBS

Osmotic Pressure

- Isotonic solutions
- Hypertonic solutions
 - Plasmolysis
 - Food preservation
- Hypotonic solutions
 - Lysis of cells
- **Extreme halophiles**
 - Require high salt concentrations for growth

- Obligate halophiles

30 % salt

- **Extreme halophiles**

- Require high salt concentrations for growth
- Facultative halophiles

2% Salt

Summary

- Most bacteria grow at 6.5 to 7.5 pH.
- Buffers are used to maintain pH in medium.
- Osmotic pressure is necessary for integrity of microbes.

TOPIC 37 Microbial Control III

❖ Various methods for microbial control

- Physical
- Mechanical
- Chemical
- Biological

❖ Physical

- Heat
- Radiation

❖ Dry Heat

- Incineration
- Dry oven
- **Heat**

Microbial Control III

Heat

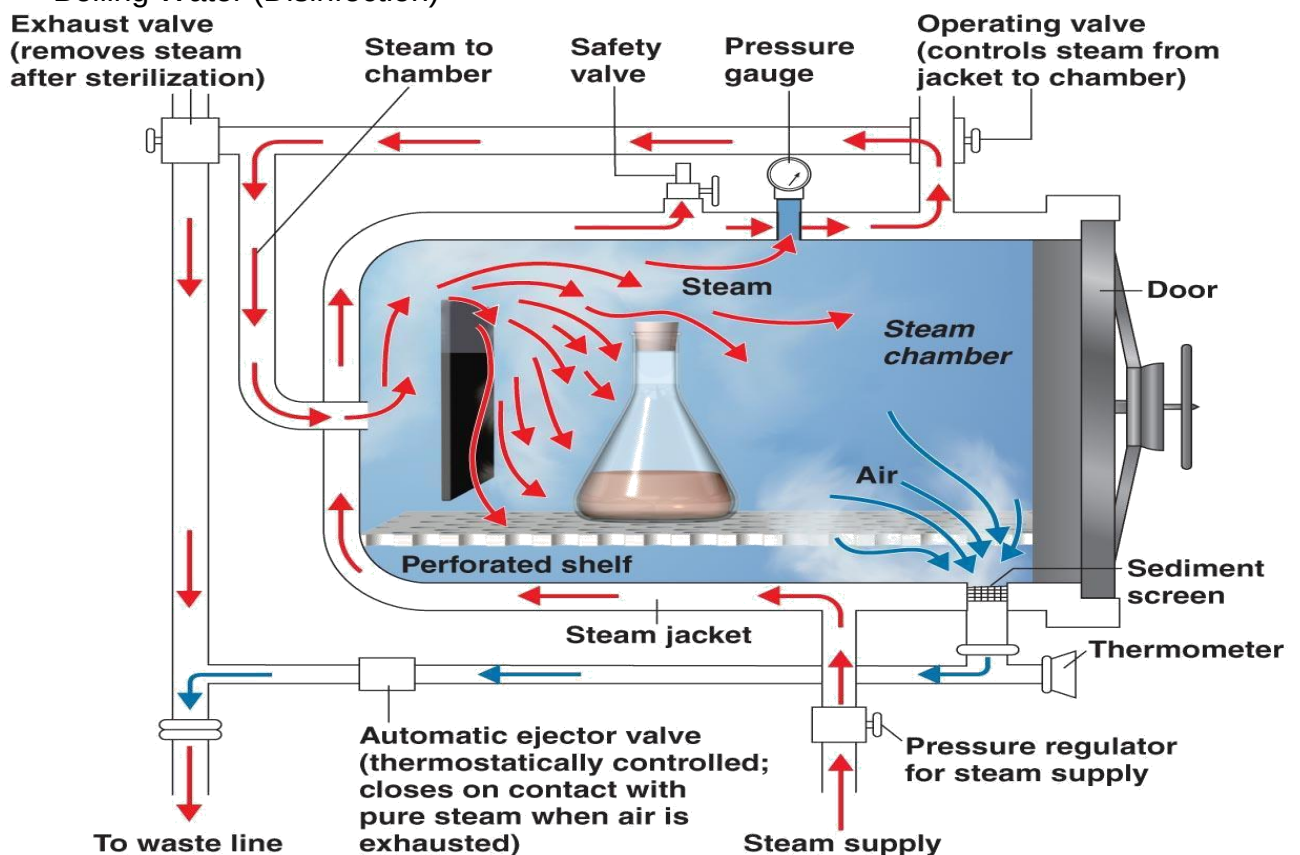
- Kills by oxidation
 - Dry heat
 - Flaming
 - Incineration
 - Hot-air sterilization

	Hot-Air	Autoclave
Equivalent Treatments	170°C, 2 hr	121°C, 15 min

- Moist Heat

Autoclaving

Boiling Water (Disinfection)



Steam must contact item's surface

- Reduces spoilage organisms and pathogens

❖ Equivalent treatments

- 63°C for 30 min
- High-temperature short-time: 72°C for 15 sec
- Ultra-high-temperature: 140°C for <1 sec
- Thermoduric organisms survive

- Physical methods for control of microbial population

- Dry Heat

- Moist Heat
- Low temp
- High pressure
- Desiccation

Other physical methods include

- Low temperature inhibits microbial growth
- Refrigeration
- Deep-freezing
- Lyophilization
- High pressure denatures proteins
- Desiccation prevents metabolism
- Osmotic pressure causes plasmolysis

TOPIC 38 Microbial Control IV

- Physical methods

– Radiation

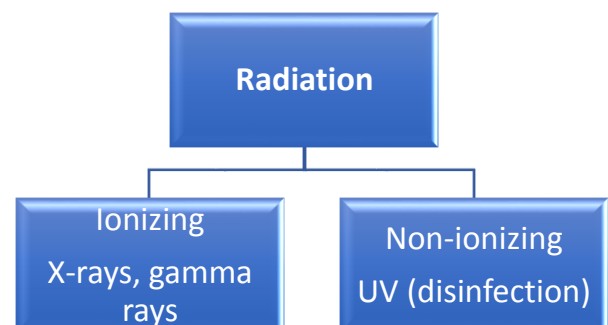
Ionizing

Nonionizin

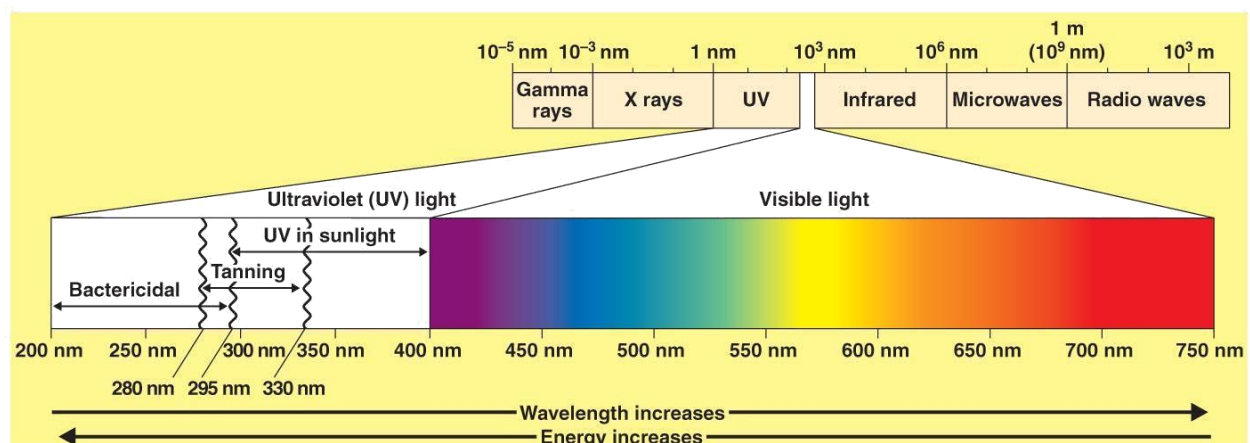
g

Mechanical Methods

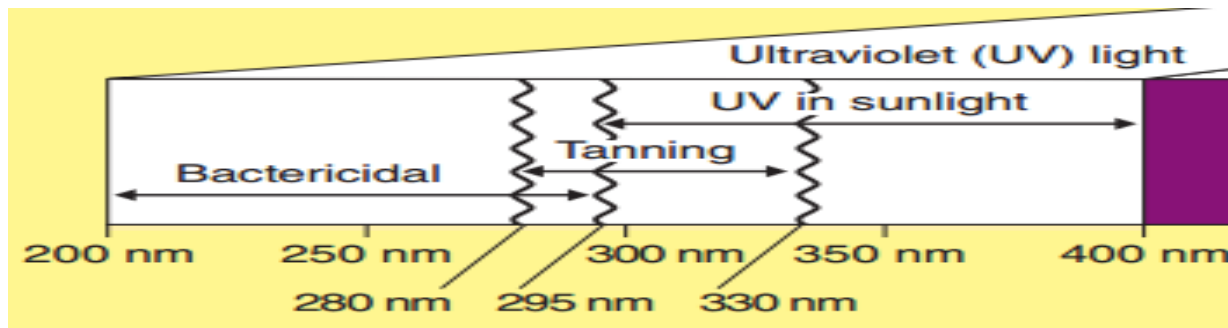
– Filtration



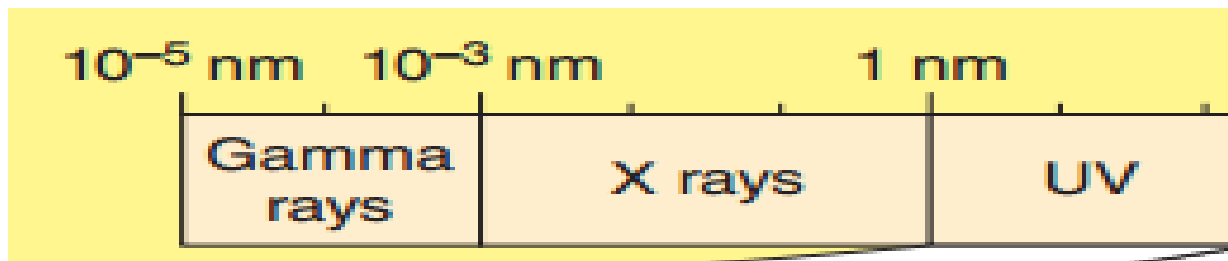
Radiation:



UV Radiation



Ionizing Radiation



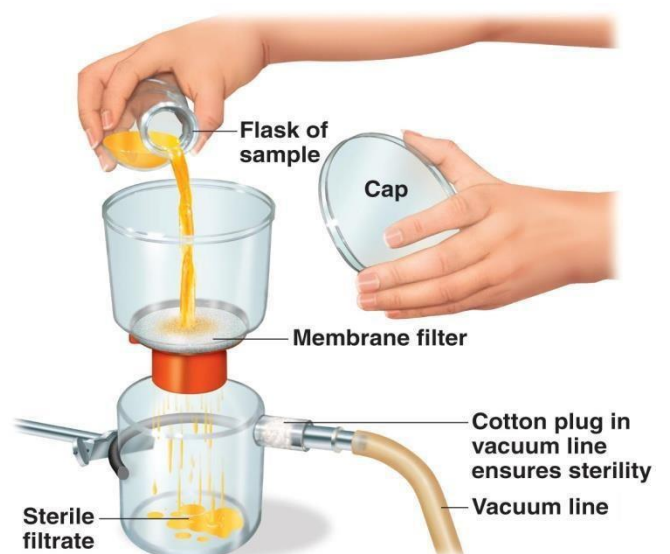
- Mechanical removal includes Filtration
 1. Air
 2. Liquid

Filtration

- HEPA removes microbes $>0.3 \mu\text{m}$
- Membrane filtration removes microbes $>0.22 \mu\text{m}$

In what situation is filtration the only practical way to eliminate undesirable microbes?

- Radiation
 - Ionizing
 - Non-ionizing
- Filtration
 - Hepa filter
 - Membrane filter



TOPIC 39 Microbial Control V

Learning Objectives

- Use of gases for microbial control

Gas

Ethylene oxide

- Many heat-sensitive items such as
- Disposable plastic petri dishes and syringes

- Heart-lung machine components
- Sutures, and catheters
- Strong alkylating agent that kills by reacting with functional groups of DNA and proteins to block replication and enzymatic activity
- It rapidly penetrates packing materials, even plastic wraps.
- EtO is explosive, supplied in a 10 to 20% concentration mixed with either CO₂.
- Ethylene oxide
- Concentration
- Humidity
- Temperature influence the rate of sterilization
- A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/l.

Ethylene Oxide is used to sterilize heat sensitive inanimate objects.

TOPIC 40 Microbial Control VI

Learning Objectives

- Chemicals as antimicrobial agents

Chemical Methods

❖ Chemicals are used to control microbial growth

- Concentration of disinfectant
- Organic matter
- pH
- Time

• Ideal Chemical

Effective at low concentration

- Nontoxic to body tissues
- Effective in organic matter
- Broad spectrum of activity

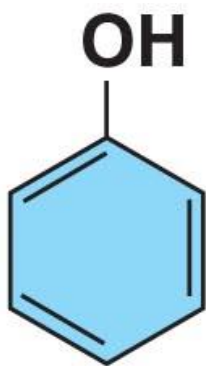
Phenol and Phenolics

First widely used antiseptics

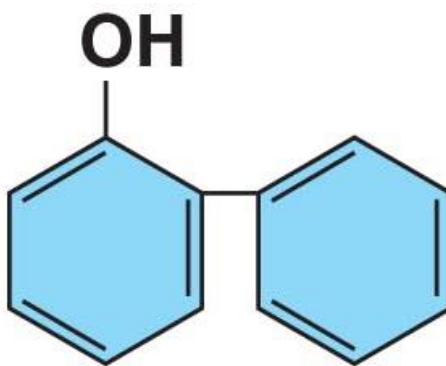
Disrupt plasma membranes

Effective in organic matter

Used for Pus, saliva, feces



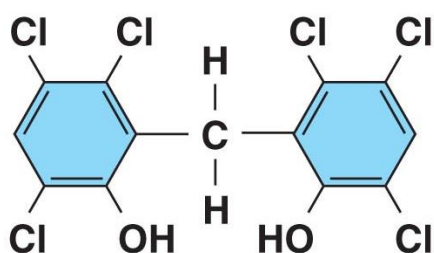
(a) Phenol



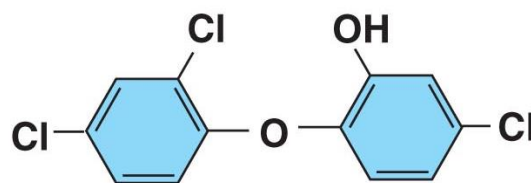
(b) O-phenylphenol

Bisphenols

- Hexachlorophene, triclosan
 - Disrupt plasma membranes (lipids)



(c) Hexachlorophene (a bisphenol)



(d) Triclosan (a bisphenol)

Biguanides

- Chlorhexidine
- Alexidine
 - Disrupt plasma membranes

Halogens

1) Iodine

- Tinctures: In aqueous alcohol
- Iodophors: In organic molecules
- Alter protein synthesis and membranes

❖ Chlorine

- Bleach: Hypochlorous acid (HOCl)
- Chloramine: Chlorine + ammonia
- Oxidizing agents
- Chemicals are used for disinfection or as antiseptics or for sterilization of inanimate objects.

TOPIC 41 Microbial Control VII

Learning Objectives

- Chemicals as antimicrobial agents

Alcohols

- Ethanol, isopropanol
 - Denature proteins, dissolve lipids
 - Require water
- 70% is more effective than 100%.

Heavy Metals

- Ag, Hg, and Cu
 - Silver nitrate may be used to prevent gonorrheal ophthalmia neonatorum
 - Silver sulfadiazine used as a topical cream on burns
 - Copper sulfate is an algicide
- Oligodynamic action
 - Denature proteins

Microbial Control VII
Surface-Active

Soap	Degerming
Acid-anionic detergents	Sanitizing
Quarternary ammonium compounds (cationic detergents)	Bactericidal, denature proteins, disrupt plasma membrane

Chemical Food Preservation

- Organic acids
- Inhibit metabolism
 - Sorbic acid, benzoic acid, and calcium propionate
 - Control molds and bacteria in foods and cosmetics
- Nitrite prevents endospore germination
 - Antibiotics
 - Nisin and natamycin prevent spoilage of cheese

Aldehydes

- Inactivate proteins by cross-linking with functional groups (–NH₂, –OH, –COOH, –SH)
- Use: Medical equipment

- Glutaraldehyde, formaldehyde, and ortho-phthalaldehyde .

A host of chemicals can be used for microbial control

Topic 42 Microbial Control 8

Learning Objectives

- Physical methods of microbial control and their mechanisms of action

Moist Heat

❖ Boiling

- Protein denaturation (PD)

❖ Autoclaving

- PD

❖ Pasteurization

- PD

Dry Heat

1) Direct flaming

- a) Burning to ashes

2) Incineration

- a) Burning to ashes

3) Hot air Sterilization

- a) Oxidation

Filtration

- Physical separation of bacteria from suspending liquid

High Pressure

- Alteration of molecular structure of proteins and carbohydrates

Desiccation

- Disruption of metabolism

Osmotic Pressure

- Plasmolysis

Radiation

- Ionizing
 - Destruction of DNA
- Nonionizing
 - Damage to DNA

TOPIC 43 Microbial Control IX

Learning Objectives

- Chemical methods of microbial control and their mechanisms of action

Phenol and Phenolics

- Disruption of plasma membrane
- Denaturation of enzymes
- Bisphenols
 - Disruption of PM

Biguanides

- Chlorhexidine
 - Disruption of PM

Halogens

- Iodine and chlorine
- Inhibit protein function
- Oxidizing agent
 - Alters cellular components

Heavy metals

- Protein denaturation

Surface Active Agents

- Soaps and detergents
 - Mechanical removal
- Acid-anionic sanitizers
 - May involve enzyme inactivation or disruption
- Quaternary ammonium compounds
- Quaternary ammonium compounds (cationic detergent)
 - Enzyme inhibition
 - Protein denaturation
 - Disruption of plasma membrane

Food Preservatives

- Organic acids

- Metabolic inhibition
- Nitrates/Nitrites
 - Inhibits iron containing enzymes of anareobes

Aldehydes

- Protein denaturation

Chemical Sterilization

- Ethylene Oxide
 - Inhibits vital cellular function
- Various mechanisms for microbial controls by chemical methods

TOPIC 44 Genetic Transfer I

Learning Objectives

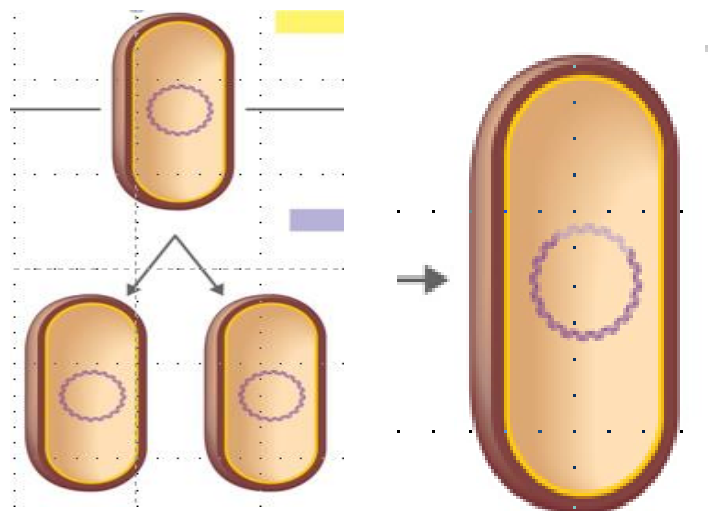
- ✓ How various genes are transferred from one microbe to another
- ✓ What is lateral gene transfer
- ✓ Types of lateral gene transfer
- ✓ Some definition

Definitions

- Genetics: The study of what genes are, how they carry information, how information is expressed, and how genes are replicated
- Gene: A segment of DNA that encodes a functional product, usually a protein
- Chromosome: Structure containing DNA that physically carries hereditary information; the chromosomes contain the genes
- Genome: All the genetic information in a cell
- Genomics: The molecular study of genomes
- Genotype: The genes of an organism
- Phenotype: Expression of the genes

Gene Transfer

- Vertical gene transfer
 - Parents to off springs
- ❖ Horizontal
 - From cells to cells within the same generation
 - From bacteria to bacteria
 - Donor cell
 - Recipient cell
 - Transformation
 - Conjugation

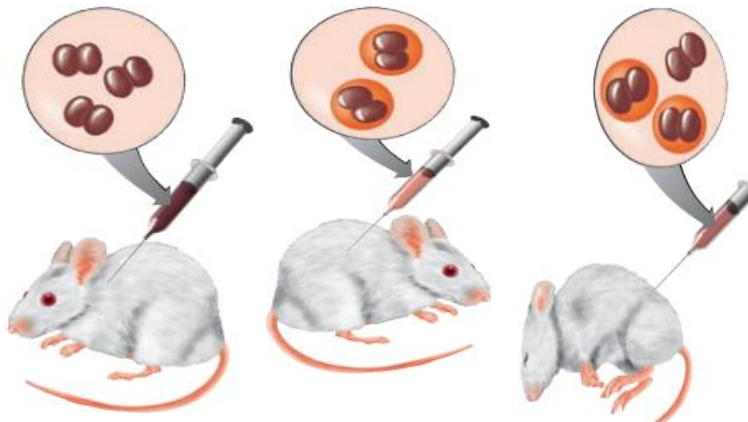


- Transduction

Transformation

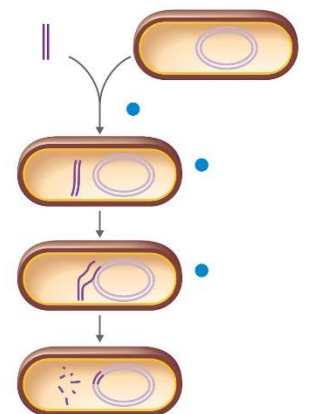
- Naked DNA is transferred
- Frederick Griffith observed this in 1928.
 - *Strep pneumoniae*

Gene Transfer



Transformation

- Transformation can occur in vitro
- In broth culture, live non-capsulated organisms cultured. Dead capsulated cells mixed with it. Capsulated bacteria were recovered from broth. Research revealed it to be DNA.
- **Competent cells: that take up the DNA**
- **Not all cells do it naturally.**



Transformation is uptake of naked DNA by microbes

TOPIC 45 Genetic Transfer II

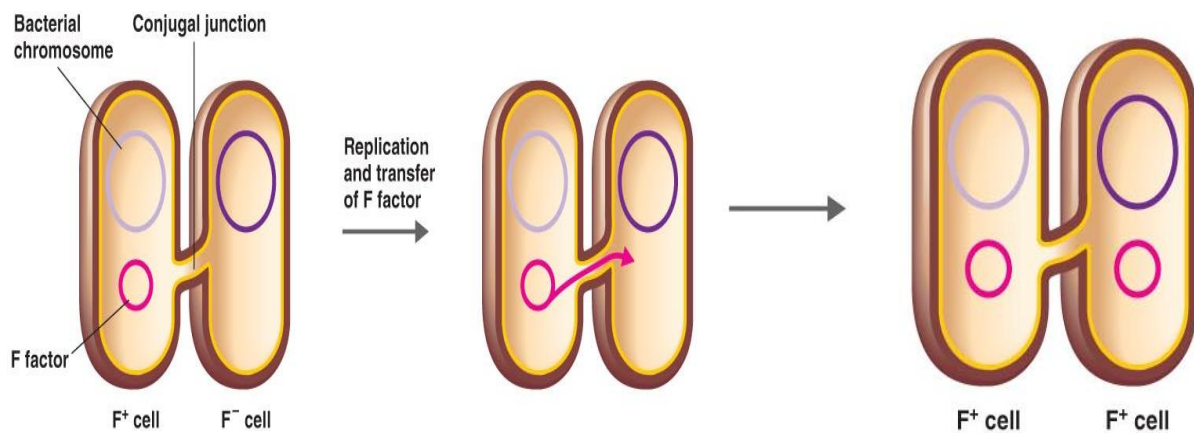
Learning Objectives

- What is conjugation

Conjugation

- Mediated by a plasmid
- Plasmid is an extra chromosomal circular DNA fragment. Replicates independently of chromosome. Genes of plasmids not important for bacterial growth.
- Requires direct cell to cell contact. Opposite mating types cells. Plasmids in G – ive cells have genes that code for sex pili. G +ve secrete sticky surface molecules
- These plasmids are transmissible from one bacterium to another during conjugation.
- Plasmid is replicated and a single copy of the plasmid is transferred to the recipient bacterium.
- Complimentary copy is then made.
- Fertility factor carrying plasmid was the first plasmid studied (F).





Conjugation is mediated by sex pili and plasmids or F factors can be transferred.

TOPIC 46 Genetic Transfer III

Learning Objectives

- High frequency recombination cells (Hfr)

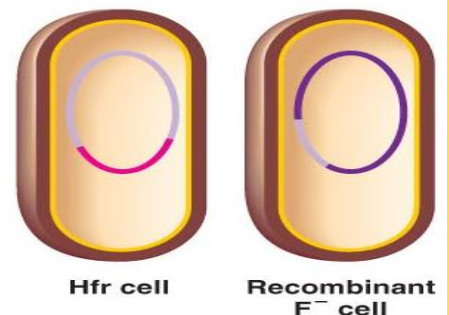
Hfr

- If F factor gets integrated into the chromosome of the recipient cells, the bacterium becomes high frequency of recombination cell (Hfr).



High Frequency Recombination

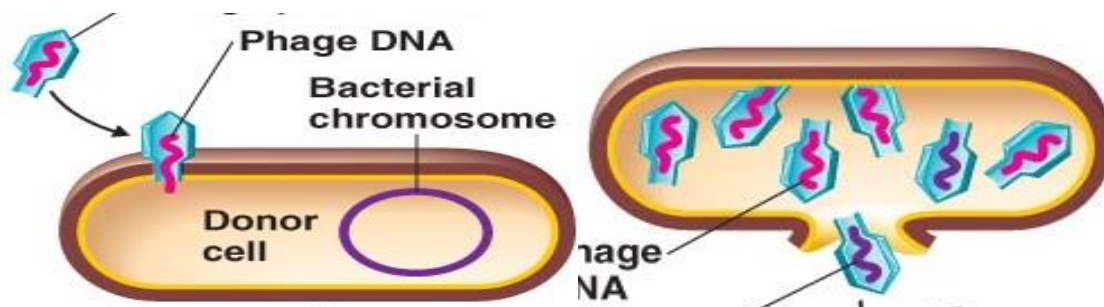
- Conjugation can be used for gene mapping on the chromosomes.
- conjugation_mapping.html
- High frequency of recombination is also a kind of conjugation.
- Can be used for mapping genes.



TOPIC 47 Genetic Transfer IV

Transduction

- Transfer of DNA by a virus called bacteriophages.



Phages can transfer DNA and the process is transduction

- Generalized
- Specialized

TOPIC 48 Classification I

Learning Objectives

- Some definitions
- Three domain system
- Taxonomic hierarchy
- Bacterial identification

Some Definitions

- **Taxonomy:** The science of classifying organisms (orderly arrangements)
- **Systematics, or Phylogeny:** The study of the evolutionary history of organisms
- **Clone:** Population of cells derived from a single cell
- **Strain:** Genetically different cells within a clone

History

- ❖ From the time of Aristotle: living organisms had two groups (plants and animals).
- ❖ Carolus Linnaeus: 1735
 - Two kingdoms
 - Plantae and Animalia
- ❖ Carl von Nägeli, 1859
 - Bacteria and fungi in kingdom Plantae
- ❖ Ernst Haeckel, 1866
 - Proposed Kingdom Protista for bacteria, protozoa, algae, and fungi.
- ❖ 1959: Fungi placed in their own kingdom
- ❖ Edouard Chatton, 1937
 - Introduced the term prokaryotes
- ❖ Robert G.E. Murray, 1968:
- ❖ Proposed kingdom Prokaryotae

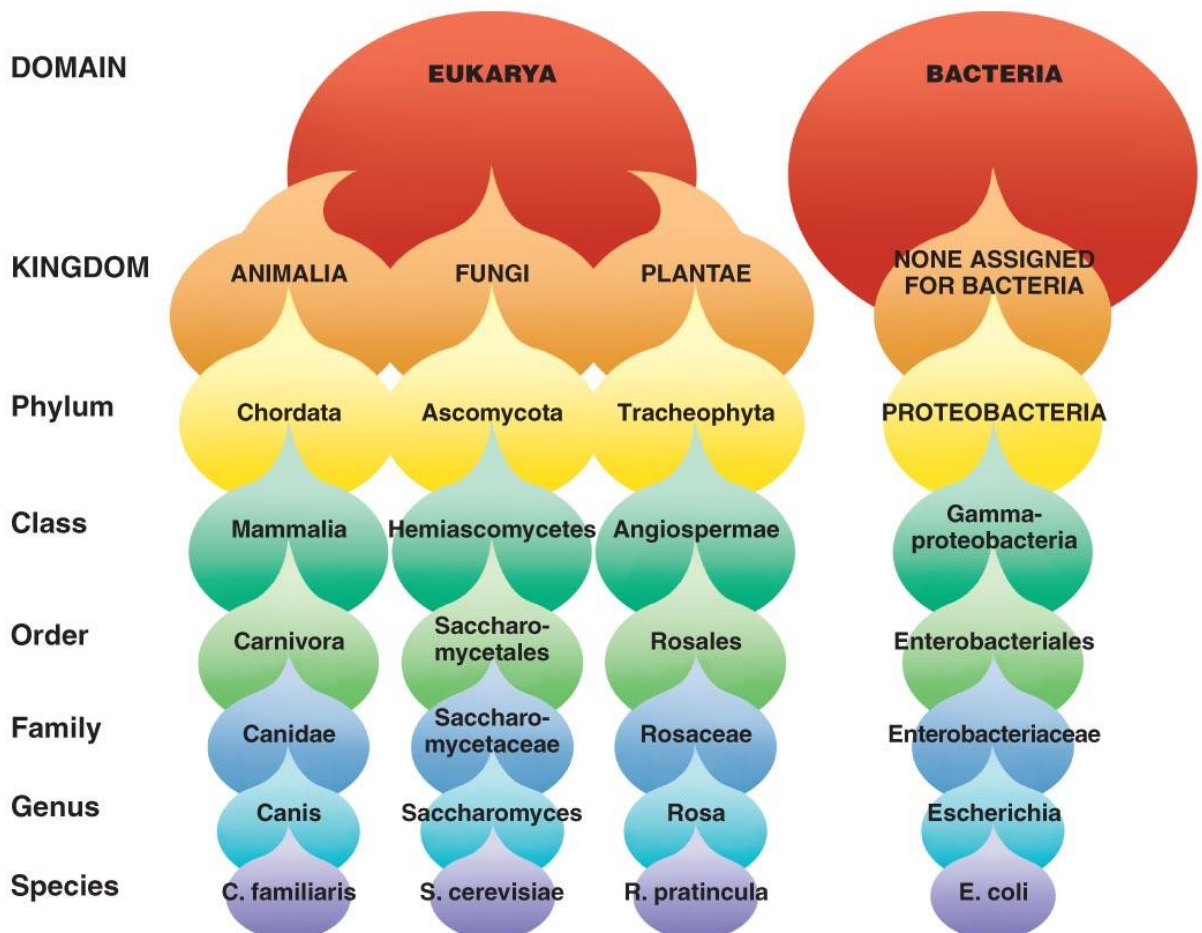
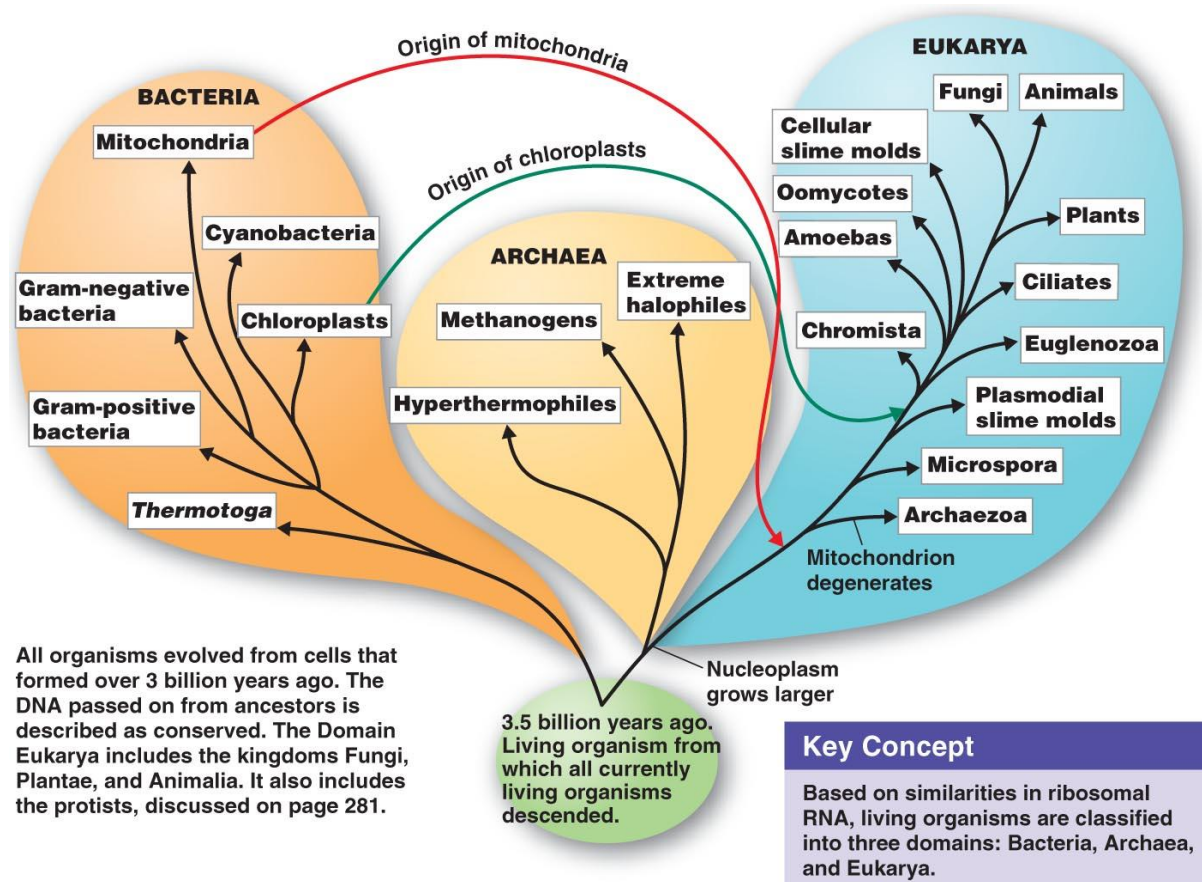
Robert H. Whittaker, 1969:

- 5 kingdom system
 - Animalia
 - Plantae
 - Fungi
 - Monera
 - Protista

Three Domain System

- ❖ Carl R. Woese, 1978
Based on ribosomal differences
- ❖ Prokaryotes:

- Archaea
- Bacteria
- ❖ Eukarya
 - Animals, plant, fungi
- ❖ All Species Inventory (2001–2025)
 - To identify all species of life on Earth (10 – 100 M)



Taxonomic Hierarchy

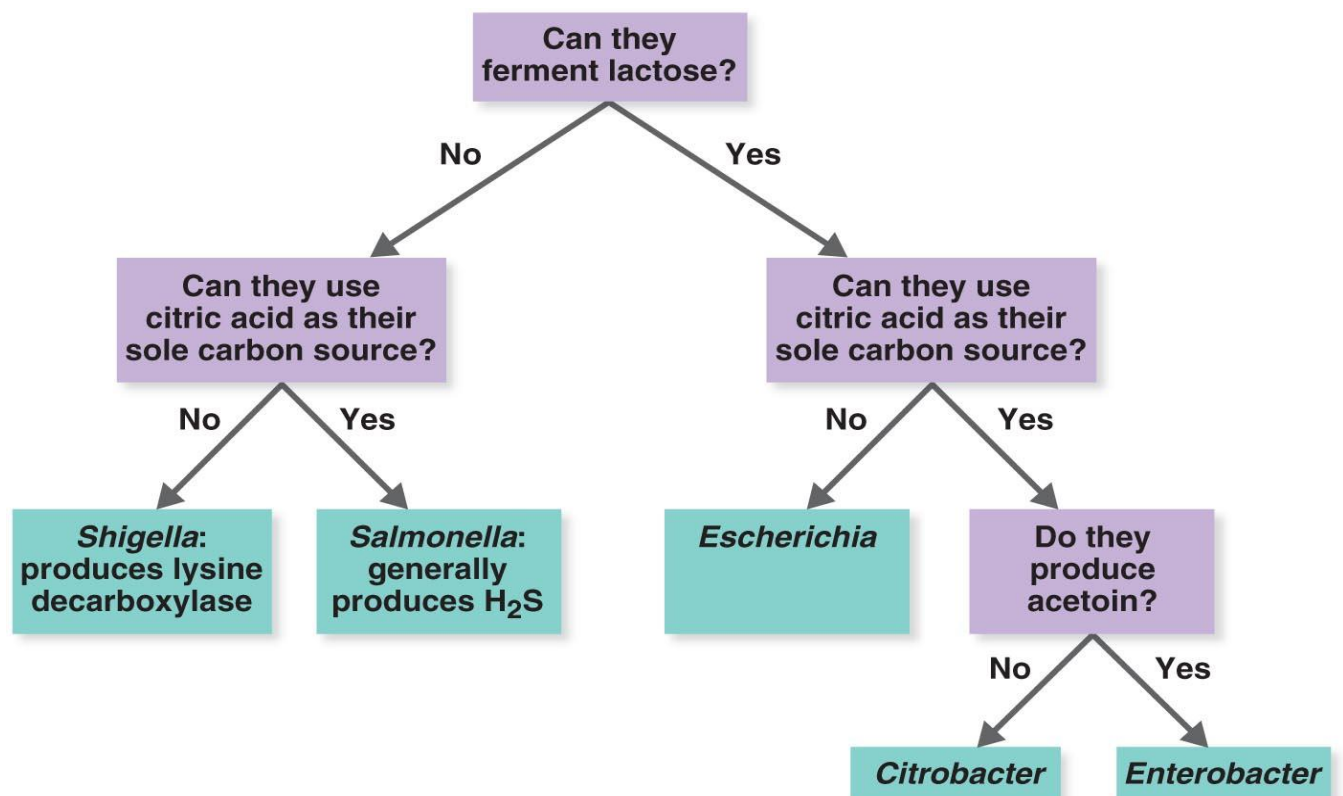
- ❖ Domain
 - Kingdom
 - Phylum
 - Class
 - ◆ Order
 - Family
 - Genus
 - Species
- **Summary**
- ❖ 3 domains
 - Archaea
 - Bacteria
 - Eukaryota

TOPIC 49 Bacterial Identification I

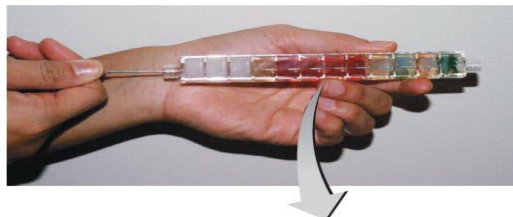
Learning Objectives

- ❖ Various microbial identification methods
- ❖ Bacterial Identification I
- ❖ Differential staining
- ❖ Morphology
- ❖ Motility
- ❖ Biochemical testing
- ❖ Serological testing
- ❖ Nucleic acid based testing
- ❖ Bacterial Identification I
- ❖ Differential staining
 - Gram staining
 - Negative
 - Positive
- ❖ Bacterial Identification I
- ❖ Morphology
 - Rods
 - Cocci
 - Spirals
- ❖ Bacterial Identification I
- ❖ Motility
 - Flagella
 - Motile
 - Non-motile
- ❖ Biochemical Testing
 - Enzymes of metabolism

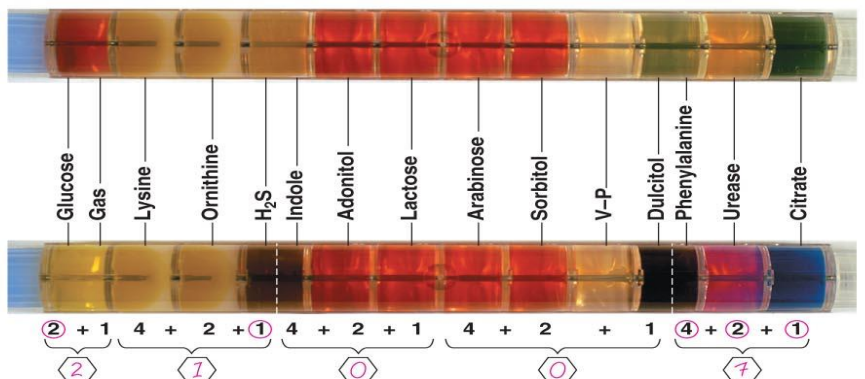
❖ G –ve, oxidase –ve, Rods



- 1 One tube containing media for 15 biochemical tests is inoculated with an unknown enteric bacterium.



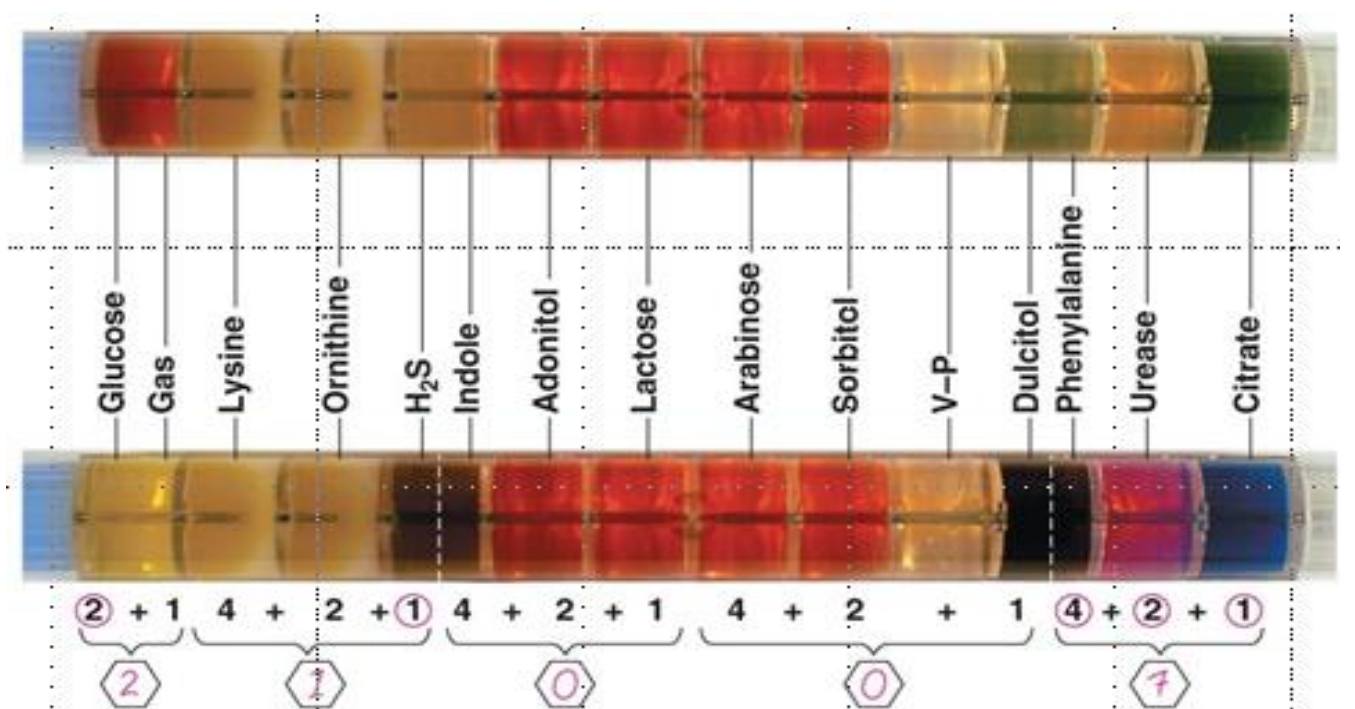
- 2 After incubation, the tube is observed for results.



- 3 The value for each positive test is circled, and the numbers from each group of tests are added to give the ID value.

- 4 Comparing the resultant ID value with a computerized listing shows that the organism in the tube is *Proteus mirabilis*.

ID Value	Organism	Atypical Test Results	Confirmatory Test
21006	<i>Proteus mirabilis</i>	Ornithine ⁻	Sucrose
21007	<i>Proteus mirabilis</i>	Ornithine ⁻	
21020	<i>Salmonella choleraesuis</i>	Lysine ⁻	



ID Value	Organism	Atypical Test Results	Confirmatory Test
21006	<i>Proteus mirabilis</i>	Ornithine ⁻	Sucrose
21007	<i>Proteus mirabilis</i>	Ornithine ⁻	
21020	<i>Salmonella choleraesuis</i>	Lysine ⁻	

Microbes identified by various methods

TOPIC 50 Bacterial Identification II

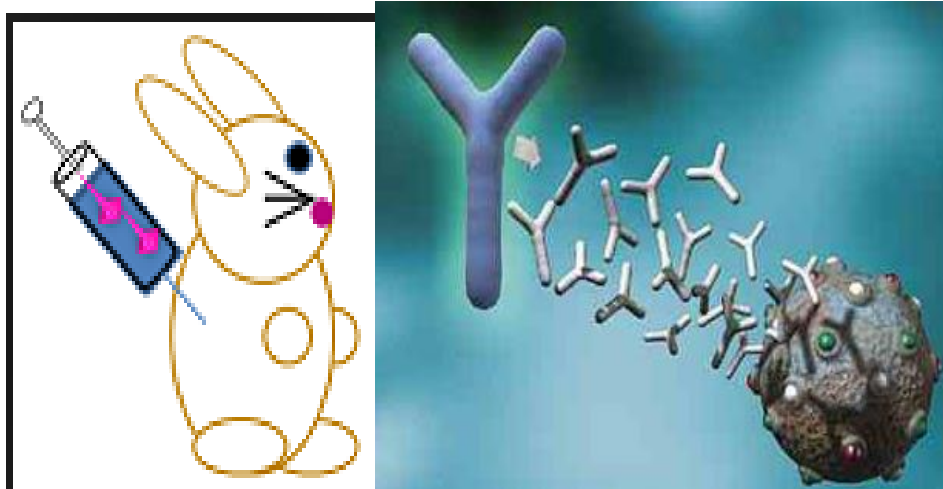
Learning Objectives

- Serological Methods for microbial identification

Serological Testing

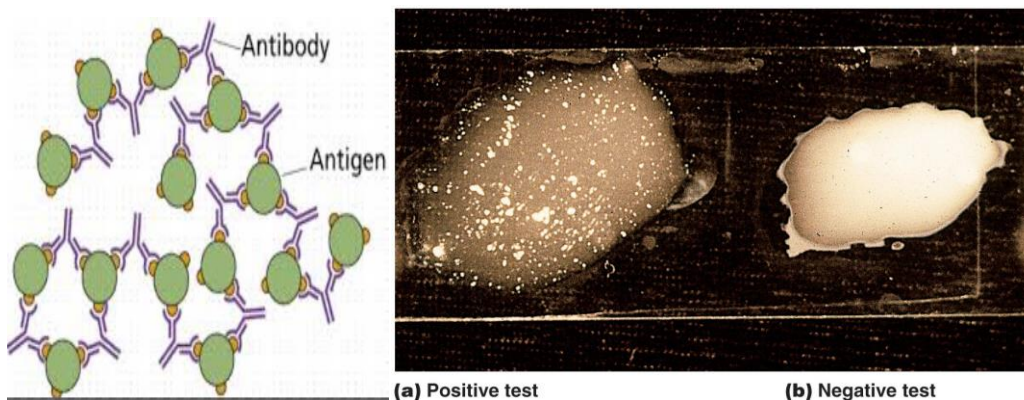
- Antigen antibody reactions

Antibody Production by Lymphocytes



Agglutination

- Agglutination is with particulate antigen



TOPIC 51 Bacterial Identification III

Learning Objectives

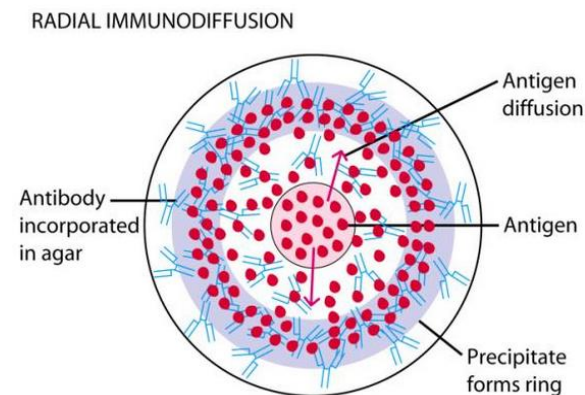
- Serological Methods for microbial identification
- Precipitation tests

- Agar gel ppt test

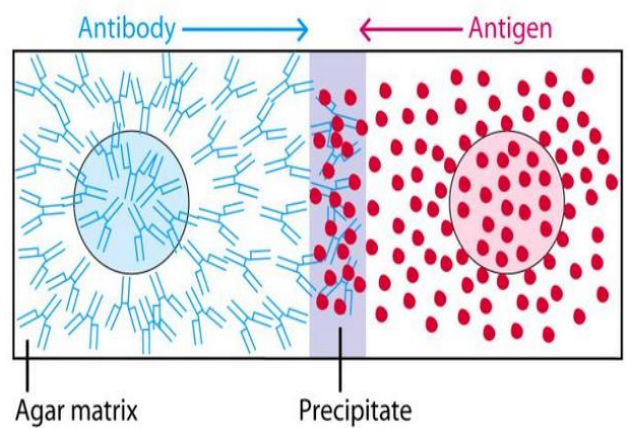
Serological Testing

- Precipitation test
- Soluble antigen binds with antibodies
- Bacterial Identification III
- Precipitation test
- Soluble antigen binds with antibodies

Radial Immunodiffusion Test



DOUBLE IMMUNODIFFUSION



Precipitation is with soluble antigen

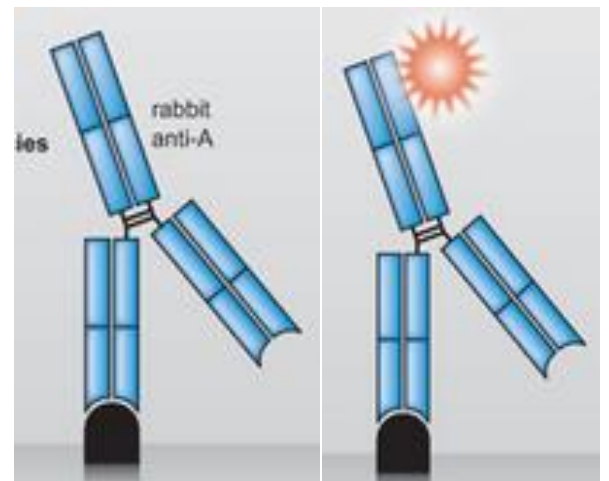
TOPIC 52 Bacterial Identification IV

Learning Objectives

- Enzyme Linked Immunosorbent Assay
 - ELISA

Serological Testing

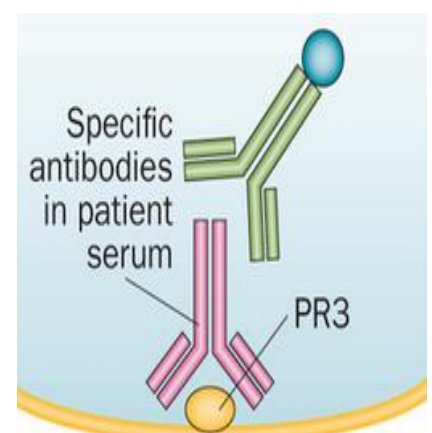
- Some antigen antibody reactions are not visible to the eyes.
- Such reactions are visualized indirectly using various probes.
- Probes include enzymes, radioisotopes etc.
- Enzymes or radioisotopes are conjugated to antibodies
- Presence of enzymes detected by incubating with substrate that forms colored compounds.
- ELISA used for antigen or antibody detection in a sample.



Anti-body structure



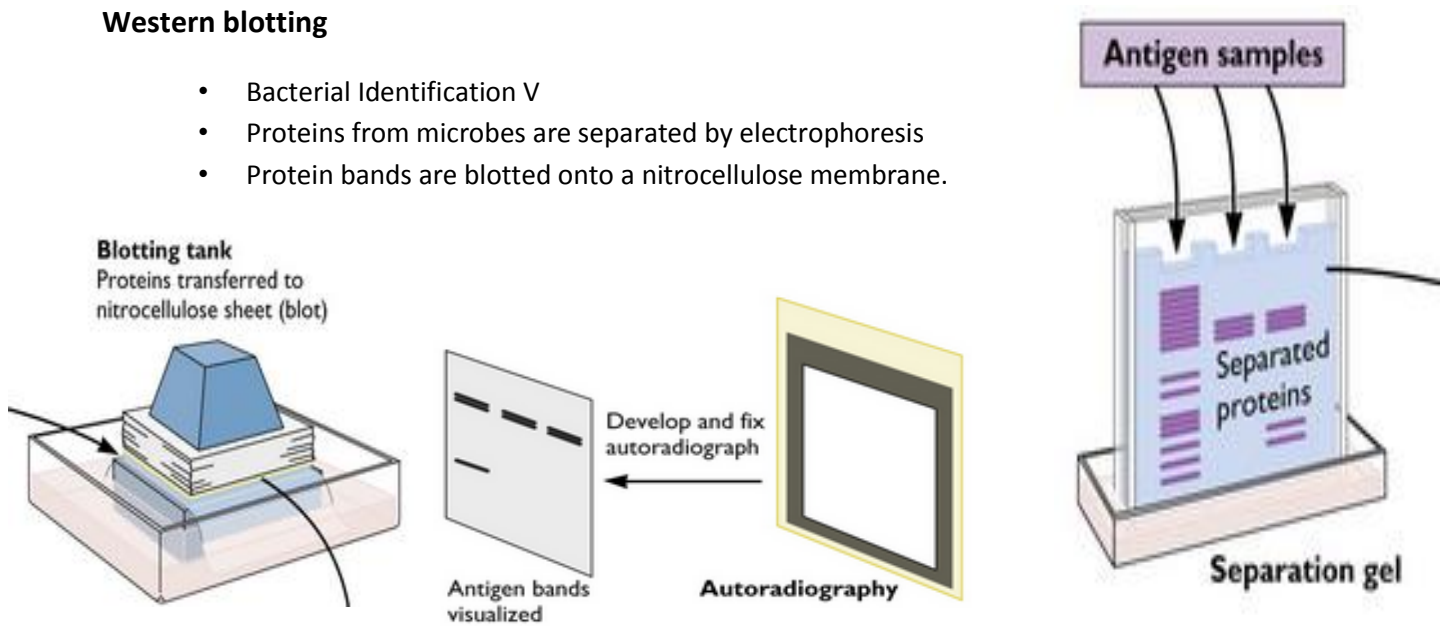
Anti-body deduction by Elisa



TOPIC 53 Bacterial Identification V

Western blotting

- Bacterial Identification V
- Proteins from microbes are separated by electrophoresis
- Protein bands are blotted onto a nitrocellulose membrane.



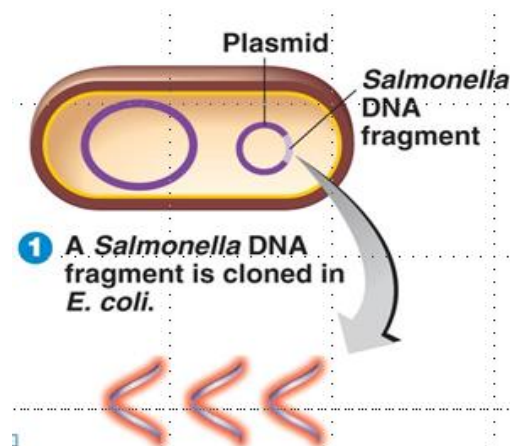
- Proteins could be detected by electrophoresis and blotting.
- Blots can be detected by specific antibodies labeled with radioisotopes etc.

TOPIC 54 Bacterial Identification VI

Nucleic acid base microbial detection methods

- DNA probes
- DNA chip
- PCR

DNA probes



DNA Chip



(a) A DNA chip can be manufactured to contain hundreds of thousands of synthetic single-stranded DNA sequences. Assume that each DNA sequence was unique to a different gene.

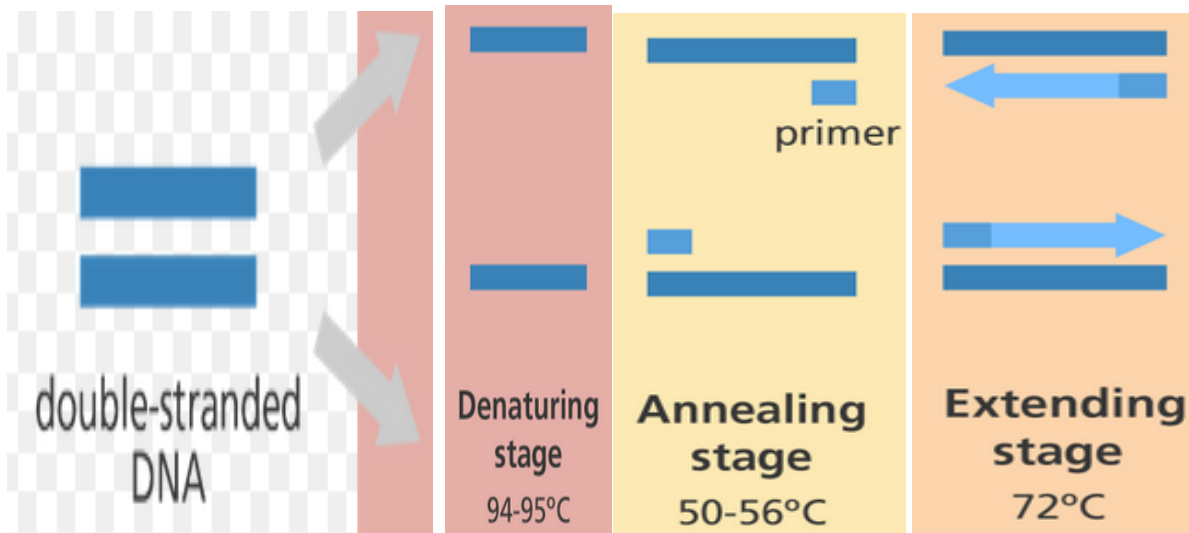
- DNA probes can be used for diagnosing various diseases.

TOPIC 56 Bacterial Identification VII

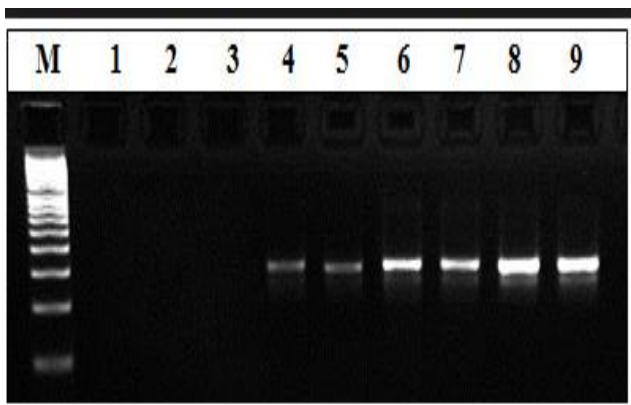
Nucleic acid base microbial detection methods

— PCR

PCR Basics



- After 30 cycles, enough targets DNA accumulate which could be detected by electrophoresis.



PCR provides a quicker way of identification of microbes.

TOPIC 56 Fungi I

Learning Objectives

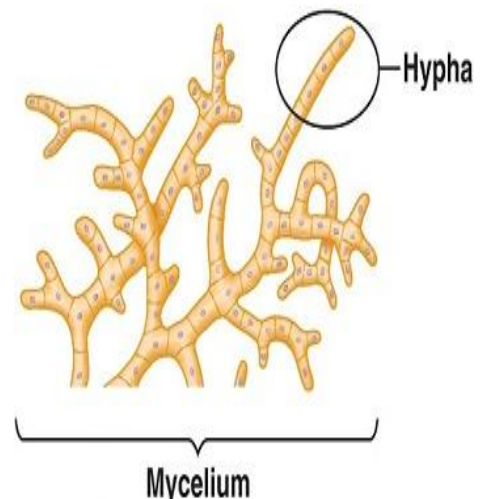
- What are fungi
- Nomenclature used to define fungi

General Features

- Kingdom: Fungi
- Chemoheterotrophs
- Acquire nutrients by absorption
- Asexual and Sexual reproduction

Definitions

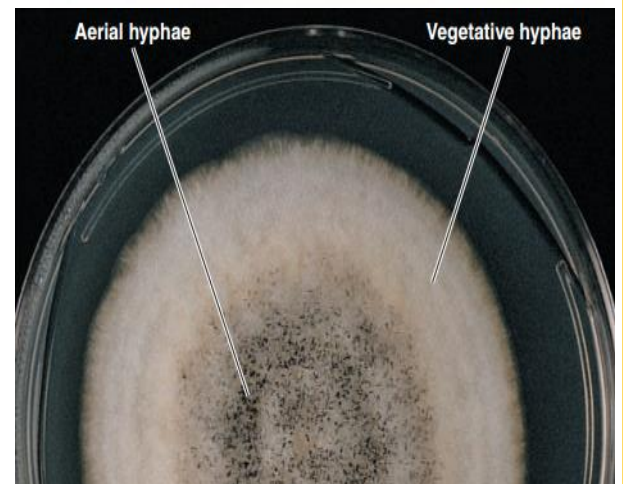
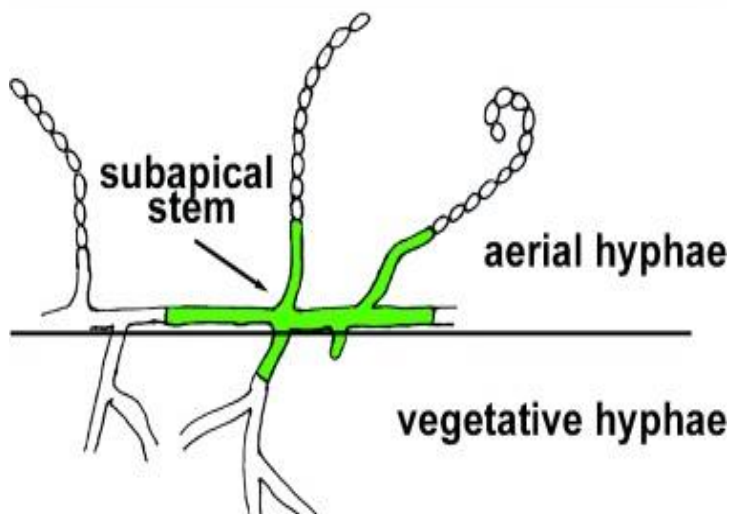
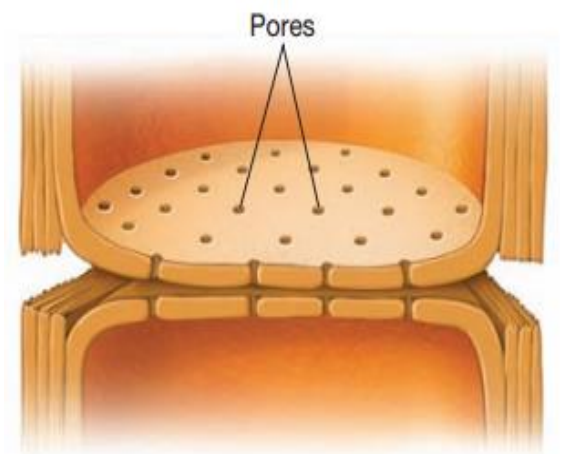
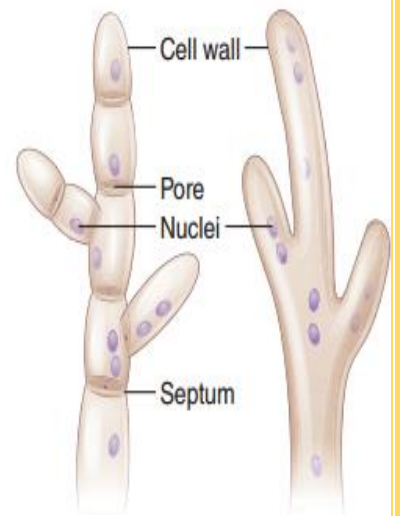
- Hyphae: Thread like filaments of a fungus
- Mycelium: Collection of hyphae



- Thallus: Collection of mycelia
- Mold: Filamentous fungi
- Yeast: unicellular fungus
- Mycology: Study of fungi

Types of Hyphae

- ❖ Septate: Cross-walls, divide hyphae into uninucleate cell-like units
- ❖ Non-septate or coenocytic:
 - No septum
- Vegetative Hypha: Absorbs nutrients
- ❖ Aerial or reproductive hypha: Bears reproductive spores
- ❖ Septal pores
- ❖ Fungi are eukaryotes
- ❖ Multicellular except yeasts
- ❖ Molds and Yeasts
 - Septate and non-septate hyphae



Summary

- Fungi are eukaryotes
- Multicellular except yeasts
- Molds and Yeasts
- Septate and non-septate hyphae

TOPIC 57 Fungi II

Reproductive spores of filamentous fungi

Types of Spores

- Asexual spores formed by mitosis of hyphal cells
- Conidiospores: Unicellular or multicellular spore not enclosed by a sac

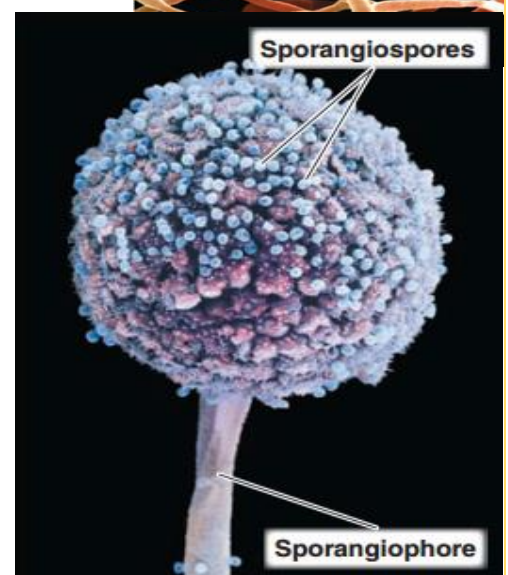
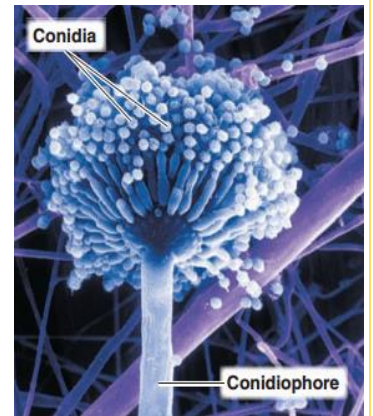
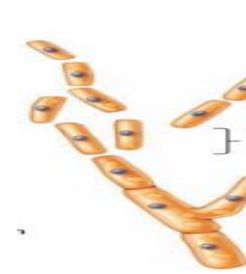
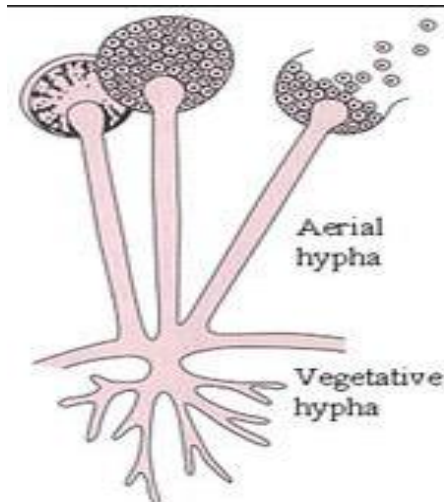
- Sporangiospores: Enclosed by a sac

Conidiospores

- ❖ Conidia: Chain of spores at the end of a conidiophore
Aspergillus
- ❖ Arthroconidia: spores formed by fragmentation of septate hypha
 - Coccidioides immitus
- ❖ Blastoconidia: spores as budding of the parent cells
 - Candida albicans
- ❖ Chlamydoconidia: spores formed by rounding and enlargement within a hyphal segment
 - Candida albicans

Sporangiospores

- Formed within a sporangium or sac at the end of an aerial hypha called sporangiophore
 - Rhizopus
- Fungi reproduce by sexual and asexual spores
- Conidiospores
- Sporangiospores



TOPIC 58 Fungi III

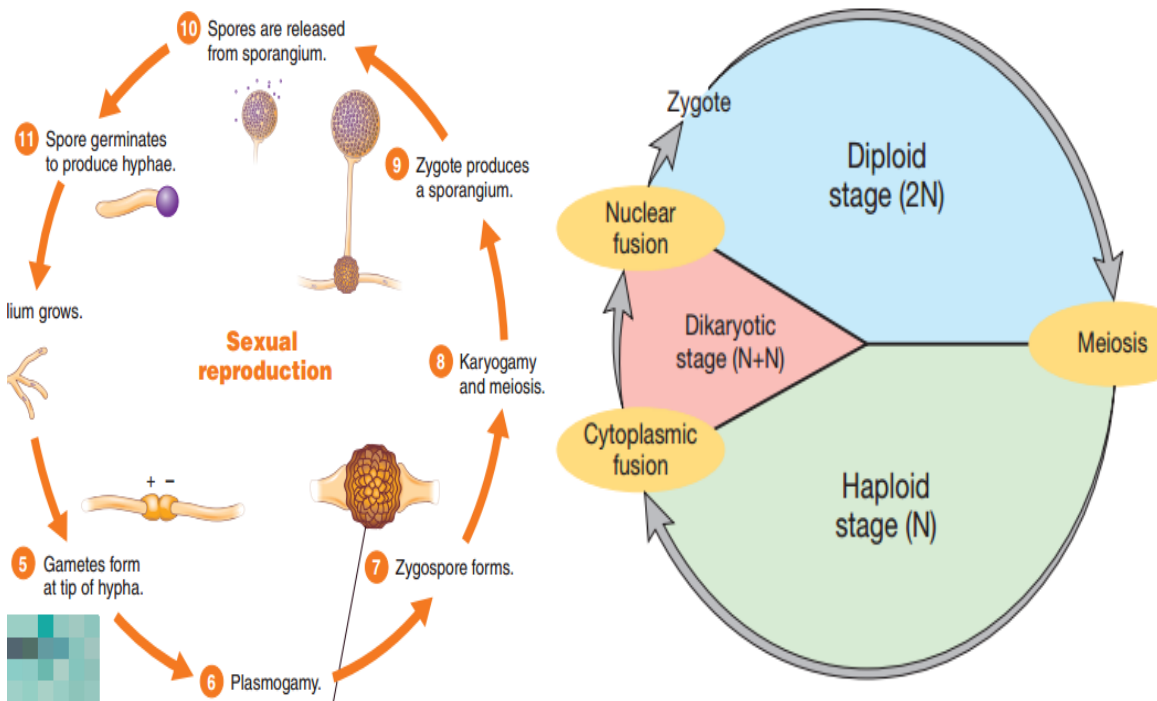
- Reproductive spores of filamentous fungi
 - Sexual spores

Sexual Spores

- Plasmogamy: A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-).
- Karyogamy: The (+) and (-) nuclei fuse to form a diploid zygote nucleus.

- Meiosis: The diploid nucleus gives rise to haploid nuclei (sexual spores)

Sexual Spores



- Fungi reproduce by sexual and asexual spores
- Plasmogamy
- Karyogamy
- Meiosis

TOPIC 59 Fungi IV

- What is a yeast
- Dimorphic fungi

Sexual Spores

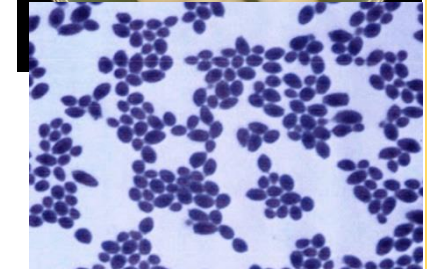
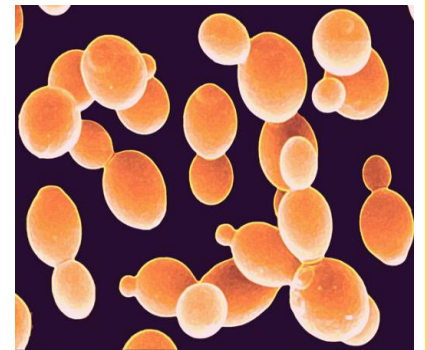
- Non-filamentous fungi, Unicellular, Reproduce by budding (asexually) or sexually, Some are dimorphic

- Yeast: at 37°C
- Molds: Room temp

Yeast are Larger than bacteria, No flagella or cilia, Facultative anaerobes

Larger than bacteria, No flagella or cilia, Facultative anaerobes, Gram positive

- Yeasts are unicellular, some are dimorphic, both sexual and asexual reproduction, Bigger than bacteria.

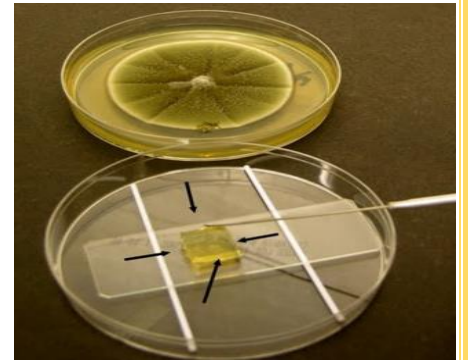


TOPIC 60 Fungi V

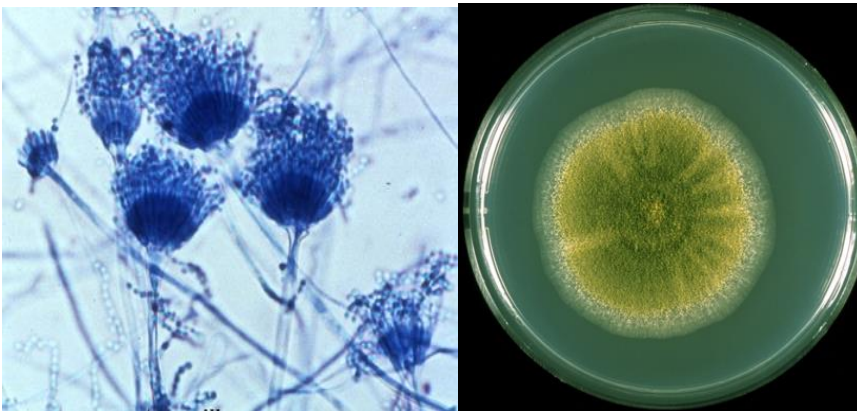
- How to study fungi

Filamentous fungi

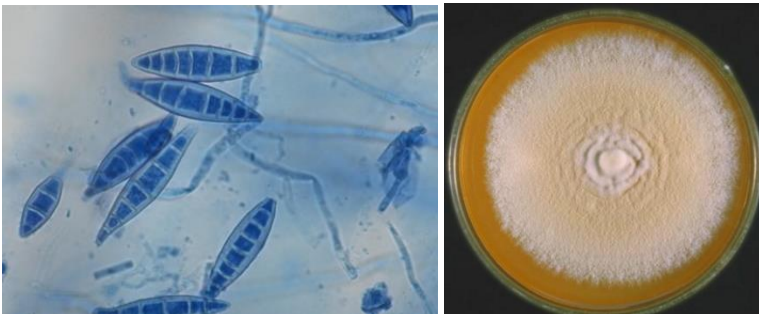
- Identified by morphology, not by biochemical testing
- Slide culture is made using Sabouraud's agar
- Spores are inoculated
- Slides stained with lactophenol cotton blue



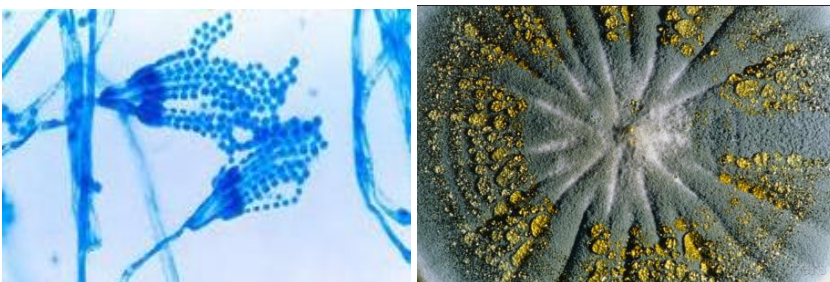
Aspergillus



Trichophyton



Aspergillus



- Yeast can ferment and can produce various products
- Biochemical testing is used for yeast identification
- Morphology is used to identify filamentous fungi
- Biochemical testing is used for yeast identification

TOPIC 61 Fungi VI

- Economic benefits of fungi
- Diseases by fungi

Uses of Yeast

- ❖ Saccharomyces for alcoholic beverages
 - Anaerobic conditions for alcohol
 - Aerobic conditions
 - Bread making

Yeast uses

- Biotechnology: Hepatitis B vaccine

Research

Fungal uses

- ❖ Citric acid: Aspergillus niger
- ❖ Cellulase: Trichoderma (plant cell digestion to clear fruit juices)
- ❖ Taxol: Taxomyces (anticancer)
 - Biological pest control: Entomophaga (kills gypsy moth)
- ❖ Penicillin: Penicillium

Diseases by Fungi

- ❖ Mycosis is a general term used for fungal diseases
- ❖ Systemic mycosis: Deep within the body
 - histoplasmosis and coccidioidomycosis
- ❖ Subcutaneous Mycosis: Beneath the skin
 - Sporotrichosis in gardeners and farmers
- ❖ Cutaneous Mycosis:
 - Trichophytosis use keratinase to degrade keratin in nails, hair and skin
 - Ringworm infections

Fungi are useful mostly

A few cause infections

62 Lichen 1

Learning Objectives

- Lichens
- Uses of lichens

Lichens

- ❖ Combination of green alga or a cyanobacterium (phycobiont) and a fungus (Mycobiont)
- ❖ Symbiotic relationship
- ❖ Partners do not grow alone
- ❖ Grow where no other microbes can grow
- ❖ Grow on bare rocks, tree trunks and house roofs and bare soils
- ❖ Typically grow extremely slowly
 - 1 mm to 3 cm per year
- ❖ Classified on the basis of the fungus

Types of Lichens

- Crustose: forms a thin crust
- Foliose: Leaf-like
- Fruticose: Finger-like projections



Lichen Thallus

- Medulla: Algal layer and fungal hyphae
- Cortex: Could be two, upper layer and lower layer
- Rhizine: Roots or Holdfasts

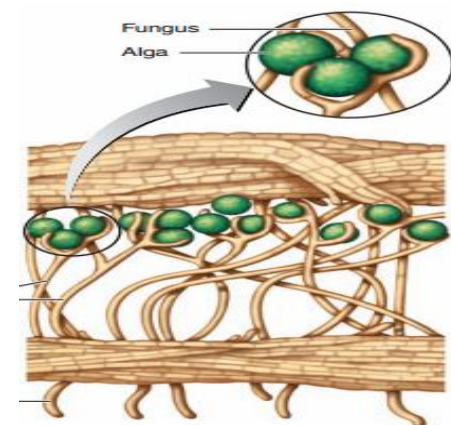


Lichen uses

- Dyes for clothes
- Usnic acid as antiseptic in China
- Erythrolitmin: for Litmus paper
- Can cause allergic contact dermatitis

Summary

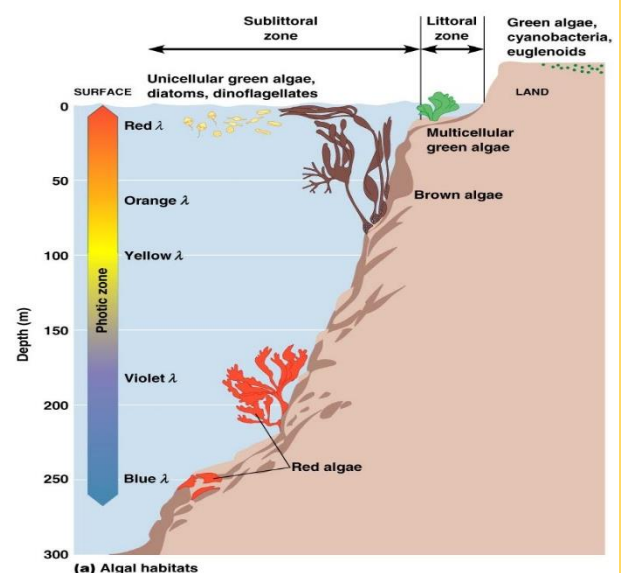
- Fungus and alga or cyanobacterium
- Cannot grow alone
- Have various uses
- Some can cause contact dermatitis



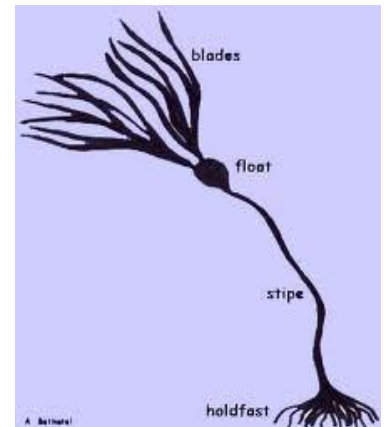
63 Algae 1

Algae

- Simple eukaryotic cells
- Some are unicellular, others are multicellular (thallus)
- Lack tissues (roots, stem and leaves) seen in plants
Absorb nutrients from water through their surfaces
- Mostly photoautotrophs, a few are chemoheterotrophs
- Mostly aquatic (water is necessary for physical support, reproduction and diffusion of nutrients)
- Cell covering thallus can carry photosynthesis
- Some algae float by gas-filled bladders

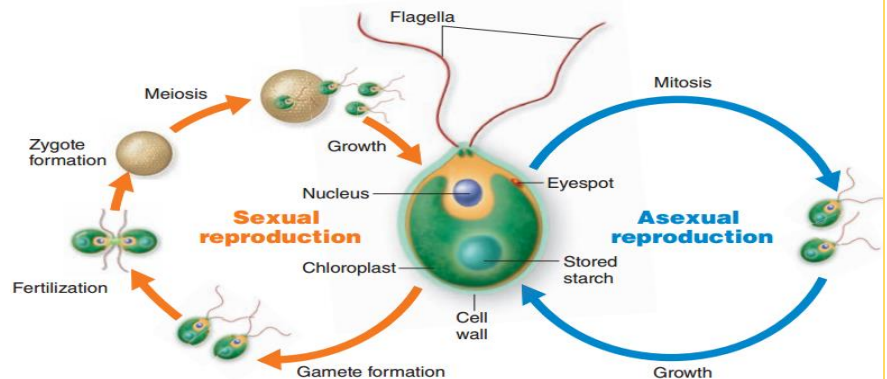


- Their location in oceans depends on the availability of nutrients, wavelength of light and the surface on which to grow.
- Body of multicellular algae is called a thallus which consists of branched holdfasts (anchor alga to rock) stemlike hollow stipes and leaflike blades.



Reproduction

- All algae can reproduce asexually
 - Multicellular algae: a fragment can form a new thallus
 - Unicellular algae: mitosis followed by cytokinesis
- Sexual reproduction:
 - Alternation of generations of haploid and diploid individuals

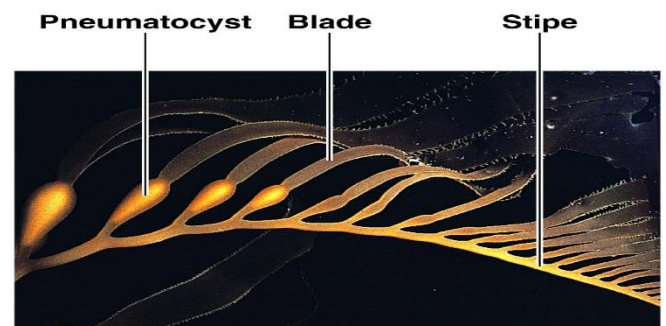


- ✓ Eukaryotes
- ✓ Unicellular and multicellular
- ✓ Lack tissues
- ✓ Mostly aquatic
- ✓ Reproduce sexually and asexually

64.Algae 2

Phaeophyta (Kelp)

- ✓ Brownish in color
- ✓ Cellulose and alginic acid cell walls
- ✓ Multicellular
- ✓ Chlorophyll *a* and *c*, xanthophylls
- ✓ Store carbohydrates
- ✓ Harvested for algin



(b) Brown alga (*Macrocystis*)

0.5 m

Rhodophyta

- ✓ Reddish in color
- ✓ Cellulose cell walls
- ✓ Most are multicellular
- ✓ Chlorophyll *a* and *d*, phycobiliproteins
- ✓ Store glucose polymer
- ✓ Harvested for agar and carrageenan



(c) Red alga (*Microcladia*)

10 cm

Chlorophyta

- Green algae
- Cellulose cell walls
- Unicellular or multicellular
- Chlorophyll *a* and *b*
- Store glucose polymer
- Believed to have given rise to terrestrial plants



(a) Multicellular green alga (*Ulva*)

10 cm

Bacillariophyta

- Brownish in color
- Pectin and silica cell walls



(a)

SEM 50 μm

- Unicellular
- Chlorophyll *a* and *c*, carotene, xanthophylls

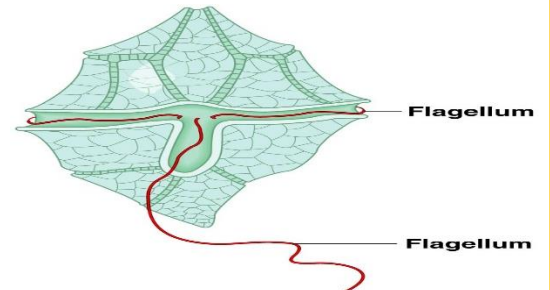
Bacillariophyta

- Store oil
- Fossilized diatoms formed oil
- Produce domoic acid



Dinoflagellata

- Brownish in color
- Cellulose in plasma membrane
- Unicellular
- Chlorophyll *a* and *c*, carotene, xanthins
- Store starch
- Some are symbionts in marine animals
- Neurotoxins cause paralytic shellfish poisoning



Oomycota

- ❖ Colorless, white
- ❖ Cellulose cell walls
- ❖ Multicellular
- ❖ Chemoheterotrophic
- ❖ Produce zoospores
- ❖ Decomposers and plant parasites
 - *Phytophthora infestans*: responsible for Irish potato blight
- ❖ Decomposers and plant parasites
 - *P. cinnamomi* infects Eucalyptus.
 - *P. ramorum* causes sudden oak death.

Role of Algae

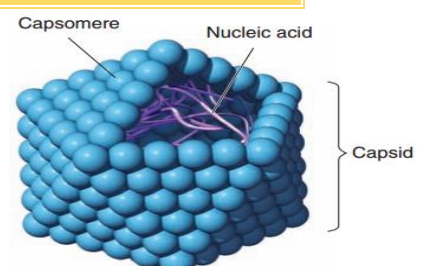
- Important part of aquatic food chain
- Fix CO₂ into organic compound
- 80% earth O₂ produced by planktonic algae
- Petroleum and natural gas are the residues (hydrocarbons) of diatoms and other planktonic organisms.
- Unicellular algae are symbiont in animals.
- ✓ Various types of algae
- ✓ Have variety of chlorophylls
- ✓ Have various uses
- ✓ Some cause plant and fish diseases

65. Virus 1

Definitions

- Virus: infectious viral particle, composed of nucleic acid, surrounded by a protein coat called capsid.

Virion



- Viruses have an extracellular and an intracellular state.
- Outside of a cell, in the extracellular state, a virus is called a virion.

Viroid

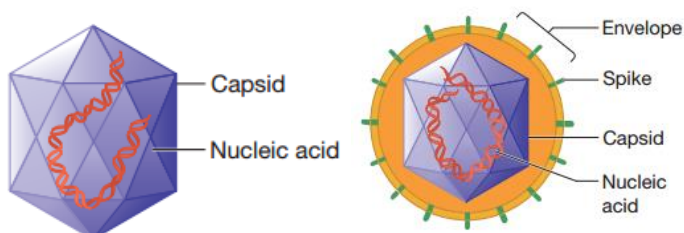
- ✓ Infectious circular RNA of plants
- ✓ Formed of only RNA
- ✓ Protein coat is absent
- ✓ Smaller in size than a typical virus
- ✓ Infects plants only

Virus Structure

- Nucleic Acid: Either DNA or RNA (never both)
 - Single or double stranded
 - Circular or linear
 - One segment or multiple segment
- Capsid: made up of protein subunits, capsomeres.
 - Capsomeres: subunit proteins can be single type or multiple types
- Capsid: made up of protein subunits, capsomeres.
 - Arrangement of capsomeres is characteristic of a particular type of virus

Virus Envelope

- In some viruses, the capsid is covered by an envelope (lipid, protein and carbs)
- May or may not have spikes (carbohydrate-protein complexes) projecting from the surface of the envelope.

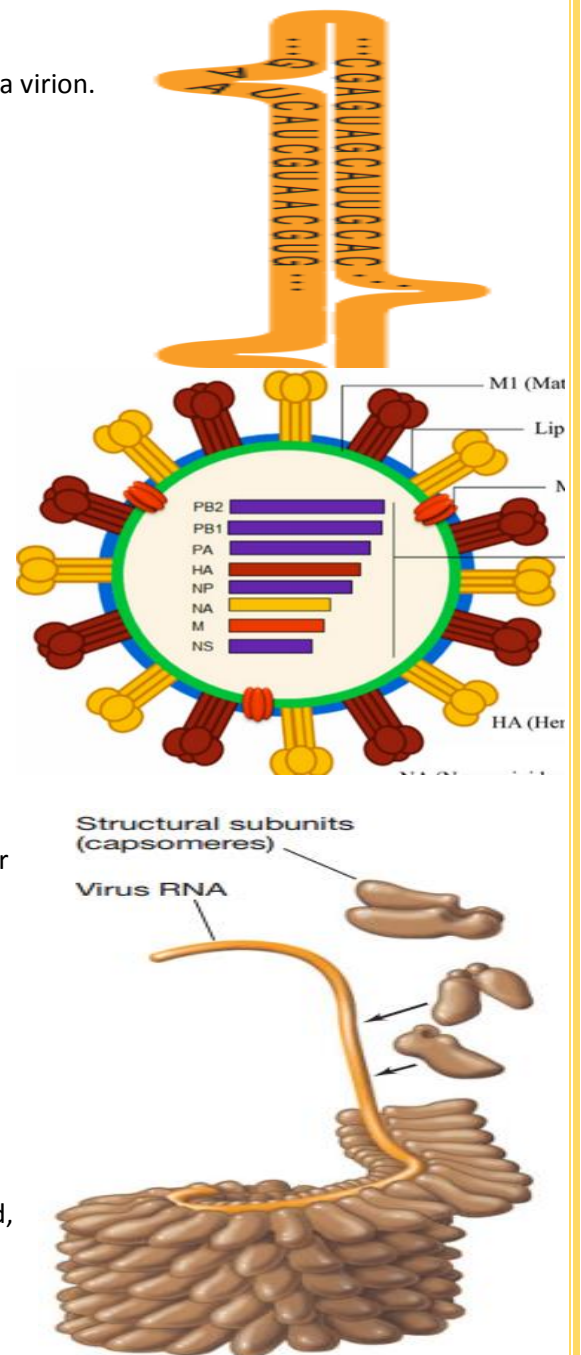


Virus host range

- Most viruses infect only specific types of cells in one host
- Host range is determined by specific host attachment sites and cellular factors

Capsid Architecture

- Helical Viruses
 - Resemble long rods, rigid or flexible
 - Rabies virus
- Polyhedral Viruses



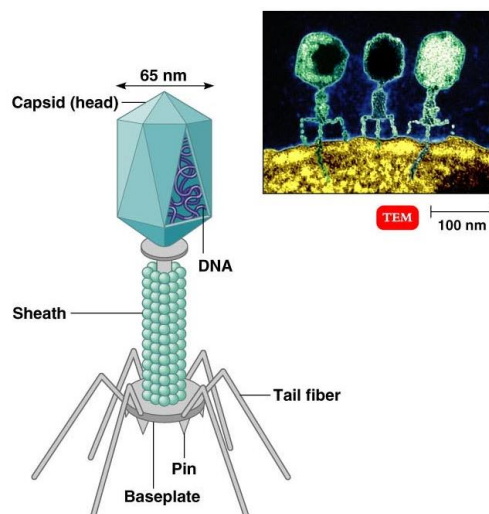
- Icosahedron: Regular polyhedron with 20 triangular faces and 12 corners
- Example: Poliovirus

Enveloped Viruses

- Enveloped Viruses
- Roughly spherical
 - Influenza virus

Complex Viruses

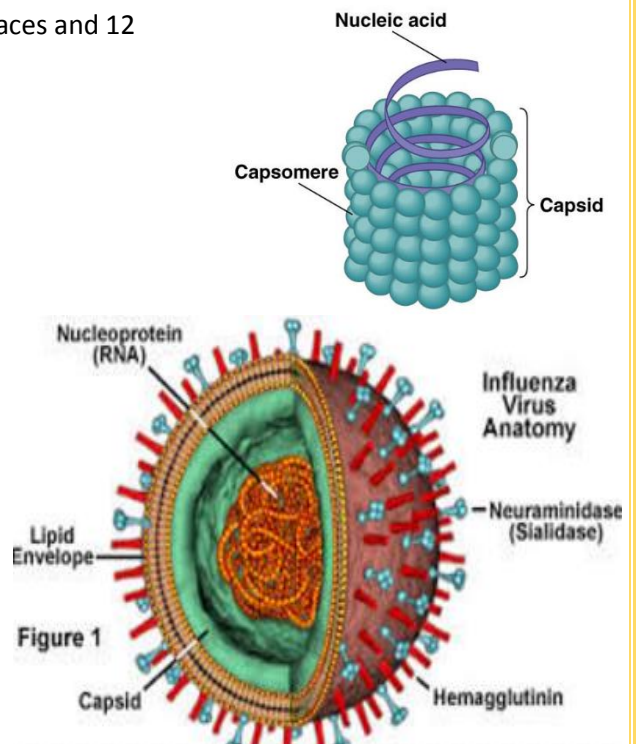
- Head is polyhedral.
 - N.Acid



- Tail sheath is helical.
 - Bacteriophage

Viral Taxonomy

- Family names end in *-viridae*.
- Genus names end in *-virus*.
 - Herpesviridae
 - *Herpesvirus*
- Viral species: A group of viruses sharing the same genetic information and ecological niche (host).
- Common names are used for species.
- Subspecies are designated by a number.
 - Human herpes virus HHV-1, HHV-2, HHV-3
- Virus, virion and viroid
- DNA or RNA
- Symmetry of viruses
- Capsomeres

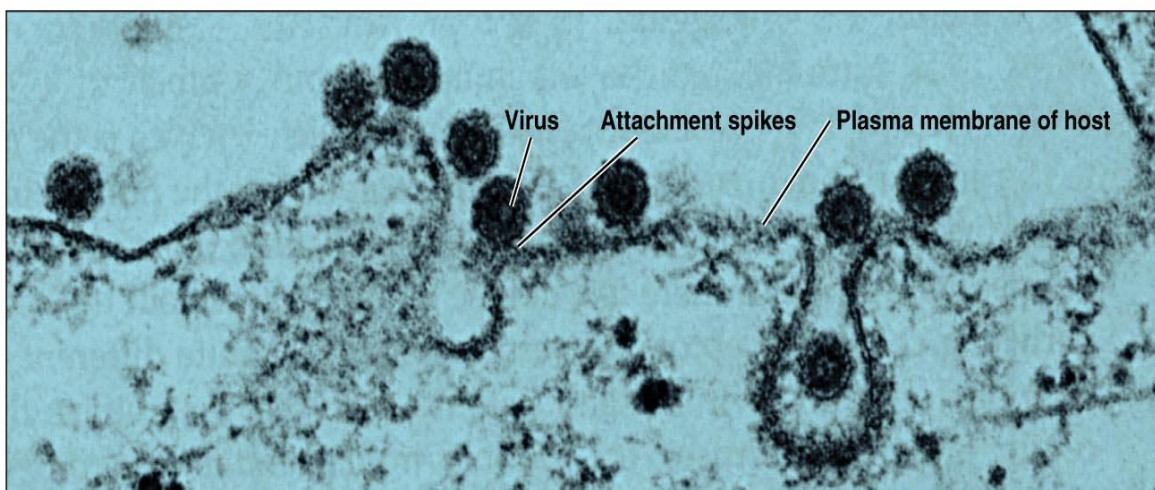


66 Virus 2

Viral Multiplication

- ❖ **Attachment:** Viruses attach to cell membrane.
- ❖ **Host cell receptors** (determines the range of infectivity)
- ❖ **Entry:** Penetration by pinocytosis (plasma membrane folds inwards to form a vesicle)
- ❖ **Fusion:** Viral envelope fuses with plasma membrane and releases the capsids into the cell's cytoplasm
- ❖ **Uncoating:** Separation of viral NA from its protein coat by viral or host enzymes.
 - (undergoes eclipse period)
- ❖ **Biosynthesis:** Production of nucleic acid and proteins.
- ❖ **Maturation:** Nucleic acid and capsid proteins assemble.
- ❖ **Release by budding** (enveloped viruses) or rupture.

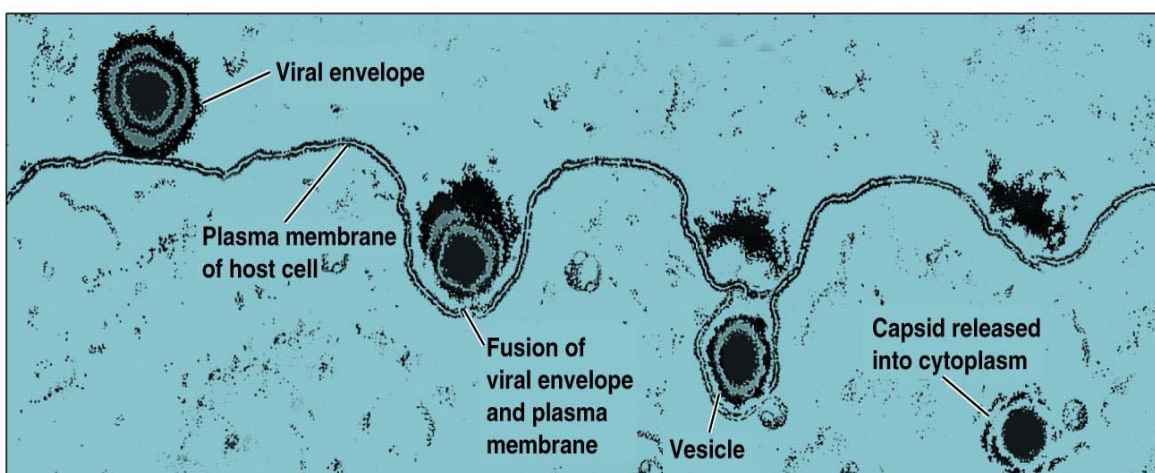
Pinocytosis



(a) Entry of togavirus

TEM | 100 nm

Fusion

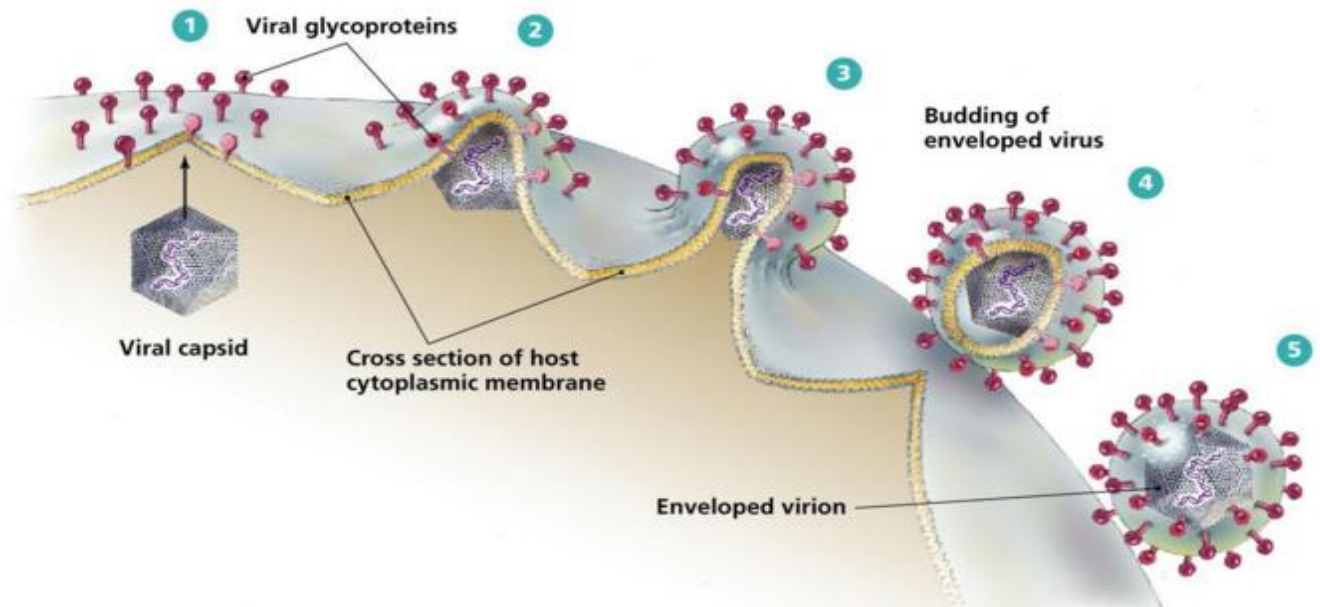


(b) Entry of herpesvirus

Biosynthesis

- Early proteins
- Late proteins
- Genome replication
- Assembly
- Release

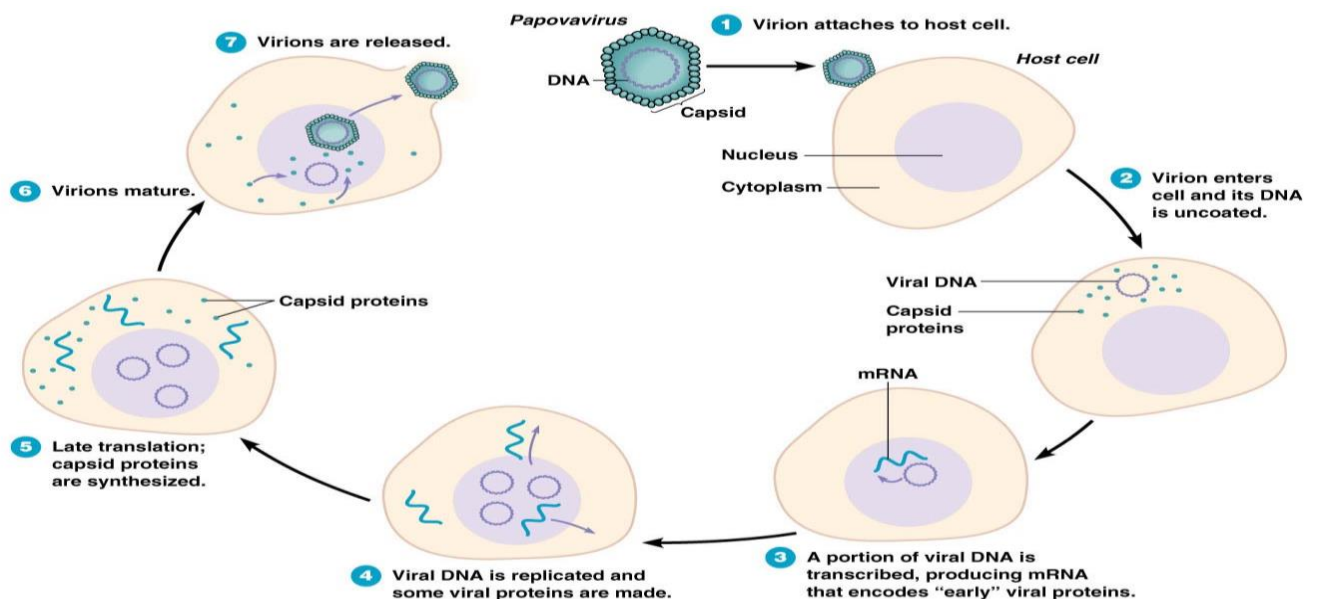
Release of virus by budding



Replication of virus has many stages

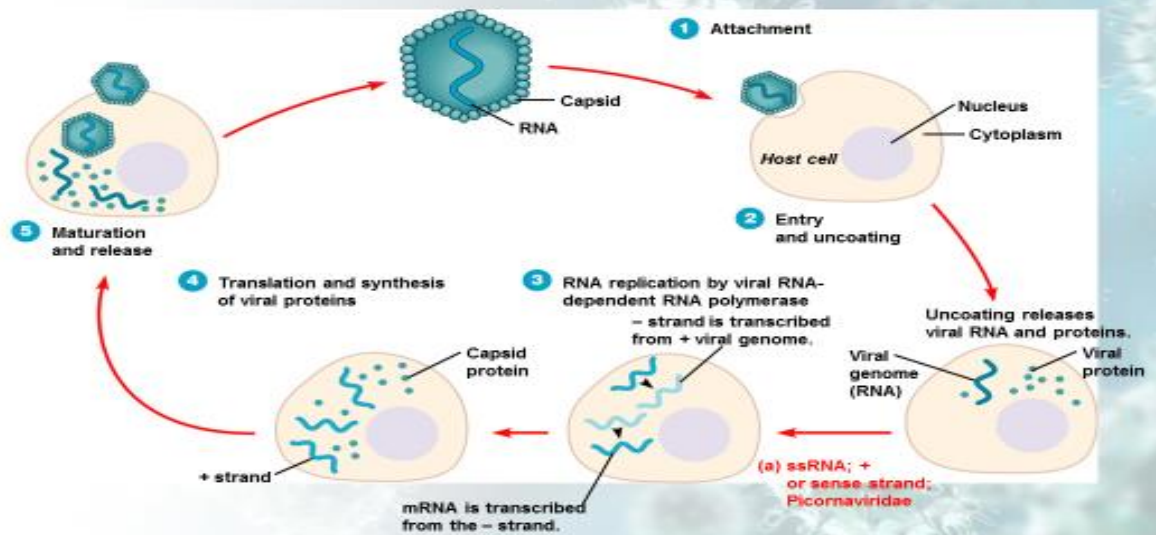
67 Virus 3

Multiplication of DNA Viruses



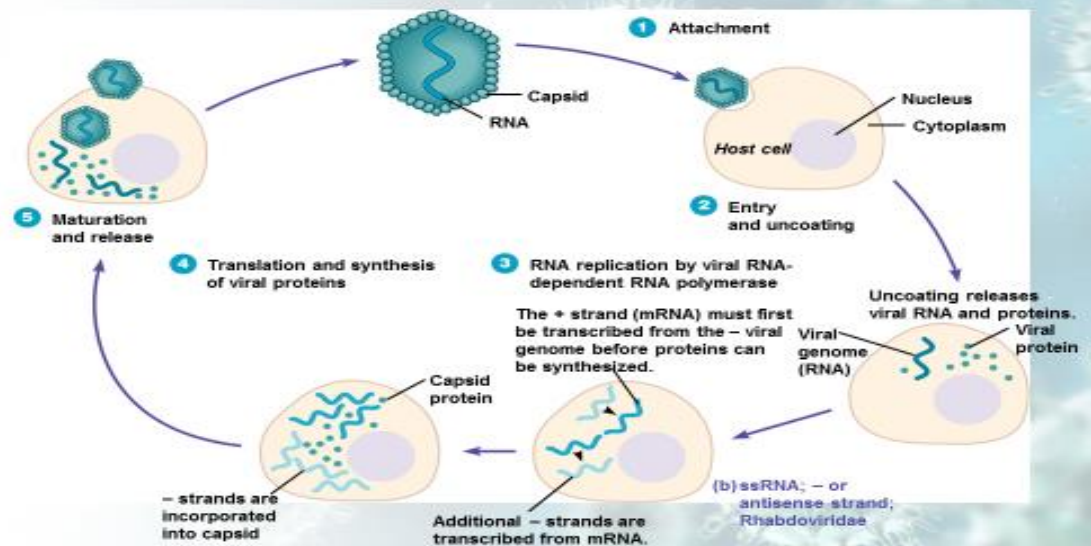
Virus III

+ or Sense Strand RNA Virus



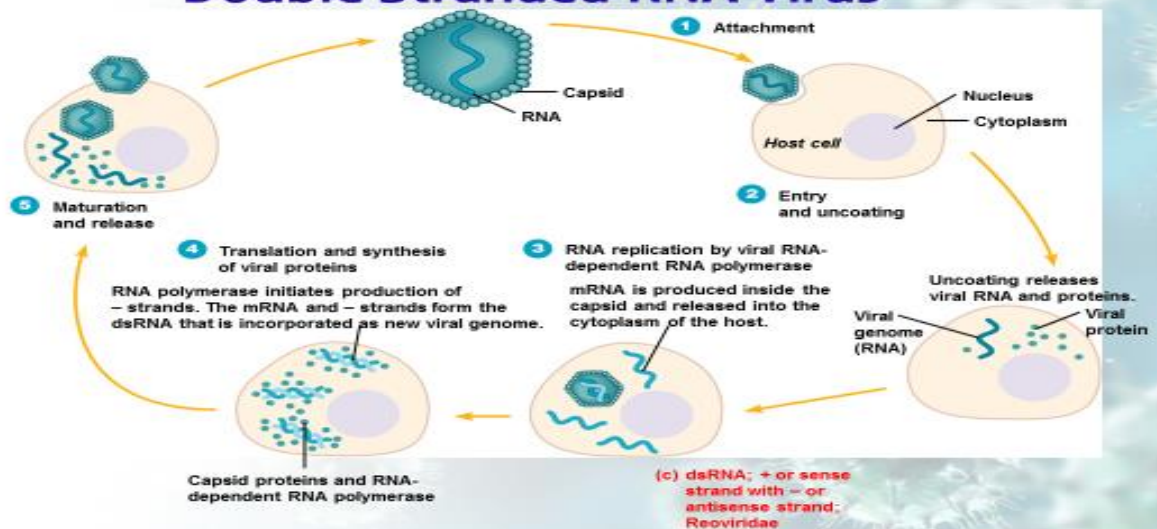
Virus III

- or Antisense Strand RNA Virus

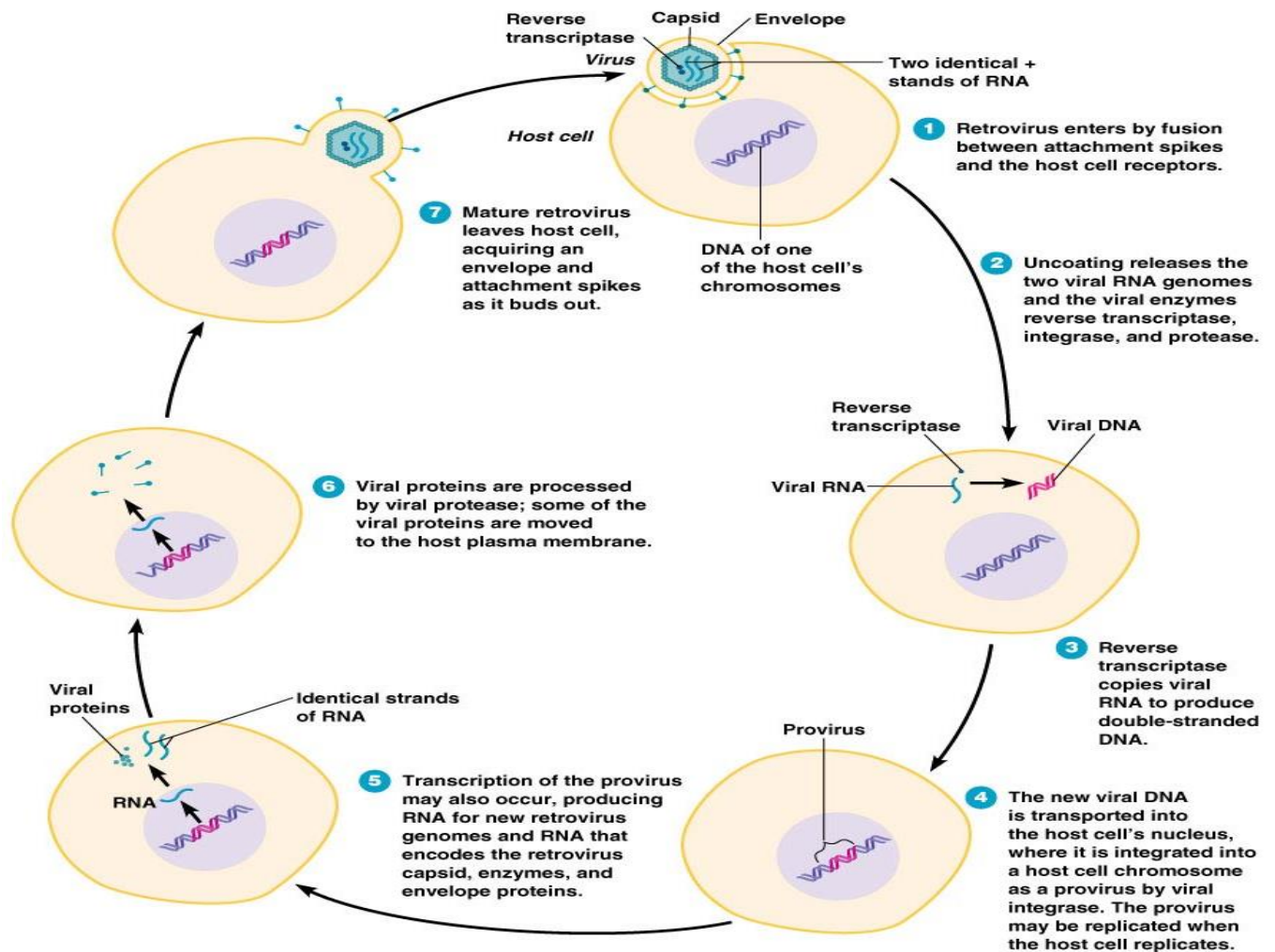


Virus III

Double Stranded RNA Virus



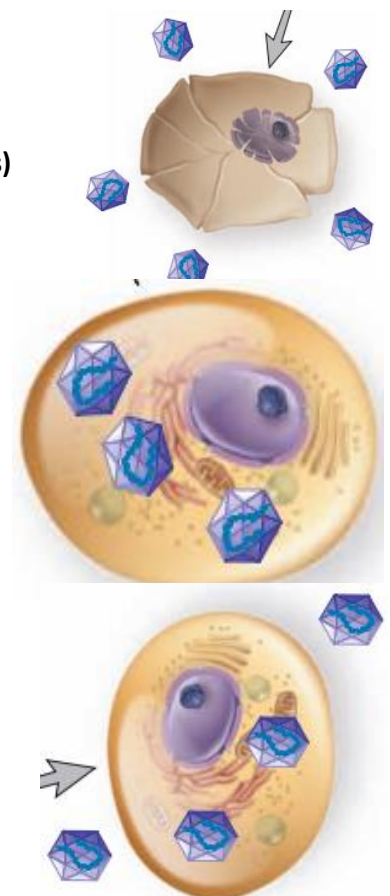
Multiplication of a Retrovirus

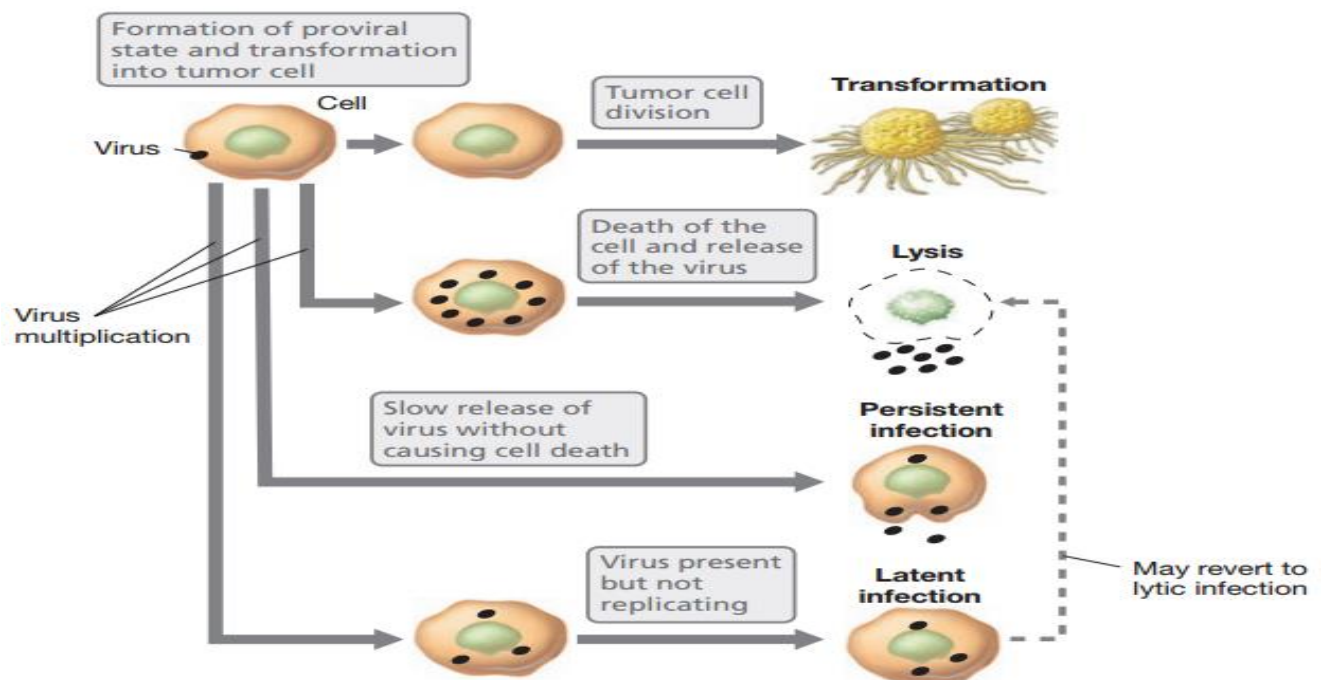
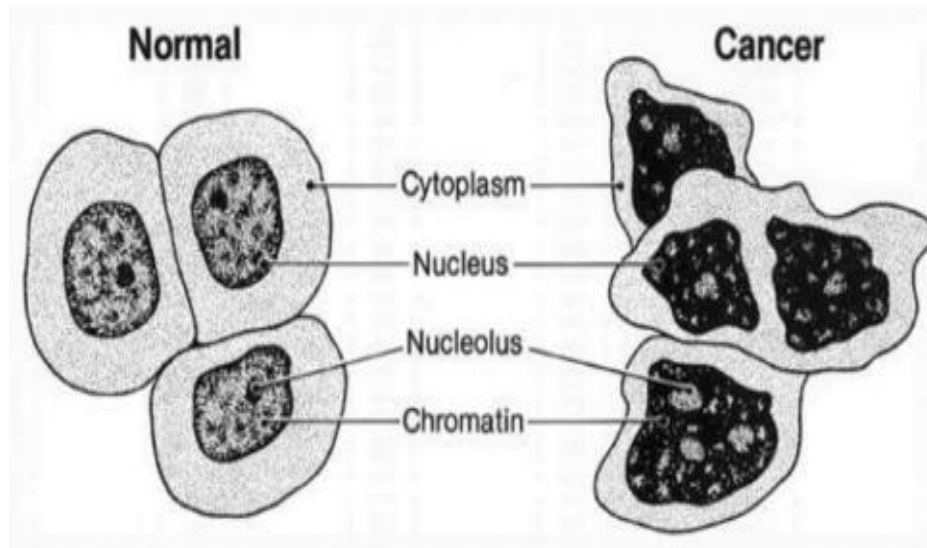


68 Virus 4

Consequences of Viral Infection

- Lysis of cells
- Microscopic and macroscopic Degenerative changes (Cytopathic Effects)
- Virulent infection: lysis of cells
- Latent infection: Virus does not replicate
- Persistent infection: Virus replicates but slowly
- Transformation: Tumor cells





69 70 Virus V

Cultivation of Viruses

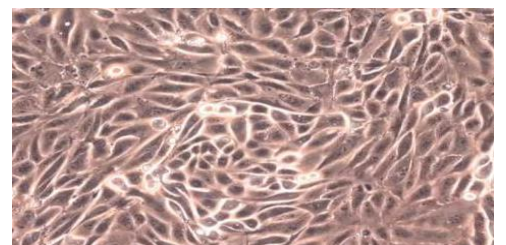
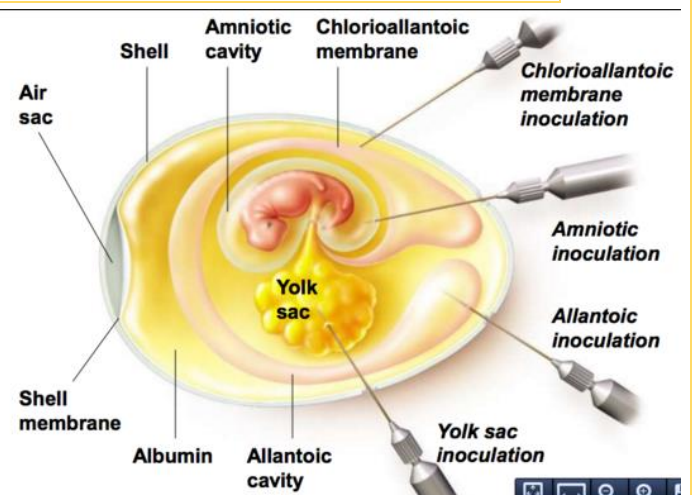
- Need live cells to grow
- Animals
- Embryonated eggs
- Cell Cultures
- Plants and animal

Embryonated Hen Eggs

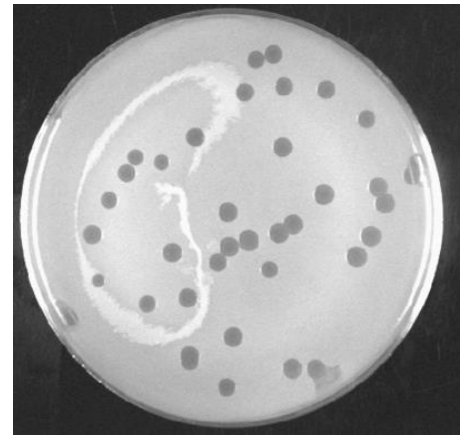
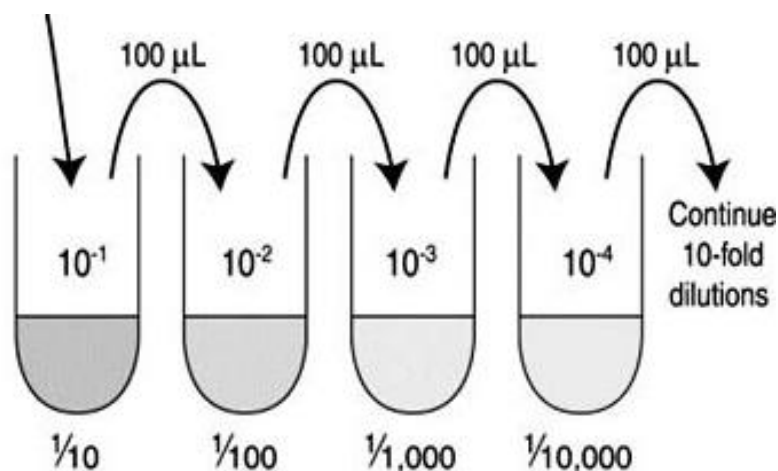
Cell Culture

Virus Enumeration

- Plaques formation:



- It is equivalent to bacterial colony
- Plaque is an area where the virus infection has lysed the cells.
- Serial Dilutions:



Enumeration

- Plaque assay
 - Plaque forming units are counted
- Infectious dose 50% (ID50)
 - TCID₅₀
- Lethal dose 50% (LD50)

Virusoids

- ✓ Also called Satellite RNA
- ✓ circular ssRNA
- ✓ Encode one or two genes products
- ✓ Need helper virus to infect host cells
- ✓ Human hepatitis D virusoid
- ✓ Uses hepatitis B virus as helper

Prions

- Proteinaceous infectious particles
- Cause neurodegenerative diseases
 - Scrapie in sheep
 - Bovine spongiform encephalopathy
- Kuru in humans
- Abnormal form of cellular proteins
- How this happens, not clear
- Genetic component involved

71 Epidemiology I

Epidemiology

- The study of where and when diseases occur and how they are transmitted in a population

Epidemiology Methods

- Descriptive: (recording the data about disease)
 - Location and time of cases of disease
 - Gender, health, age of patients etc.

Analytical: (To determine the cause of disease)

- Analysis of data
- Mode of transmission and means to prevent disease
- Experimental Epidemiology:
 - Hypothesis of a disease is tested
 - Application of Koch's postulates

Epidemiology

- Case reporting: Health care workers report specified disease to local, state, and national offices
 - Incidence and prevalence of disease
 - Incidence and prevalence of disease
 - Origin and spread of a disease
 - Data helpful for control measures
- Nationally notifiable diseases: Physicians are required to report occurrence
 - Highly communicable
 - Highly communicable Associated with greater economic losses
 - Belong to diseases that have been eradicated
- Morbidity: Incidence of a disease
- Morbidity rate: Number of people affected in relation to the total population in a given time period
- Mortality: Deaths from a disease
- Mortality rate: Number of deaths from a disease in relation to the population in a given time
- Pathogenicity: The ability to cause disease
- Virulence: The extent of pathogenicity

72 Epidemiology II

Learning Objectives

- ✓ Language of disease
- ✓ Microbiota
- ✓ Factors that determine the microbiota

- ✓ Symbiosis
- ✓ Microbial antagonism

Definitions

- ❖ **Pathology: Study of diseases**
 - Cause of the disease
 - Pathogenesis: how disease establishes itself
 - Structural and functional changes
- ❖ **Infection: invasion by the microbes**
- ❖ **Disease: dysfunction**
- ❖ **Microbiome: Microbial communities that live in or on humans**
- ❖ **Normal microbiota or flora: permanent residence but do not cause diseases under normal conditions**
- ❖ **Transient microbiota: present for several days, weeks or months and then disappears**

Microbiota

- What determines the distribution and composition of it?
 - Nutrients
 - Physical and chemical factors
 - pH, O₂, CO₂
 - Host defenses
 - Stress, age

Symbiosis

- Living together is symbiosis
- Mutualism: both are benefited
- Parasitism: one is harmed
- Commensalism: One is benefited, other is unharmed

Microbial antagonism

- It is a competitive exclusion.
 - Compete for nutrients
 - By chemicals
 - Bacteriocins by E. coli suppresses Salmonellae
- Clostridium difficile:
 - Normal microbiota keeps it low but antibiotics can result in diarrhea

Opportunistic pathogen

- Normal microbiota under stress conditions can cause infections.

73 Epidemiology III

Learning Objectives

- ✓ Description of diseases
- ✓ Stages of diseases
- ✓ Sources of infection

Definitions

- ✓ Endemic: consistently present, but with low incidence
 - Infected ones are reservoir of infection
- ✓ Epidemic: within an area, unusually high incidence of a disease
- ✓ Pandemic: World wide epidemic
- ✓ Incidence: # of new cases in a given time period in a population
- ✓ Prevalence: New + existing cases

Disease Progression

- Infection: colonize and grows in body
- Incubation period: time b/w infection and diseases
- Acute period: height of disease
- Decline period: Disease symptoms subside
- Convalescent period: patient returns to normal

Definitions

- ❖ Reservoir: Sites where organisms of infections remain viable
 - Act as source of infection
- ❖ Inanimate objects: saprophytic (Cl. tetani)
 - Difficult to eradicate
- ❖ Animate: live in the body
 - Spread from person to person
 - By direct or indirect contact
- ❖ Single host organisms
 - Can be eradicated or controlled

Zoonosis

- Primarily an animal infection, but can be transmitted to humans
- Can assume epidemic proportions in humans
- Difficult to control or eradicate

Carrier

- A living carrier is a pathogen infected individual who does not show any signs or symptoms of the disease.
- A potential source of infection for others
 - Typhoid Mary story

74 Epidemiology V

Learning Objectives

- How diseases get transmitted
- Various vehicles of infection

Contact Transmission

- Direct: person to person
 - Physical contact
 - Influenza
- Indirect: A nonliving object is involved
 - Fomite: any nonliving object (towel, bedding)

- Droplet: mucus droplets

Vehicle Transmission

- Transmission by a medium
- Waterborne
- Foodborne
- Airborne
- Blood, other body secretion

Vectors

- Arthropods:
 - Mechanical transmission (Passive)
 - Biological transmission (Active)

Definitions

- Nosocomial Infections

75 Pathogenicity I

Learning Objectives

- How disease occurs
- Factors that aid in setting up of an infection

How disease occurs?

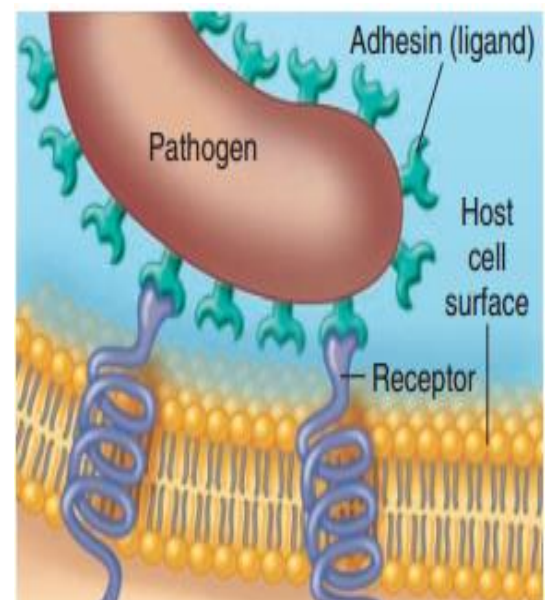
- When the microbe overpower host's body defenses, disease occurs.
- Pathogen must get access to the host.
- It must adhere to host tissues.
- It must penetrate or evade host defenses.
- It must damage the host tissues.

Portal of Entry

- Mucus membrane:
- Tubal organs
- Respiratory system and digestive system as largest portals
- Skin: It is the largest organ of the body in terms of the surface area.
- Parenteral route: By injury
- Preferred route of entry: Streptococci inhaled vs ingested

Invading Organisms

- Number: Few vs many
- ID50: Virulence can be expressed in terms of ID50
 - Depends upon the route of entry
- Bacillus anthracis:
 - Skin: ID50 is 10-50 spores
 - Inhalation: 10 -20 thousands spores
 - GI anthrax: ID50 is 250 to 1000 thousands spores
- LD50: Potency of toxin is expressed in LD50
 - Botulinum toxin in mice is 0.03 ng/kg
 - Shiga toxin: 250 ng/kg
 - Staph enterotoxin: 1350 ng/kg



76 Pathogenicity II

Learning Objectives

- How infectious organisms enter the body
- How organisms evade body defenses

Adherence

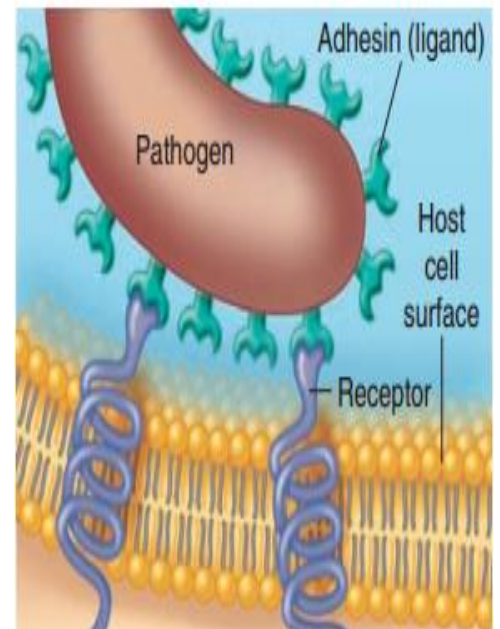
- Adhesion is mediated by adhesins or ligands to the receptors.
 - Adhesins present on glycocalyx, pili, fimbriae, or flagella.
 - These are glycoproteins or lipoproteins.

Biofilms

- Dental plaques is an example of biofilm.

Defense Evasion

- ❖ Capsule: Impairs phagocytosis
 - Antibodies can help
 - Only a few bacteria produce capsule
 - *Strep pneumoniae*
 - *Klbesiella pneumoniae*
- ❖ Cell wall components:
 - M protein
 - Found on cell surface and fimbriae
 - Resists phagocytosis
 - *Strep pyogenes*
- ❖ Cell wall components:
 - Mycolic Acid:
 - Resists phagocytosis
- ❖ Enzymes: (exoenzymes)
 - Coagulase: blood clotted
 - Bacteria is trapped or hides itself in the clot
 - Staphs produce it.
- ❖ Hyaluronidase: Dissolves CT
 - Bacteria can spread easily.
- ❖ Collagenase: Breaks down collagen
- ❖ IgA protease: Digests IgA antibodies
- ❖ Antigenic variation:
 - Influenza virus
 - *Neisseria gonorrhoeae*



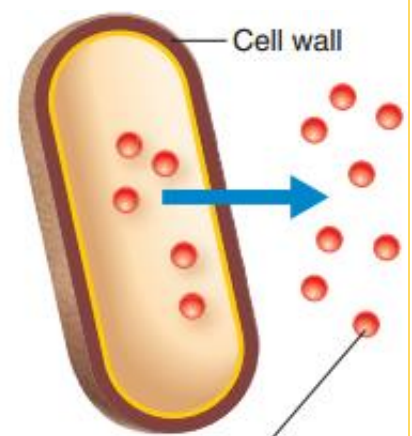
77 Pathogenicity III

Damage by Microbes

- ❖ Nutrient depletion
 - Siderophores for iron
- ❖ Direct damage by invasion
- ❖ Toxins
- ❖ Hypersensitivity reactions
- ❖ Nutrient depletion
 - Siderophores for iron
 - Lactoferrin
 - Transferrin
 - ferritin
- ❖ Direct damage:
- ❖ Bacteria that grows in host cells rupture the cells and spread further.

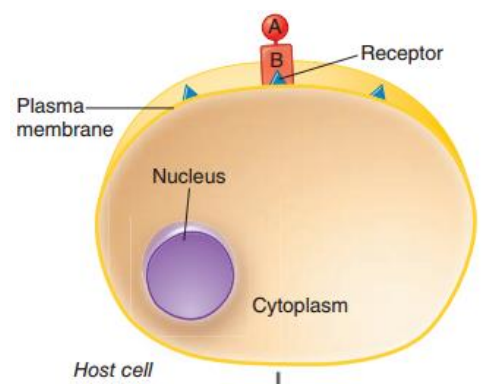
Toxin production:

- Exotoxins
 - Antitoxins
 - Toxoid
- A-B toxins



Toxin Types:

- ❖ A-B toxins:
 - diphtheria toxin
- ❖ Membrane disrupting toxins:
 - Cause lysis of cells
 - Leukocidins
 - Hemolysins
- ❖ Superantigens:
 - Provoke intense immune response
 - Cytokines are released by T cells.



Toxin production:

- Endotoxins
 - Fever
 - Shock
 - Limulus amoebocyte lysate assay (LAL)
 - Hemolymph from horseshoe crab

78 Innate Immunity I

Definitions

- ✓ **Immunity:** Ability of the body to protect itself from harmful effects of diseases.
- ✓ **Susceptibility:** lack of immunity

Types of Immunity

- a) **Innate:** Present at birth
- b) **Adaptive:** Acquired by experience

Innate Immunity

- ✓ **Present at birth**
- ✓ **Rapid to respond**
- ✓ **Nonspecific**
- ✓ **Do not increase in response with repeated exposures to the same agent**
- ✓ **Have no memory**
- ✓ **Innate Immunity**
- ✓ **First line of defense: Physical barriers (skin, mucus membrane)**
- ✓ **Second line of defense: Phagocytic cells and their secretions**

Adaptive Immunity

- a) **Specific**
- b) **Have memory**
- c) **Increase with repeated exposure**
- d) **Slower to respond**
- e) **Humoral immune response**
- f) **Cellular immune response**

79 Innate Immunity II

Innate Immunity

- **Toll like receptors (TLRs):** Protein molecules on defensive cells
- **Pathogen associated molecular patterns (PAMPs):** Formed by LPS, flagellin, peptidoglycan, DNA, RNA
- **Cytokines** are released as a result of defense cell stimulation by PAMPs and TLRs interactions.
- **Cytokines** recruits more immune cells and also stimulate adaptive immune response.

1st Line of Defense

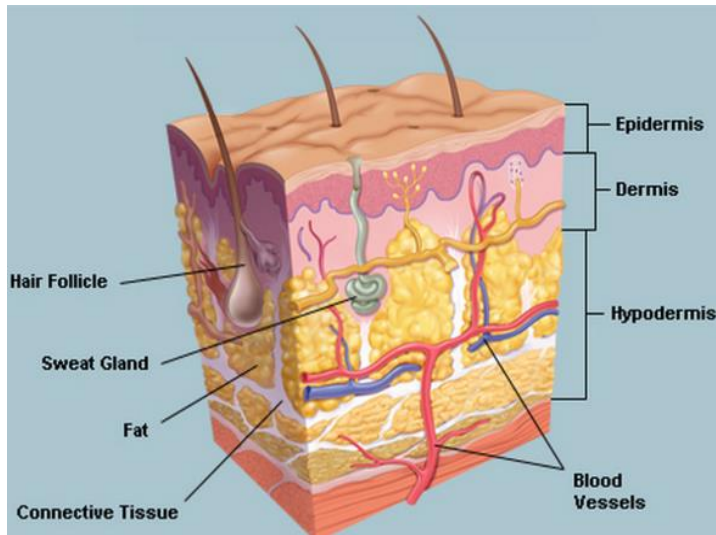
- **Physical Factors:**
 - **Skin**
 - **Mucus membrane**
 - **mucus**
 - **Lacrimal apparatus**
 - **Cilia**

Physical Factors:

- a) **Urine**
- b) **Earwax**
- c) **Peristalsis**
- d) **Vomiting**
- e) **Diarrhea**
- f) **Defecation**

Skin

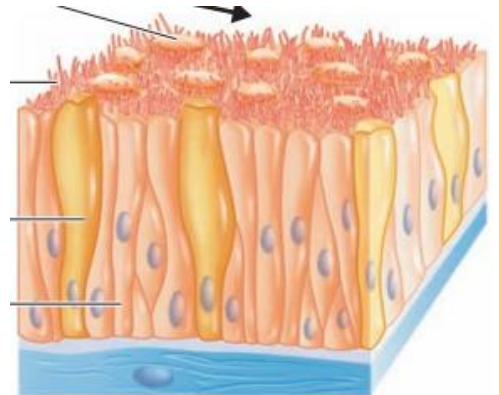
Mucus



Cilia

Chemical Factors:

- ✓ Cebum
- ✓ pH of the skin (3-5)
- ✓ Lysozyme in sweat, tears, saliva, body fluids and urine
- ✓ Acidic pH of stomach



2nd Line of Defense

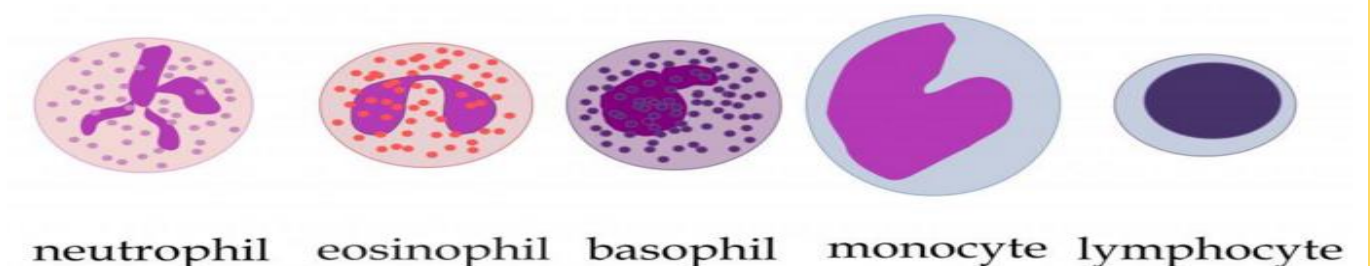
Leukocytes

- (1) Granulocytes
 - (i) Neutrophils
 - (ii) Eosinophils
 - (iii) Basophils
- (2) Agranulocytes
 - (i) Monocytes

Lymphocytes

- (1) Natural killer
- (2) B cell
- (3) T cell

Leukocytes



Summary

- ✓ Various cells of innate immunity
- ✓ Some secretions or molecules of innate immunity

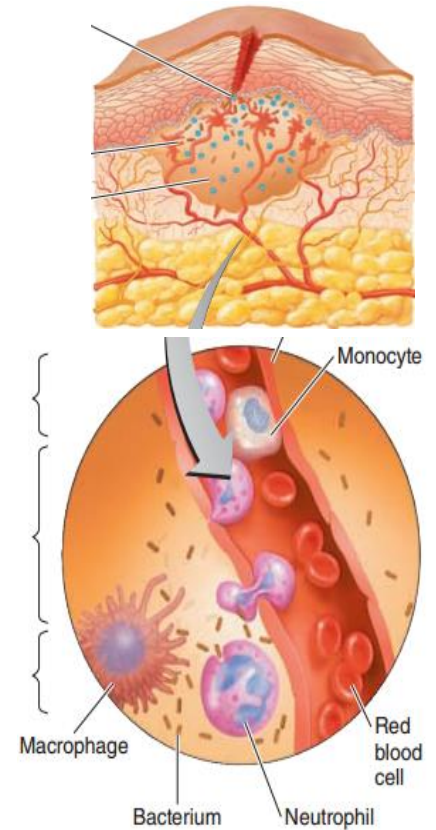
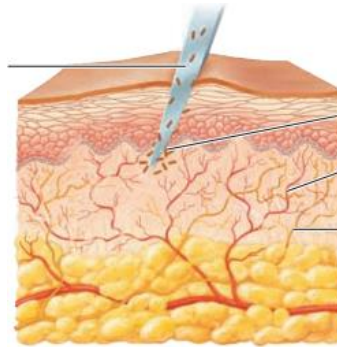
80 Innate Immunity III

Inflammation

- Damage to body tissues triggers a local defensive response

- Redness
- Pain
- Swelling
- Heat

- ✓ Histamine
- ✓ Kinins
- ✓ Prostaglandins by damaged cells
- ✓ Leukotrienes by mast cells
- ✓ Cytokines are released (TNF-alpha)
 - Causes release of acute phase proteins from liver.



Fever

- Systemic response
- Controlled by hypothalamus
- Pyrogens by microbes
- IL-1, TNF alpha
- Growth of microbes arrested

Complement System

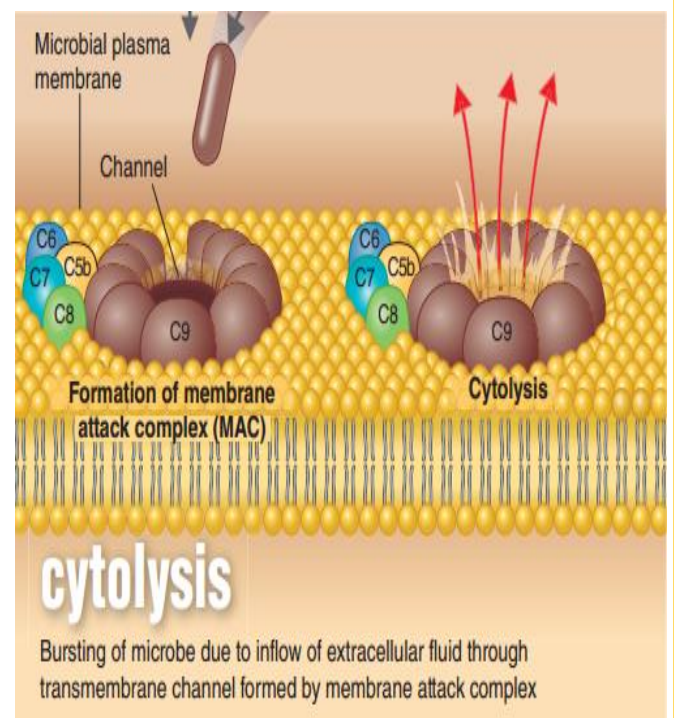
- More than 30 different proteins make complement system
- It complements the cells of the immune system in destroying microbes.

Interferons

- Antiviral proteins
- Produced by macrophages and lymphocytes in response to viral infections (fibroblasts)
- Interfere with viral replication
- Species specific
- Not viral specific
- Types
 - Alpha (infected cells)
 - Beta (infected)
 - Gamma (lymphocytes)

Antimicrobial Peptides

- 12 - 50 amino acid long peptides
 - Defensins by neutrophils
 - Dermicidin by sweat glands
 - Thrombocidins by platelets
- 1) Secretions of innate immunity
 - 2) Cytokines
 - 3) Complement
 - 4) Interferones



81 Adaptive Immunity I

Adaptive Immunity

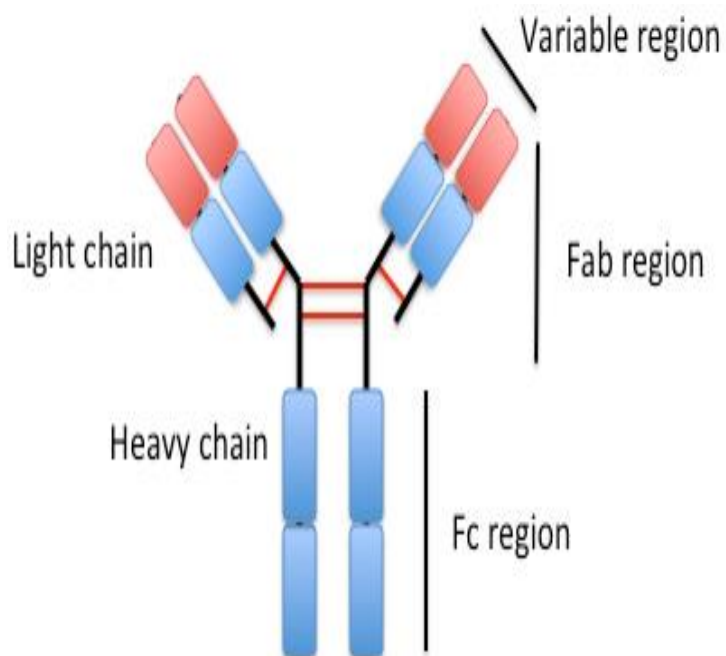
- 1st line of defense
 - Skin
- 2nd line of defense
 - Macrophages, neutrophils
- 3rd line of defense
 - B and T cells
 - Composed of specialized cells
 - B cells: humoral immunity
 - T cells: cellular immunity
 - This immunity is induced.
 - Observations: Individuals once recovered from smallpox, measles, or chickenpox become immune to it.
- Experiments led to antibodies discovery in serum and vaccination.

Adaptive Immunity I

Immunity Transfer Experiment



Antibodies by B cells



Thymus and skin grafting



Adaptive Immunity

- T cells were discovered
- Responsible for cellular immunity

B cells responsible for humoral immunity

T cells, for cellular immunity

82 Adaptive Immunity II

Adaptive Immunity

- ❖ **Antigen:** A foreign substance that provokes immunity
- ❖ More specifically called as Immunogen
- ❖ Protein in nature
- ❖ Could be large polysaccharides
- ❖ **Epitopes:** Specific regions on an antigen
 - Also called as antigenic determinants
- ❖ Most antigens have MW >10,000 D

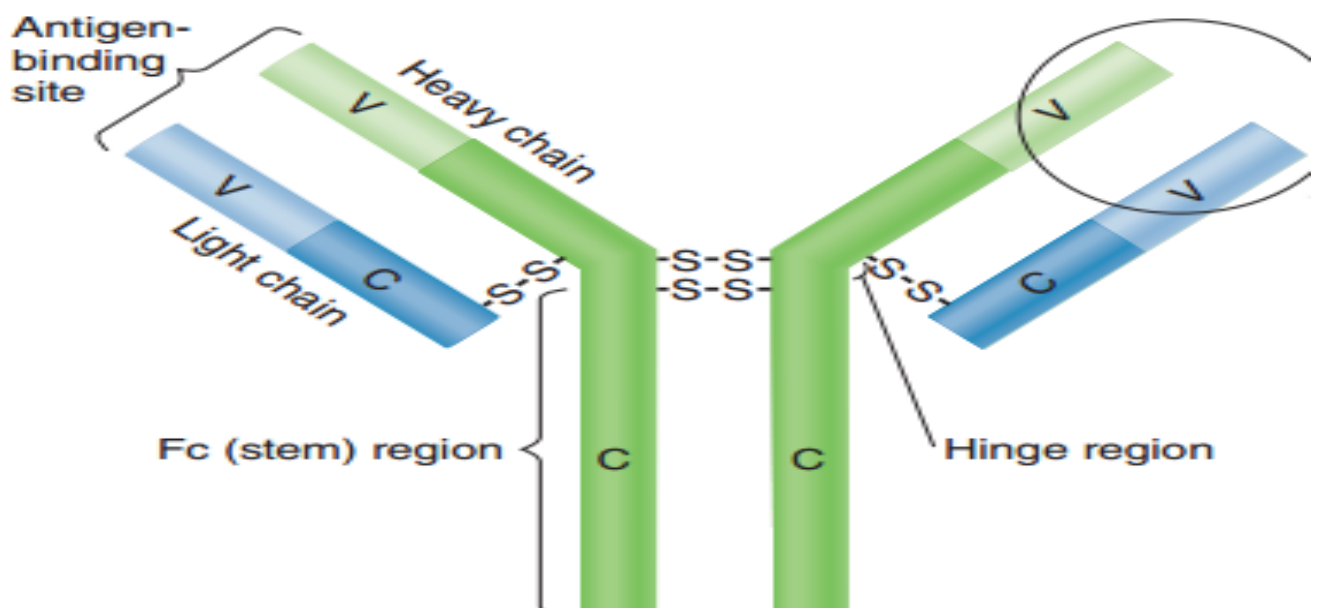
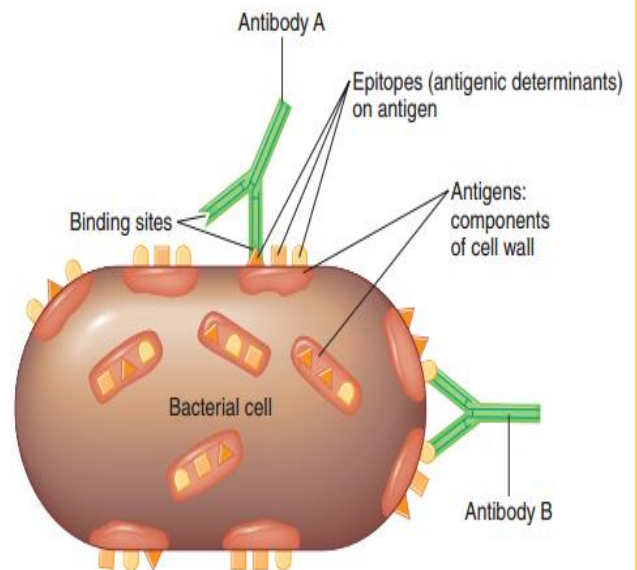
- < 10,000 D molecules are poor antigens
 - Haptens
- Need a carrier proteins to make antibodies
- Once Abs are formed, they can bind haptens.
 - Penicillin

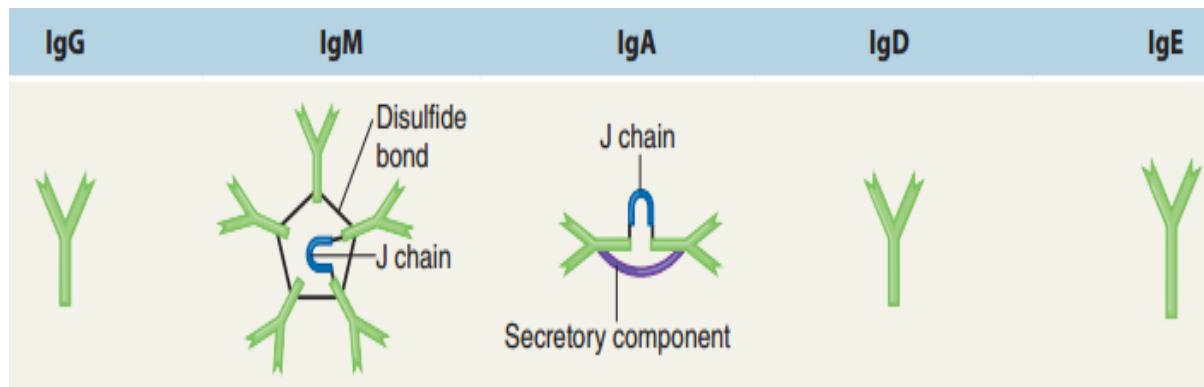
Antigen Characteristics

- High MW
- Complex in nature
- Protein are better
- Foreign to the body
- Degradable

Antibodies

- Glycoproteins in nature
- Present in gamma globulins of serum
- IgM: blood
- IgG: serum and body secretions
- IgA (mucus membrane)
- IgE (allergy) and IgD



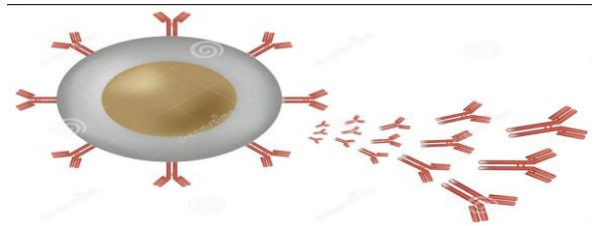


- Proteins are good antigens
- They have antigenic determinants
- Antibodies are glycoproteins
- Five classes of antibodies

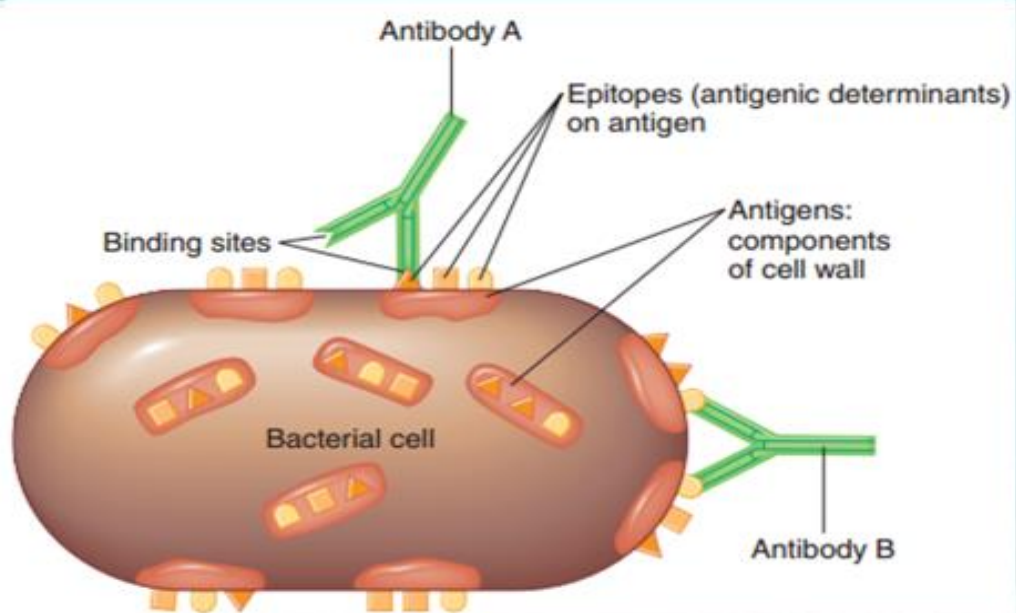
83 Adaptive Immunity III

Adaptive Immunity

- 1) B cells: Each B cell has unique antibody molecules against one epitope only.
- 2) B cell never changes its specificity.
- 3) May change its class



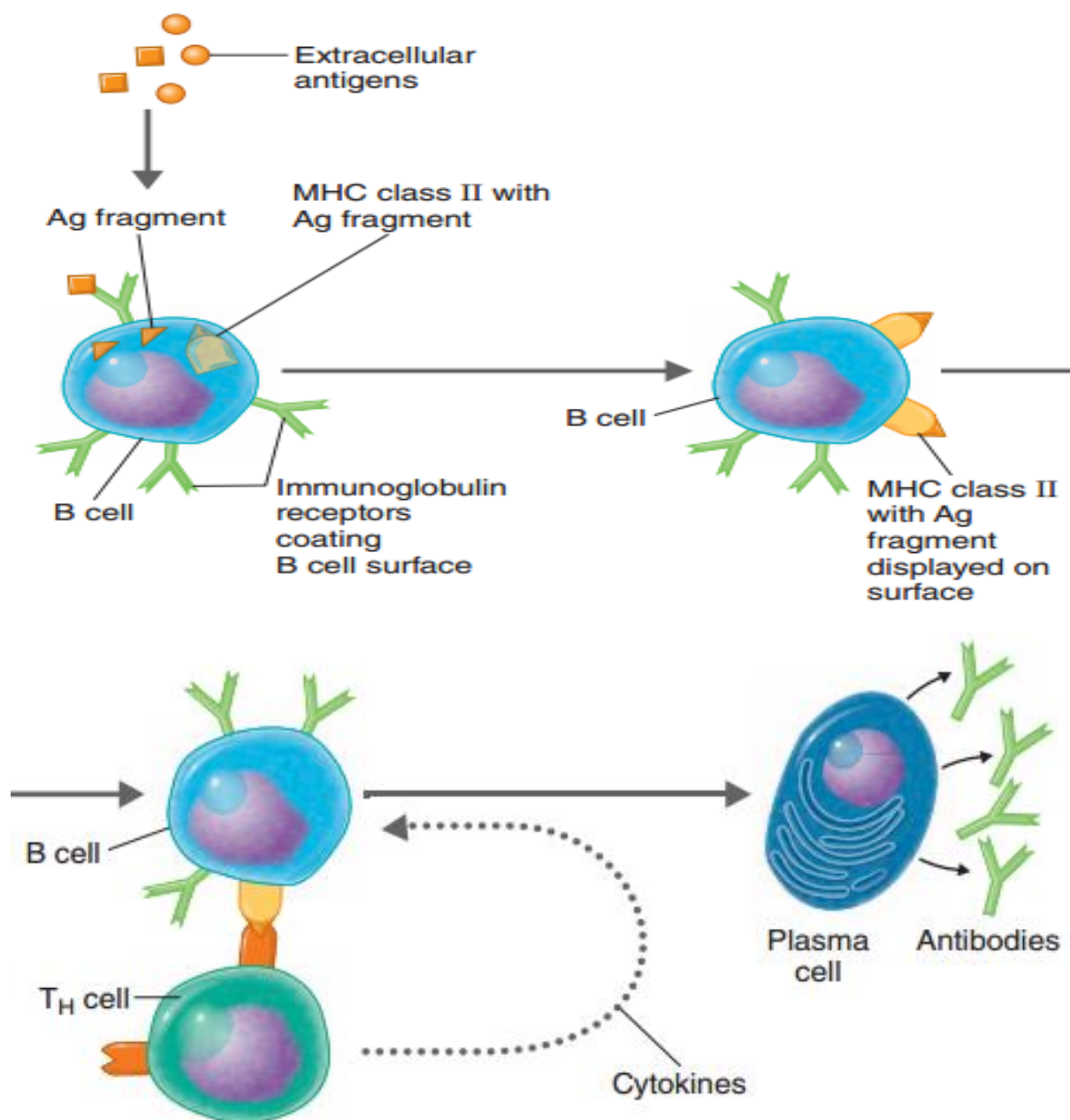
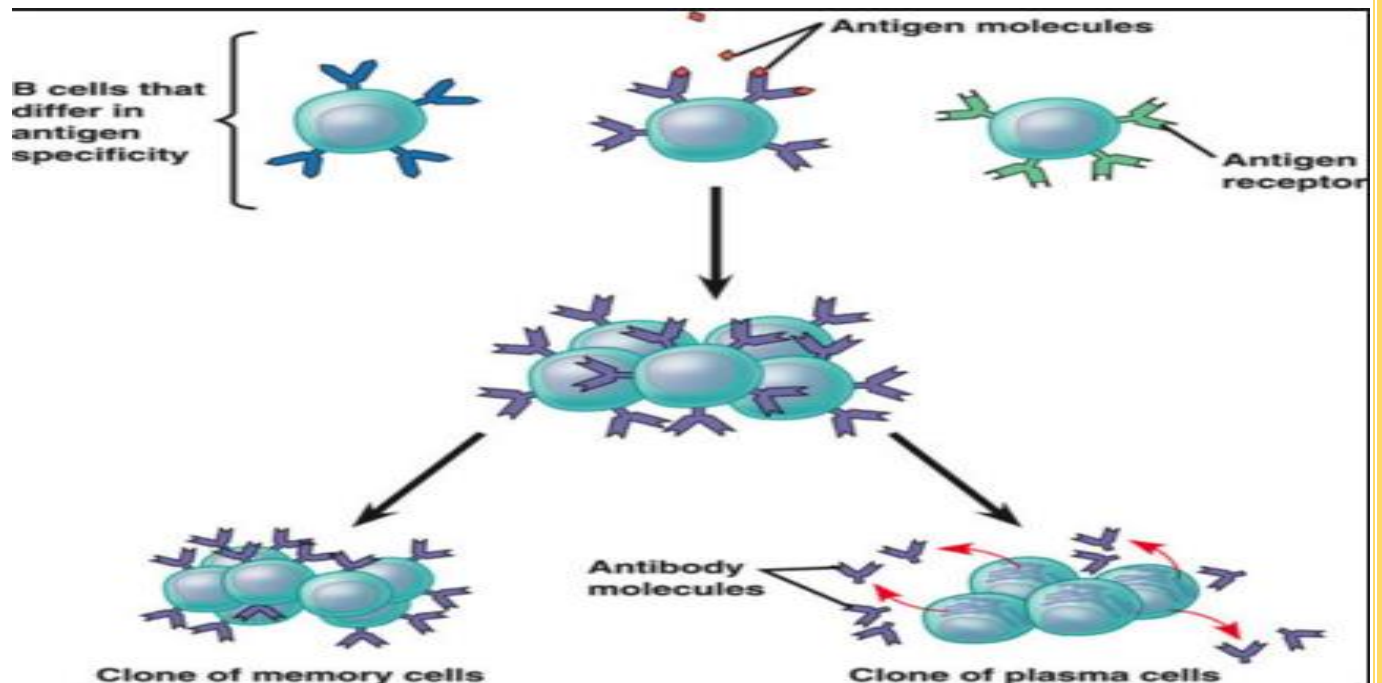
Epitopes on an Antigen



Adaptive Immunity

- Important: B cells in bone marrow create antibodies without even seeing the antigens.
- It is like choosing a shoe or a shirt from a shop!

Clonal Selection



- Clonal selection
- B cells are selected by the antigen to secrete antibodies

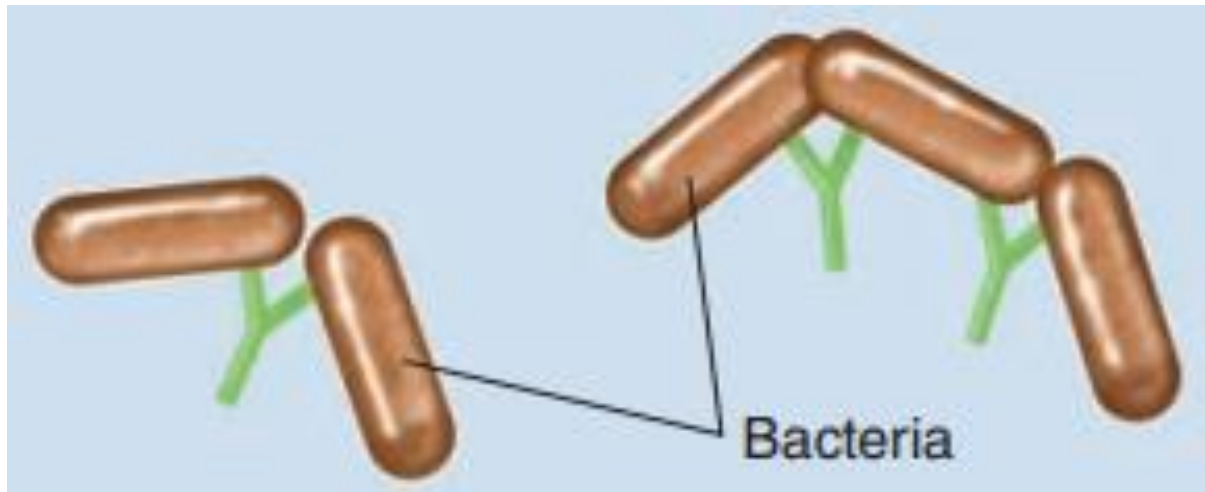
84 Adaptive Immunity IV

Ag-Ab Binding

1) Agglutination

- 2) Opsonization
- 3) Complement Activation
- 4) Ab-dependent cell-mediated cytotoxicity
- 5) Neutralization

Agglutination



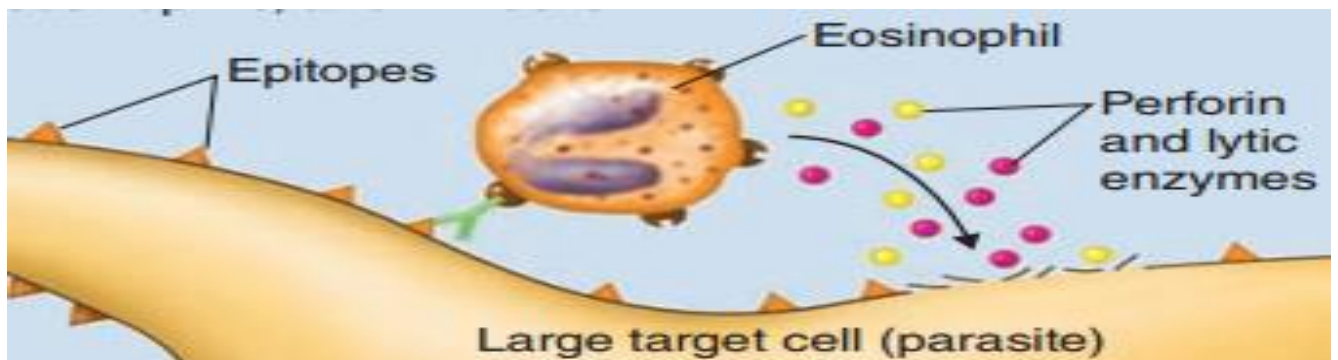
Opsonization



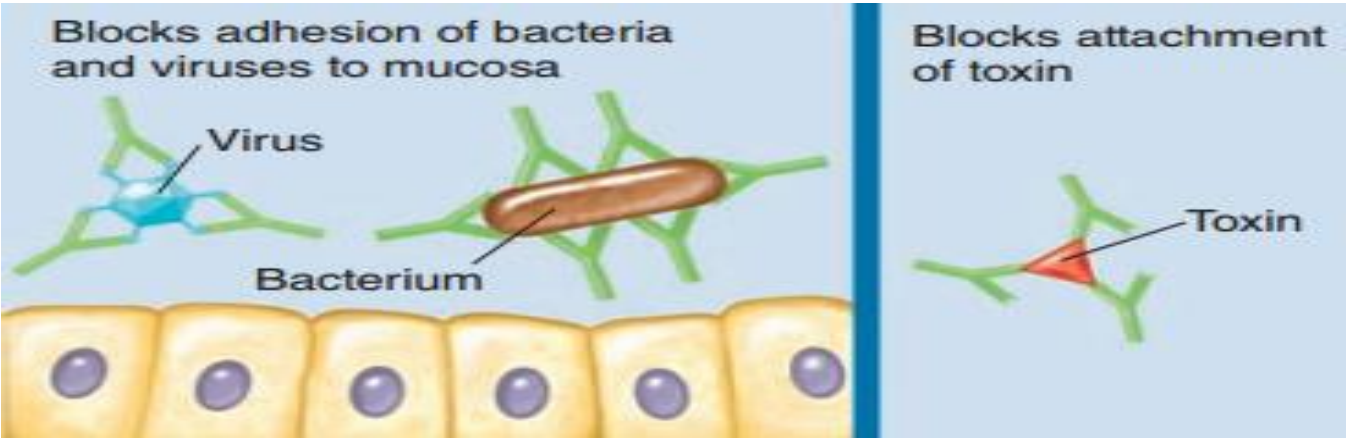
Complement Activation



Ab-Dependent Cell-mediated Cytotoxicity



Neutralization



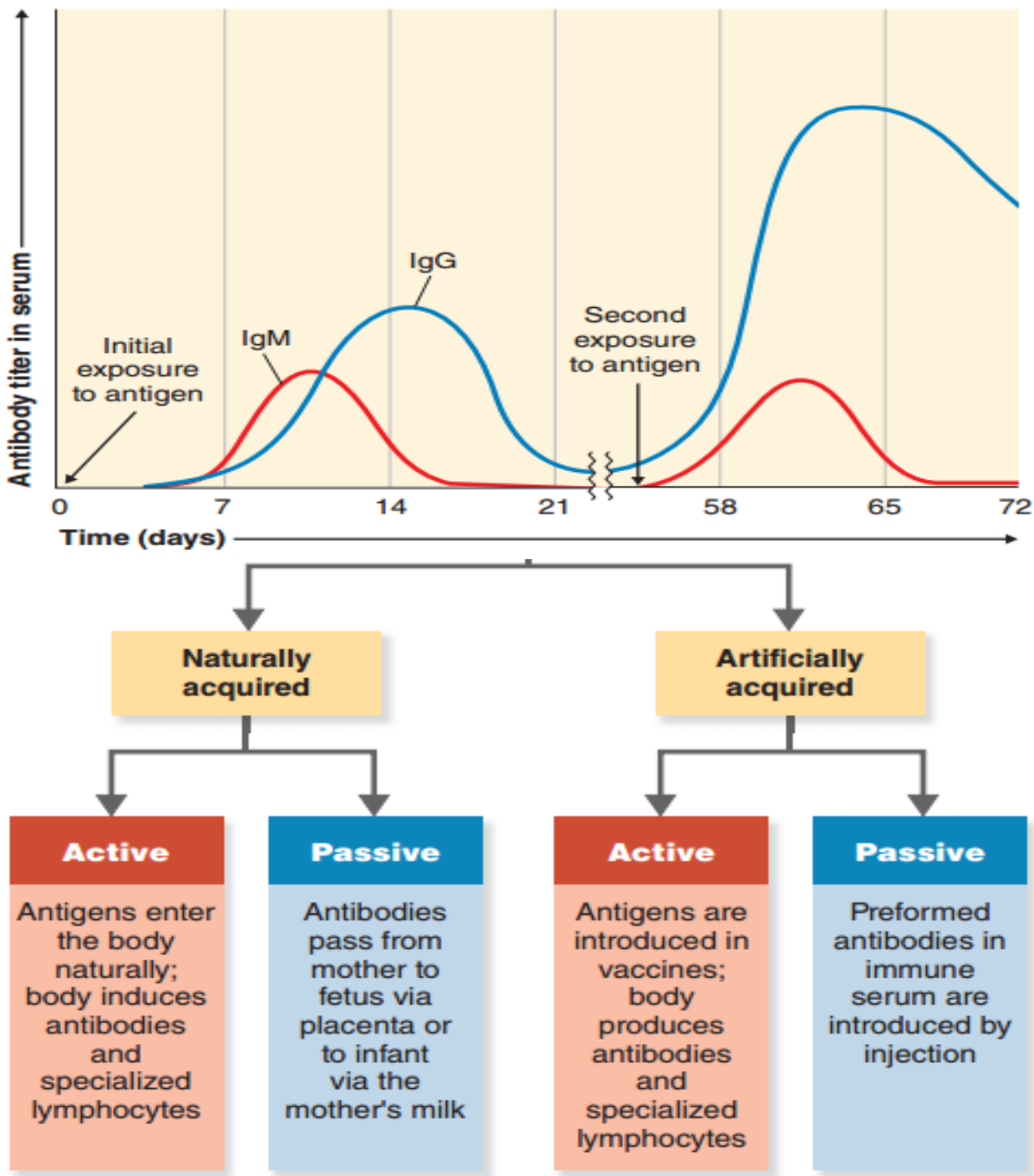
Antibodies are used by 5 methods to protect the body from microbes etc.

85 Adaptive Immunity V

missing

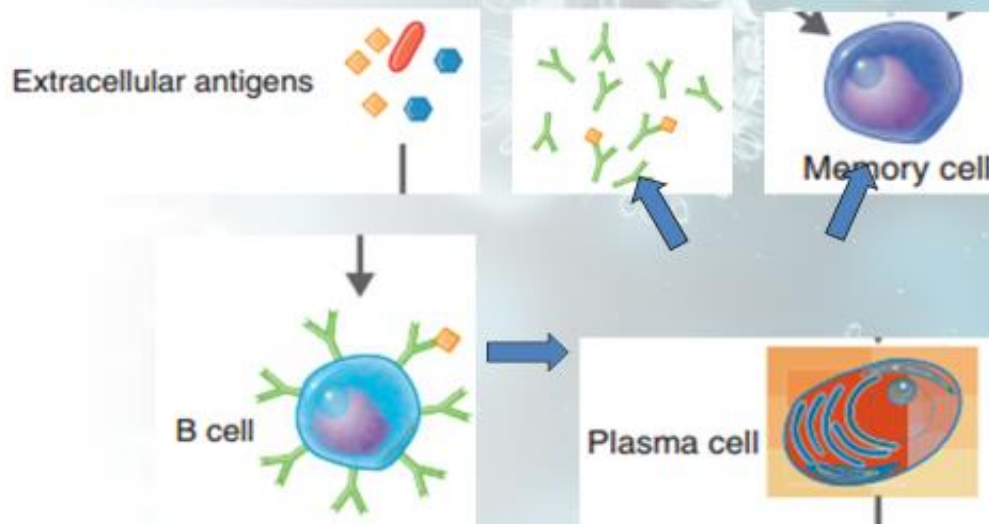
86 Adaptive Immunity VI

Primary and Secondary Immune Response



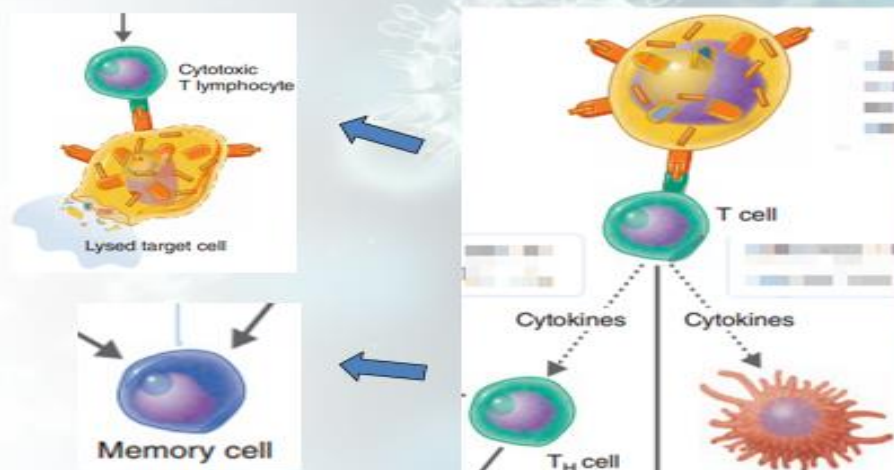
Adaptive Immunity VI

Humoral Immune Response Summary



Adaptive Immunity VI

Cellular Immune Response Summary



MHC Molecules

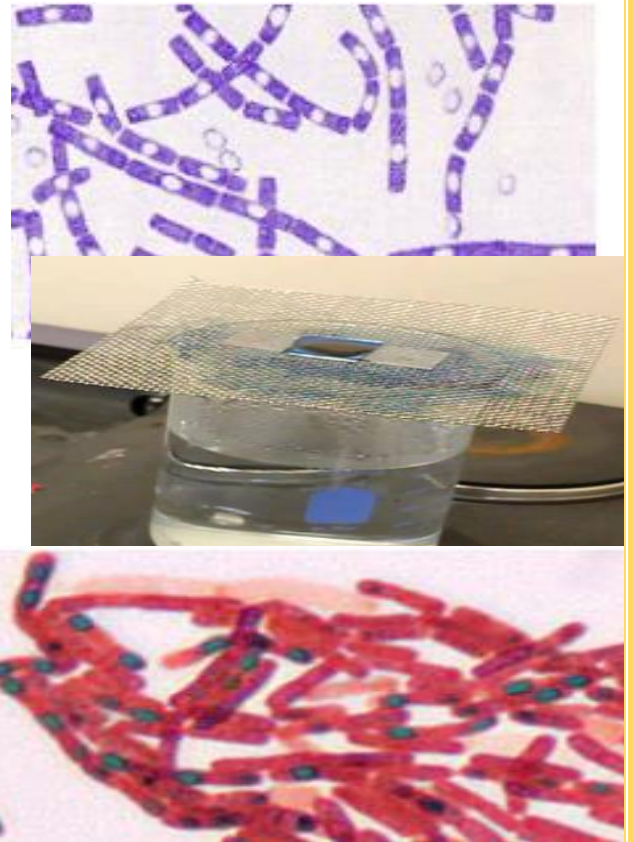
- 1) MHC I: Present on all nucleated cells
- 2) MHC II: Present only on APCs
- 3) Antigen Presenting Cells

Primary and secondary immune responses. Their importance and applications in vaccinations

87 Special staining I

Spore Staining

- Spores contain dipicolinic acid + Ca^{++}
 - Spores become heat resistant
 - So, strong stains are used with heat
 - Make a smear and air dry it.
 - Fix the smear by heat.
 - Heat water in a beaker.
 - Place slide with smear side up.
-
- Pour malachite green on the slide.
 - Keep it there on the boiling water beaker for 5 min.
 - Wash the smear with dH_2O .
 - Counterstain with safranin for 30 sec.
 - Observe under oil-immersion lens
-
- Spore can be seen with special stains.
 - Heating is needed for the stain to penetrate the cell.



88 Special staining II

Flagella Stain

- Transfer a drop of fresh culture on one end of a slide.
- Allow the drop to trickle down slowly
- Air dry the smear. Do not heat fix it.
- Cover the smear with mordant (tannic acid 10% in 5% NaCl) for 10 to 30 min.
- Rinse the smear gently with H_2O .
- Add carbol fuchsin and let it sit for 5-15 min.
- Rinse off the stain with water
- Air dry the smear
- See under the microscope

Flagella can be seen with or without the stain (by observing motility).

89 Special Staining III

Acid Fast Staining

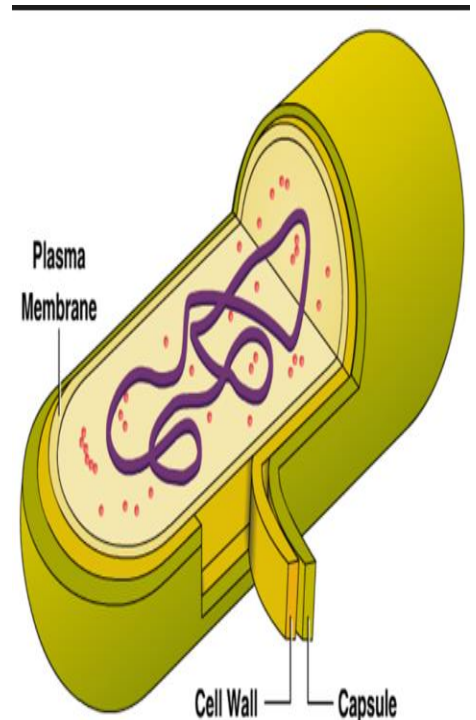
- Differential stain
- 1882, Paul Ehrlich observation on Mycobacterium
- Not easily stained
- Once stained do not lose the stain even with acid alcohol
- Mycobacterium have mycolic acid (waxy)
- Makes cell wall impermeable to most stains
- Ziehl Neelsen method is commonly used
- Make a smear
- Carbol fuchsin on slides
- Heat the slide for 5-10 min
- Wash with water and then with acid alcohol for 1 min.
- Counterstain with methylene blue for 1 min.
- See under the microscope.

Acid fast bacteria have mycolic acid that can be stained by heating the organisms with the stain.

90 Special Staining IV

Capsular Staining

- It is extracellular material (glycocalyx).
- When regular and thick, called capsule
- When loose and not organized, called slime
- Thickness of the capsule depends upon the culture conditions.
- Mostly are water soluble.
- Capsular organisms usually make the broth viscous and stringy
- Colonies look moist, glistening, and sticky.
- Involves in virulence
- Those lacking capsule are rough strains.
- Prepare a thick smear in a loopful of congo red (1%) stain
- Fixation in acid alcohol for 15 sec
- Wash with dH₂O
- Cover it with acid fuchsin for 1 min.
- Wash with water
- Bacteria stains red
- Capsule colorless
- Dark blue background



Capsule can be seen with staining.

Helps in identifying organisms.

91 Lymphatics

missing

92 Hypersensitivity I

Hypersensitivity?

- Abnormal antigenic response
- Allergy: another name
 - Those antigens are called allergens.
 - Immune system acts in a damaging manner.

Types of Hypers

- 1) Type I (Anaphylactic):
- 2) Type II (Cytotoxic):
- 3) Type III (Immune complex):
- 4) Type IV (delayed cell-mediated):

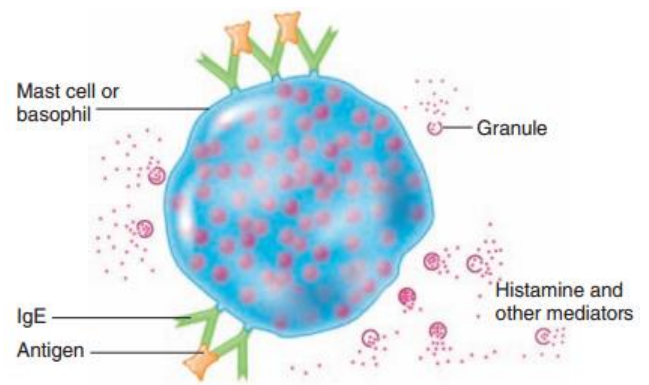
Anaphylaxis?

- How to recognize it?
- ✓ Difficulty in respiration

- ✓ Shock
- ✓ Hypotension
- ✓ Increase in heart rate

Anaphylactic

- ❖ Happens in <30 min.
- ❖ IgE is involved which binds to mast cells
- ❖ Mast cells degranulate and secrete mediators of inflammation.
 - Histamine, leukotrienes, PG



Anaphylaxis

- ✓ Shock from drug reactions
- ✓ Venoms
- ✓ Common allergens causing asthma
- ✓ Diagnosis by demonstration of IgE by i/d injections of allergens one by one.
- ✓ Quantitation of IgE by ELISA
- ✓ Rx: Epinephrine & antihistaminic drugs

Anaphylaxis involves IgE antibodies. Histamine and other mediators mediate the reaction. Could be treated by antihistaminic drugs.

93 Hypersensitivity II

Type II: Cytotoxic

- Involves
 - Antigenic cell
 - IgG or IgM
 - Complement
- Blood transfusion reaction is an example.

Illustration	Plasma Antibodies
	Neither anti-A nor anti-B antibodies
	Anti-A
	Anti-B
	Anti-A and Anti-B

Drug induced cytotoxicity

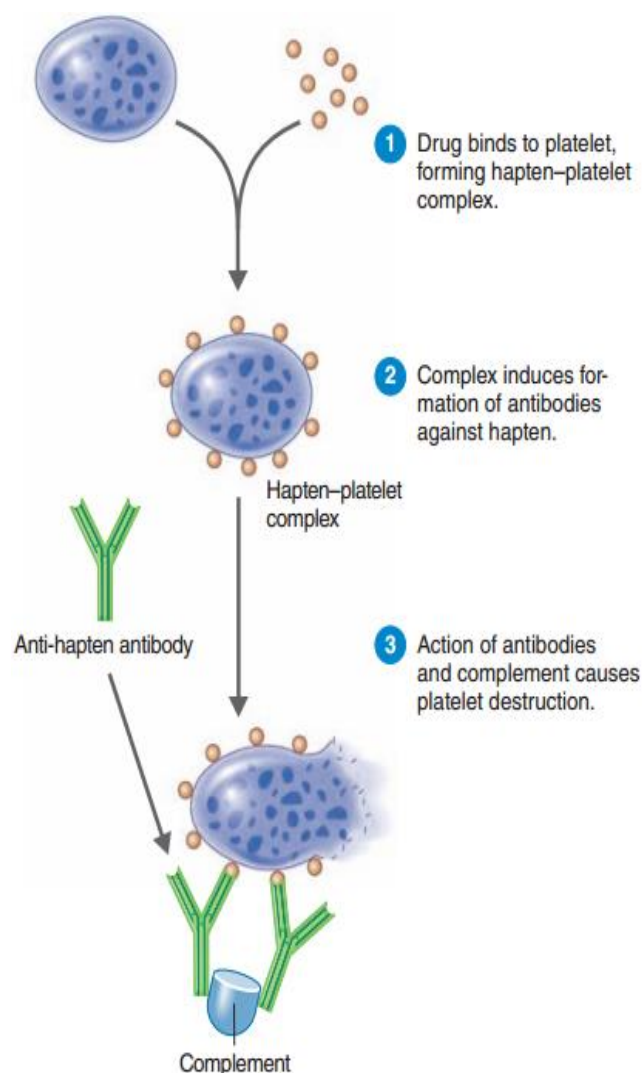
Diagnosis

- Cell deficiencies
- Low number of RBCs due to hemolysis
- Thrombocytopenia if platelets are involved.

Rx

- Avoid those drugs that cause it.
- Blood transfusion with correct blood type
- Symptomatic treatment

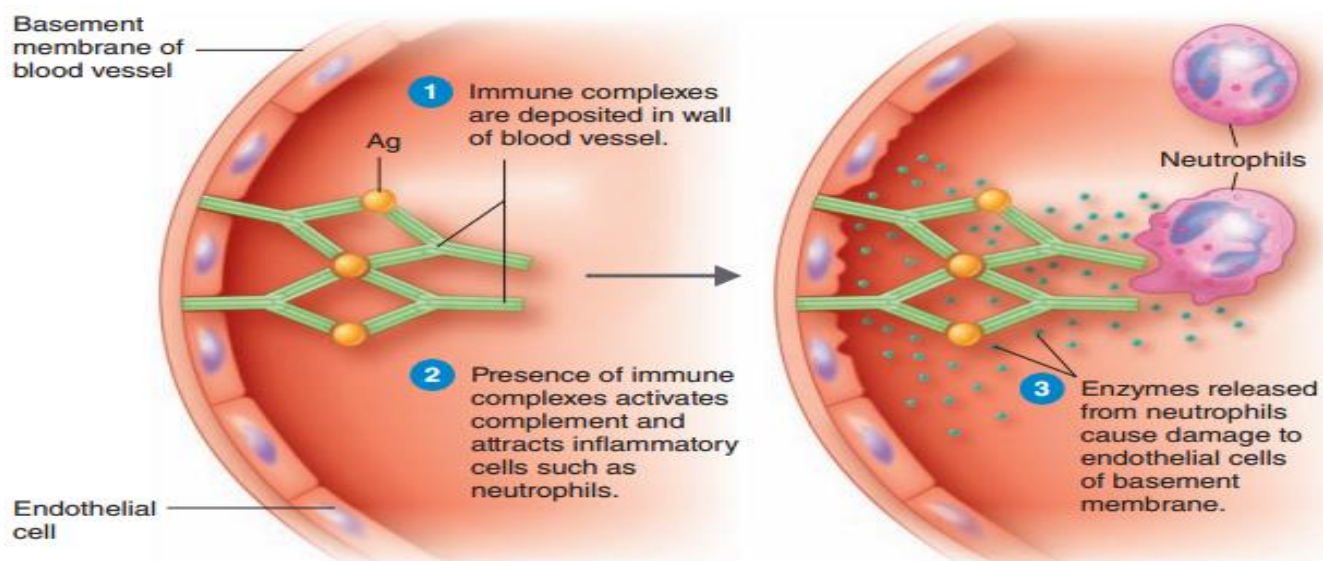
IgG or IgM involved Causes damage to the cells.Treated according to the cause.



94 Hypersensitivity III

Immune complex

- Involves
 - Soluble Antigen circulating in the serum
 - IgG or sometimes IgM
 - Complement



- Diagnosis by history and symptoms
 - Glomerulonephritis

- Confirmed by biopsy and testing of presence of IgG in kidney cells
- Rx. Corticosteroids

Immune complexes are formed which when get stuck in capillaries cause damage by compliment activation.

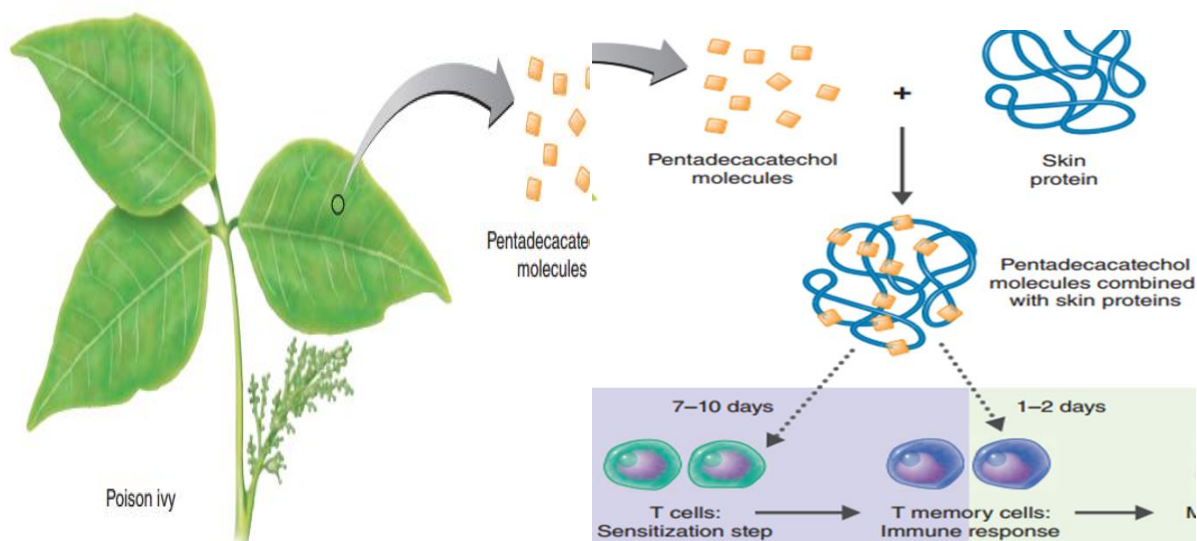
95 Hypersensitivity IV

Type IV

- Originally called tuberculin reaction
- Robert Koch performed the test. Contact dermatitis is the most common example.

Involves:

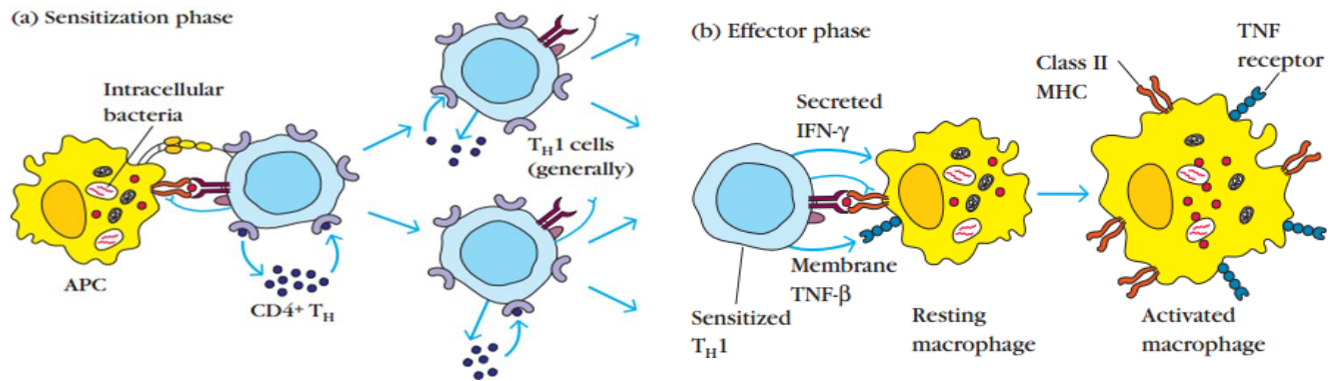
- Antigen
- T cells
- Macrophages
- Transplant rejection is good example.
- Reaction to hair dye



Type IV: Ivy & Latex Glove Reaction



- How it happens:
 - Macrophages take up the antigen
 - Present it to CD⁴⁺ Th1 cells
 - Th1 cells secrete cytokines that activate macrophages



Diagnosis:

- Skin testing
- Rx: symptomatic
- Avoid contact

T helper cells are involved mostly. Rx. Avoid the contact or symptomatic Rx

96 Vaccine

Concept of Vaccines

- People recovered from infections are immune to them.
- Vaccine: Suspension of organism or fraction of organism that induces immunity

Principle of Vaccines

- It mimics the infection by the organism but without causing the disease

Types of Vaccines

- Live attenuated:
 - Weakened organisms
 - Louis Pasteur was the first to do that.
 - *Pasteurella multocida*

Benefits of Live Vac

- Better immunity: mimics actual infection
 - Humoral
 - Cellular
- Virus replicates increasing the Ag dose
- Long term immunity

Types of Vaccines

- Inactivated Killed:
 - Organism killed with formalin or phenol
 - But why??
- Cannot be weakened
- Need frequent boosters

Inactivated Killed

- Examples

- Rabies
- Polio
- Influenza
- Mostly induce humoral immunity

Other Vaccines

- ❖ **Toxoids:** Toxins are inactivated and used as a vaccine.
 - Tetanus toxoid
- ❖ **Conjugated Vaccines:**
 - Poor antigens attached to proteins
 - *Haemophilus influenzae* type b
- ❖ **DNA vaccines:**
 - DNA injected into cells
 - Proteins are expressed which stimulate immunity
 - West Nile Virus
- ❖ Polysaccharides have poor success

Modern Vaccines

- **Subunit vaccines:**
 - Portion of viral or bacterial proteins that can induce immunity
- Also called recombinant vaccines

Modern Trends

- Organisms in bulk are needed to make vaccines.
- Bacteria can be grown in flasks by providing medium of growth
- Cell culture for viral vaccines
- Chicken embryos can be used to grow viruses
 - Influenza vaccine
- Animals can be used to grow viruses
- Plants can be used in recombinant technology

Vaccines mimic infection but without harmful effects. Various types are available. Booster is required for better protection.

97 Antimicrobials I

A Little History

- Paul Ehrlich coined the word “chemotherapy”
- His observation: Organisms stained without staining the background.
- 1928: Alexander Fleming working with *Staph aureus* observed colony of a mold with inhibitory effect.
 - *Penicillium notatum*
 - Later, *P chrysogenum*
- Disinfectant:
 - Toxic to all
- Antimicrobials used for chemotherapy:
 - Selectively toxic to microbes sparing body cells



Microbe	Antibiotics
Bacillus subtilis	Bacitracin
Paenibacillus polymyxa	Polymyxin
Streptomyces nodosus	Amphotericin B
Streptomyces venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Saccharopolyspora erythraea	Erythromycin
Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpurea	Gentamicin

Antibiotic Discovery

- Must not be toxic to the body
 - Many antibiotics are produced by microbes, but only a few are selectively toxic to microbes.

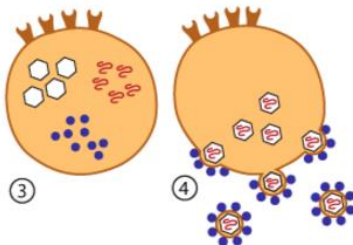
Antibiotics are products of microbes against microbes.

Must not be toxic to the body.

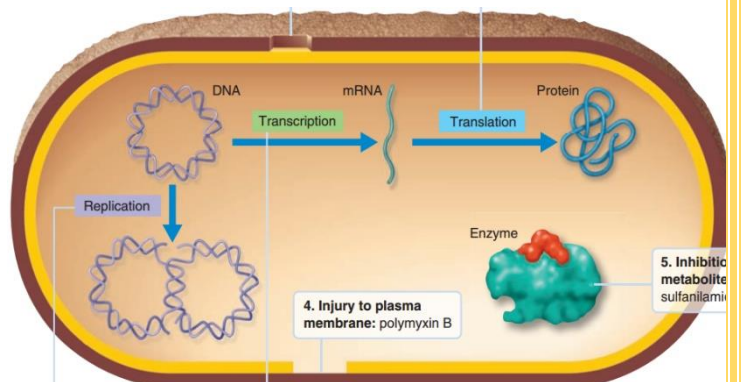
98 Antimicrobials II

A Little History

- Developing drugs against prokaryotes is relatively easy
- Against eukaryotes is difficult
 - Viruses and fungi



- Problem is at the cellular level
- Spectrum of Drugs
- Narrow: targets only a group of organisms
 - Penicillin for G+ve
- Wide: Targets many groups of microbes
 - Tetracycline
- Narrow: well targeted
 - Spare microflora
- Wide: Targets every organism
 - Destroy normal microflora as well
- Candida albicans
 - Grows in the absence of normal microflora esp. in children
- Drug Targets



Target: Cell Wall

- Inhibition
 - Penicillins
 - Cephalosporins
 - Bacitracin
 - Vancomycin

Target: Protein Synthesis

- Inhibition
 - Chloramphenicol
 - Erythromycin
 - Tetracyclines
 - Streptomycin

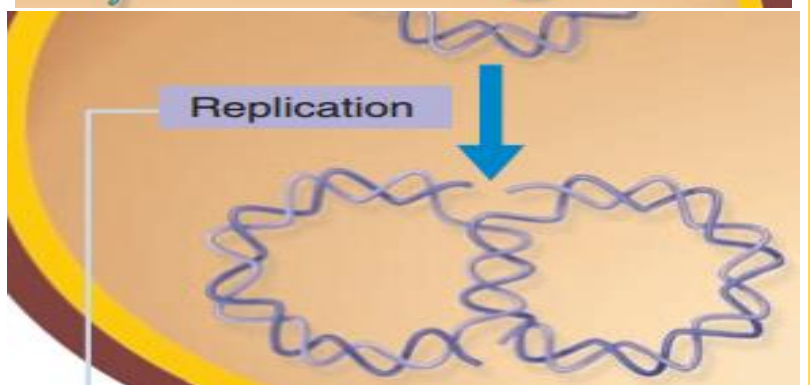
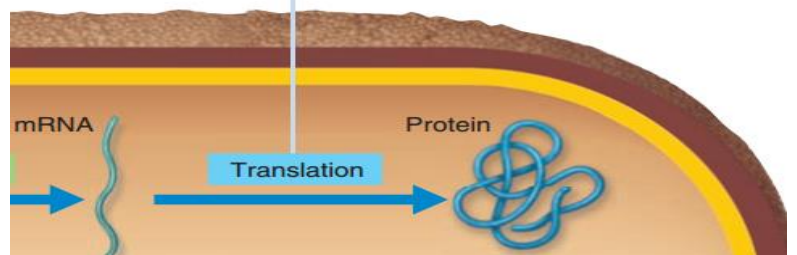
Target: DNA Replication

- Inhibition
 - Quinolones
 - Rifampin

1. Inhibition of cell wall synthesis: penicillins, cephalosporins, bacitracin, vancomycin



2. Inhibition of protein synthesis: chloramphenicol, erythromycin, tetracyclines, streptomycin



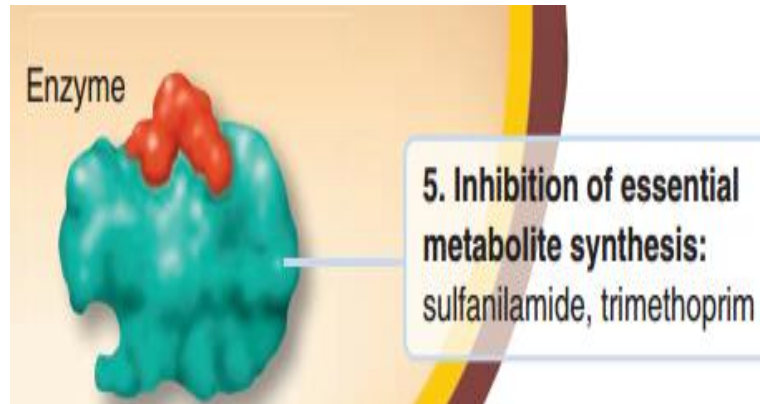
Target: Plasma Membrane

- Injury
 - Polymyxin B

Target: Ess. Metabolite

- Inhibition:
 - Sulfanilamide
 - Trimethoprim

Antibiotics selectively inhibit various components of microbes.



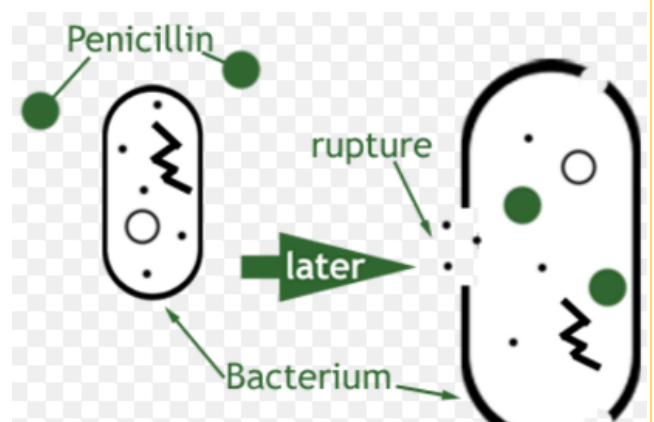
99 Antimicrobial's III

Terminology

- ❖ Bactericidal:
 - Kill microbes directly.
- ❖ Bacteriostatic:
 - Prevent growth of microbes

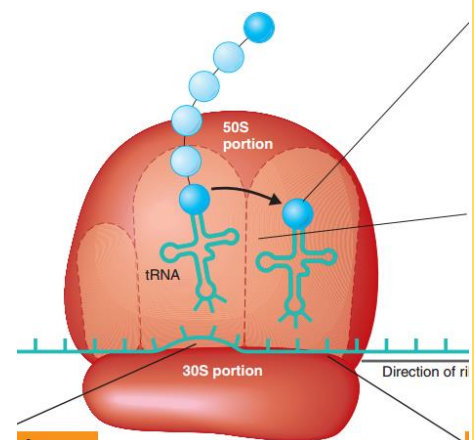
Cell Wall Inhibition

- ❖ Target is peptidoglycan
 - Cell wall does not get synthesized
 - Cell lysed as a result (cidal).
 - Targets growing cells
 - Penicillin



No Protein Synthesis

- Based on ribosome structural differences in pro and eukaryotes
 - Eukaryotes: 80S
 - Prokaryotes: 70S
- Chloramphenicol:



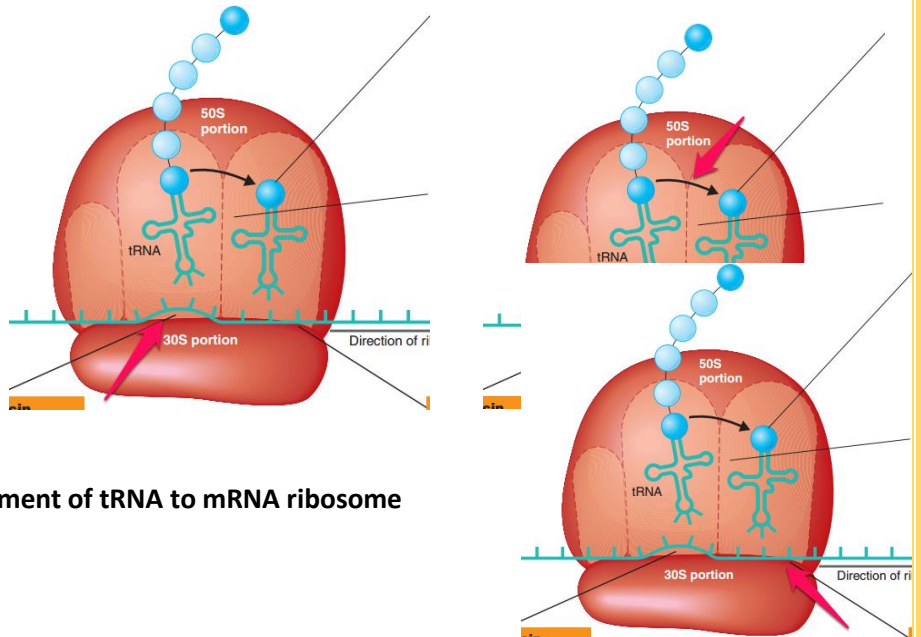
- ✓ Binds to 50S
- ✓ Inhibits peptide bonding
- ✓ Bacteriostatic

- **Streptomycin:**

- ✓ Changes shape of 30S
- ✓ Misreading of RNA codes
- ✓ Bacteriostatic

- **Tetracyclines:**

- Interfere with attachment of tRNA to mRNA ribosome complex
- Bacteriostatic



Plasma Membrane

- ❖ Polypeptide antibiotics brings changes in the permeability of plasma membrane
- ❖ Result in the loss of imp metabolite from the cell
- ❖ Antifungal drugs are examples
 - Amphotericin B
 - Ketoconazole
- ❖ Combine with sterols in the plasma membrane
- ❖ Bacteria lack sterol

Nucleic Acid Synth

- Interfere with DNA replication and transcription
 - Very limited b/c can interfere with body cells

Essential Metabolites

- Sulfanilamide and para-aminobenzoic acid (PABA)
- PABA is a substrate for an enzyme that is involved in folic acid synthesis
- FA is needed for purine and pyrimidine synthesis

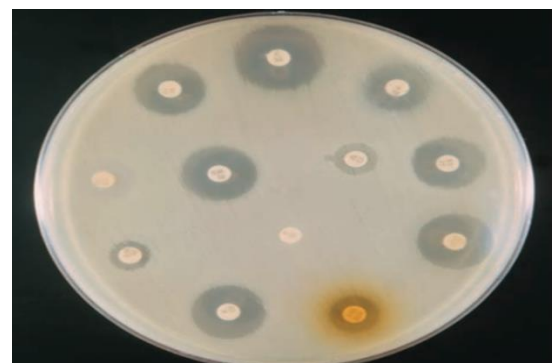
100 Antimicrobials IV

- **Drug Sensitivity**

- ❖ Physicians usu prescribe drugs that they know will work.
- ❖ If the organism has been identified, selection of drug is easy.

- **Disk Diffusion Method:**

- ❖ Standardized inoculum spread on agar plate
- ❖ Filter paper disks soaked with antibacterials
- ❖ Zone of inhibition is recorded
- ❖ Zone compared to a standard Table



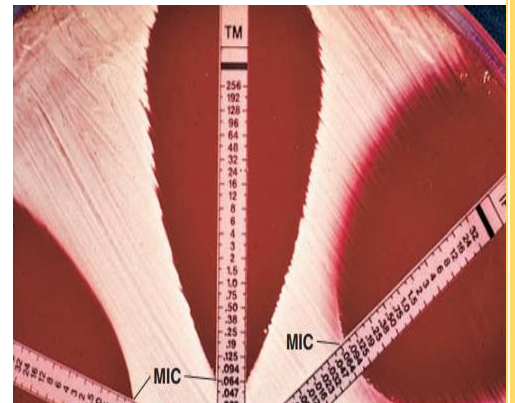
❖ **Report:**

- Sensitive
- Intermediate
- Resistant

❖ **Minimum Inhibitory Concentration (MIC):**

❖ Also called E test:

- The lowest conc. that inhibits visible growth



❖ **Broth Dilution Method:**

❖ Diffusion method has limitation

- Does not tell you if the drug is bacteriostatic or bactericidal

❖ A broth dilution test is the answer.

❖ Various dilutions of the drug made and tested with test organism

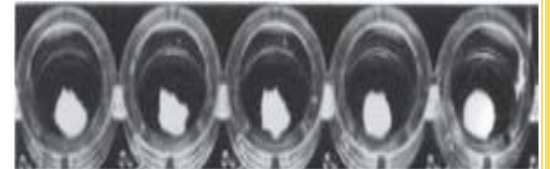
❖ Broth Dilution Method:

❖ Tells both MIC and minimal bactericidal concentration (MBC)

❖ The wells that do not show growth are important.

❖ Culture the well that is one well higher in concentration than MIC.

- If growth occurs, the drug is bacteriostatic, otherwise cidal.



101 Antimicrobials V

Cell Wall

- ❖ Penicillin
- ❖ Cephalosporins
 - Cefixime (4th gen)
- ❖ Polypeptide antibiotics
 - Bacitracin (topical)
 - Vancomycin (Pen-R)
- ❖ Antimycobacterials (inhibit mycolic acid synthesis)
 - Isoniazids
 - Ethambutol

Protein Synthesis

- ❖ Chloramphenicol (potentially cytotoxic)
- ❖ Aminoglycosides
 - Streptomycin
 - Neomycin (topical)
 - Gentamycin (Pseudomonas)

❖ Tetracyclines

❖ Tetracyclines

- Against chlamydias and rickettsias
- Animal feed additives

❖ Macrolides

- Erythromycin
- Azithromycin (ssyn)

Plasma Membrane

- Polymixin B
 - Topical for G-ve

Nucleic Acid Syn

❖ Rifampin

- Inhibits synthesis of mRNA
- Rx for tuberculosis

❖ Quinolones and Flouroquinolones

- Nalidixic acid
- Norfloxacin
- Ciprofloxacin

- Used preferably for urinary tract infections

Essential Metabolites

- **Sulfonamides**
 - ✓ Sulfa drugs
 - ✓ Trimethoprim
 - ✓ Sulfamethoxazole
 - ✓ Common combination used

Various antibiotics target various components of the cell.

102 Antimicrobials VI

Antifungal Drugs

- ❖ Fungal Sterols (PM)
 - Polyenes
 - Amphotericin B
 - Used for systemic infections
 - Azoles (inhibits synthesis of PM)
 - Clotrimazole (topical)
 - Ketoconazole
 - Voriconazole
 - Crosses BBB
- ❖ Nucleic Acids
 - Flucytosine
 - Inhibits synthesis of RNA and DNA in fungal cells
- ❖ Other Antifungals
 - Griseofulvin
 - Inhibition of mitotic microtubules
 - Rx of skin infections
- ❖ **Antiviral Drugs**
- ❖ Nucleoside and nucleotide analogs
 - Acyclovir
 - Ganciclovir
 - Ribavirin
 - Used mostly for herpesviruses
- ❖ **Attachment and uncoating (flu virus)**
 - Zanamivir
 - Inhibits neuraminidase
 - Amantadine
 - Zimantadine

- Inhibits uncoating

Drugs can selectively kill fungi and viruses

Not all viruses could be killed by drugs

103 Antimicrobials VII

Antiprotozoan Drugs

- ❖ Chloroquine
 - Inhibits DNA synthesis
 - Used against malaria
- ❖ Metronidazole
 - Interferes with anaerobic metabolism
 - Used for giardiasis, amebiasis, trichomoniasis

Antihelminthic Drugs

- ❖ Praziquantel
 - Alters permeability of PM
 - Used for tapeworm and fluke infections
- ❖ Albendazole, Mebendazole
 - Inhibit absorption of nutrients
 - Used against intestinal roundworms
- ❖ Ivermectin
 - Paralyzes worms
 - Used against intestinal roundworms
 - Mites and lice

Protozoa and helminths can be killed too with drugs

104 Antimicrobials VIII

Drug Resistance

- ✓ Resistance begins with mutations
- ✓ Resistance transmitted by conjugation and transduction
- ✓ Blocking Entry:
- ✓ Inactivating by enzymes:
- ✓ Alteration of target molecule
- ✓ Efflux of antibiotics

Superbugs

- ❖ Bacteria resistant to many antibiotics
 - MRSA (most commonly)
 - *Staph aureus*
 - *Enterococcus faecium*

- *Klebsiella pneumoniae*

Summary

- Microbes can develop various ways to resist antimicrobials
 - Superbugs are resistant to many drugs
-