# Morphology of Bacteria

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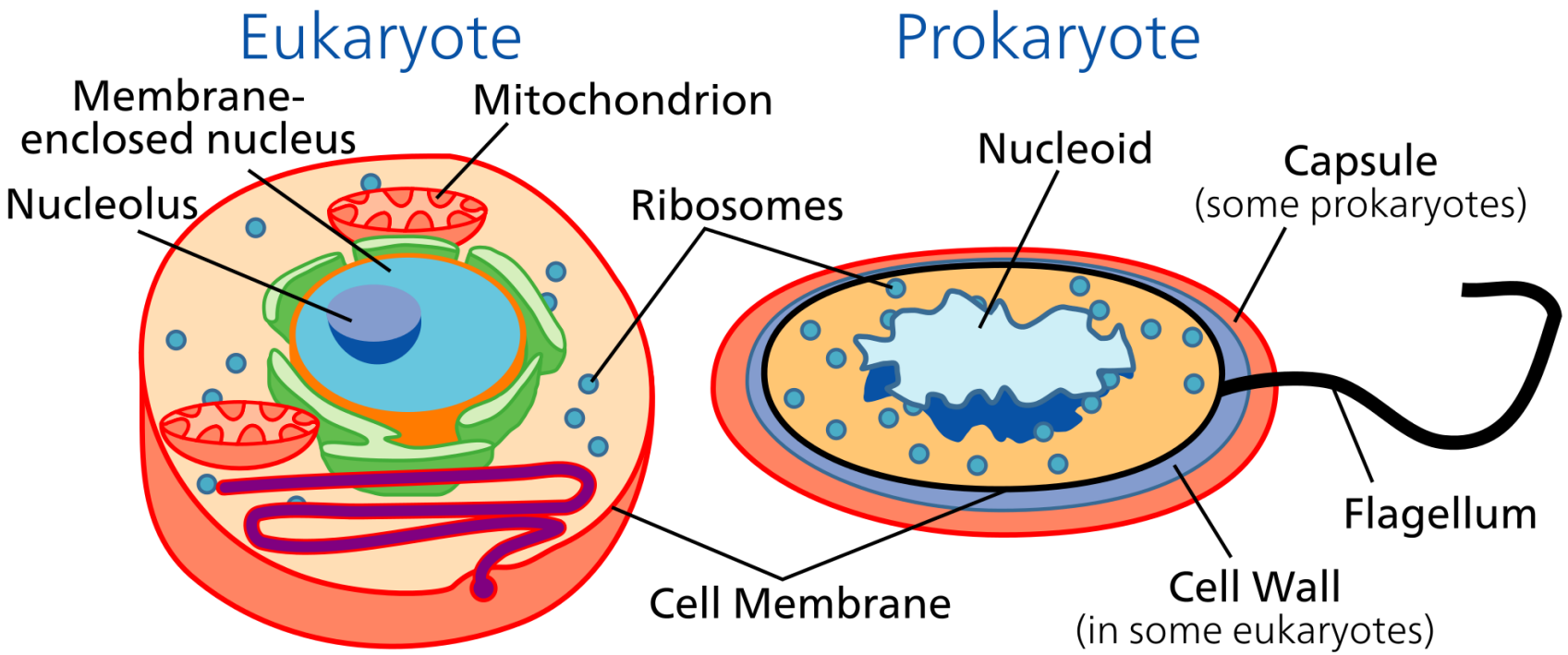
# OBJECTIVES

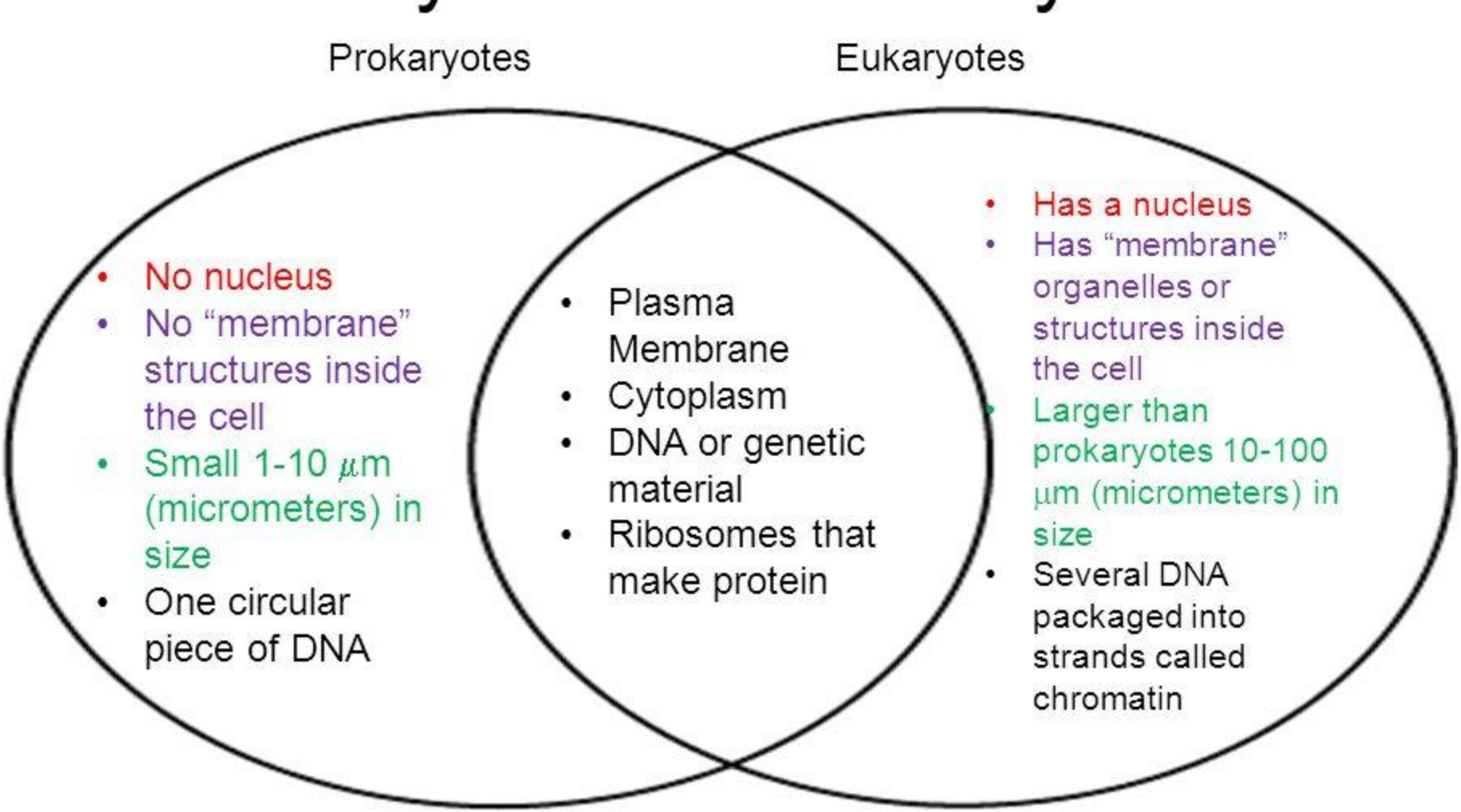
- The size, shape, arrangement of bacteria
- Microscopy
- Staining methods
- Different bacterial cell structures

# INTRODUCTION

- Microorganisms are a diverse group of several distinct classes of living beings.
- Classified under Kingdom Protista
- Further classified into prokaryotes and eukaryotes.
- Bacteria and blue-green algae are prokaryotes,
- Fungi, other algae, slime moulds and protozoa are eukaryotes.

# Difference between Prokaryotic and Eukaryotic Cells



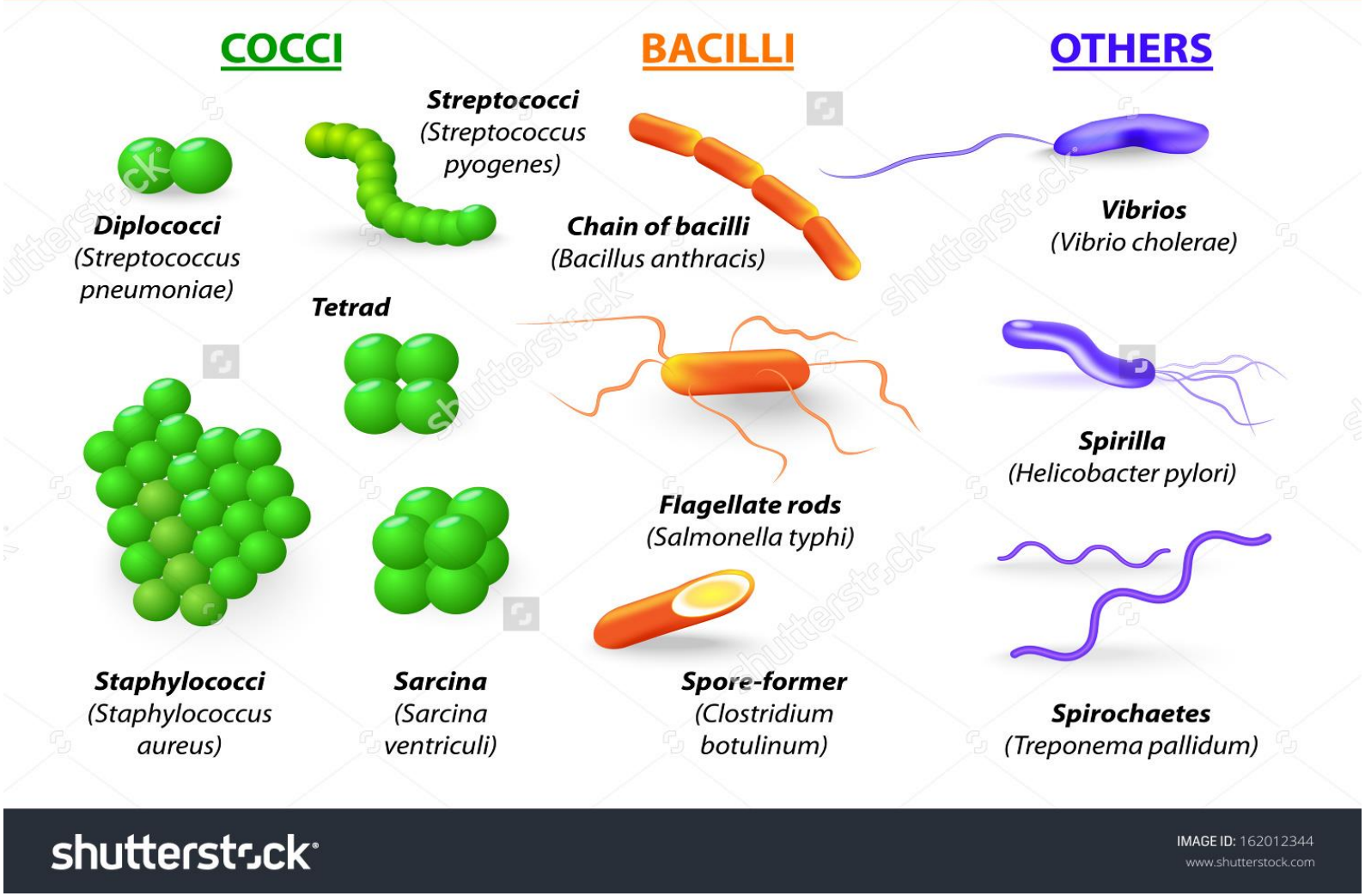

Prokaryotes
Eukaryotes
No nucleus
No "membrane" structures inside the cell
Small 1-10 μm (micrometers) in size
One circular piece of DNA
Plasma Membrane
Cytoplasm
DNA or genetic material
Ribosomes that make protein
Has a nucleus
Has "membrane" organelles or structures inside the cell
Larger than prokaryotes 10-100 μm (micrometers) in size
Several DNA packaged into strands called chromatin

# BACTERIA

The major characteristics of Bacteria are based on their size, shape and arrangements

- Size –
  - Bacteria of medical importance measure 0.2 – 1.5 µm in diameter and about 3–5 µm in length – viewed under microscope
  - The unit of measurement used in bacteriology is the micron (micrometer)
  - 1µm = one thousandth part of a mm

# SHAPES OF BACTERIA



# Microscopy

- Antony van Leeuwenhoek first observed bacteria using hand ground lenses

- The types of microscope used are
  (i) Light or optical microscope
  (ii) Phase contrast microscope
  (iii) Dark field/ Dark ground microscope
  (iv) Electron microscope

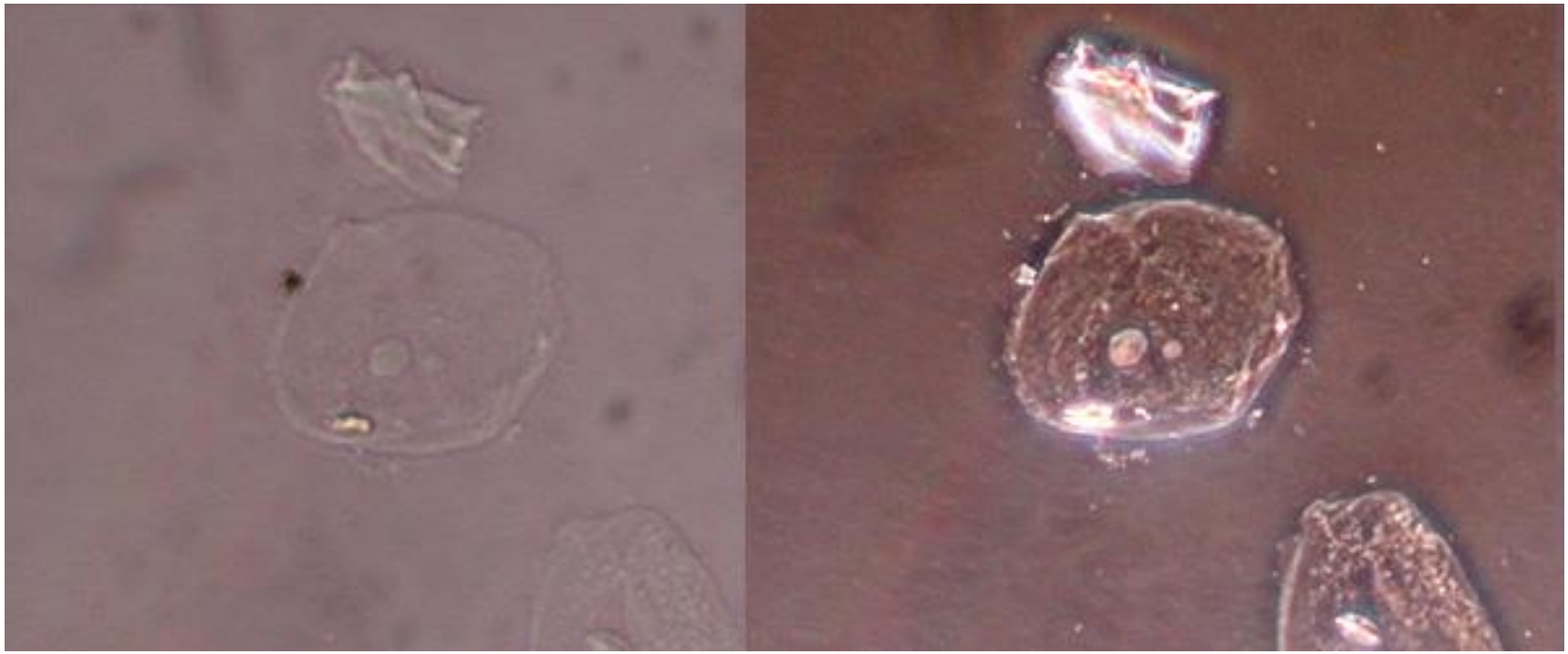
# Light or optical microscope

- Simple Microscope consists of a single lens. A hand lens is an example of a simple Microscope.
- Compound Microscope consists of two or more lenses in series. The image formed by the first lens is further magnified by another lens.
- Usually stained preparations required
- Max magnification 1000X

# Phase – contrast microscopy

- Can be used to study internal structures in living microorganisms
- No need to fix or stain
- Principle of phase-contrast microscopy is based on slight variations in refractive index
- Light rays passing through the specimen are diffracted differently and travel in different pathways
- These phase differences are seen through as different degrees of brightness

# Phase contrast microscope



# Dark field Microscopy

- Dark field microscope is used for examining live microorganisms which are invisible in the ordinary light microscope, that can not be stained
- dark field condenser has a opaque disc that

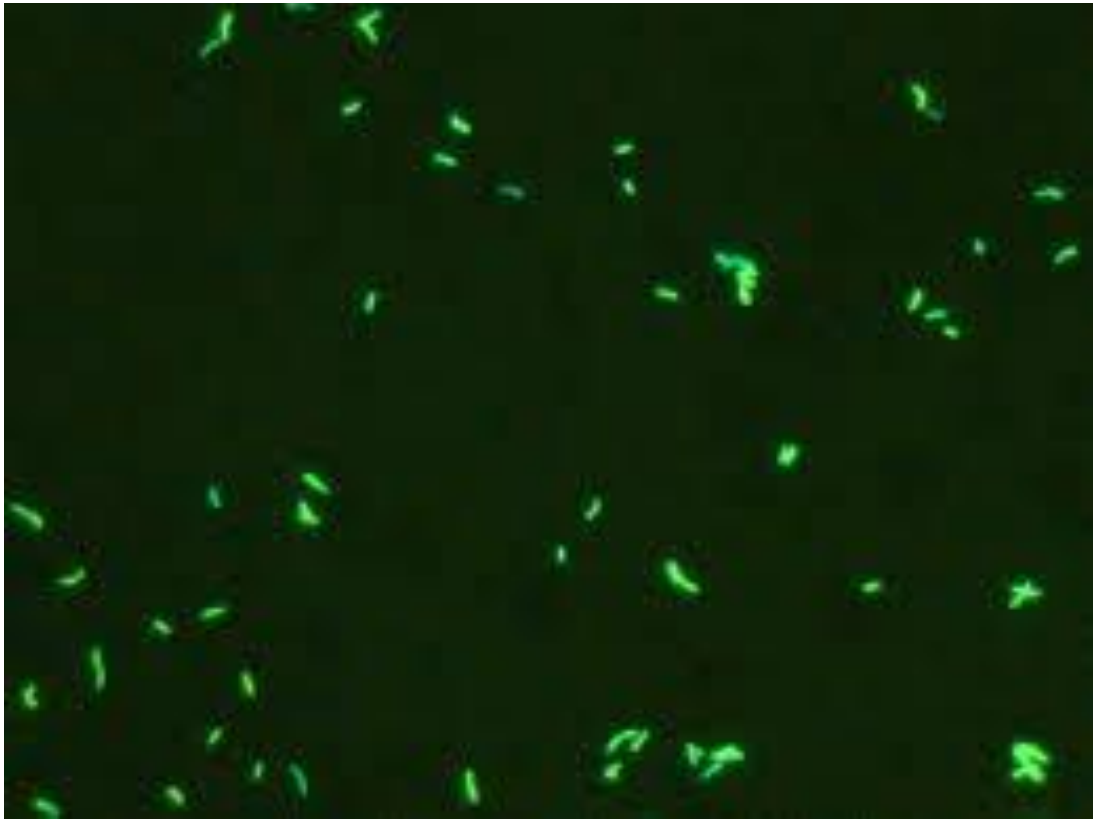
  blocks light that would enter the objective directly
- light that is reflected off the specimen reaches the objective lens
- specimen appears bright against a black background

# Dark field microscope

# Fluorescence Microscope

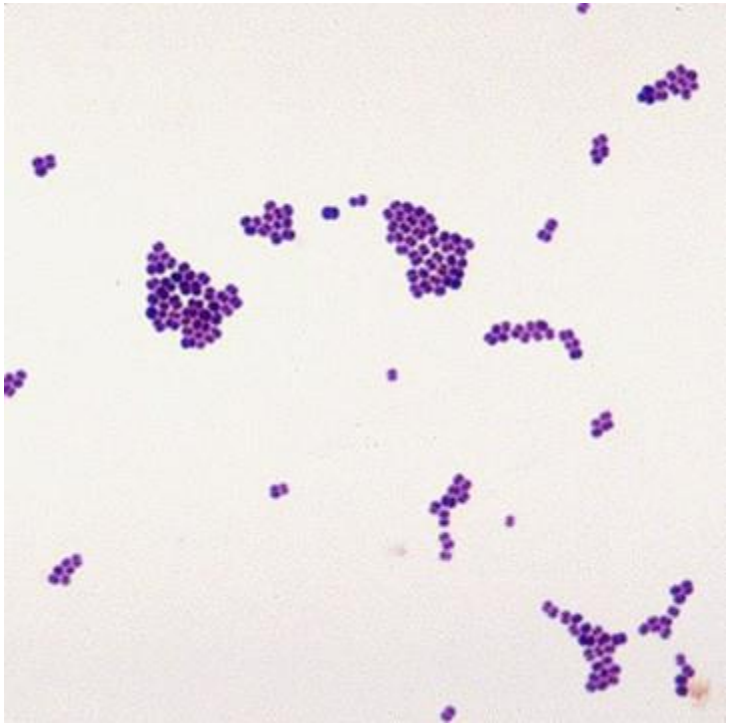
- Fluorescence microscope exposes a specimen to ultra violet light
- Usually a mercury vapour arc lamp produces an intense beam
- The light passes through an exciter filter
- A dark field condenser provides a black background against which the fluorescent objects glow
- Usually the specimens are stained with fluorescent dyes, called fluorochromes
- The fluorescence microscope can be used for direct detection of microorganisms fluorescent antibody techniques direct microscope counts (DMC)

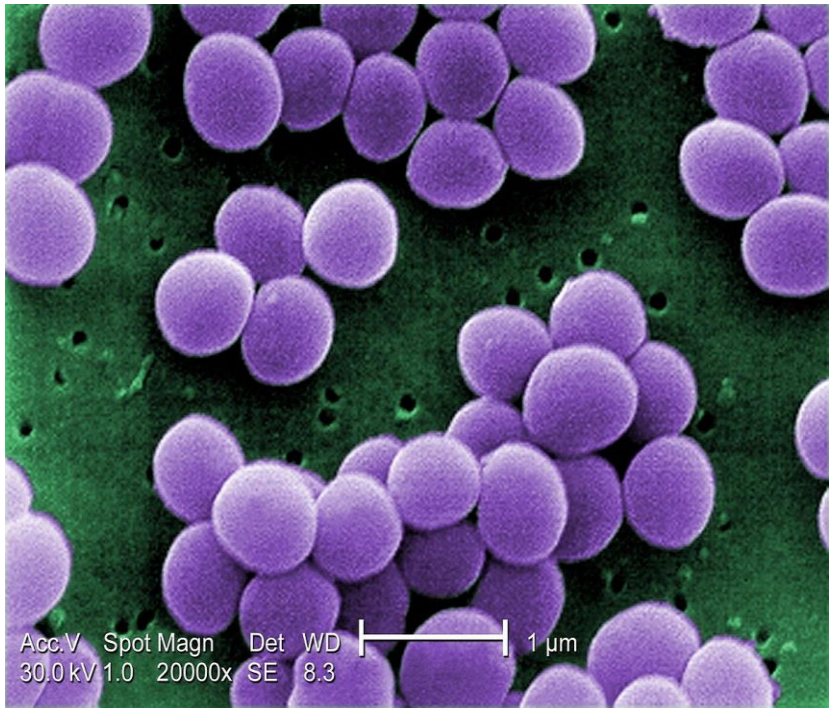
# Fluorescent Microscope



# Principles of Electron Microscopy

- Uses electromagnetic lenses, electrons and fluorescent screen to produce image
- Resolution increased 1,000 fold over brightfield microscope
  - To about 0.3 nm ($1x10^{-9}$)
- Magnification increased to 100,000x
- Two types of electron microscopes
  - Transmission
  - Scanning




Acc.V Spot Magn Det WD 1 µm
30.0 kV 1.0 20000x SE 8.3

# Stained Preparations

- Live bacteria do not show the structural detail under the light microscope due to lack of contrast.
- Hence staining techniques are used to produce color contrast.
- Routine methods of staining of bacteria involve dying and fixing smears – procedures that kill them.
- Bacteria have an affinity to basic dyes due to acidic nature of their protoplasm.

# The commonly used staining techniques are

- Simple Stains
  - methylene blue or basic fuchsin are used for simple staining.
  - They provide color contrast,
  - but impart the same color to all bacteria.

- Negative Staining

  Indian ink or Nigrosin stain background against which the unstained bacteria stand out in contrast.

- Impregnation Methods

  Cells and structures too thin to be seen under ordinary microscope may be rendered visible if they are impregnated with silver on the surface. These are used for demonstration of spirochetes and bacterial flagella.

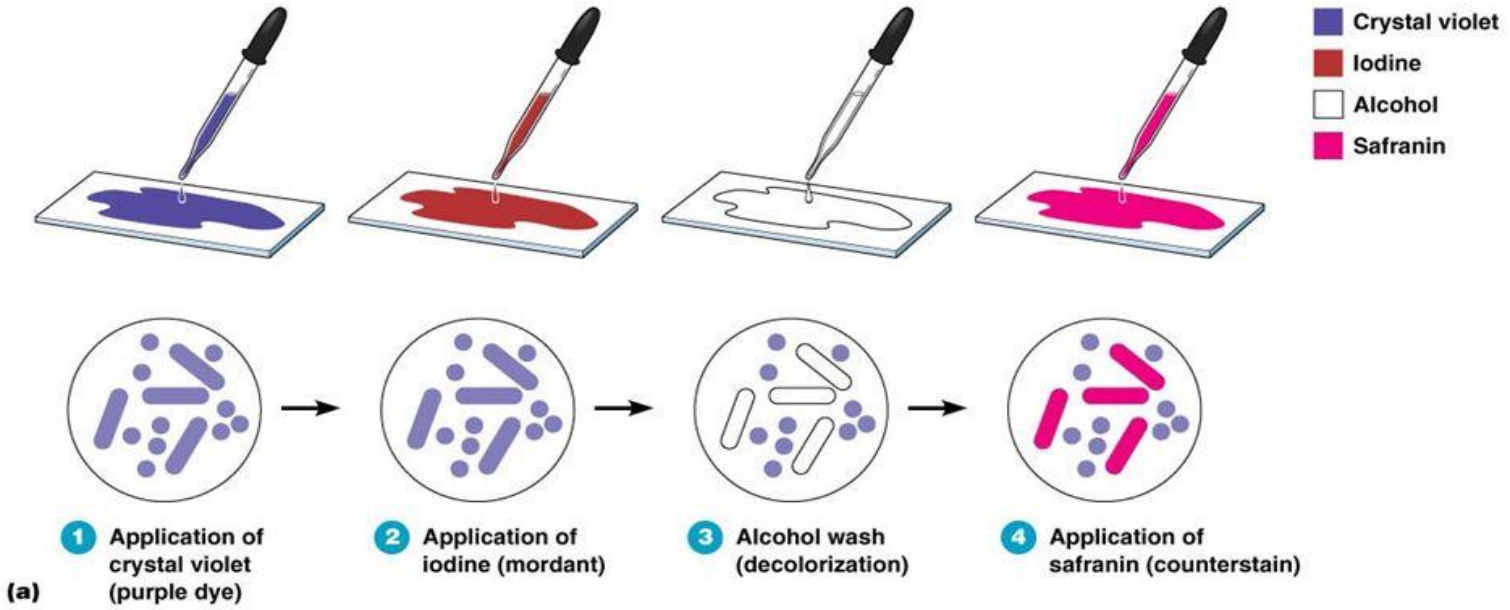
# Differential staining

- These stains impart different colors to different bacteria
- Commonly used differential stains – Gram stain and Acid fast stain.
- Gram staining technique discovered by Christian Gram

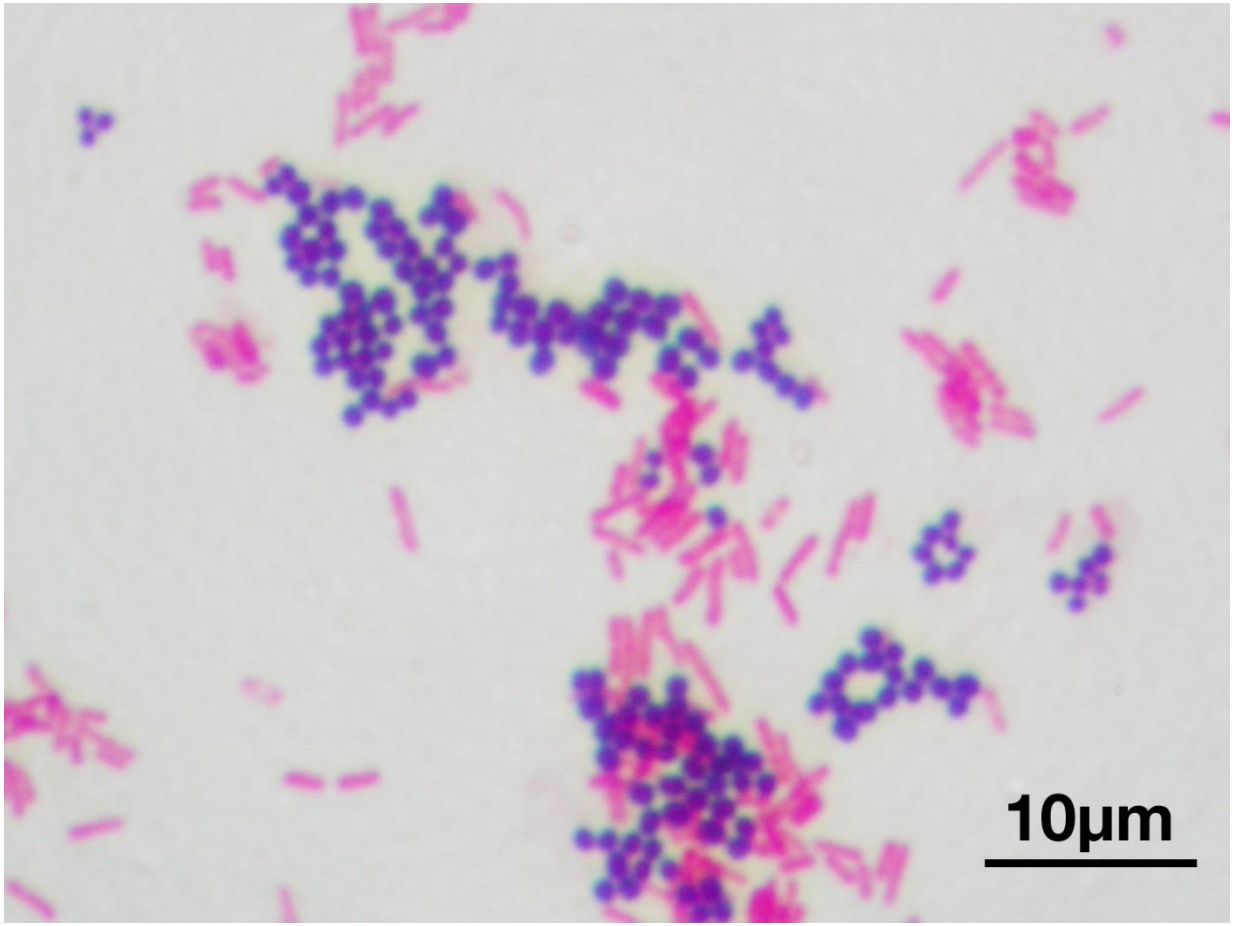
# Principle Of Gram's Staining

- The structure  of the organism 's cell wall determines whether the organism is gram positive or negative.
- When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting declorization while other get decolorized by decolorizer.
- Those bacteria which retain the primary stain are called Gram positive.
- Those bacteria which get decolorized and then get counterstained are called Gram negative.

# Gram Staining Procedure



# Gram Positive Bacteria- violet in colour
# Gram Negative Bacteria – pink in colour



# Acid Fast Stain

- Discovered by Ehrlich
- Tubercle bacilli resist decolonization with aniline dyes
- Modified Ziehl Neelsen
- Acid fastness in tubercle bacilli  is due to high content of lipids, fatty  acids, and higher alcohols

# PRINCIPLE OF ZIEHL–NEELSEN STAIN

- Acid fastness of acid-fast bacilli is attributed to the presence of large quantities of unsaponifiable wax fraction called mycolic acid in their cell wall and also the intactness of the cell wall . The degree of acid fastness varies in different bacteria.
- In this staining method, application of heat helps the dye to penetrate the tubercle bacillus.
- **Once stained, the stain cannot be easily removed.** The tubercle bacilli resist the decolorizing action of acid-alcohol which confers acid fastness to the bacteria.
- The other microorganisms, which are easily decolorized by acid-alcohol, are considered non-acid fast . The non-acid fast bacilli readily absorb the colour of the counter stain appearing blue, while the acid fast cells retain the red colour of primary stain.

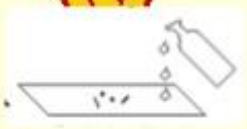
# Procedure ZN staining

## Ziehl-Neelsen (Z-N) Stain

- Carbol-fuchsin dye

- Heat

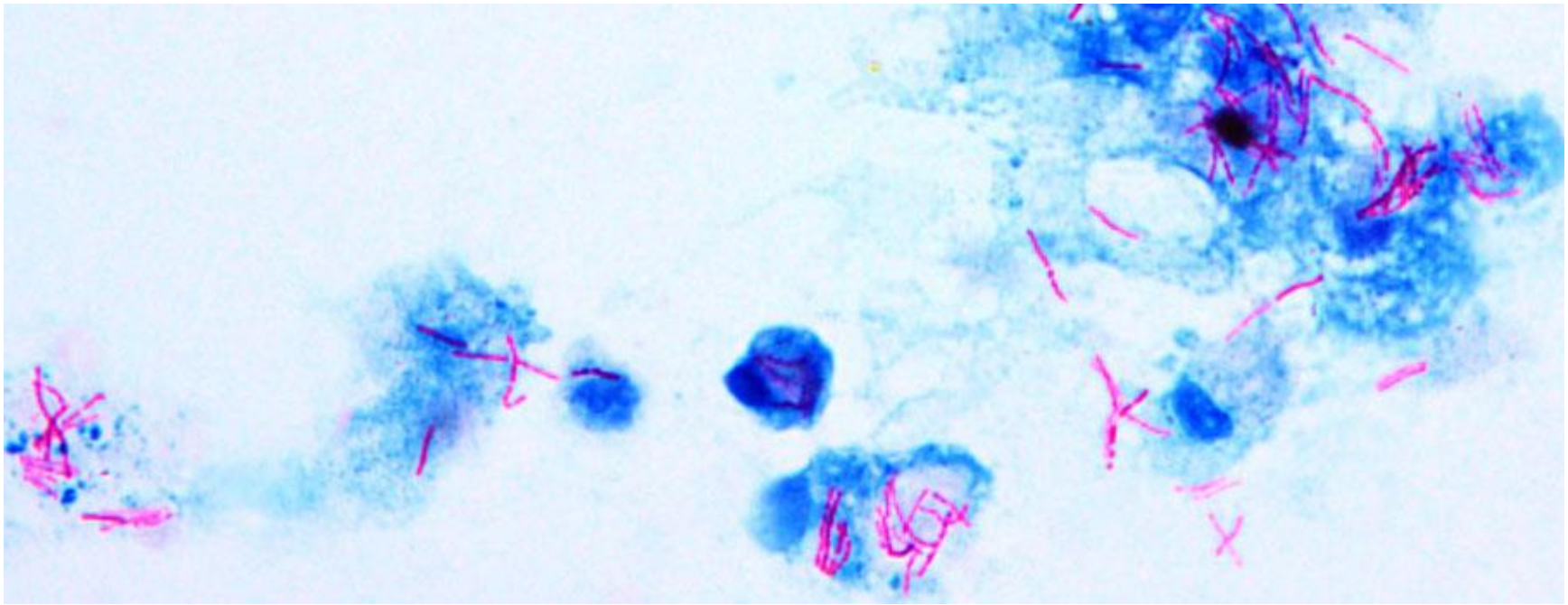
- Acid-alcohol (decolourization)



- Methylene blue

- **Acid-fast = Mycobacteria**

# Tubercle bacilli in stained smear



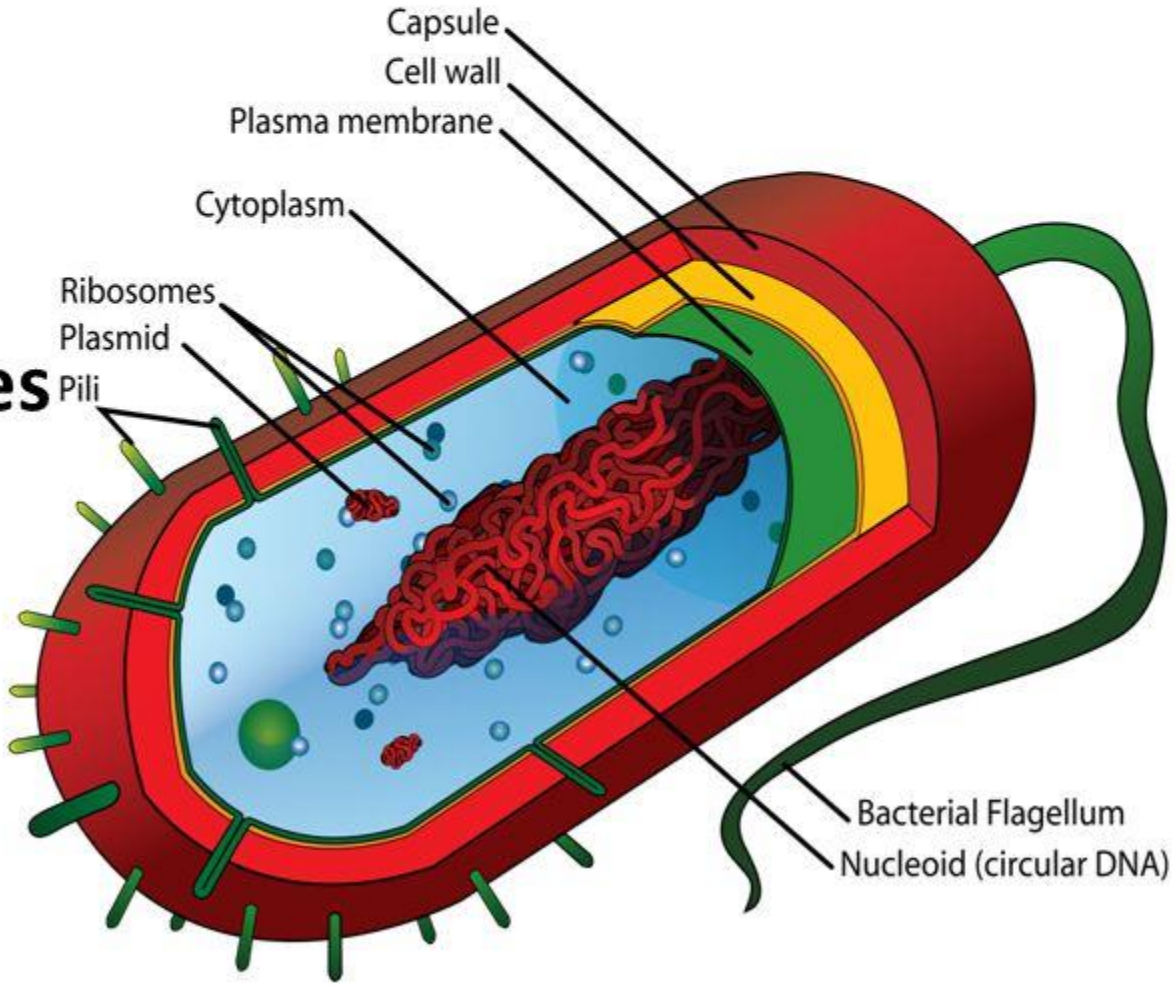
# *Structure of Bacteria*

**Essential structure**

- Cell wall
- cell membrane
- Cytoplasm
- Nuclear material

**Particular structures**

- Capsule
- flagella
- pili
- Spore

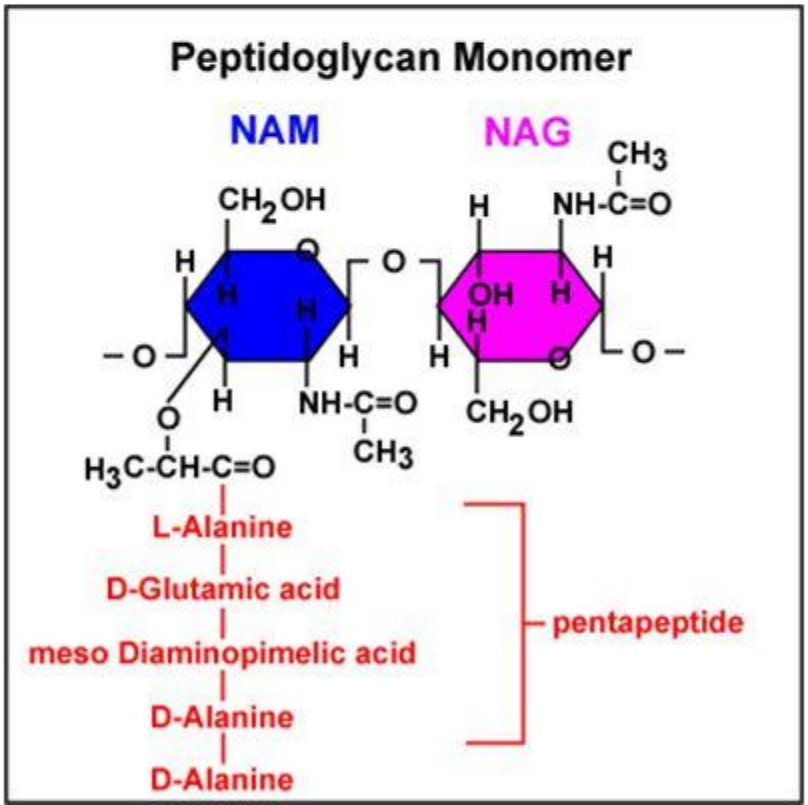


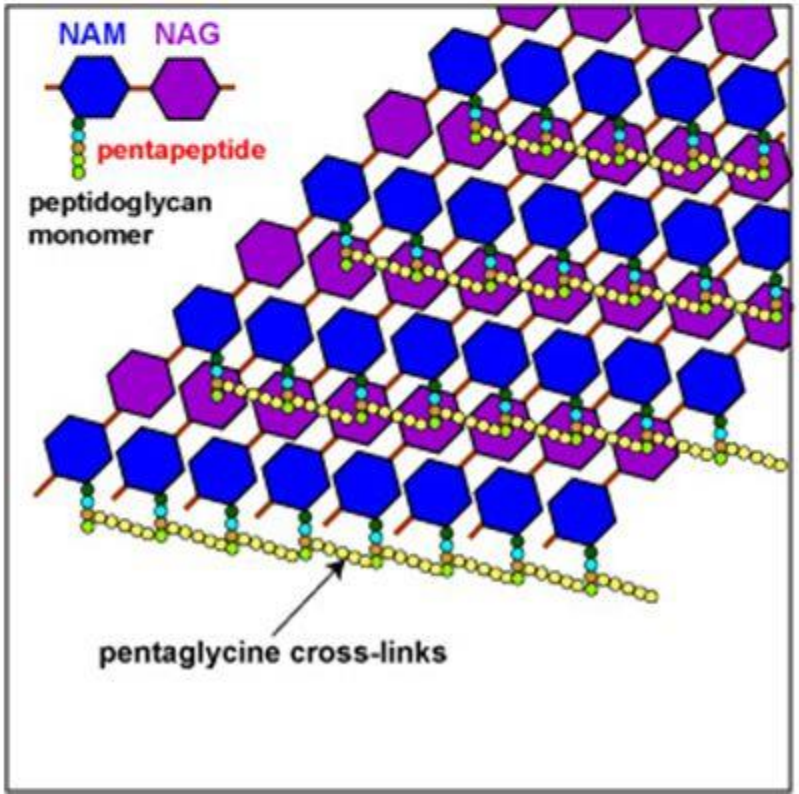
# Bacterial Cell Walls

- The cell wall is a rigid structure that lies outside the plasma membrane; it creates characteristic shapes for the bacteria and protects from osmotic lysis and toxins, often increasing pathogenicity.
- Overview of bacterial cell wall structure
  - The cell walls of most bacteria contain peptidoglycan (murein).
  - The cell walls of gram-positive bacteria and gram-negative bacteria differ greatly, but both have the periplasmic space between the cell wall material and the plasma membrane

# Peptidoglycan layer

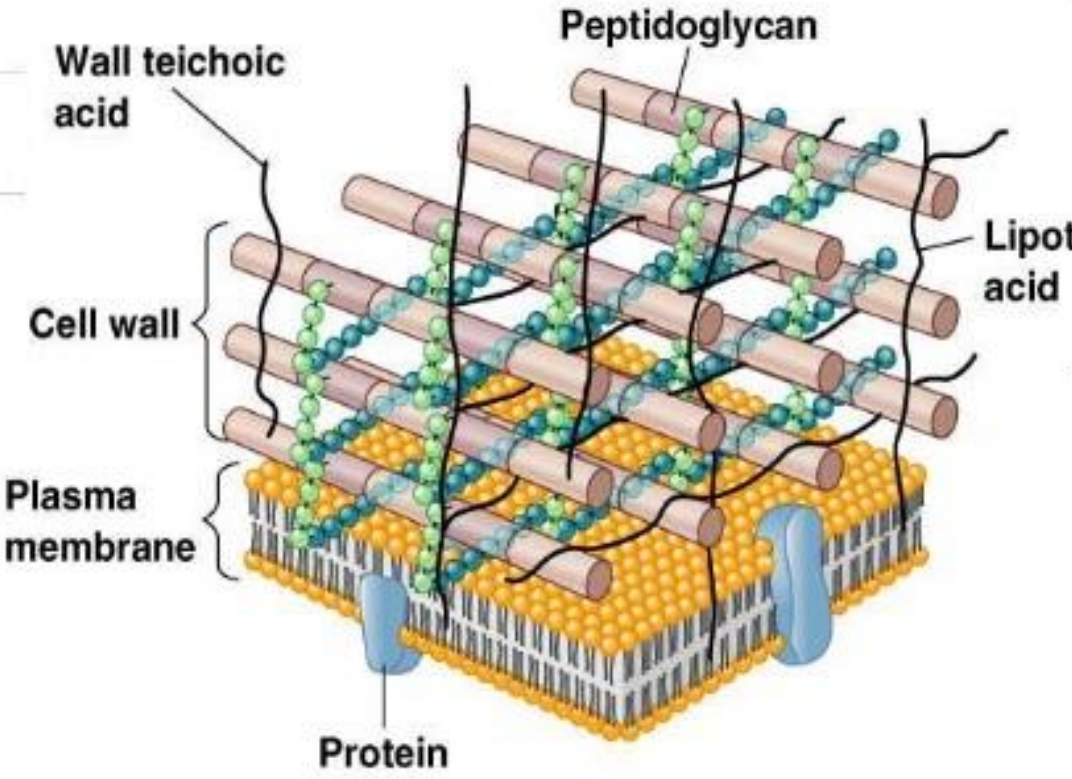
- This layer is responsible for the rigidity of the cell wall, composed of N-Acetylglucosamine (NAG) and N-acetylmuramic (NAM) acids and a small group of amino acids.
- Glysine chains held together with peptide bonds between amino acids to form a sheet



# GRAM POSITIVE WALL

- Relatively thick layer of peptidoglycan
  - As many as 30
    - Regardless of thickness, peptidoglycan is permeable to numerous substances




- Teichoic acid component of peptidoglycan; composed of glycerol and phosphate
- Lipoteucholic acid is attached to the lipids of cytoplasmic membrane
  - Gives cell negative charge

# Gram-negative cell wall :-

Gram-negative bacteria cell wall consist of many layers , plasma membrane, cell wall, outer membrane sometimes capsule.

A. Lipoprotein layer- connects the peptidoglycan to outer membrane.

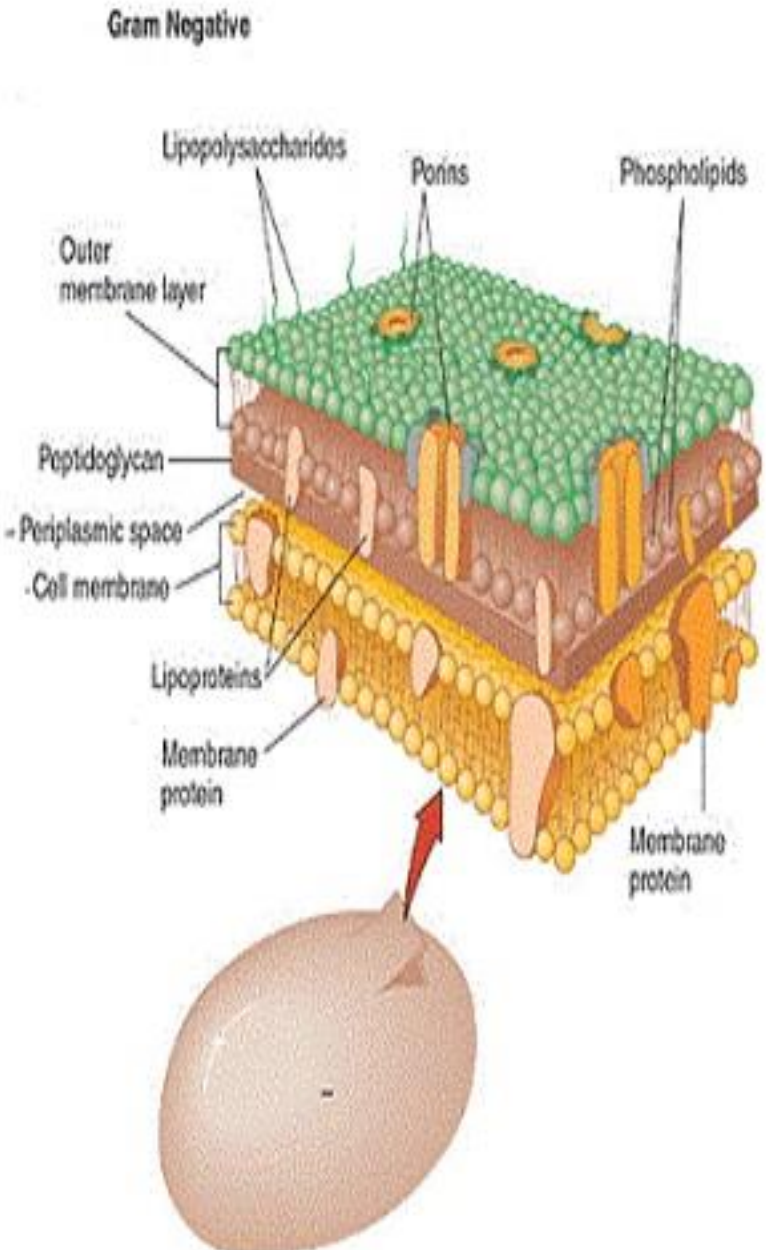
B. Outer membrane- Outer membrane proteins- target site for antibiotics.

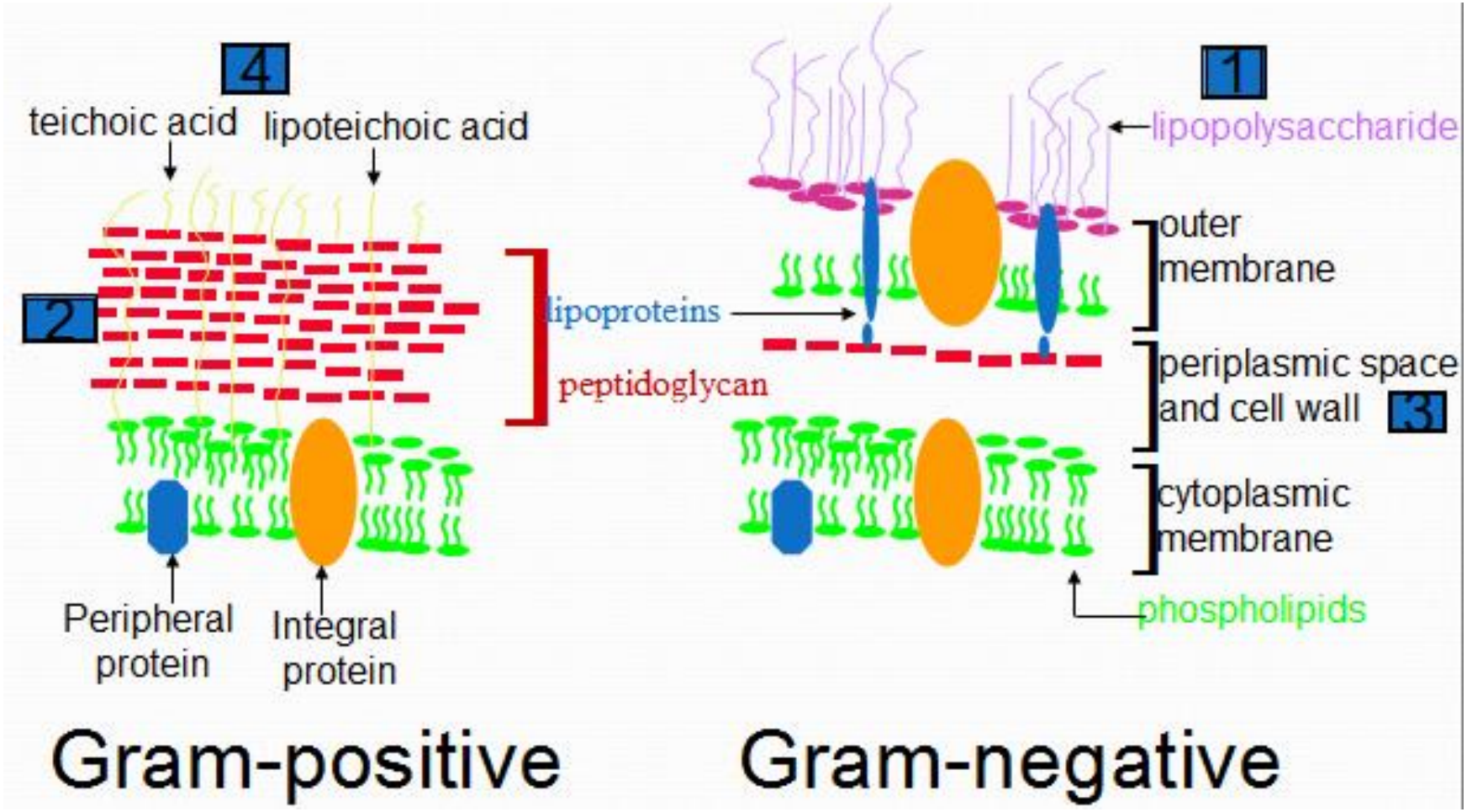
C. Lipopolysachharides- This layer consists of lipid A to which is attached a polysaccharide .

D. Periplasmic space- Space between inner membrane and outer membrane.

E. Peptidoglycan thin of few layers (7 to 8 nanometers)

The dry weight only 10% of Gram-negative strains.




4
teichoic acid
lipoteichoic acid
2
lipoproteins
peptidoglycan
Peripheral protein
Integral protein
Gram-positive
1
lipopolysaccharide
outer membrane
periplasmic space and cell wall
3
cytoplasmic membrane
phospholipids
Gram-negative

- Protoplast – bacterium without cell wall.
  - Lysozyme enzyme destroys peptidoglycan.
  - is metabolically active but unable to reproduce.

- Spheroplasts – bacterium with a damaged cell wall
  - action of toxic chemical or an antibiotic,
  - they show a variety of forms
  - Retains normal form when the toxic agent is removed

# Cytoplasmic membrane

- Cytoplasmic membrane is present immediately beneath the cell wall,
- Boundary for cytoplasm.
- It acts as a semi permeable membrane controlling the flow of metabolites to and from the protoplasm.

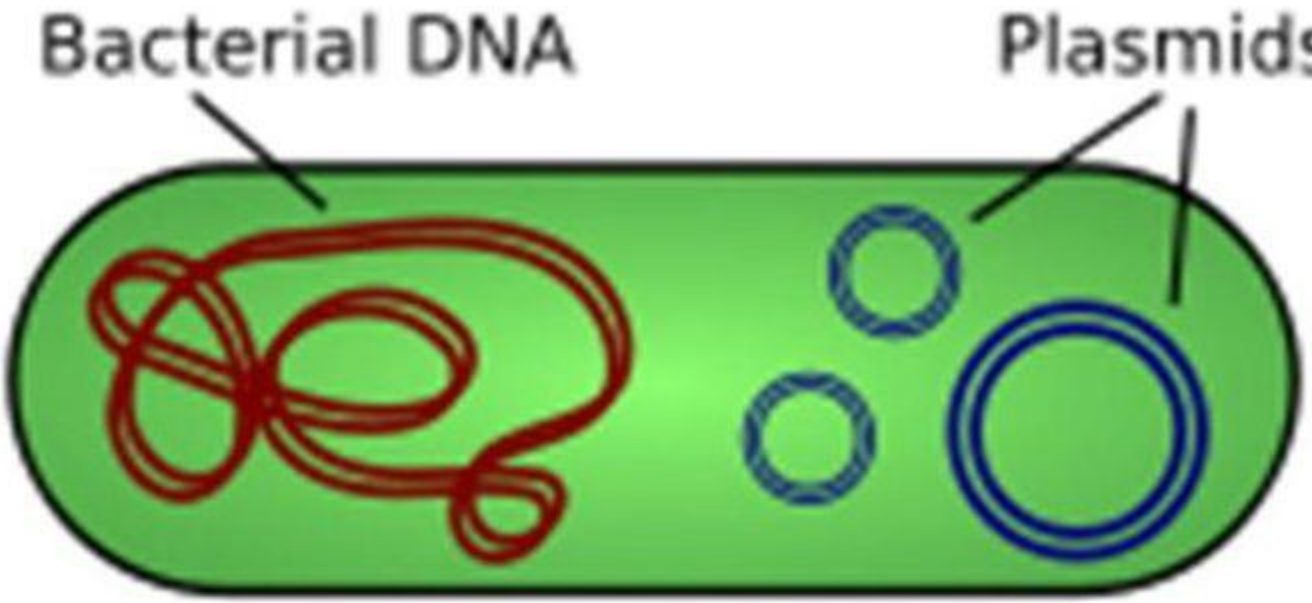
**Mesosomes -** They are vesicular, convoluted tubules formed by invagination of plasma membrane into the cytoplasm.
-respiratory enzymes
-help with cell reproduction

# Cytoplasm

- Colloidal system containing a variety of organic and inorganic solutes containing 80% Water and 20% Salts, Proteins.
- Prokaryotic cytoplasm is not mobile
- Stains uniformly

- **Nuclear Material** – Single molecule of ds DNA arranged in the form of coil, No nuclear membrane
- replicates by simple fission
- **Plasmid** – extra nuclear DNA which infers special characters to the bacterial cell
- **Ribosomes** - protein synthesis

# What are Plasmids?




- Plasmids are circular pieces of bacterial DNA that often contain genes not related to basic life functions
- Often contain antibiotic resistance genes
- Humans often cut open plasmids…attach a desired gene…reinsert the plasmid to the bacteria
- When complete, the bacteria will contain a new gene (instructions) to create a desired protein such as insulin.

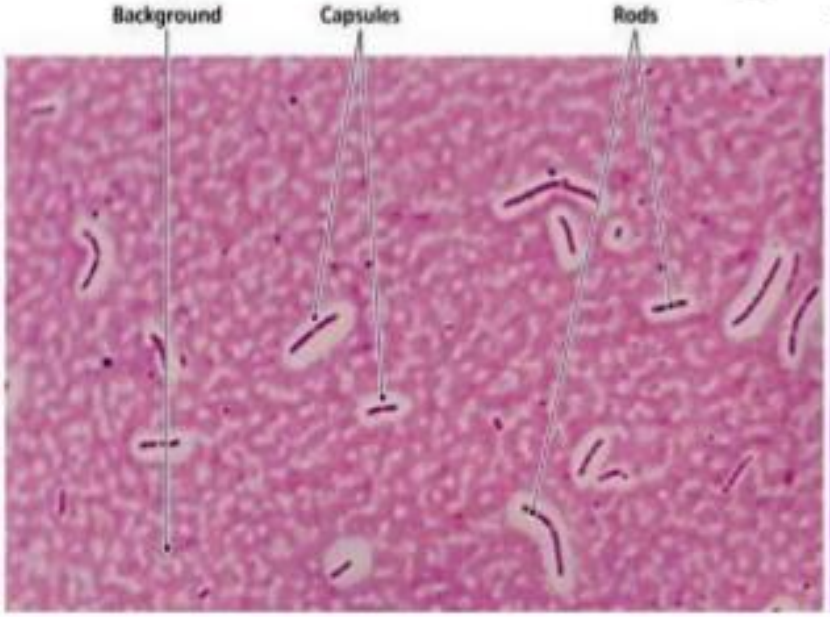
- ***Cytoplasmic inclusions:***
- **Volutin granules (metachromatic granules)**

  **Source of stored energy (polymetaphosphate), found in diphtheria bacillus with methylene blue dye or with Albert and Neisser staining.**
- **Lipid-granules: act as carbon and energy storage product.**
- **Polysaccharide granules: either starch or glycogen.**

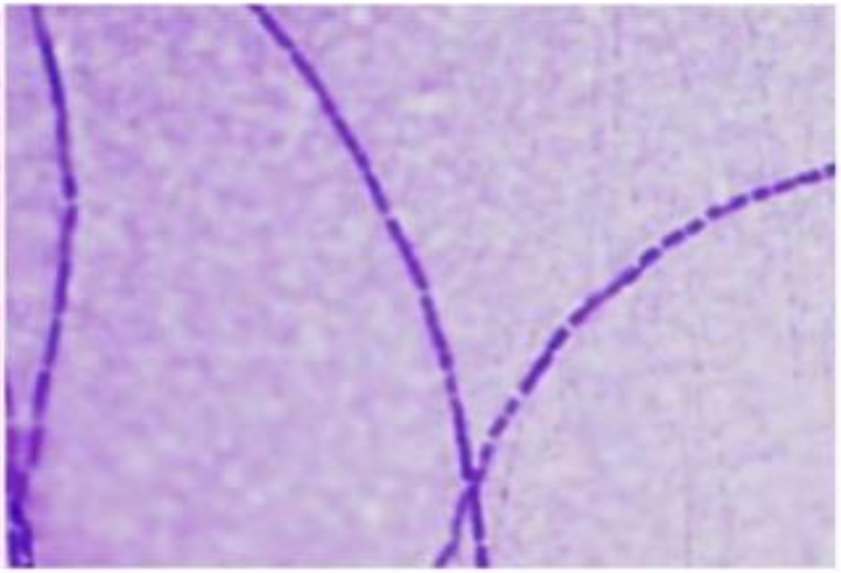
# Capsule

- Capsule is the outer most layer of the bacteria (extra cellular).
- It is a condensed well defined layer closely surrounding the cell.
- They are usually polysaccharide
- The Capsule protects against phagocytosis and lytic enzymes, drying and desication
- Seen under negative staining with India Ink

# Capsule Staining



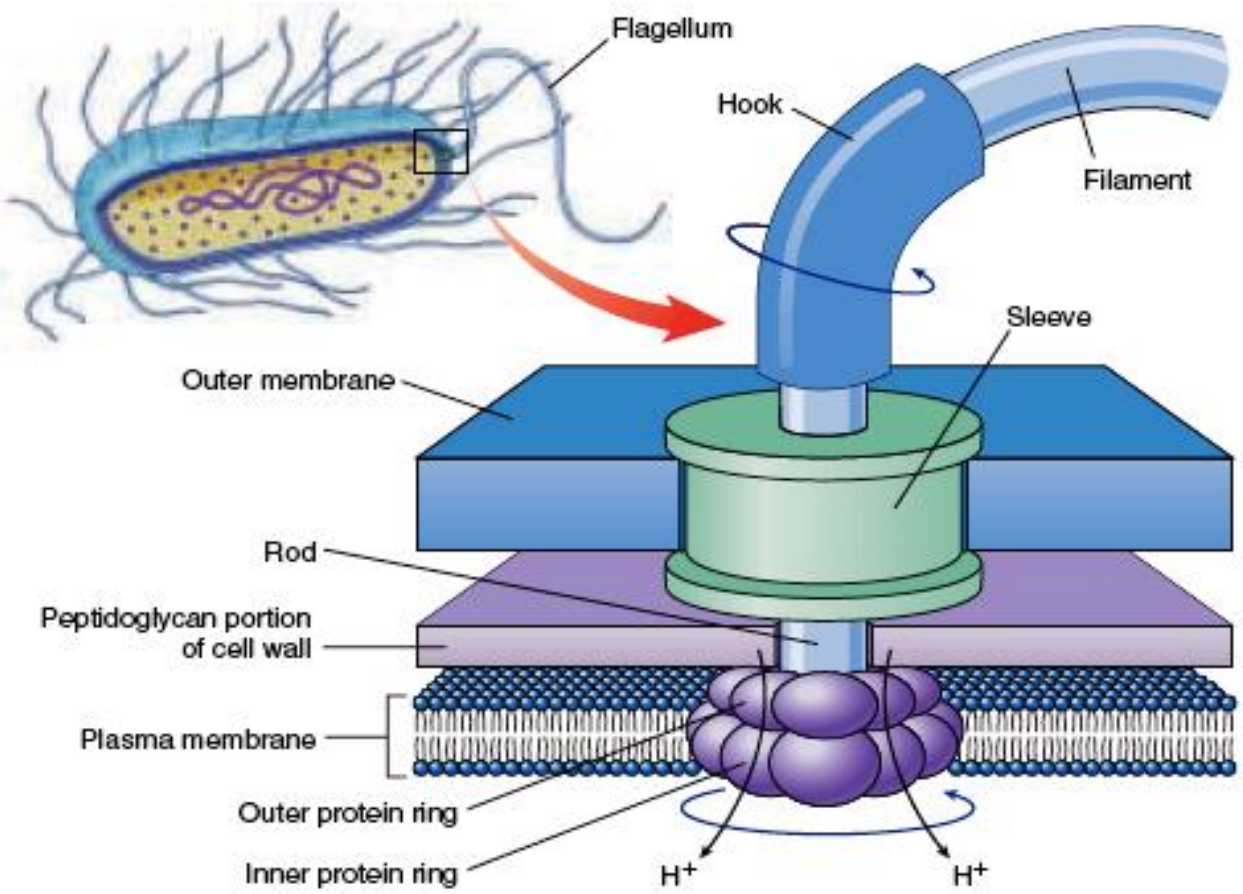

## Non Capsulated Bacterium



# Flagella

- Flagella are long hair like helical filaments extending from cytoplasmic membrane to exterior of the cell.
- Imparts mobility to the bacterium
- Antigenic–induce high antibody response not protective but imp in serodiagnosis
- Parts of flagella are the
  - Filament – external to cell wall
  - Hook –
  - basal body – Embedded in Cell wall
  - Flagellin protein subunits make up Filament and hook

# Structure of Flagella

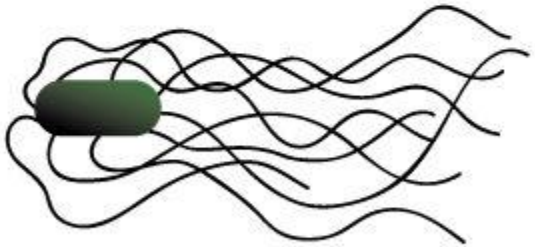


# Types of flagellar arrangement

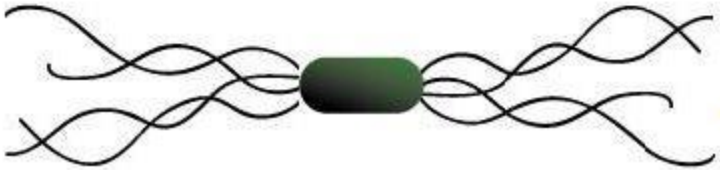
Polar/ Monotrichous – single flagellum at one pole

Lophotrichous – tuft of flagella at one pole

Amphitrichous – flagella at both poles



Peritrichous – flagella all over



Amphilophotrichous – tuft of flagella at both ends

# Pili / Fimbriae

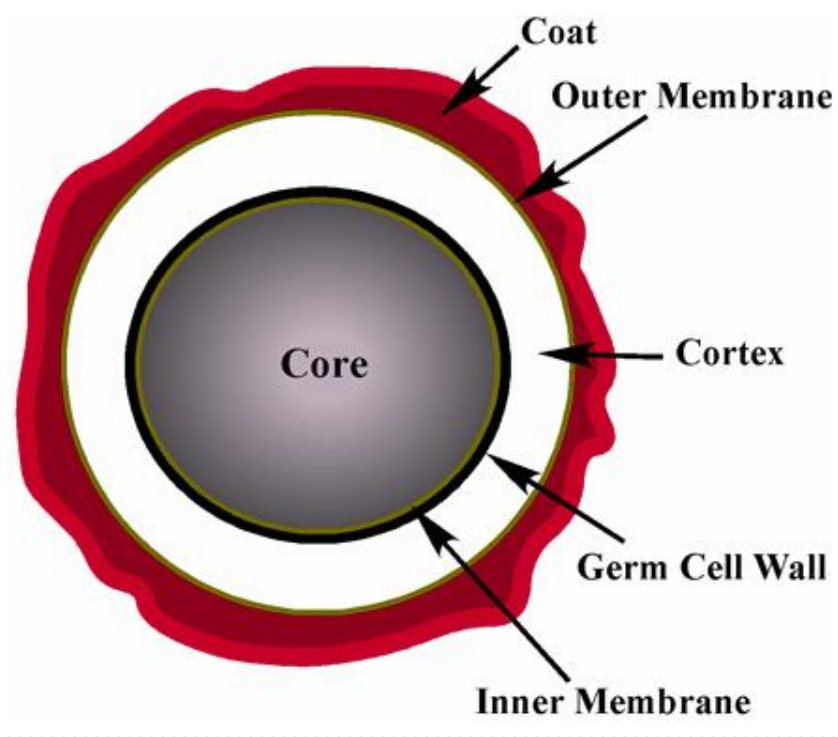
- Hair-like proteinaceous structures
- They are thinner, shorter and more numerous than flagella
- do not function in motility.
- The fimbriae is composed of a subunit called pilin.
- Helps in adhesion
- They are anitegenic
- There are two types pili
  - Non-sex pili (fimbriae or type IV)
  - Sex pili helps in conjugation.

# Spore

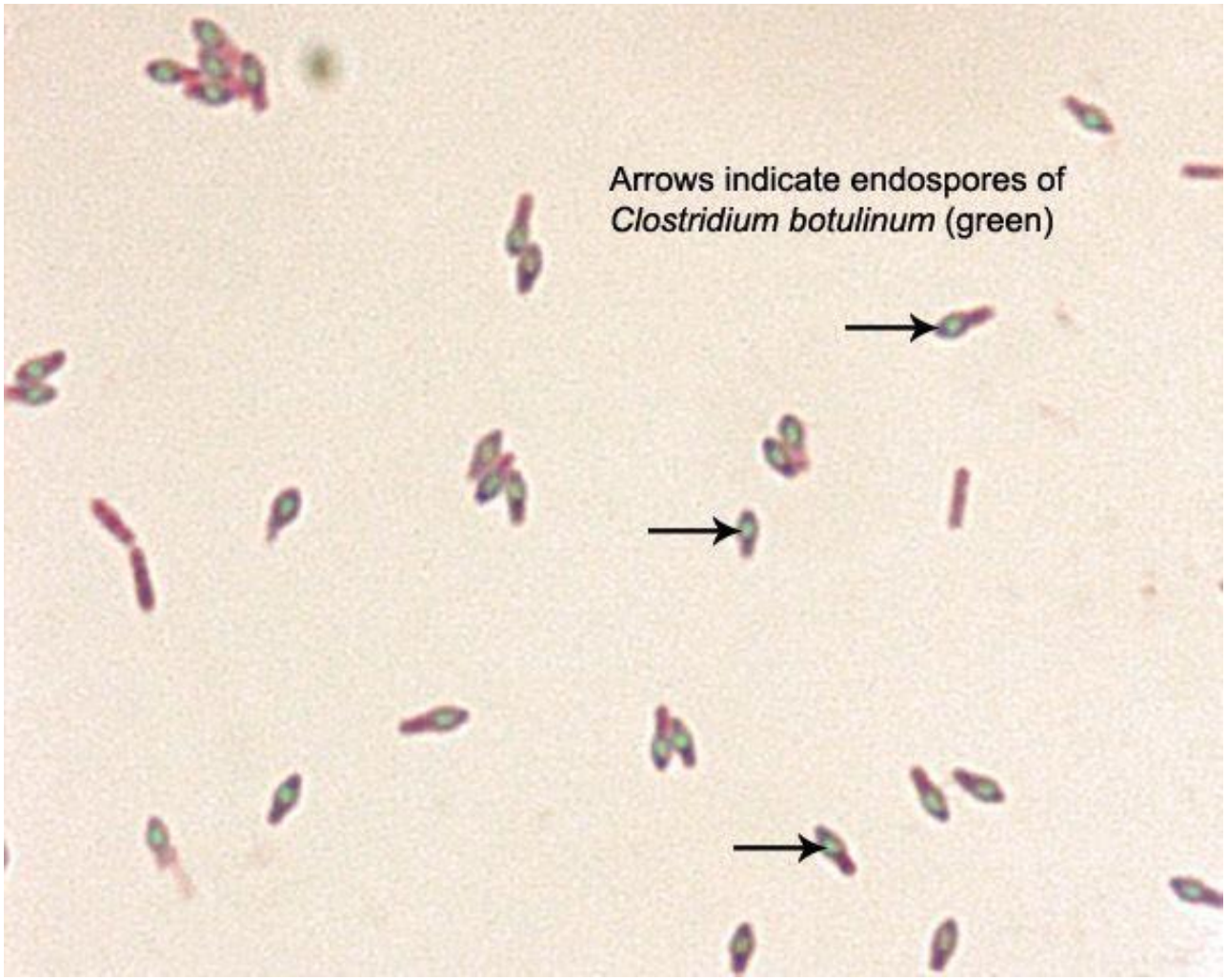
- Highly resistant resting stage
- Helps them to overcome adverse environmental conditions
- These spores are resistant to bactericidal agents and adverse physical conditions.
- Each spore can give rise to only one endospore which play a role in heat resistance.
- Spores of *Cl. tetani* remain viable in soil for years

# Spore Structure

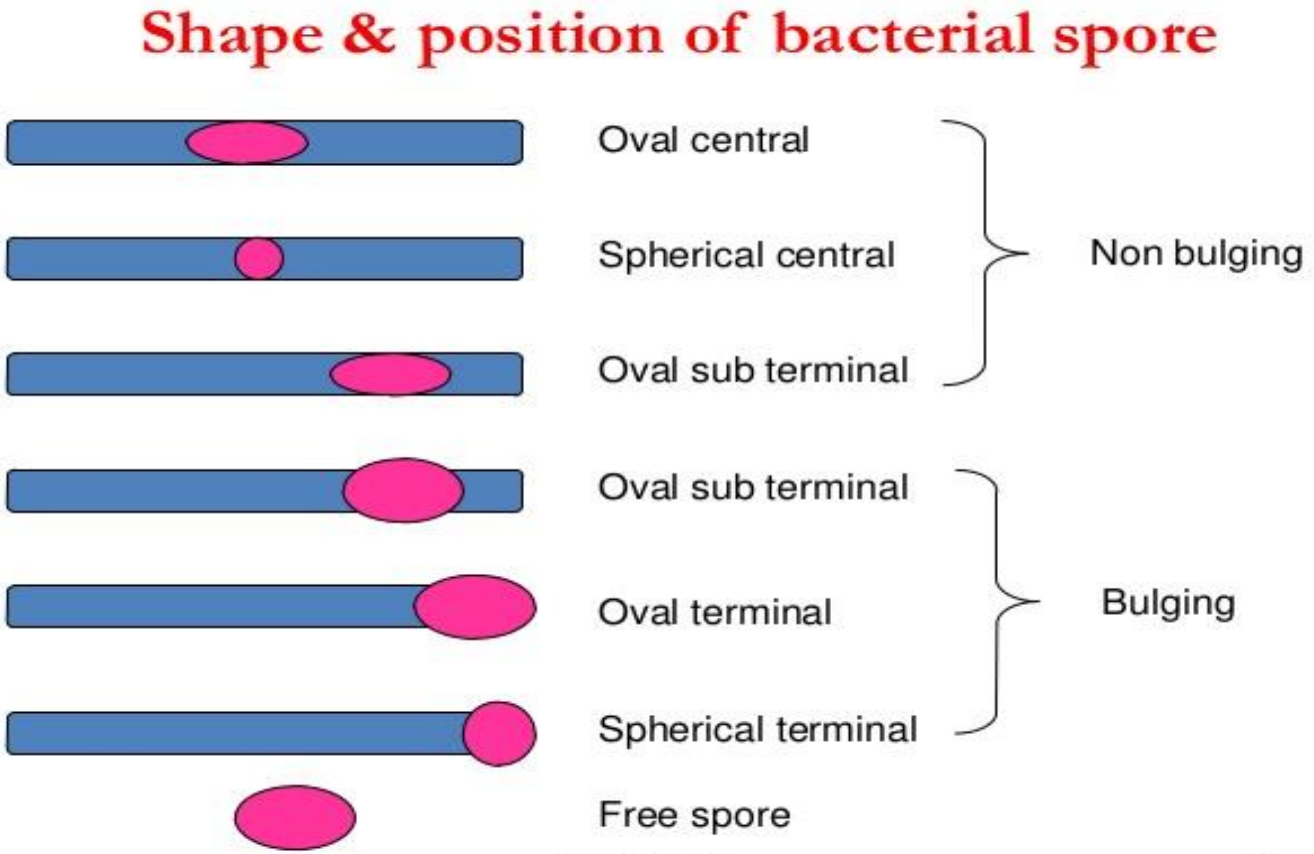
Spores consists of three layers namely core, cortex and spore coat



# Spore staining



# Spore arrangement



# Summary

- Difference between prokaryotes and eukaryotes
- Types of Microscopy
- Types of staining procedures
- Bacterial Cell structure

# Microbial Physiology

# Bacterial Growth and multiplication

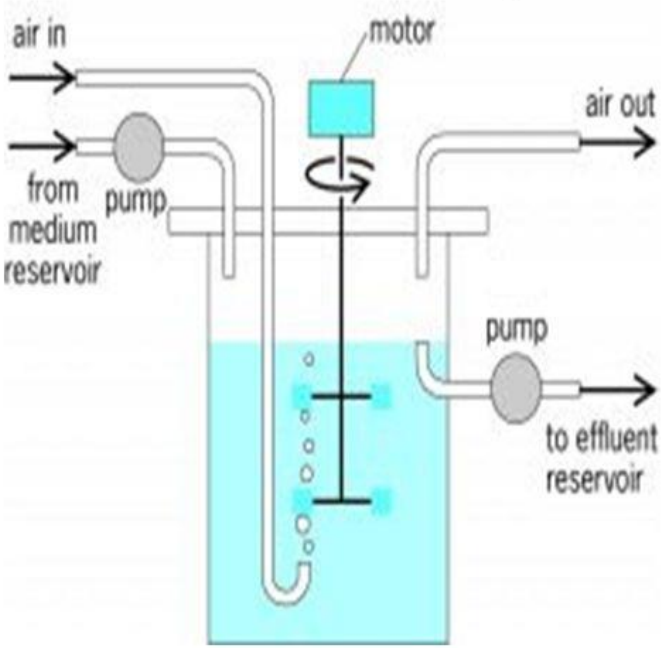
- Multiplication – Bacterial Cell divides by binary fission
- Generation time – Time required for a bacteria to divide into two
  - E.coli – 20 mins
  - Tuberculi bacilli – 20 hrs
  - Lepra bacilli – 20 days

# Growth

- Batch culture –
  Bacterial growth – liquid medium,
  Hindered – nutrients ↓, toxic products ↑
- Continuous Culture – Growth is replenished and dead bacteria removed (Turbidostat, chemostat)
- Pathogenic bacteria in human body nutrition plenty – growth may be limited by defense of body
- Colony – Bacterial growth on solid media
  Clone of cells derived from single parent cell

# Chemostat

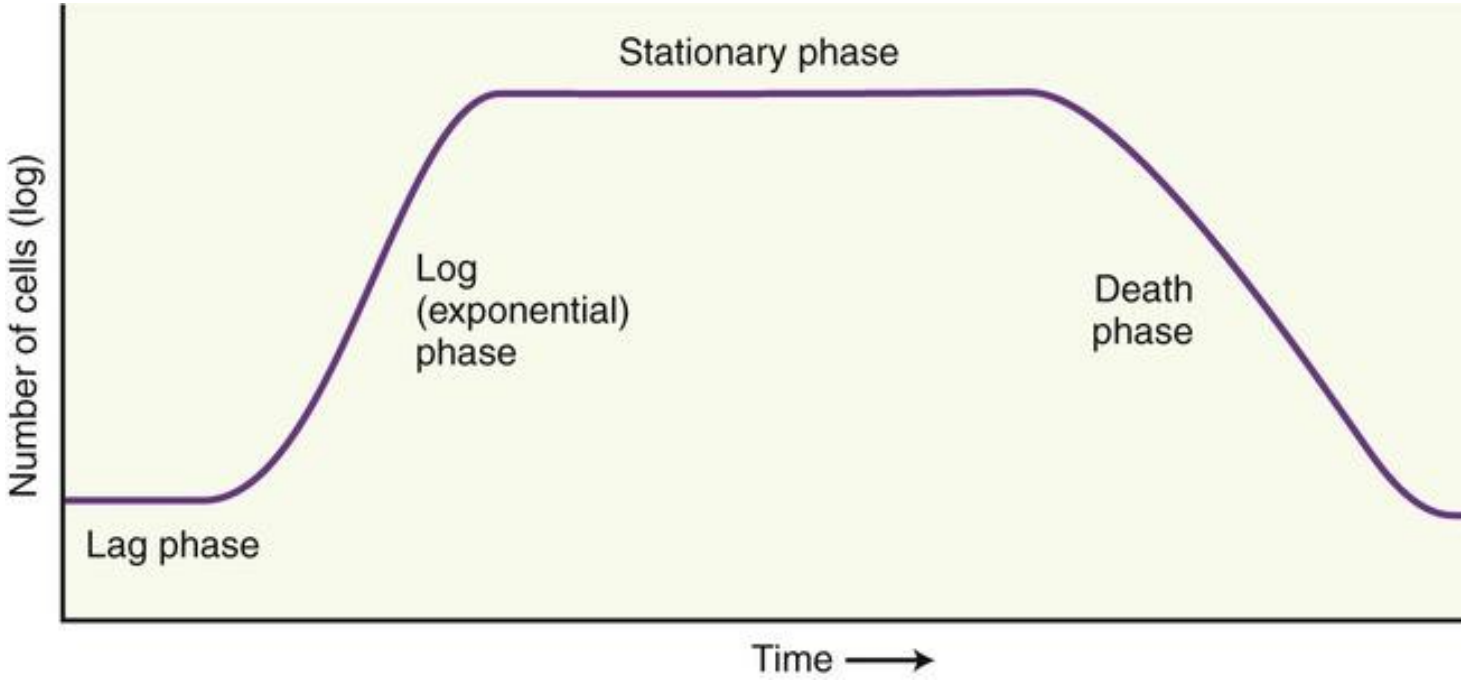
Chemostat schematic diagram




- **Chemostat:**
  - –Continuous culture system that admits a steady stream of nutrients
  - –Siphons off used media and old cells to stabilize growth rate
  - –Maintains the culture in a biochemically active state

# Bacterial Growth Curve

Bacterial counts made at specific intervals and plotted in relation to time a growth curve is obtained



# Bacterial Growth Curve

- Lag phase: adaptation to the environment
- Exponential logarithmic growth: machine in full run
- Stationary phase: nutrition exhausted, toxin increased
- Decline: cell die (steady biomass) or lysis (decrease biomass)
- Dormant as spore, non-viable state

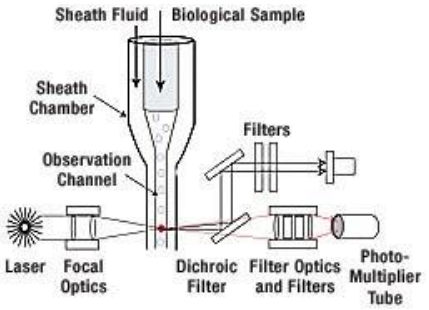
# Growth measurement

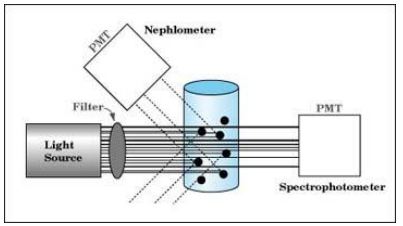
**Total Count**

- **Cell count: microscopic observation;**
- **flow cytometer-cell counter (direct)**
- **Turbidity opacity measurement - Nephlometer (indirect)**

**Viable count**

- **Colony formation: Measure the living cell (direct)**



# Bacterial Nutrition

- Water constitutes 80% of the total weight of bacterial cells.
- Proteins, polysaccharides, lipids, nucleic acids, mucopeptides & low molecular weight compounds makeup the remaining 20%.
- For growth & multiplication, the minimum nutritional requirements are water, a source of carbon, a source of nitrogen & some inorganic salts.

# Classification of Bacteria Based on Nutritional Requirement

- **Phototrophs : derive energy from sunlight**
- **Chemotrophs : obtain energy from chemical reactions**
- **Autotrophs : synthesize all organic compounds**
- **Heterotrophs : unable to synthesize and depend on performed organic compounds.**

# Growth Factors

- Some bacteria require certain organic compounds in minute quantities – Growth Factors OR Bacterial Vitamins.
- It can be :
- Essential – when growth does not occur in their absence.
- Accessory – when they enhance growth, without being absolutely necessary for it.

# Growth Factors

- Identical with mammalian nutrition
- Vitamin B complex –
- thiamine
- riboflavine
- nicotinic acid
- pyridoxine
- folic acid &
- Vit.B 12

# Factors Affecting Bacterial Growth

- **Oxygen requirement**
- **Temperature**
- **Moist & drying**
- **H-ion concentration**
- **Light**
- **Osmotic effect**
- **Mechanical and sonic stress**

# Oxygen requirement

- **Division is based on influence of oxygen into growth.**
- **Aerobic: require $O_2$ for growth**
- **Anaerobic: growth in absence of oxygen**
- **Microaerophilic: grow best in low oxygen tension.**
- **Facultative anaerobes: ordinarily aerobic but can grow in absence of oxygen.**
- **Aerobic obtain energy through oxidation**
- **Anaerobes use hydrogen than oxygen**

# Temperature

- **Optimum temperature for growth: (37$^{o}$C)**
- **Mesophilic: Bacteria grow best at 25-40$^{o}$C.**
- **Psychrophilic: grow best at temperature below 20$^{o}$C eg.(-7$^{o}$C)**
- **Thermophies: grow best at higher temperature.(55-80$^{o}$C).**
- **Extreme thermophilic bacteria can grow at 250$^{o}$C.**

# Moist and drying

- **Water is essential to bacterial protoplasm and drying is lethal**
- **Treponema is highly sensitive to drying while other withstand drying for months.**
- **Spore may survive in dry state for several decades.**

# H-ion concentration

- **Bacteria are sensitive to variation in pH.**
- **Bacteria grows best at neutral or slightly alkaline (pH-7.2-7.6).**
- **Acidophiles: "Acid loving"**
  - **Grow at very low pH (0.1 to 5.4) Lactobacillus produces lactic acid, tolerates mild acidity**
- **Neutrophiles:**
  - **Grow at pH 5.4 to 8.5**
  - **Includes most human pathogens**
- **Alkaliphiles: "Alkali loving"**
  - **Grow at alkaline or high pH (7 to 12 or higher)**
  - **Vibrio cholerae and Alkaligenes faecalis optimal pH 9**
  - **Soil bacterium Agrobacterium grows at pH 12**

# Light

- **Bacteria (except phototropic) grow well in dark.**
- **They are sensitive to UV light and radiations.**
- **Cultures die if exposed to sunlight.**

# Osmotic effect

- **More tolerant to osmotic variation than most other cells due to strength of their cell wall.**
- **Sudden exposure to hypertonic solution - shrinkage of protoplasm (plasmolysis) due to water withdrawal.**
- **Sudden transfer hypotonic solution - swell - plasmoptysis (leading to rupture of cells.) osmotic inhibition**

# Mechanical and sonic stress

- **Bacteria have tough cell walls, they may be ruptured by mechanical stress such as grinding or vigorous shaking by glass beads.**

# Summary

- Bacteria multiply by binary fission
- Bacterial growth curve
- Bacterial nutritional requirements
- Factors affecting bacterial growth