Lecture (3-4) Bacteria

1. Structures
2. Essential Structures
3. Non Essential Structures
4. Bacterial Shapes
5. Binary Fission Results
6. Identifying Bacteria
   Motility
7. Classification and Identification
8. Clinical Lab Identification

Structures

1. Cell wall.
2. Cytoplasmic membrane.
3. Cytoplasm.
4. Nuclear body.
5. Capsule.
6. Flagella
7. pili.
8. Inclusion granules

Classification and Identification

Bacterial Shapes

Cocci

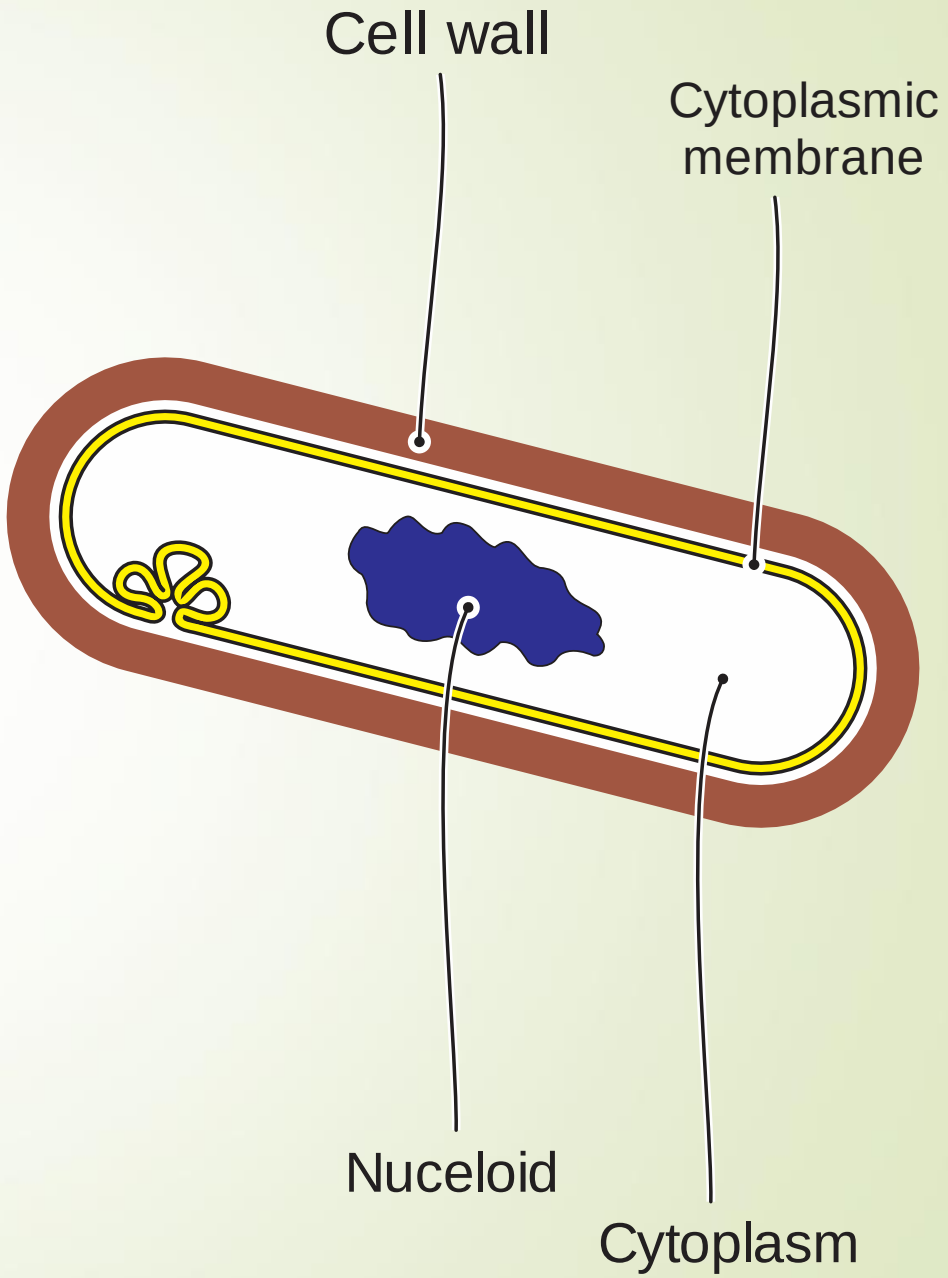
Bacillus

spiral

Essential structures

Any bacterial cell is composed of the following structures (Essential structures):

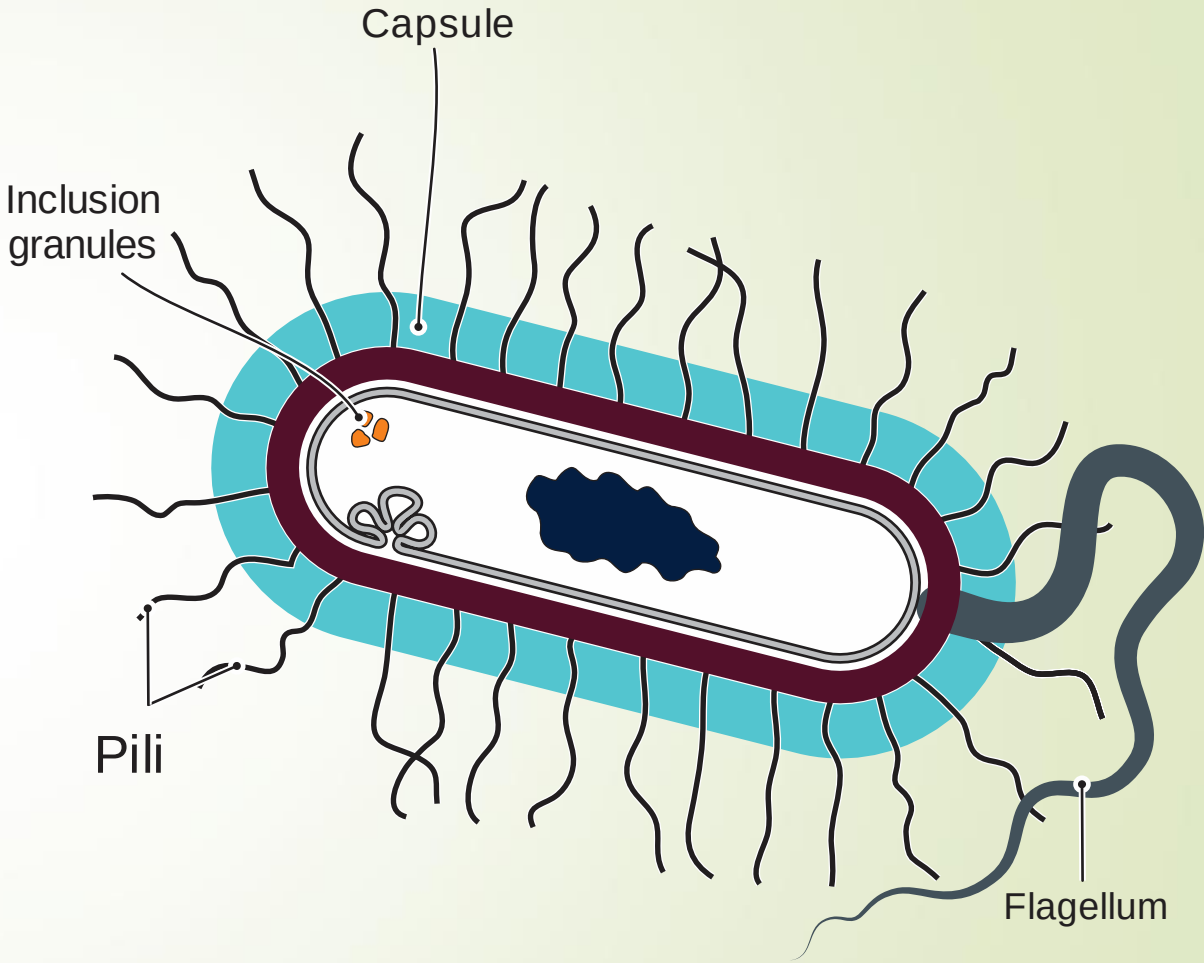
1. Cell wall.
2. Cytoplasmic membrane.
3. Cytoplasm.
4. Nuclear body.



Non Essential Structures

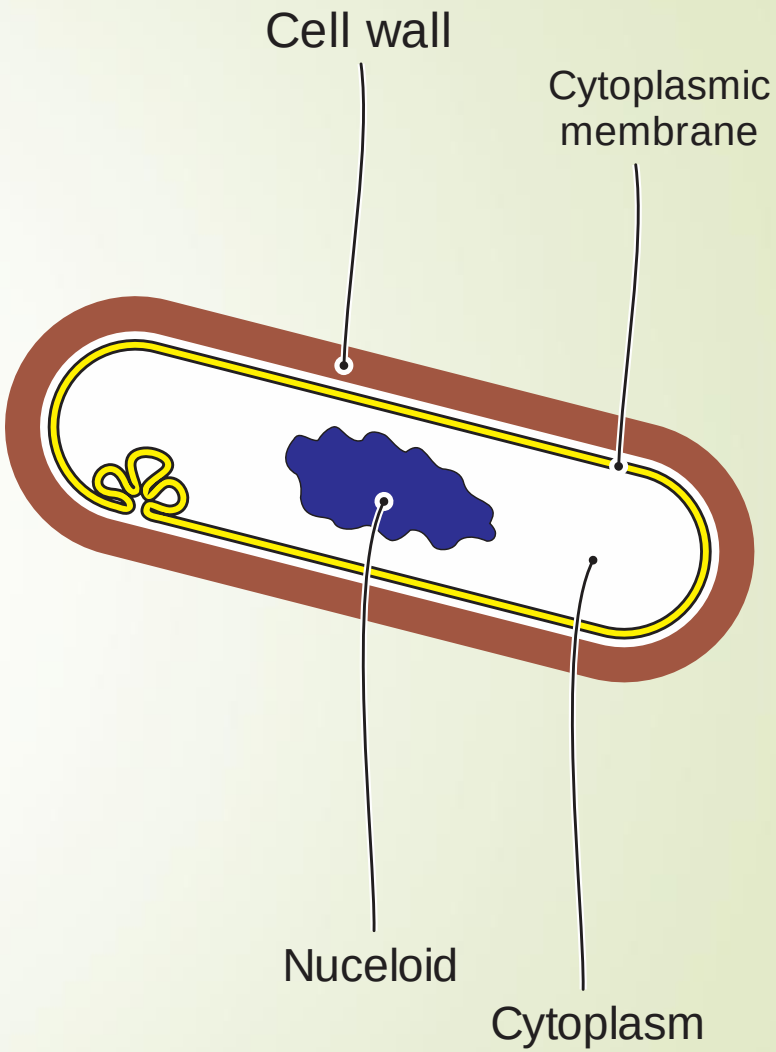
Some (Not all) bacteria may contain one or more of the following structures:

1. Capsule.
2. Flagella
3. Fimbria (pili).
4. Inclusion granules



THE CELL WALL

The cell wall is a ***rigid structure*** that ***surrounds*** the bacterial cell just outside of the plasma membrane.



Cell wall Structure

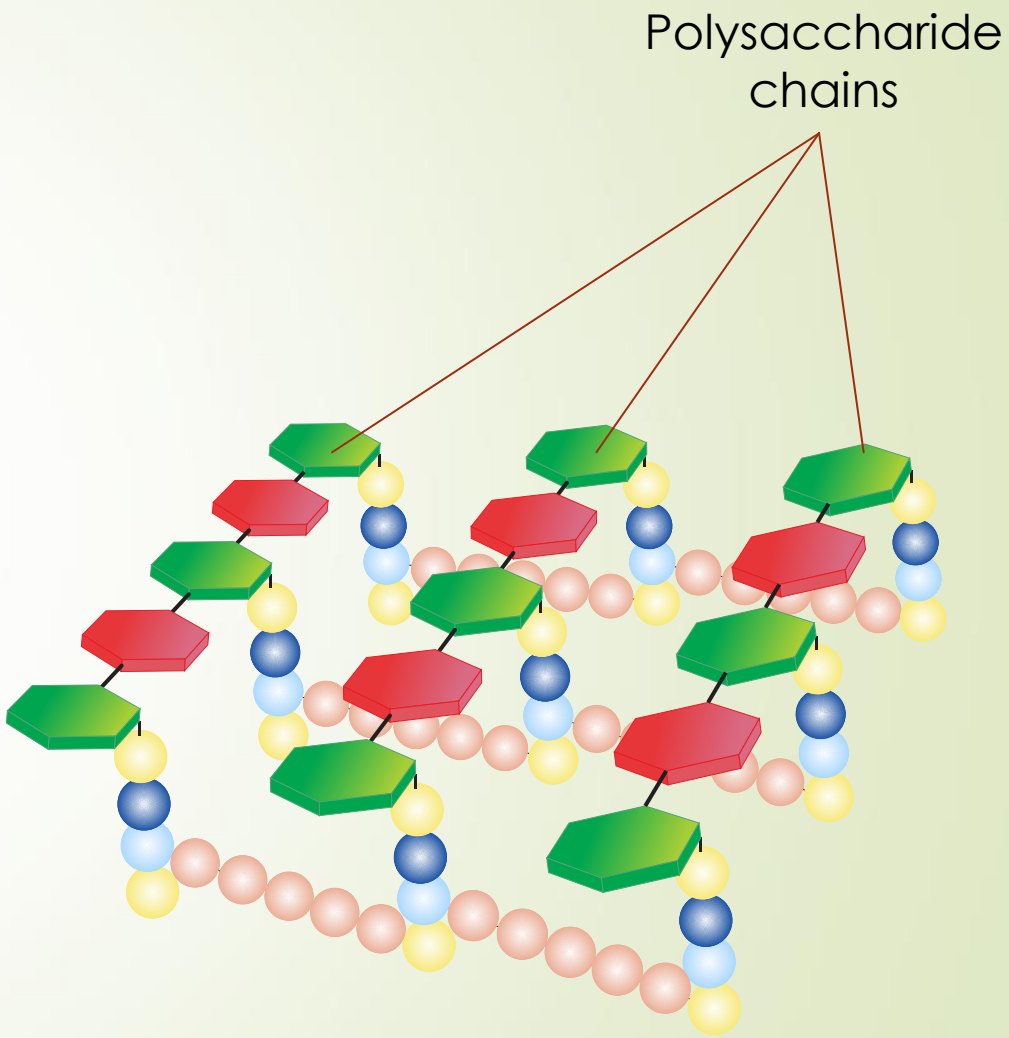
Bacteria are classified according to their cell wall as **Gram positive** or **Gram negative**.

Components of cell wall

Peptidoglycan	• The main structural component of the cell wall. • Peptidoglycan is formed of carbohydrate + protein.
Outer membrane	• It is found in gram-negative cells. • contains lipopolysaccharide (LPS).
Lipopolysaccharide	• It is found in the outer leaflet of the outer membrane of gram-negative cells. • it is toxic (endotoxin). The toxicity is due to the lipid fraction . • It is antigenic.
Periplasmic space	• It is found in gram-negative cells • It is the area between the cell membrane and the outer membrane.

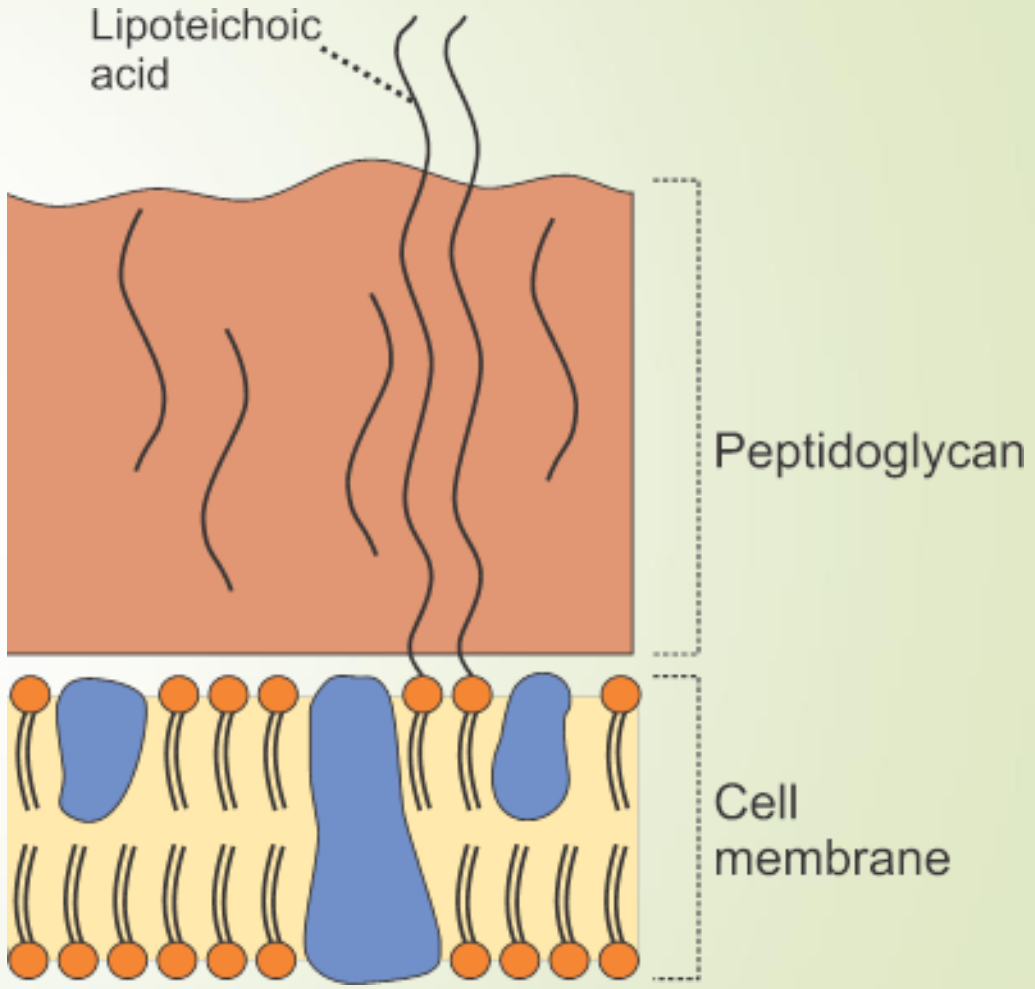
Peptidoglycan

- The main structural component of the cell wall.
- Peptidoglycan is formed of carbohydrate + protein.
- It consists of long polysaccharide **chains** that are **cross-linked** by amino acid **bridges**



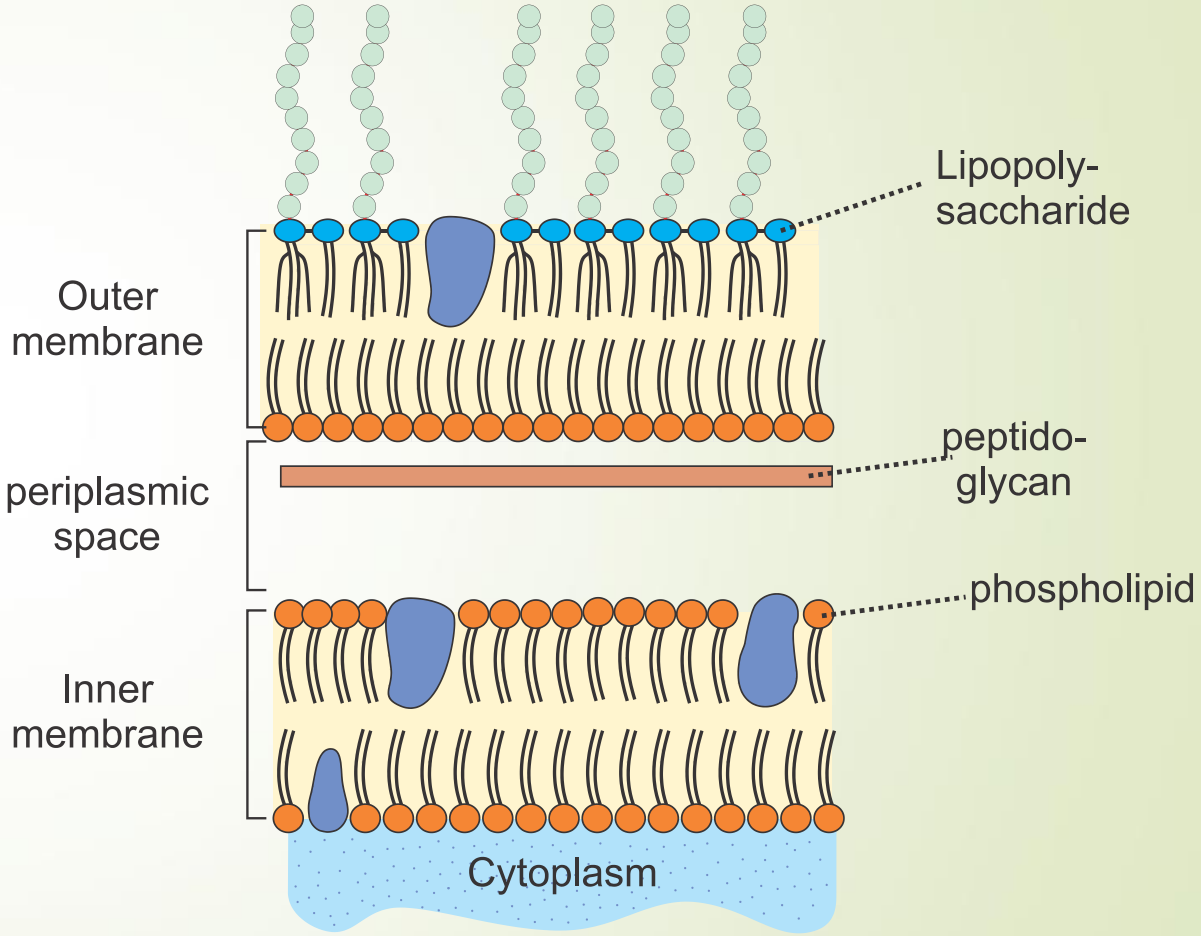
Gram positive

- In Gram-positive bacteria the peptidoglycan forms a thick layer external to the cell membrane.



Gram negative

- In Gram-negative species, the peptidoglycan layer is **thin** and is overlaid by an **outer membrane**.
- The space between the inner and outer membranes, is called the **periplasmic space**

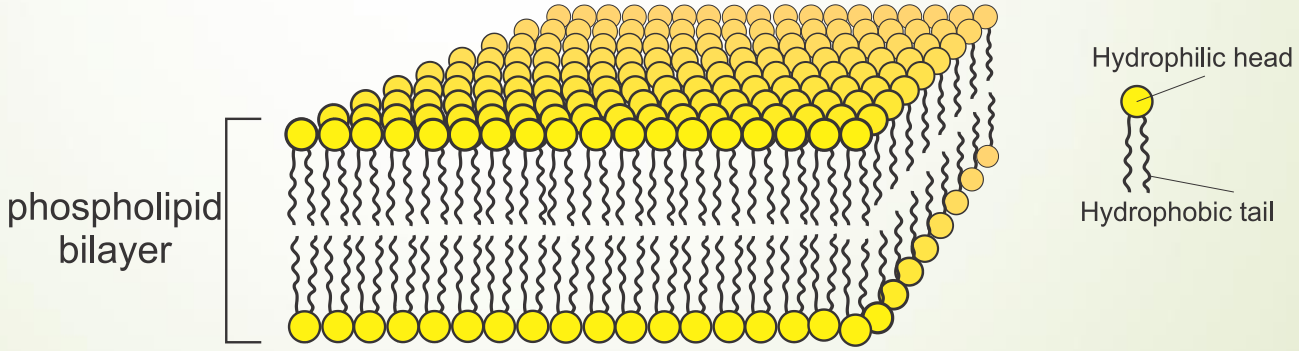


Function of the cell wall

- Determines the **shape** of the bacterial cell.
- Protection of bacterial cell against **osmotic lysis**.
- Responsible for **staining properties** of bacteria.

CYTOPLASMIC MEMBRANE

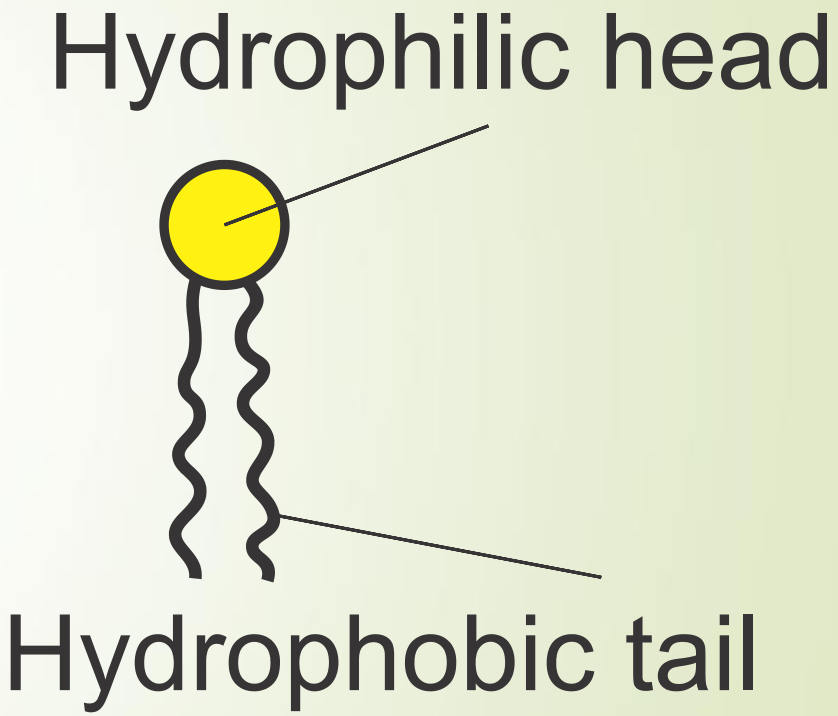
- Surrounds the cytoplasm
- The cytoplasmic membrane is a **bilayer** of **phospholipids**



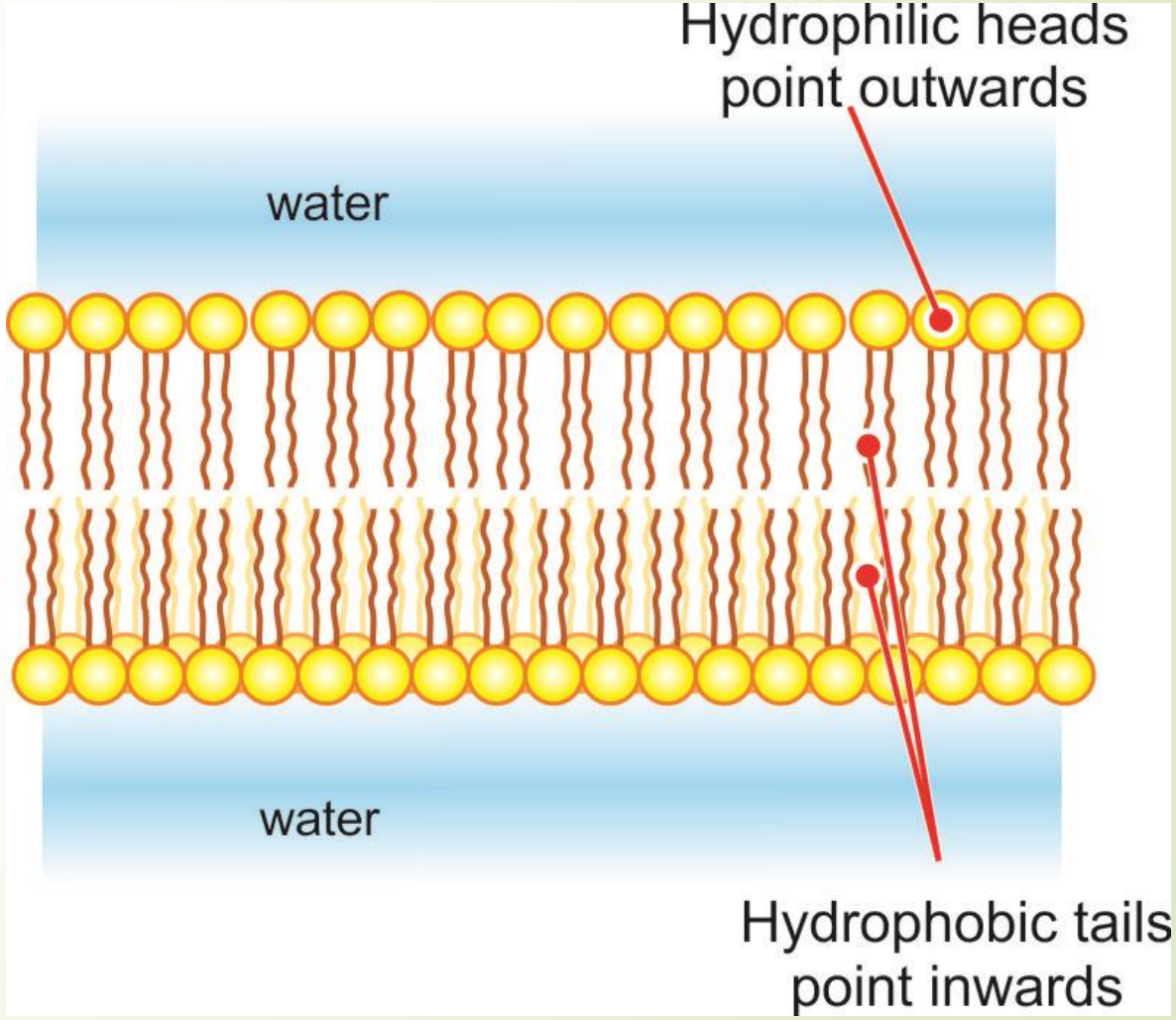
Phospholipid

Phospholipids are composed of two parts:

- Hydrophilic head ("water-loving").
- Hydrophobic tails ("water-fearing").



- The **Hydrophilic heads** points outwards (facing the aqueous medium on both sides of the bilayer).
- The **hydrophobic Tails** points inwards.



Functions of cell membrane

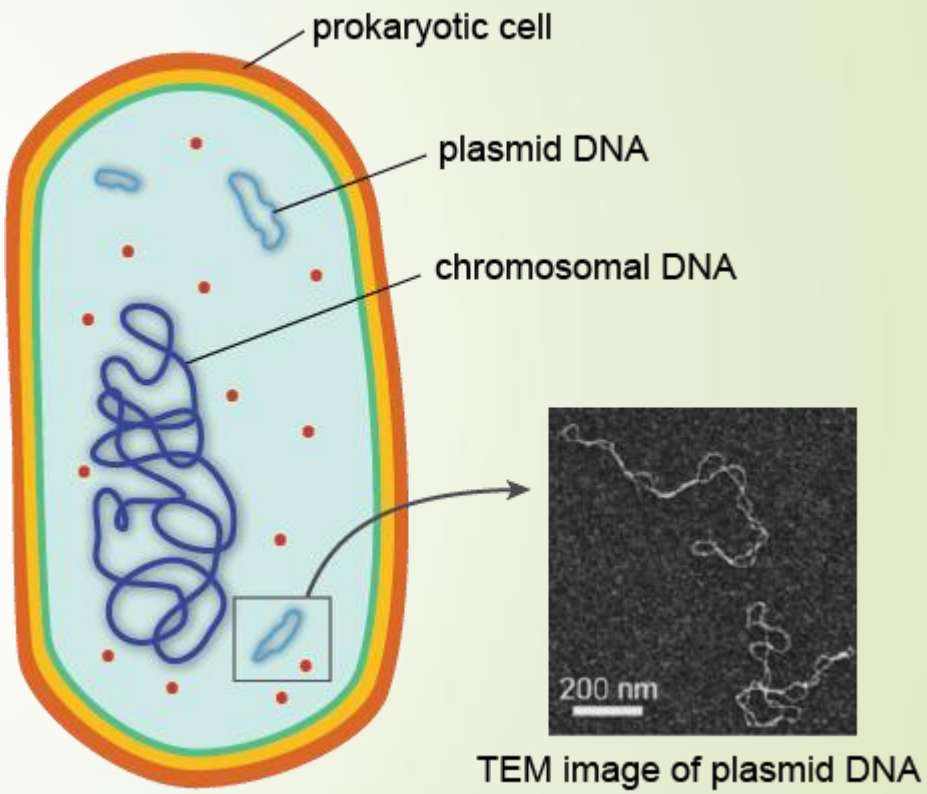
- **Selective permeability** = selectively transport nutrients to the cell, and waste products outside the cell.
- **Active transport** of ions and molecules to the inside of cells.
- **Excretion** e.g. hydrolytic exoenzymes

Cytoplasm

- A homogeneous soft gel mass inside the cell.
- The cytoplasm of prokaryotes has **no organelles**.
- It contains:
  - **Nuclear body.**
  - **Plasmids.**
  - Ribosomes.
  - Enzymes.
  - Storage granules

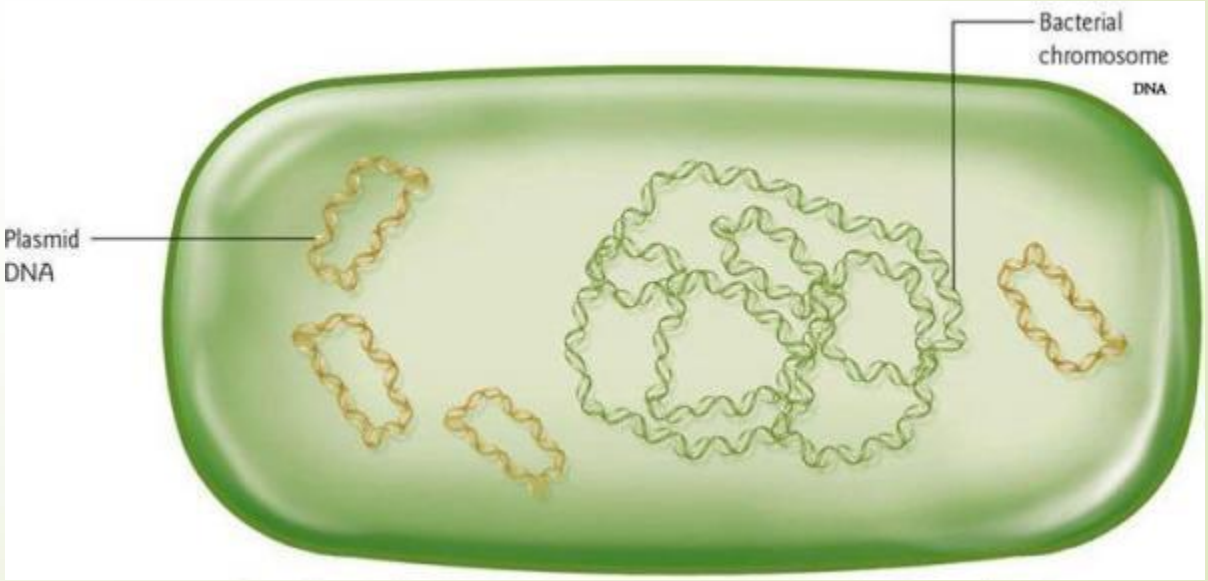
THE NUCLEAR BODY

- Consists of **single circular** DNA molecule coiled to form a mass.
- It carries all **essential** genetic information of the cell.
- There is no nuclear membrane and no nucleolus.



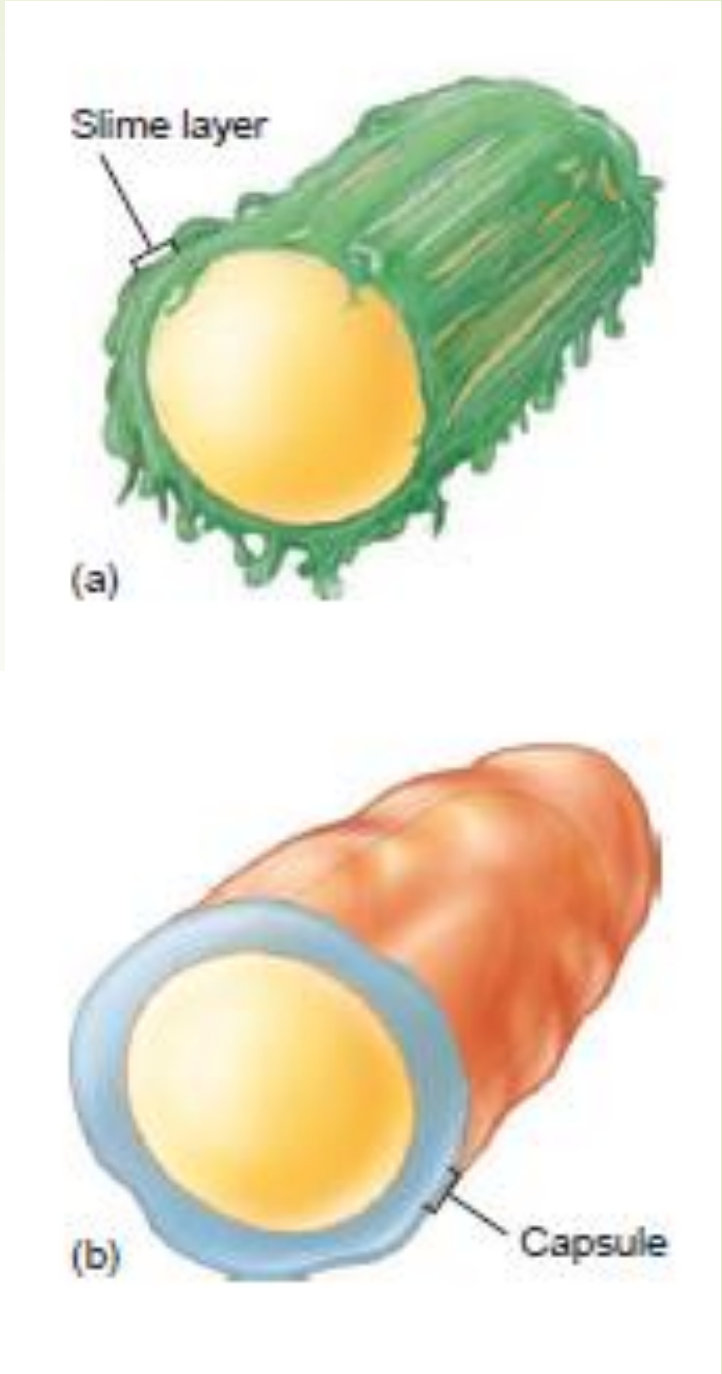
Plasmids

- Plasmids: are **extrachromosomal** pieces of circular DNA.
- Plasmids carry **non-essential genes** such as antibiotic resistance genes.



Capsule and slime layer

- Capsule is a **well-defined** structure of **polysaccharide** surrounding and **firmly attached** to bacterial cell and is external to the cell wall.
- **Slime layer** : A slime layer is a zone of **diffuse**, unorganized material, **loosely associated** with the cell wall **and can be easily removed**.

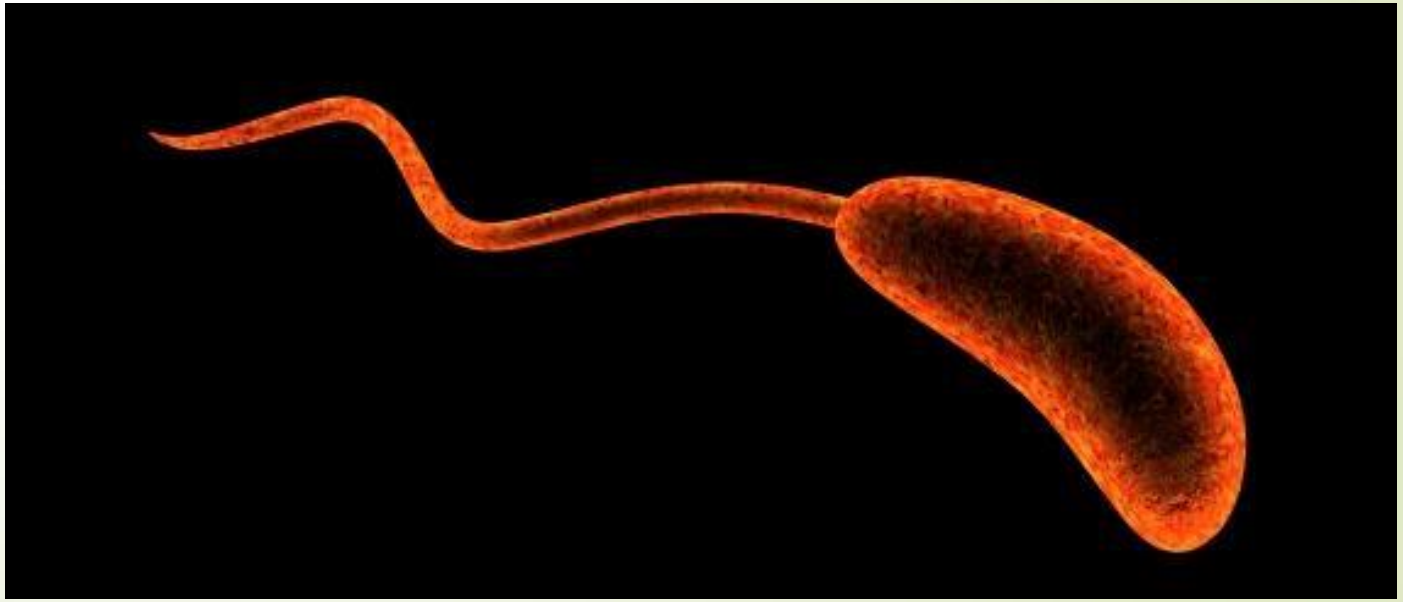


Function

- Protection of bacteria against **phagocytosis**.
- Protect cells from **drying**.
- Help bacteria in **adherence** to surfaces.

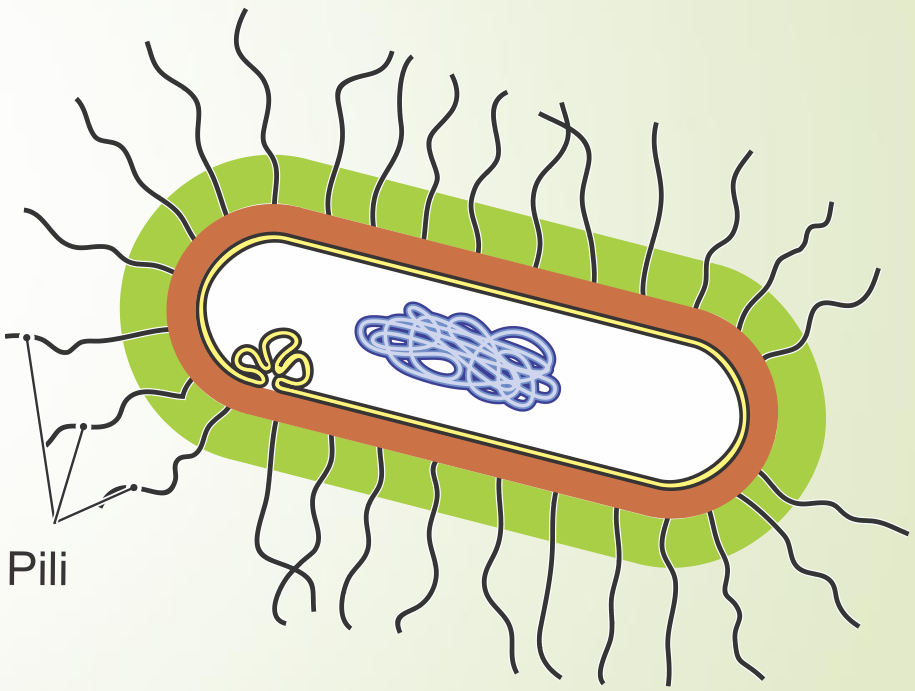
Flagella (single Flagellum)

- Flagella are long protein appendages
- Responsible for motility.



Pili (single → pilus)

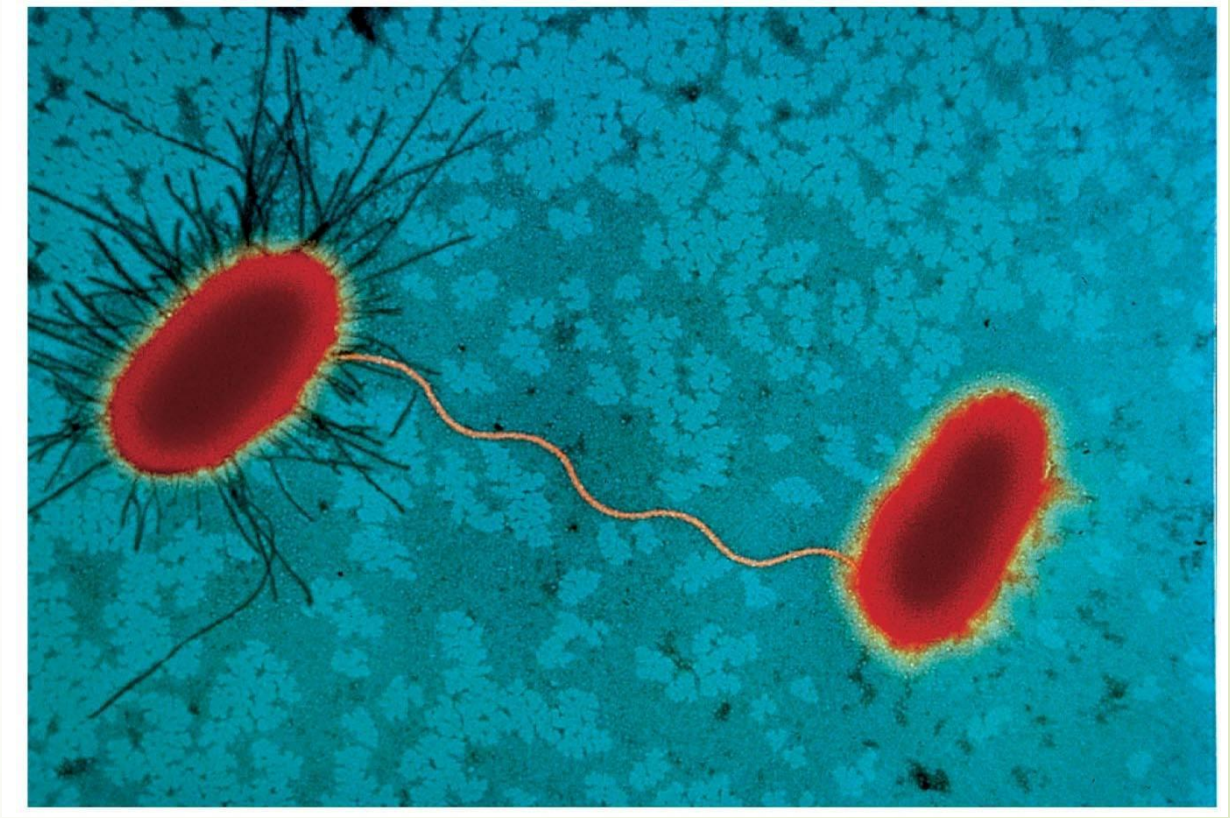
Pili are short hair-like surface appendages.



Pili (single → pilus)

Pili exist in two classes:

- **Ordinary pili**, involved in bacterial **adherence.**
- **Sex pili**, involved in transfer of genetic material (**conjugation).**

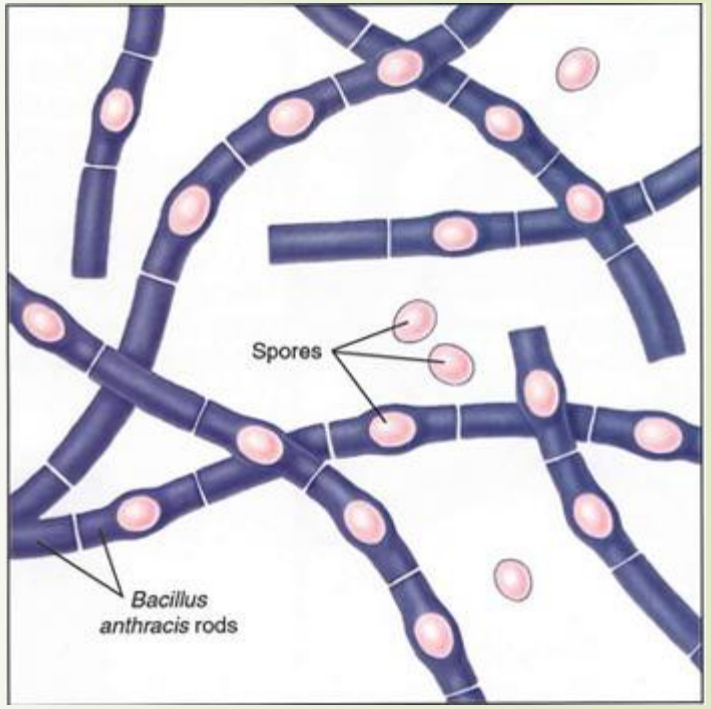


INCLUSION GRANULES

- **Function**: **Storage** of **energy** or as a reservoir of **structural building** blocks.
- **Site:** In cytoplasm.
- **Example:** Volutin granules in diphtheria bacilli (reservoir of inorganic phosphate).

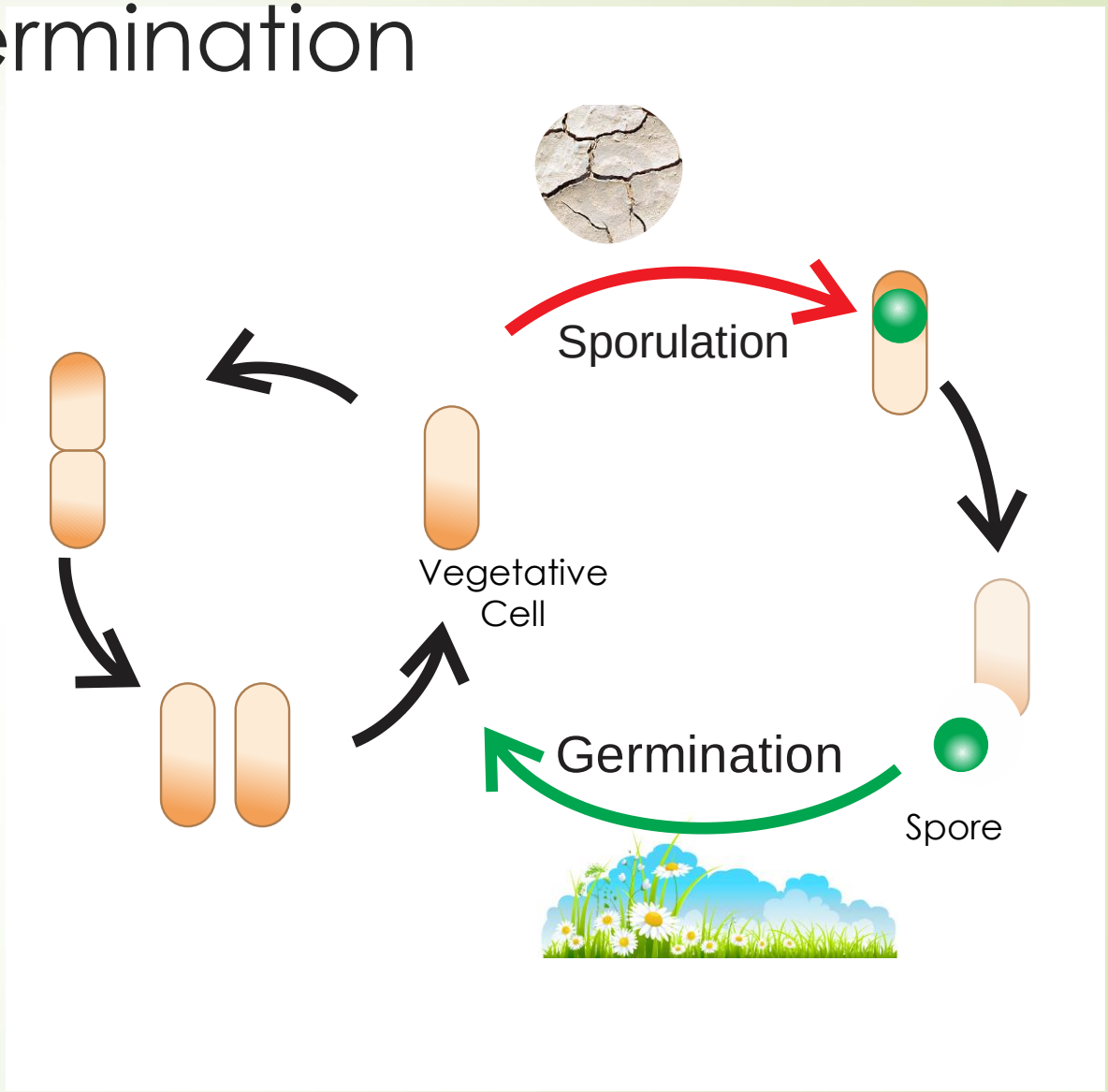
Spores

- ***Spores*** are highly resistant resting forms of some bacteria.
- Spores are formed on exposure to **<u>unfavorable conditions</u>** e.g. dryness, heat and depletion of nutrients.



Sporulation / Germination

- ***Sporulation (sporogenesis):*** *the process of formation of* ***spores from vegetative cells****.*
- ***Germination****: opposite to sporulation i.e. formation of* ***vegetative cells from spores*** *in favorable conditions.*

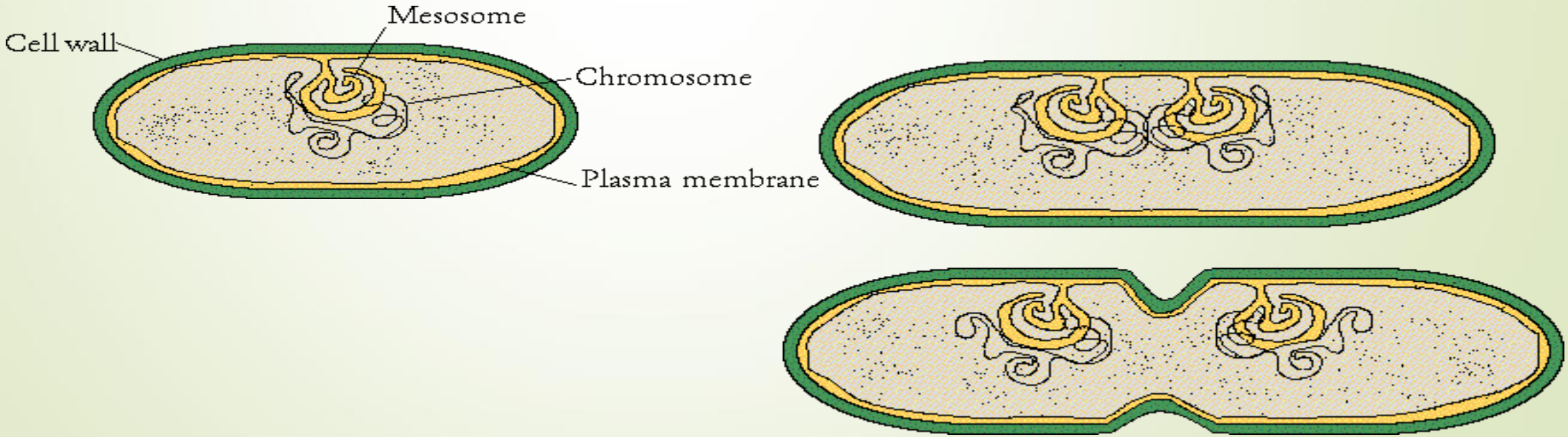


Medical Importance of spores

- The medical importance of spores lies in their **extraordinary resistance to killing by heat** and ***chemicals***.
- As a result of their resistance to heat, **sterilization** cannot be achieved by ordinary methods such as boiling.
- **Steam heating under pressure** (autoclaving) at 121°C, usually for 30 minutes, is required to ensure the sterility of products for medical use.

Bacterial Shapes

- Result of Asexual Reproduction
  - Binary Fission
    - Cross wall divides
    - Daughter cells +/- separate



Bacterial Shapes

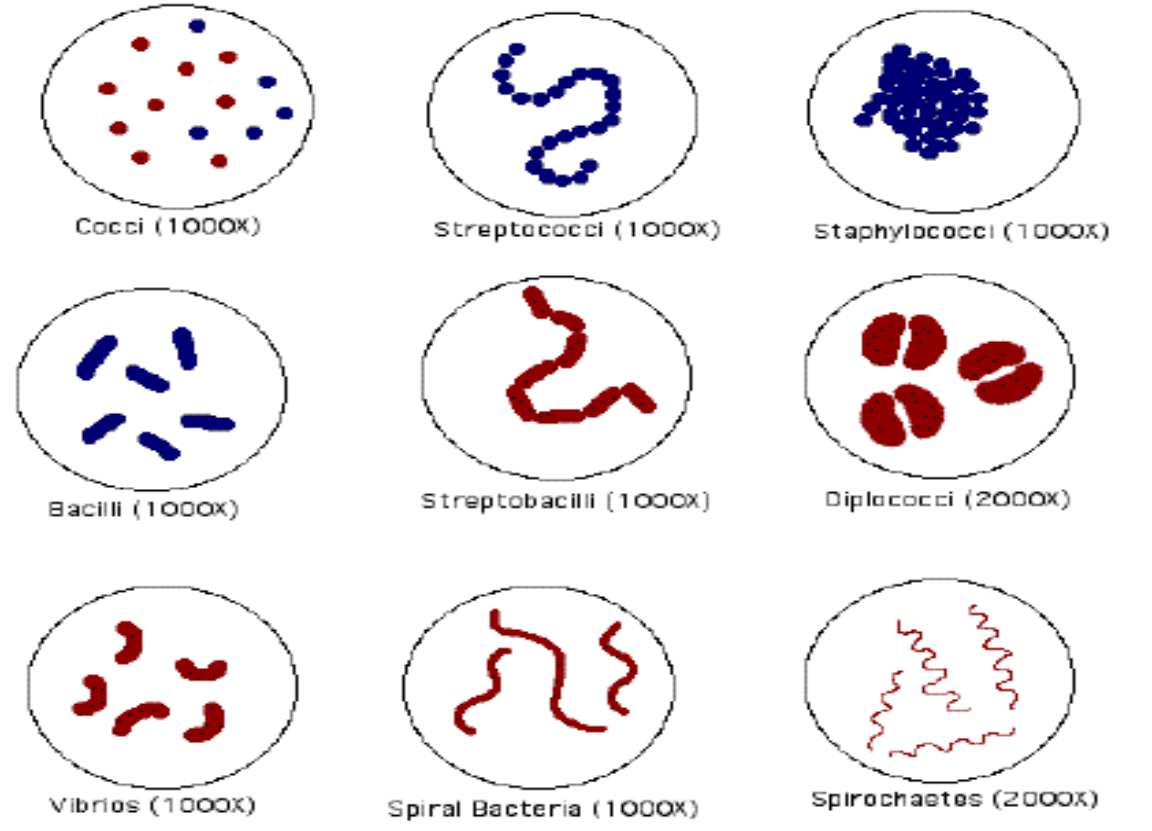
- Result of Asexual Reproduction
  - Snapping Division
    - Inner cell wall divides
    - Daughter cells hinged

  - Budding
    - Outgrowth of original cell

Binary Fission Results

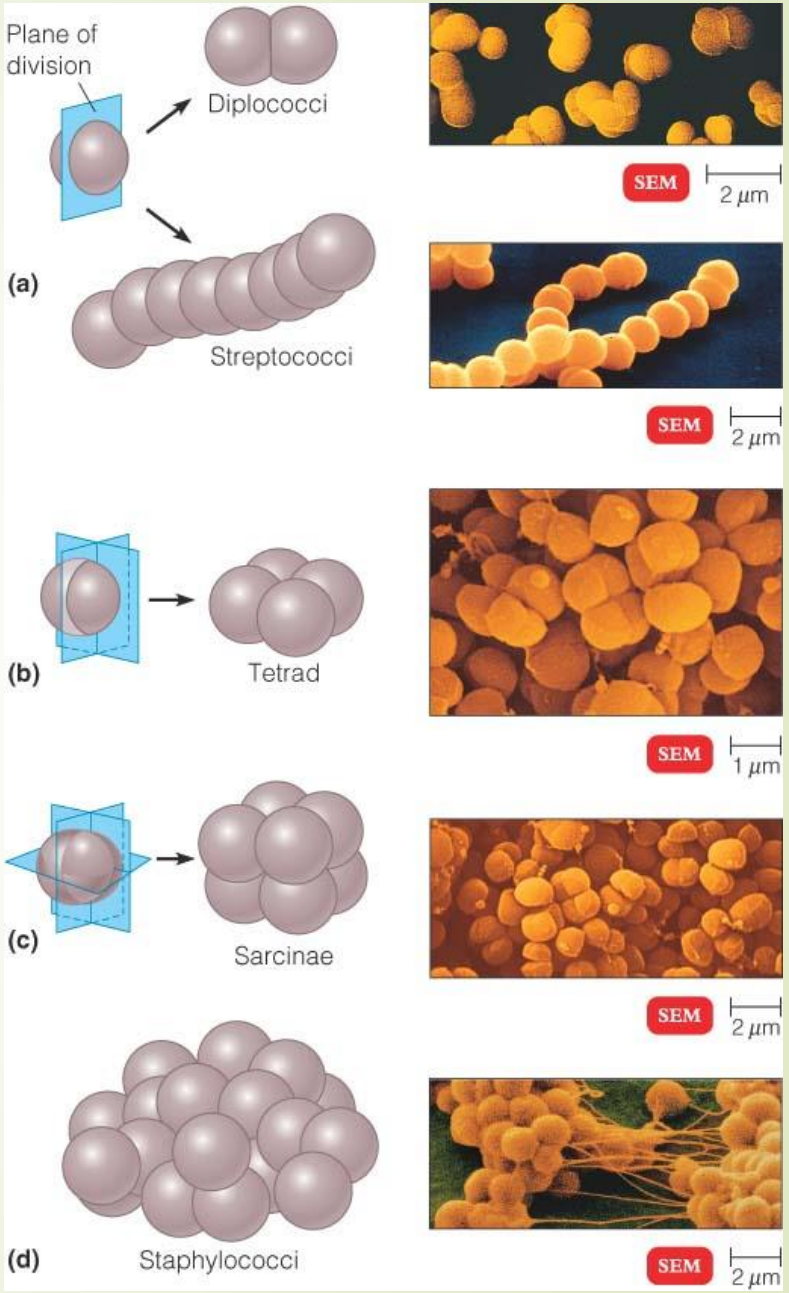
- Cocci
  - Pairs
  - Chains
  - Tetrads
  - Cubes
  - Clusters
- Bacillus
  - Separate
  - Pairs
  - Chains

Bacterial Shapes



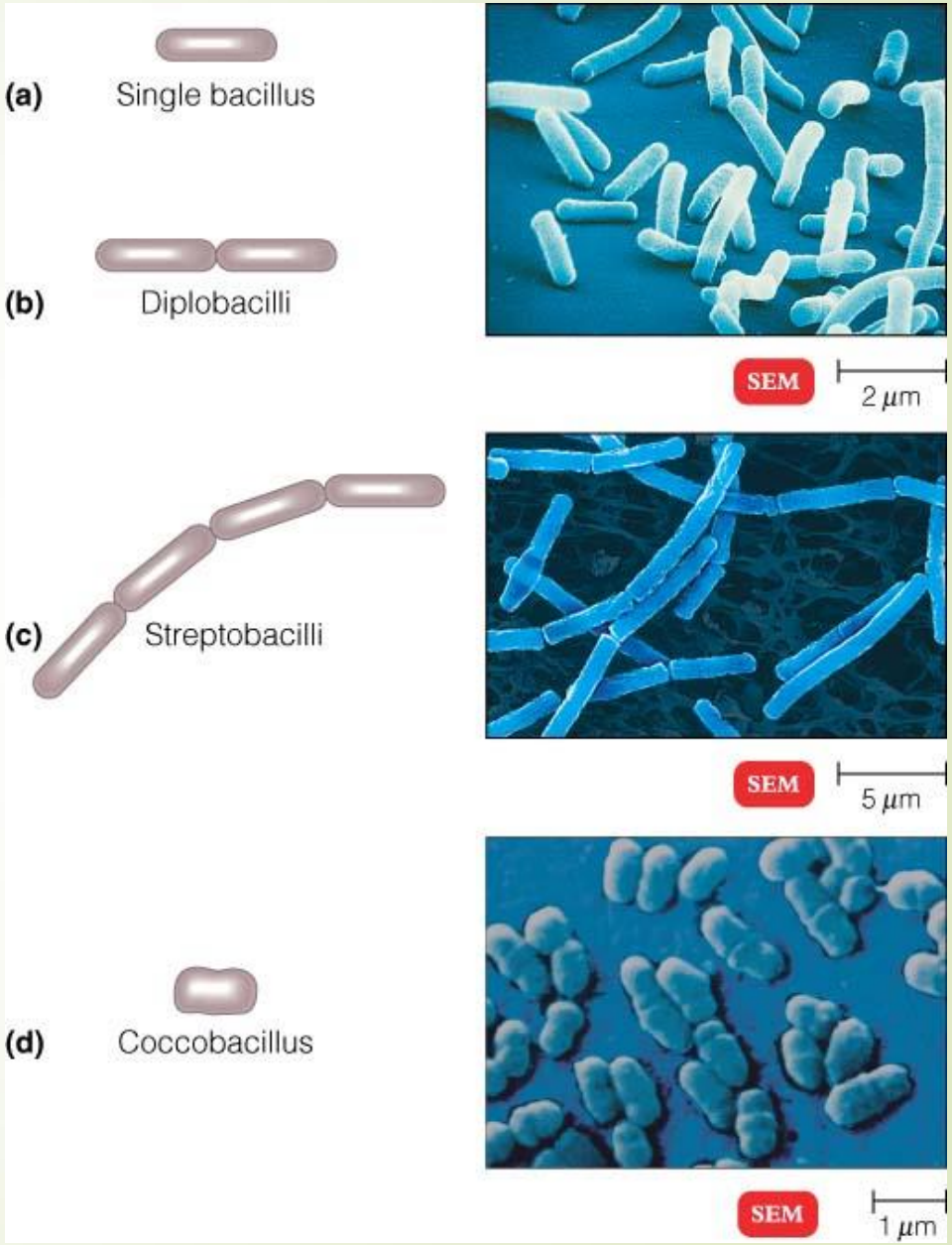
Arrangement of cocci

- Cocci that remain in pairs after dividing are called diplococci.
- Cocci that remain in chains after dividing are called streptococci.
- Cocci that divide in two planes and remain in groups of four are called tetrads.
- Cocci that divide in three planes and remain in groups cube like groups of eight are called sarcinae.
- Cocci that divide in multiple planes and form grape like clusters or sheets are called staphylococci.



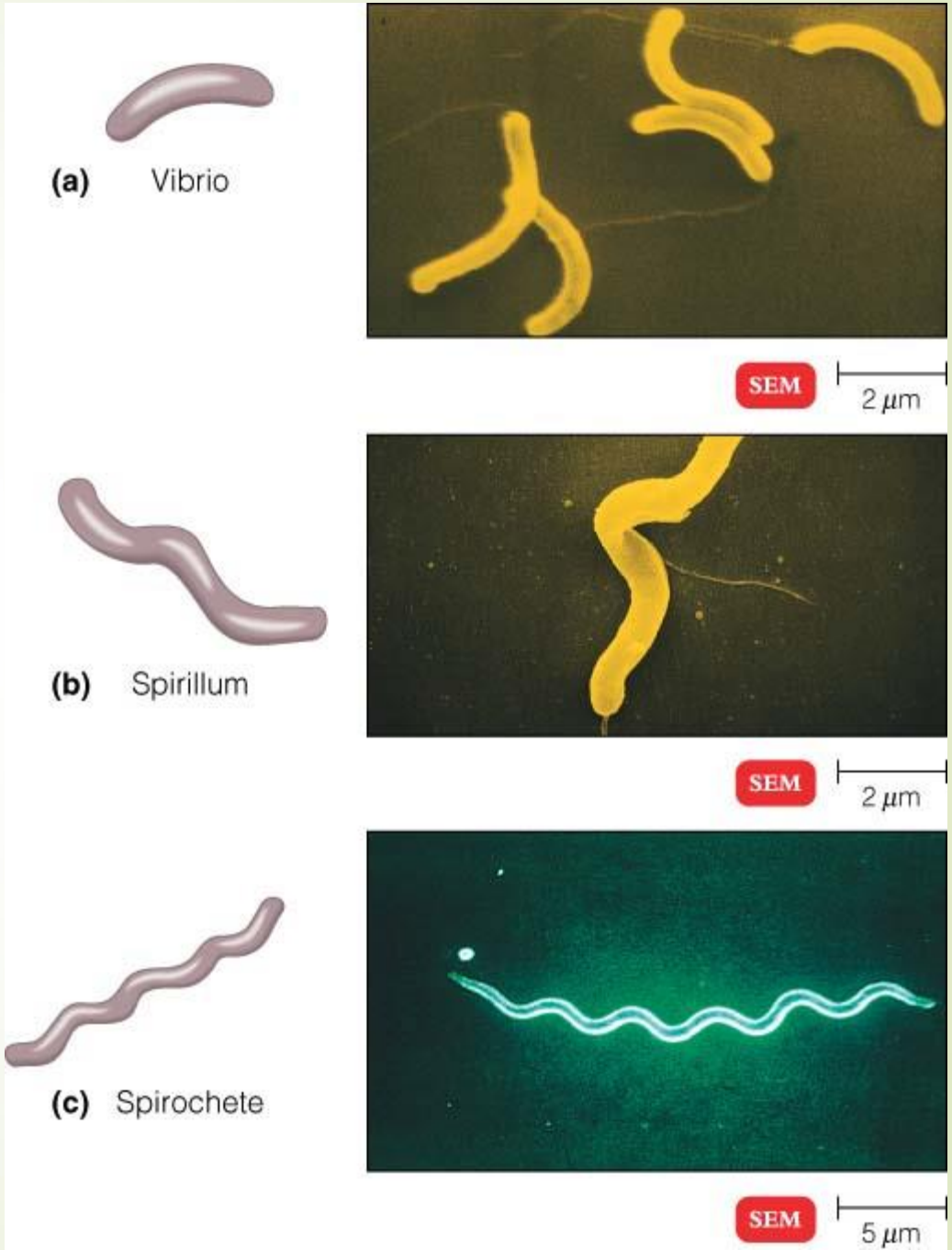
Bacilli

- Most bacilli appear as single rods. Diplobacilli appear in pairs after division.
- Streptobacilli appear in chains after division.
- Some bacilli are so short and fat that they look like cocci and are referred to as coccobacilli.

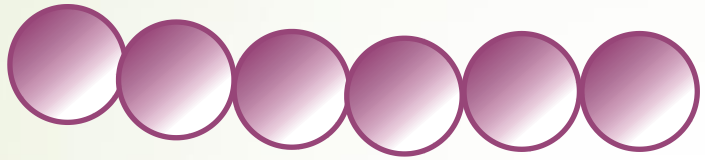


Spiral bacteria

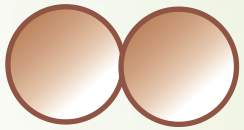
- Spiral bacteria have one or more twists.
  - Vibrios look like curved rods.
  - Spirilla have a helical shape and fairly rigid bodies.
  - Spirochetes have a helical shape and flexible bodies. Spirochetes move by means of axial filaments, which look like flagella contained beneath a flexible external sheath.



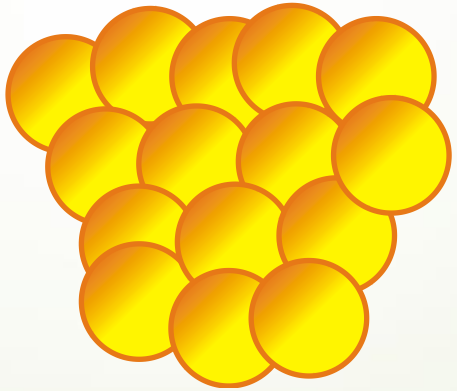
Bacterial arrangement



Streptococci

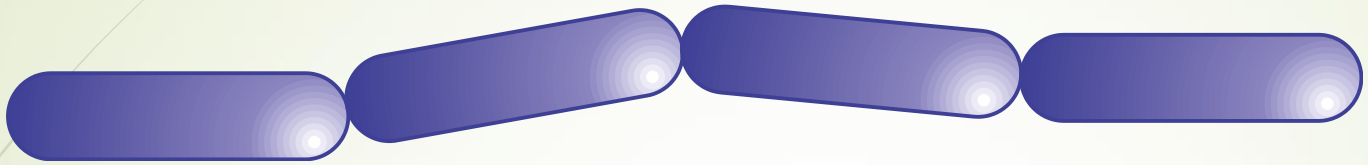


Diplococci

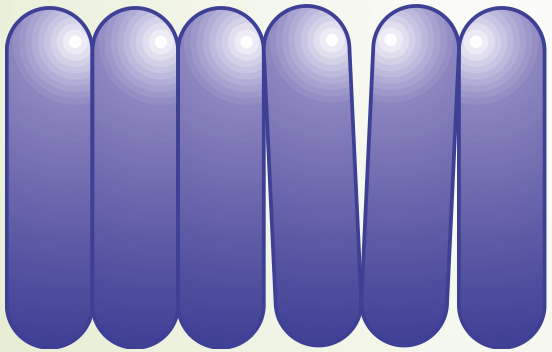


Staphylococci

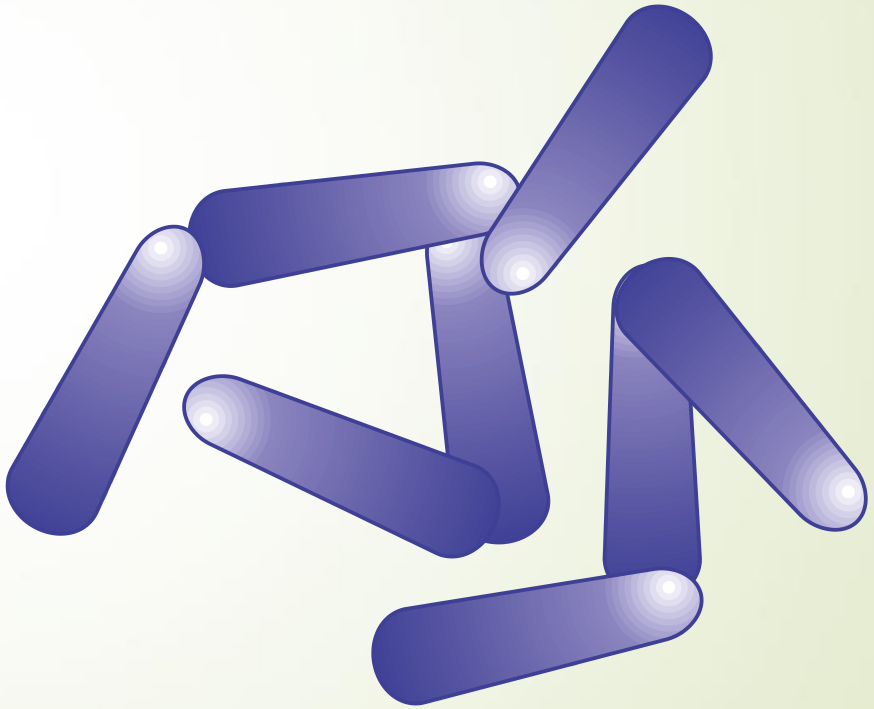
Arrangement of bacteria: Bacilli



Streptobacillus

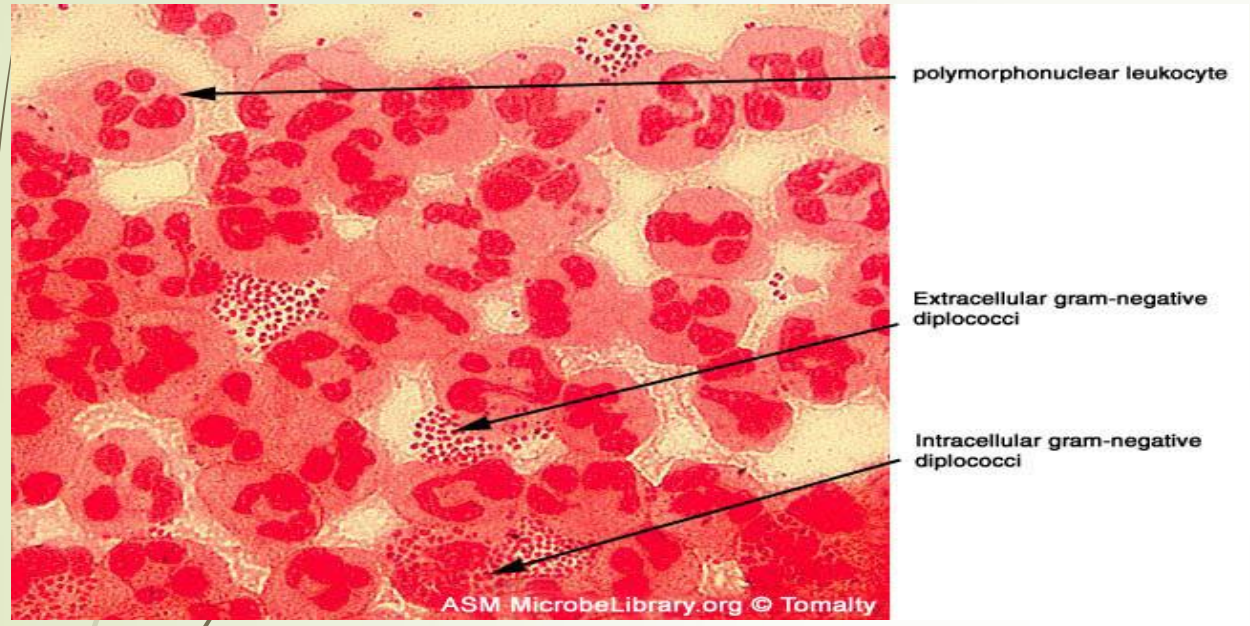


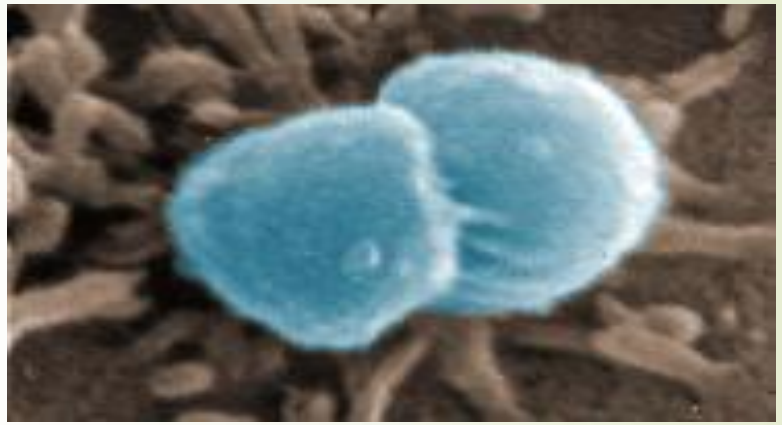
palisades



Chinese letters

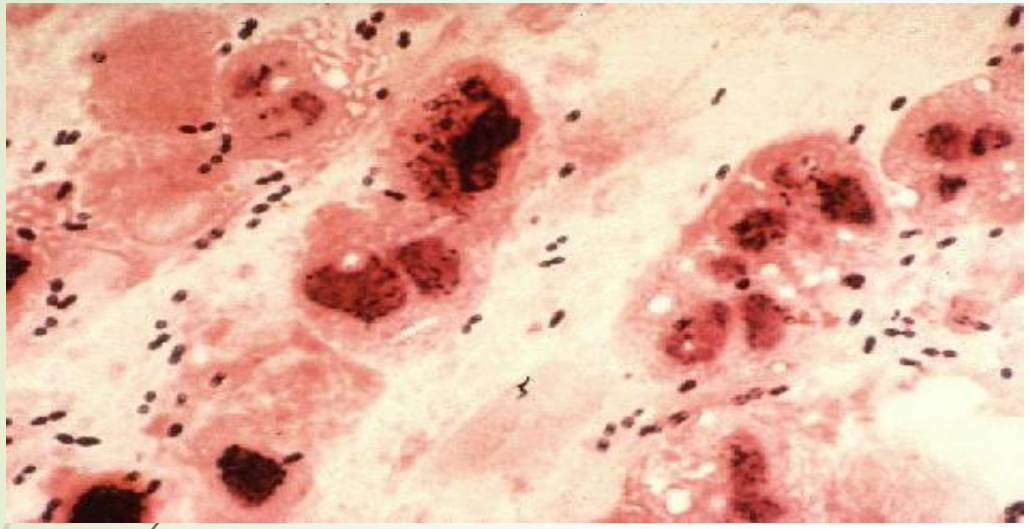
Cocci: Pairs



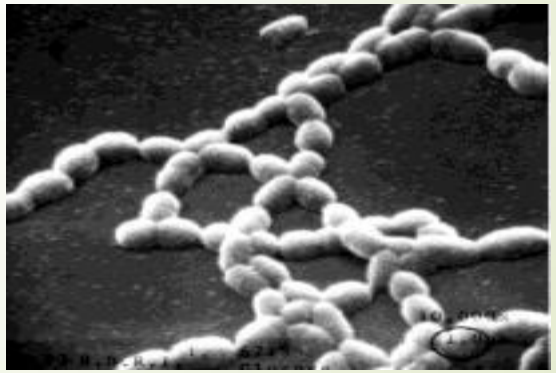


- Division in one plane
  - Diplococci
    - *Neisseria*

Cocci: Chains

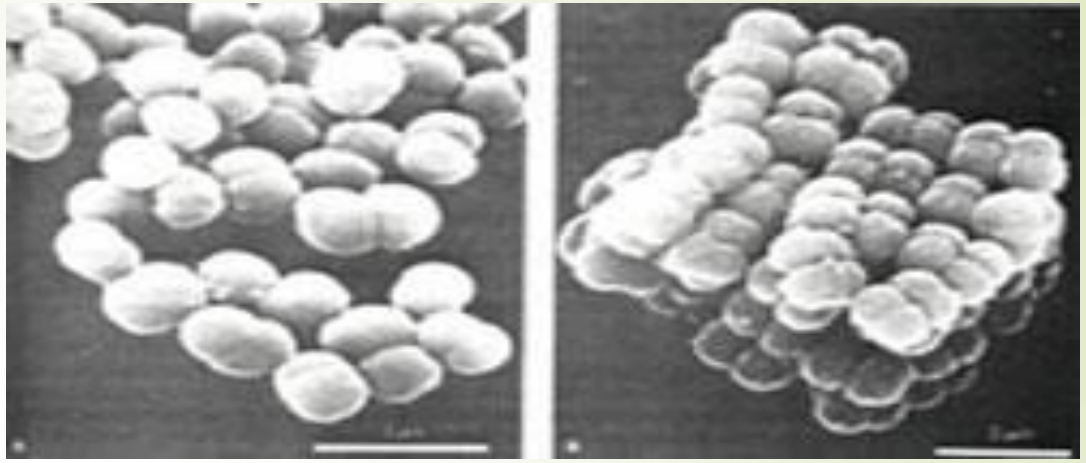


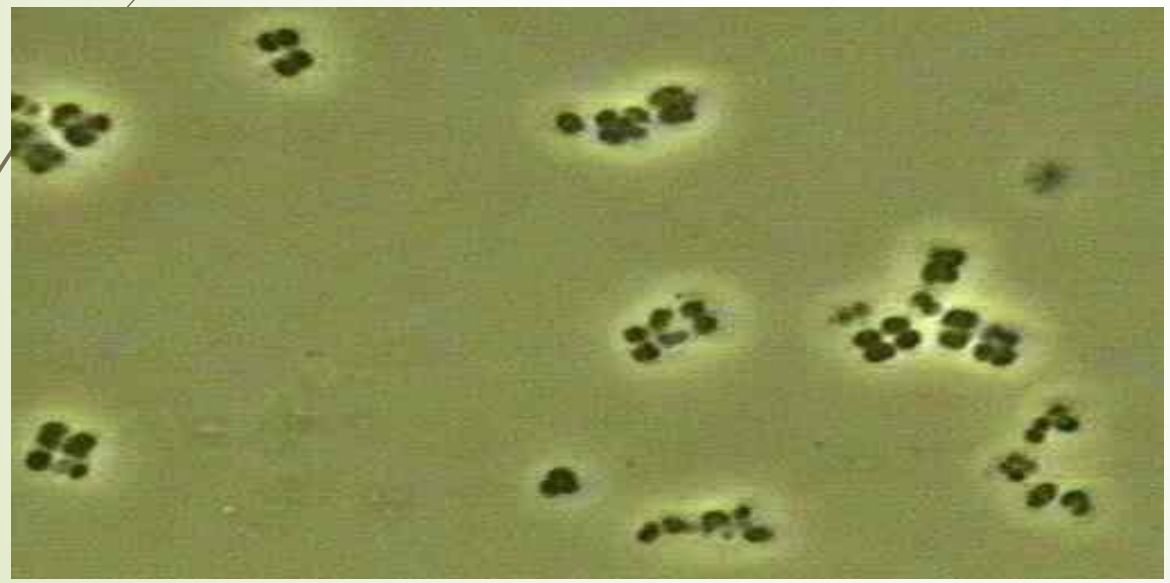
- Division in 2 Planes
  - *Streptococcus*



Cocci: Tetrads

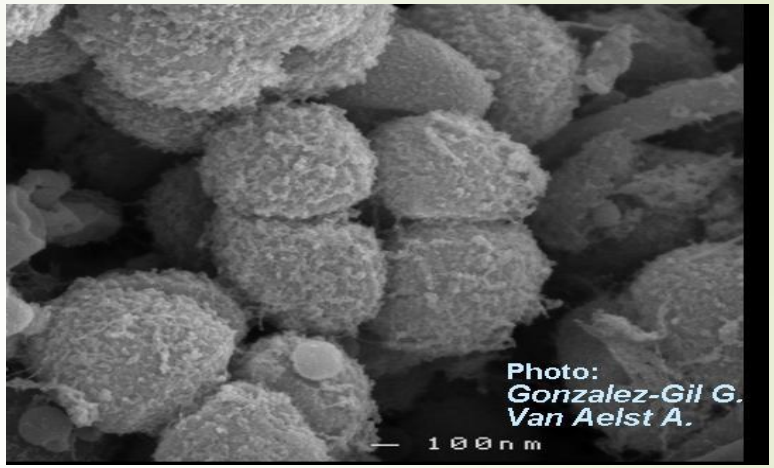
- Division in three planes
  - *Micrococcus*

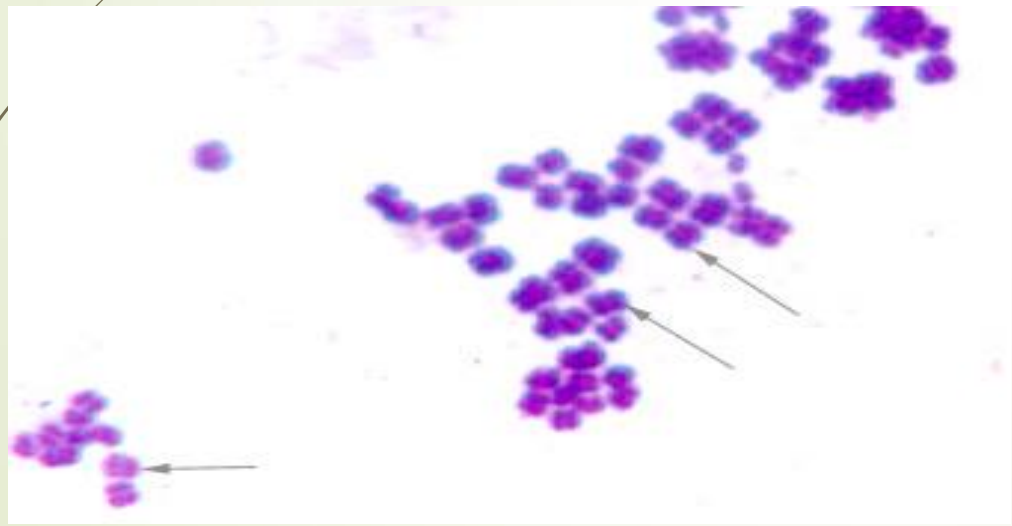




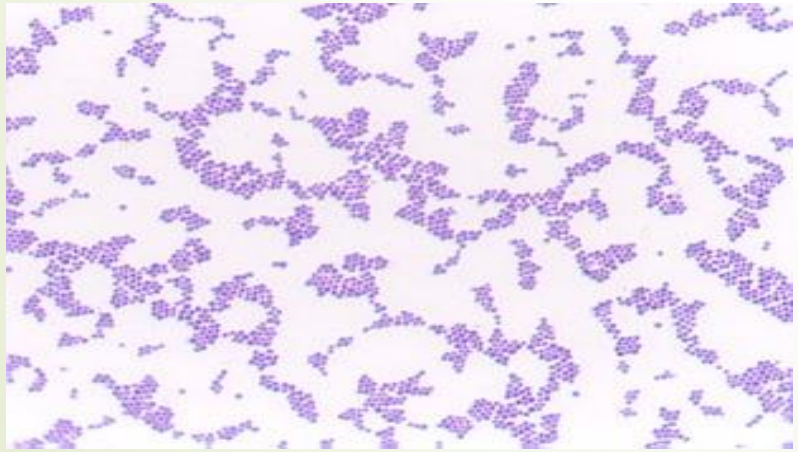
Cocci: 8-cell group

- Divides in 3 planes
  - *Sarcina*

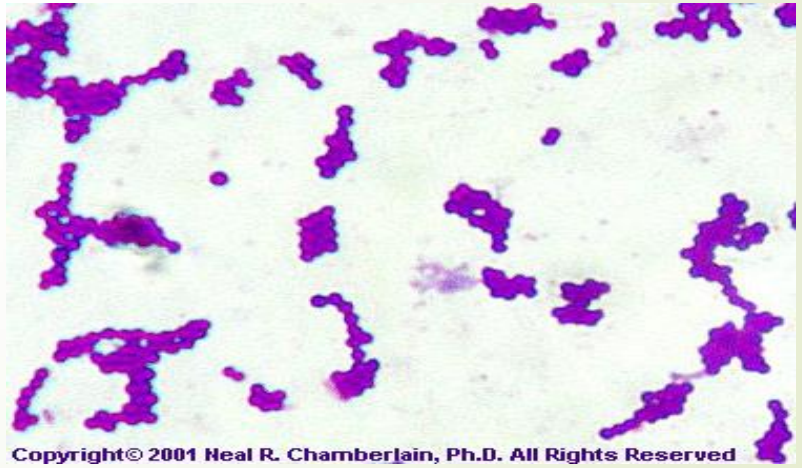




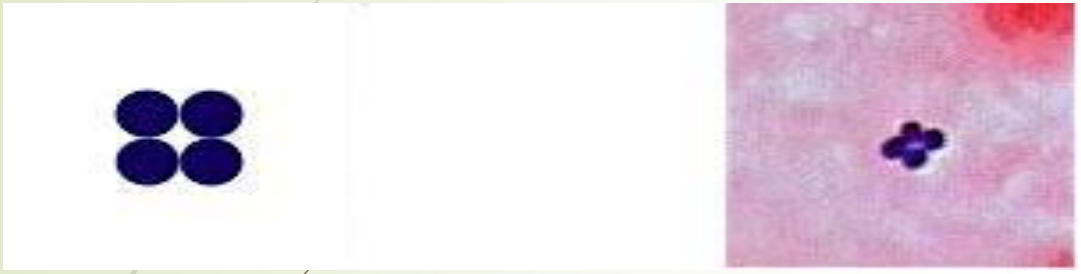
Cocci: Clusters

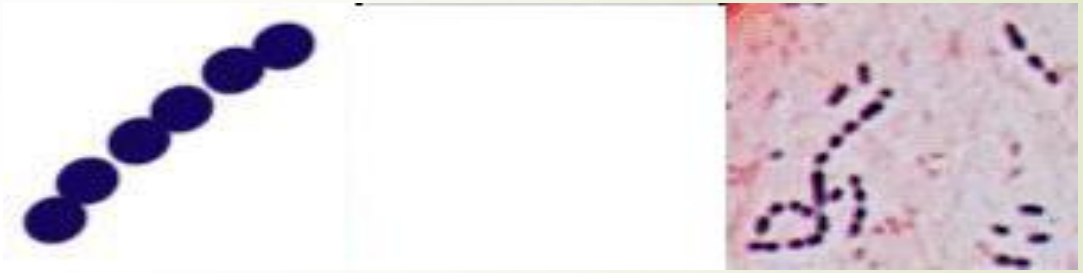


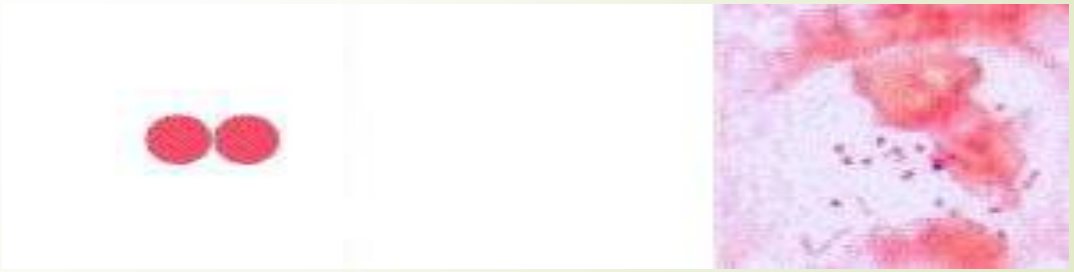
- Division in 3 planes
  - *Staphylococcus*



Cocci Summary

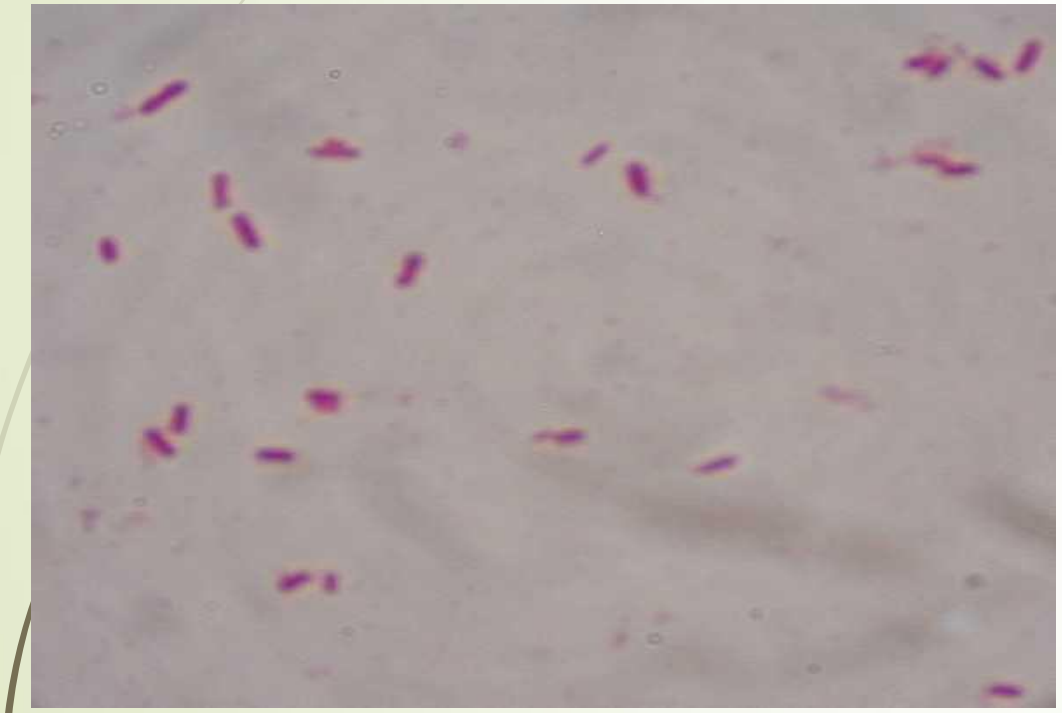


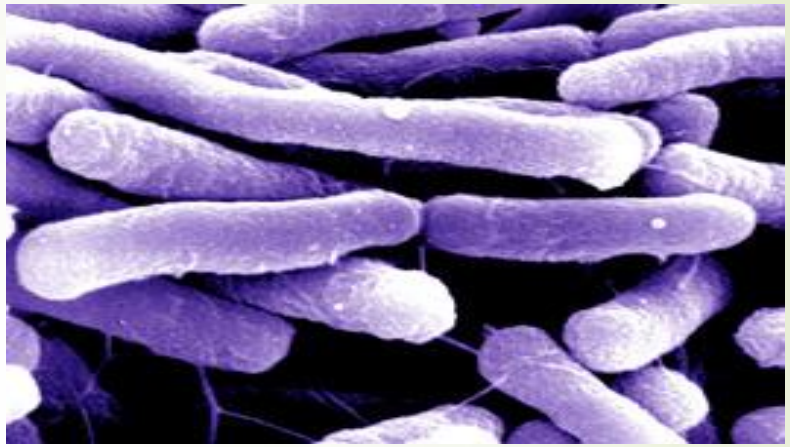




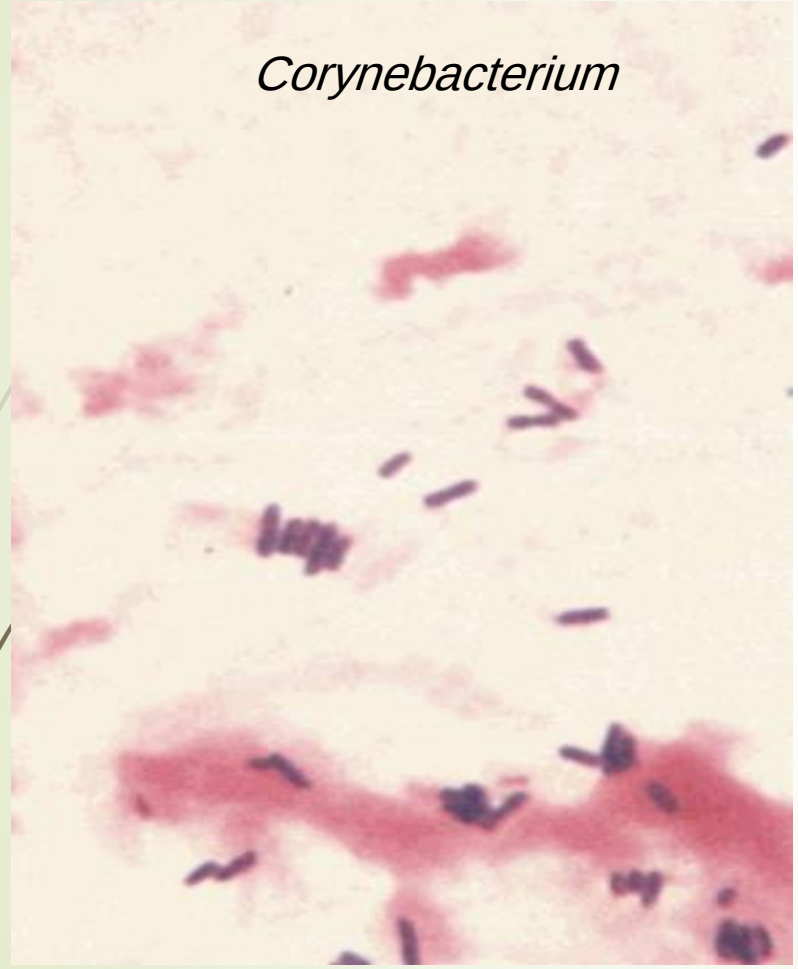
Rods: Straight

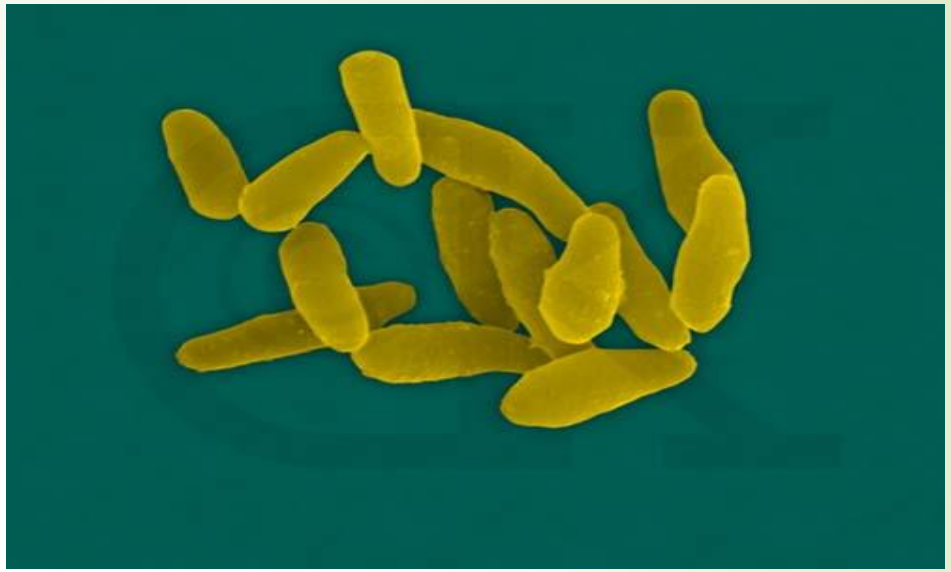
E. coli

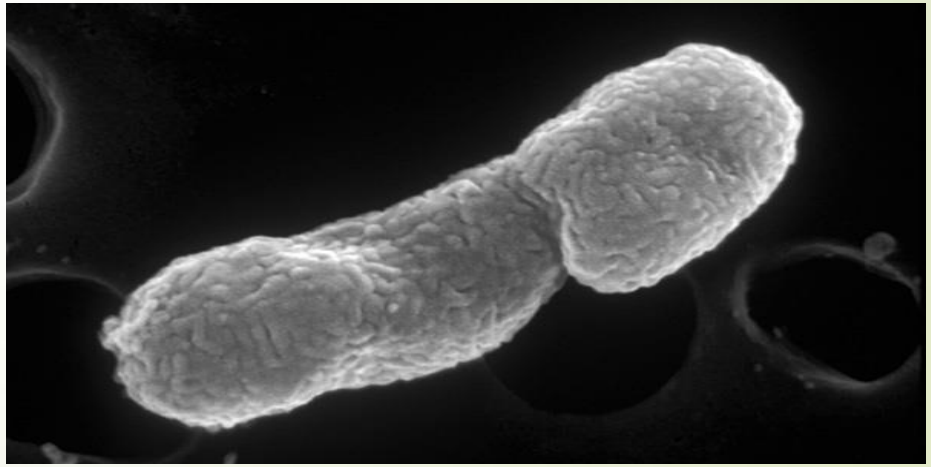




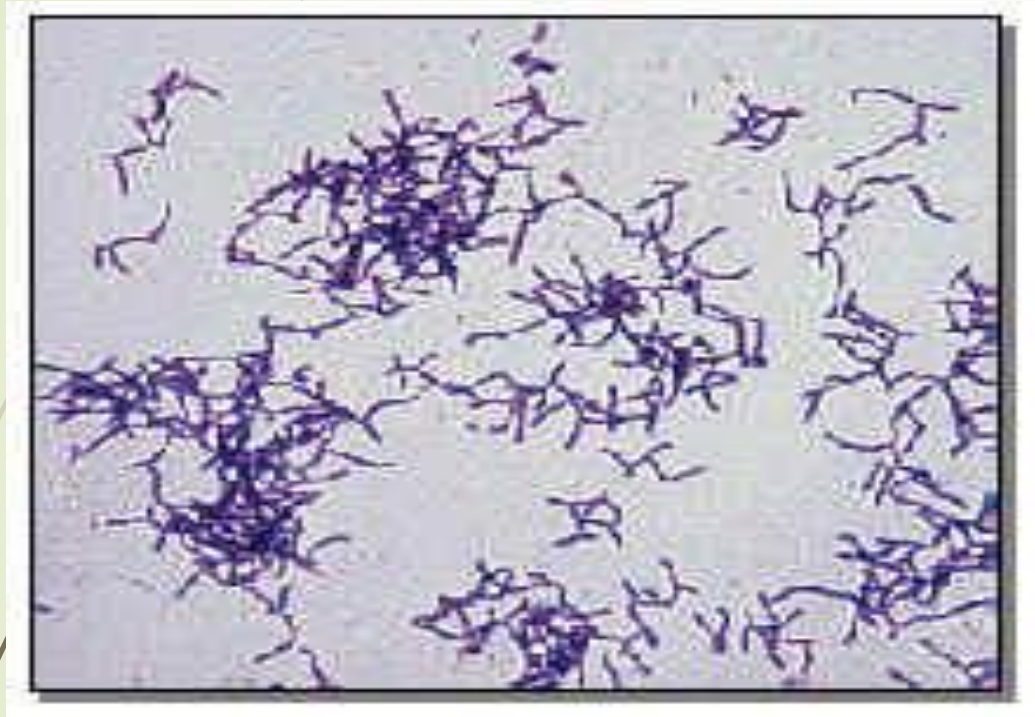
Rods: Club-Shaped



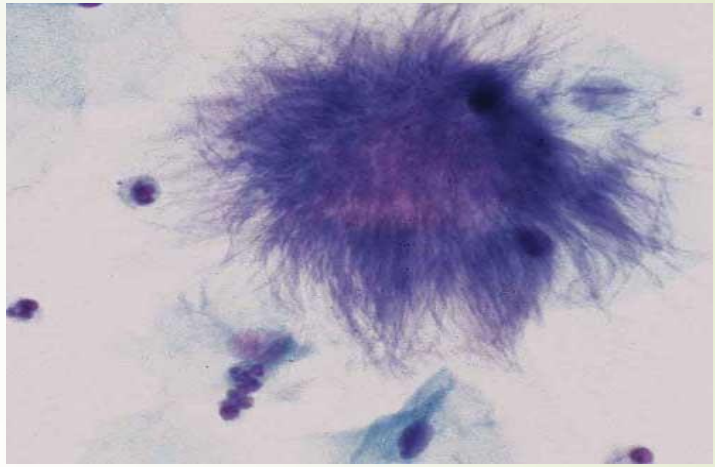


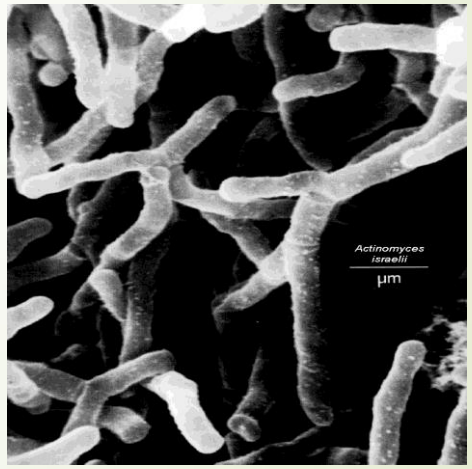


Rods: Branching

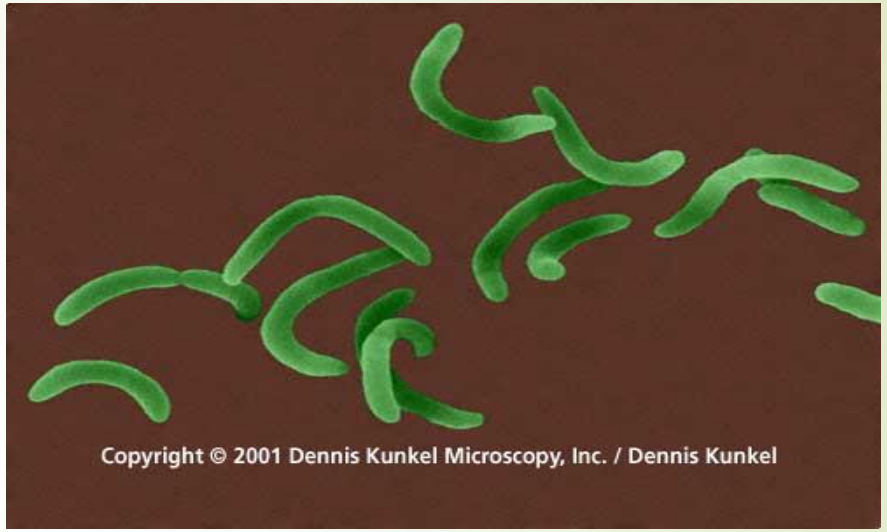


Actinomyces



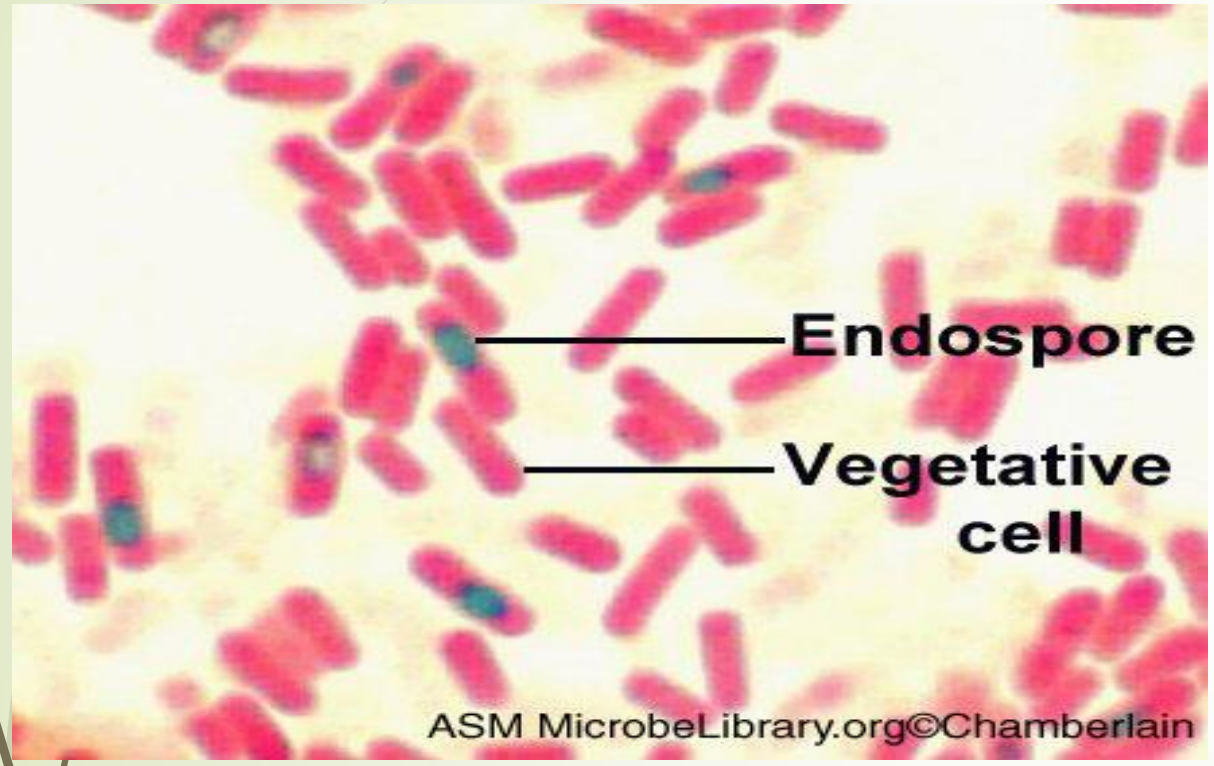


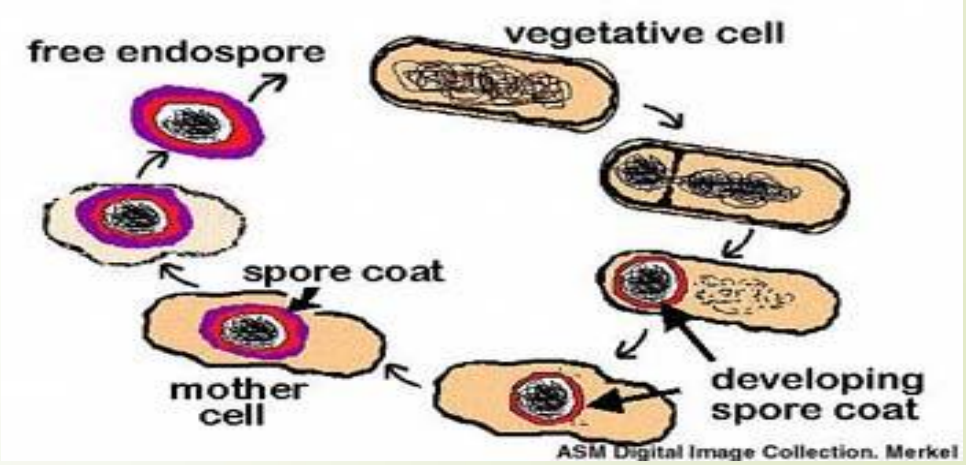
Rods: Comma form



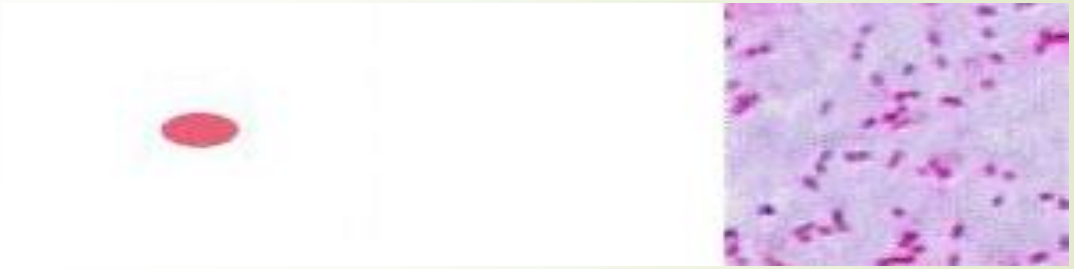
Vibrio

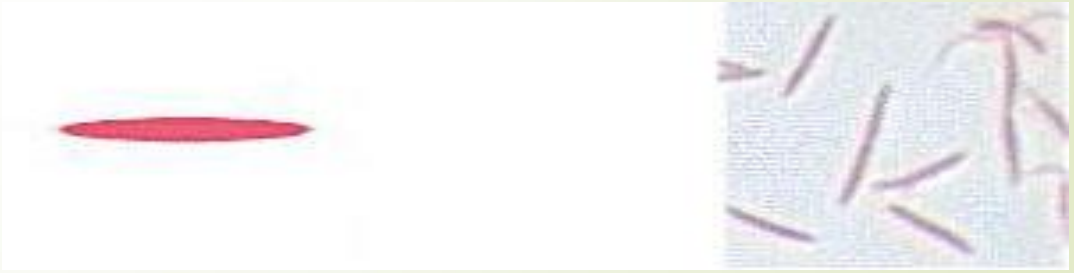
Rods: Spore Formers



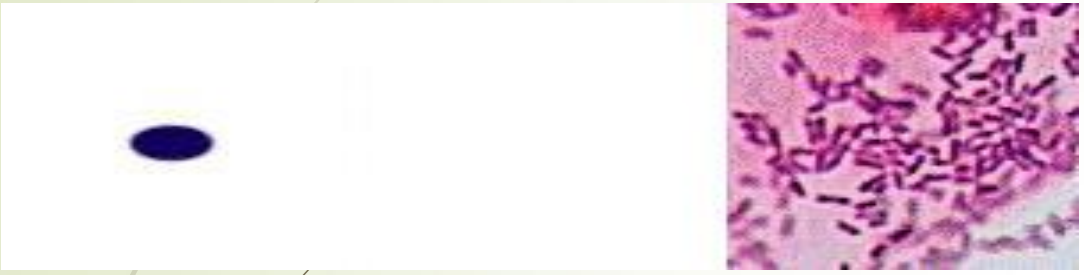


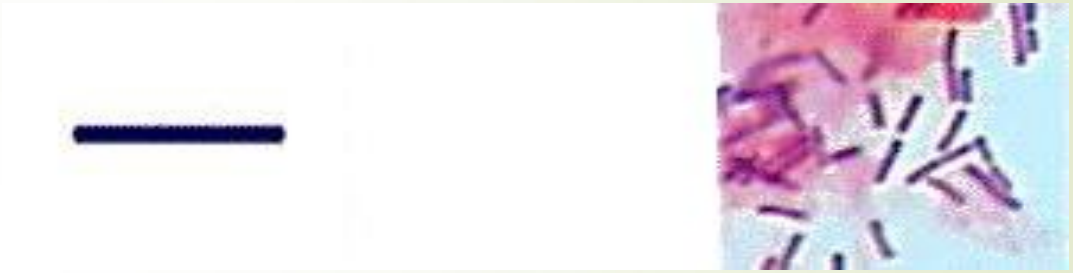
Gram Negative Rod Summary

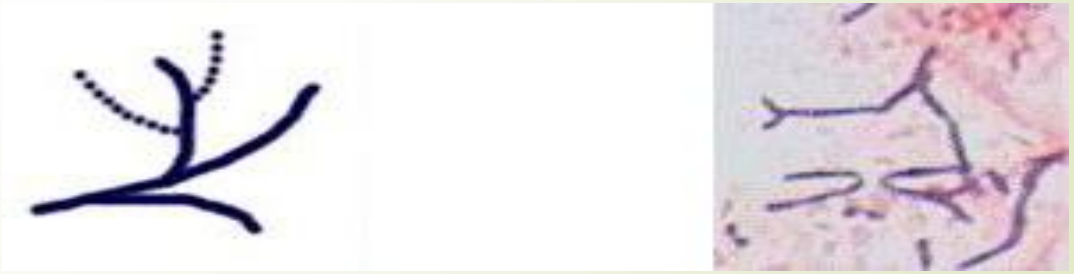




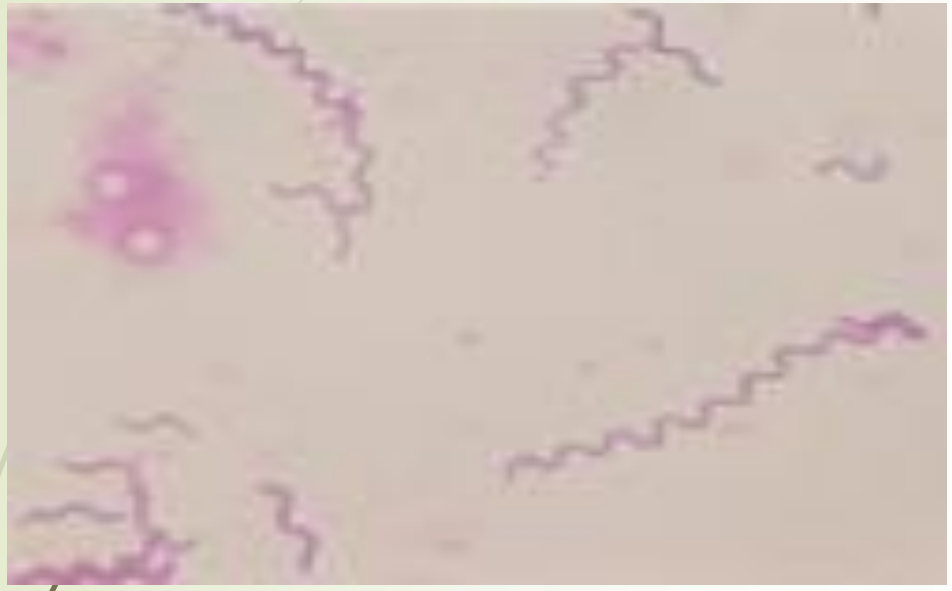
Gram Positive Rod Summary

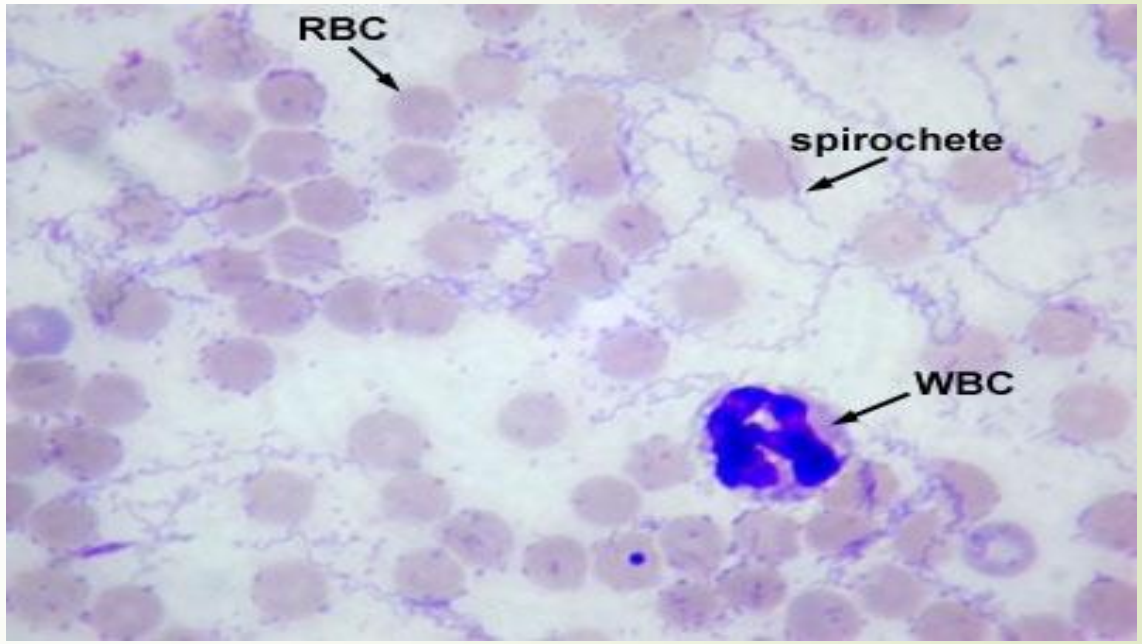




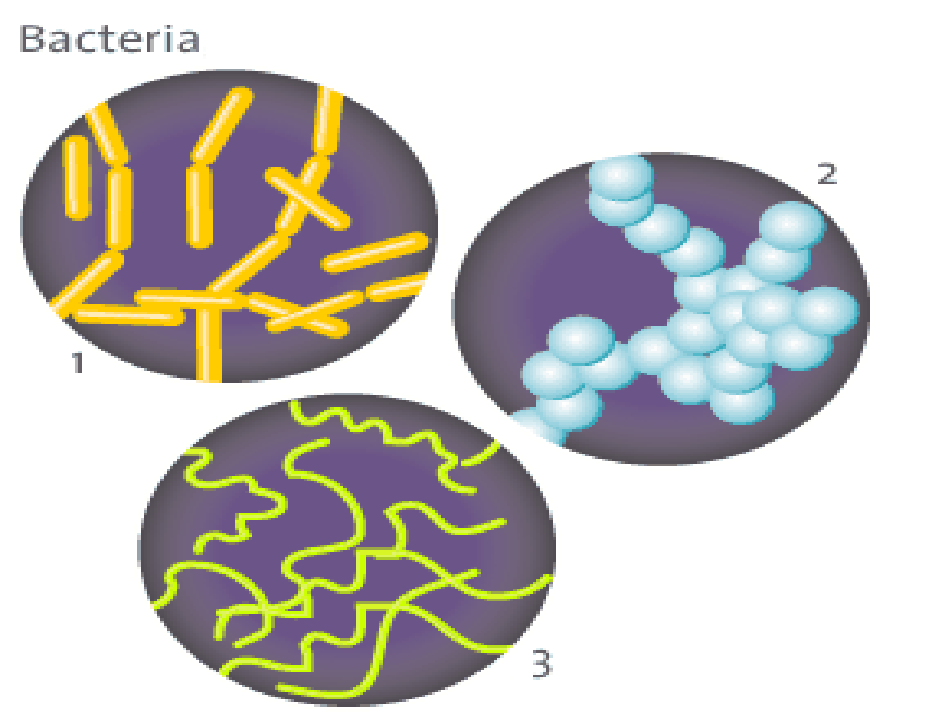


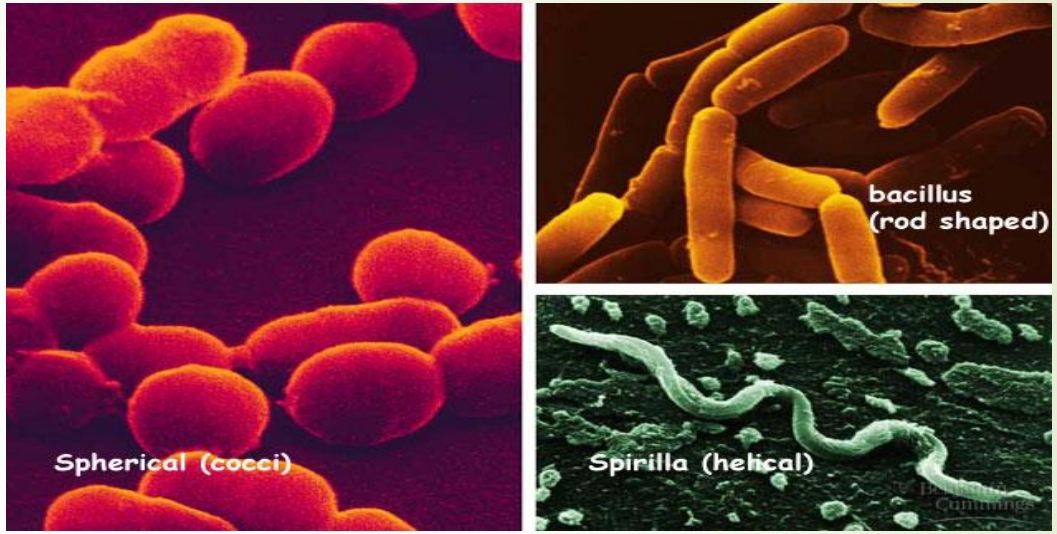
Spiral Forms

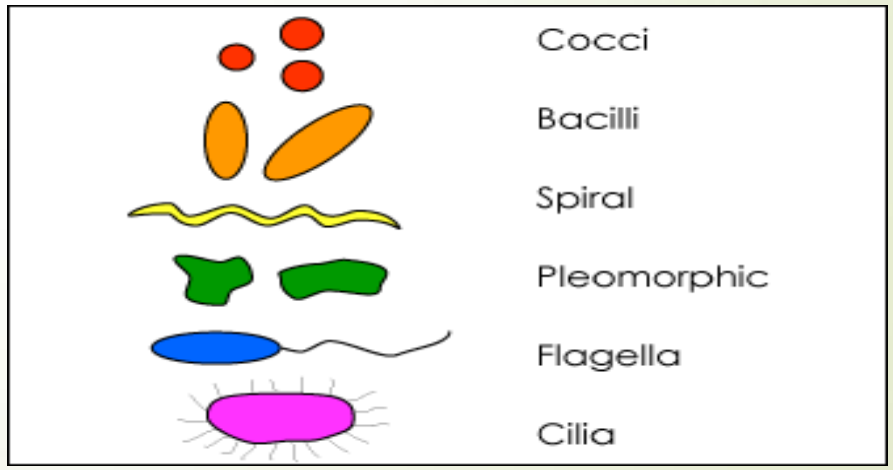




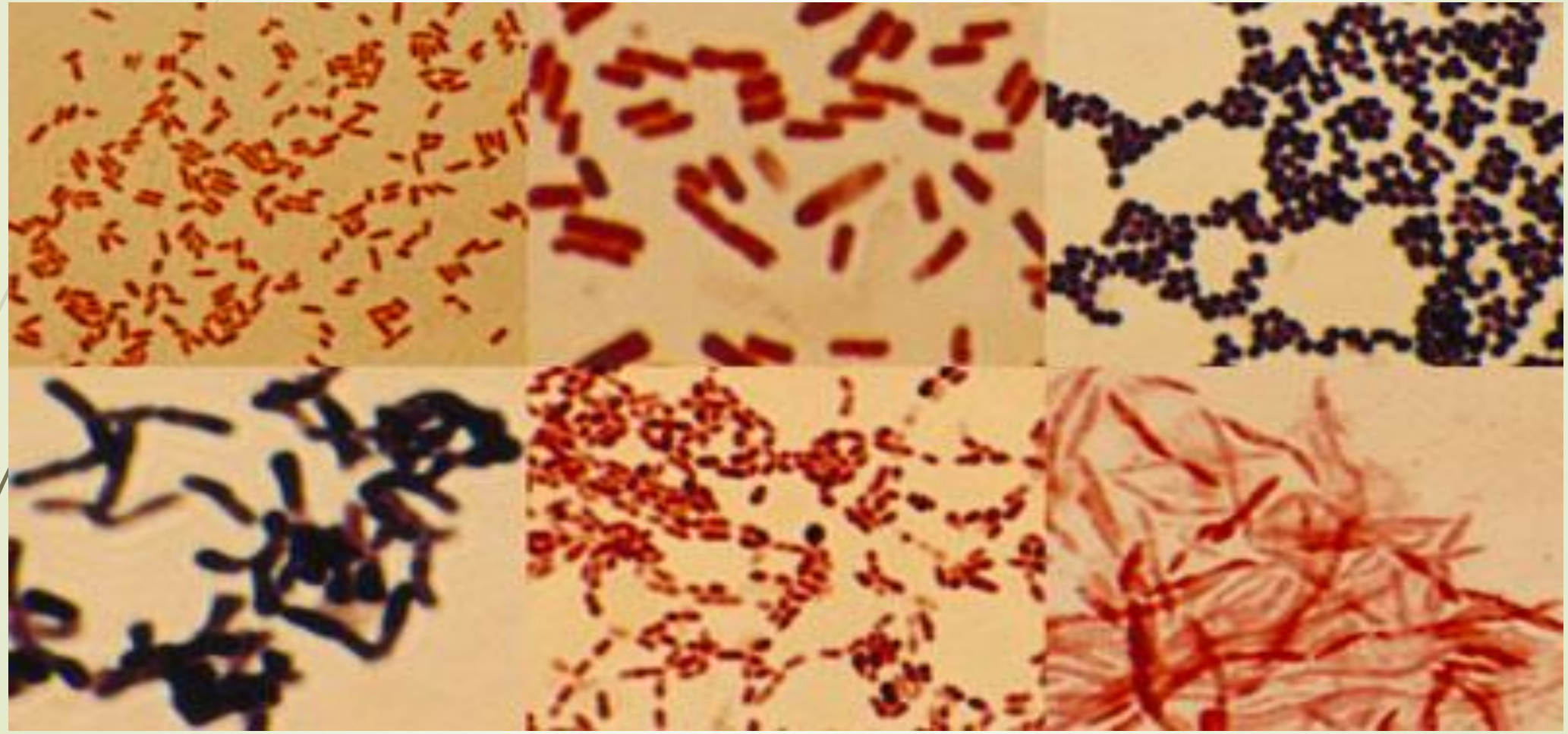
Bacterial Shapes Review



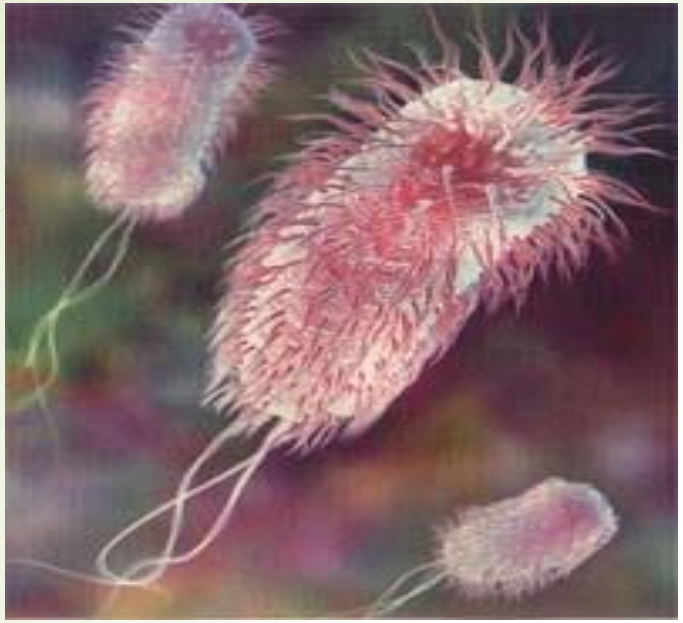


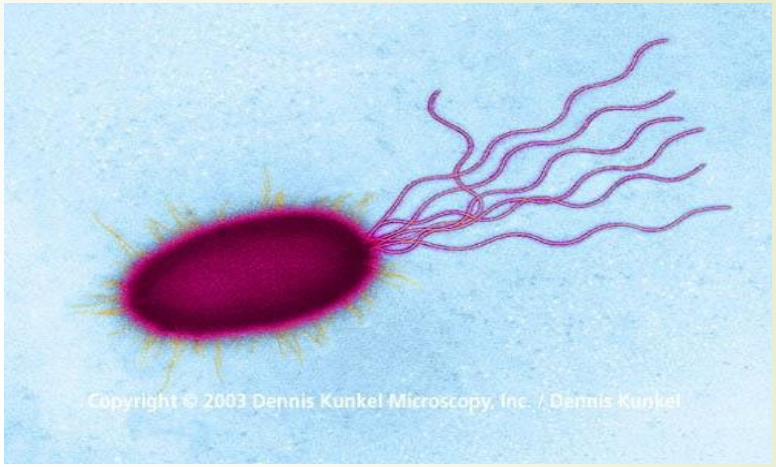


Bacterial Shapes Review



Motility

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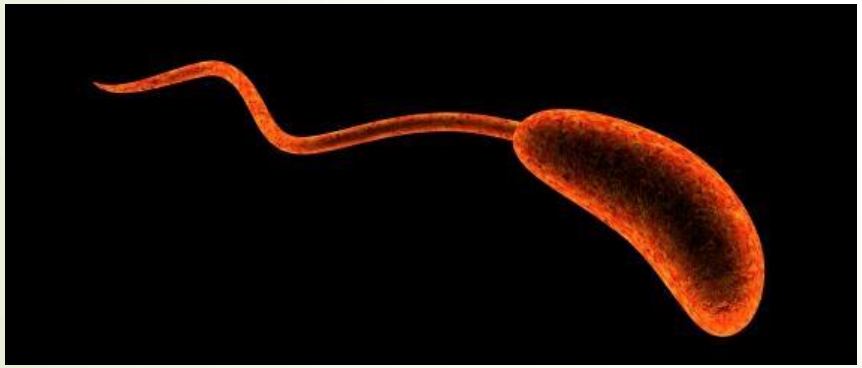

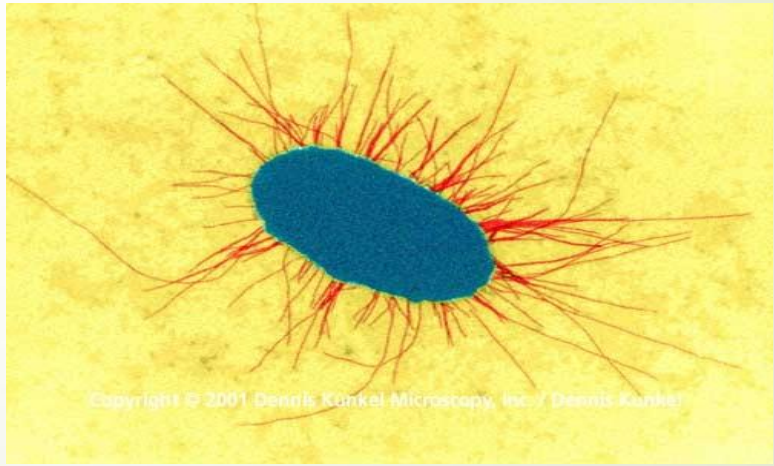
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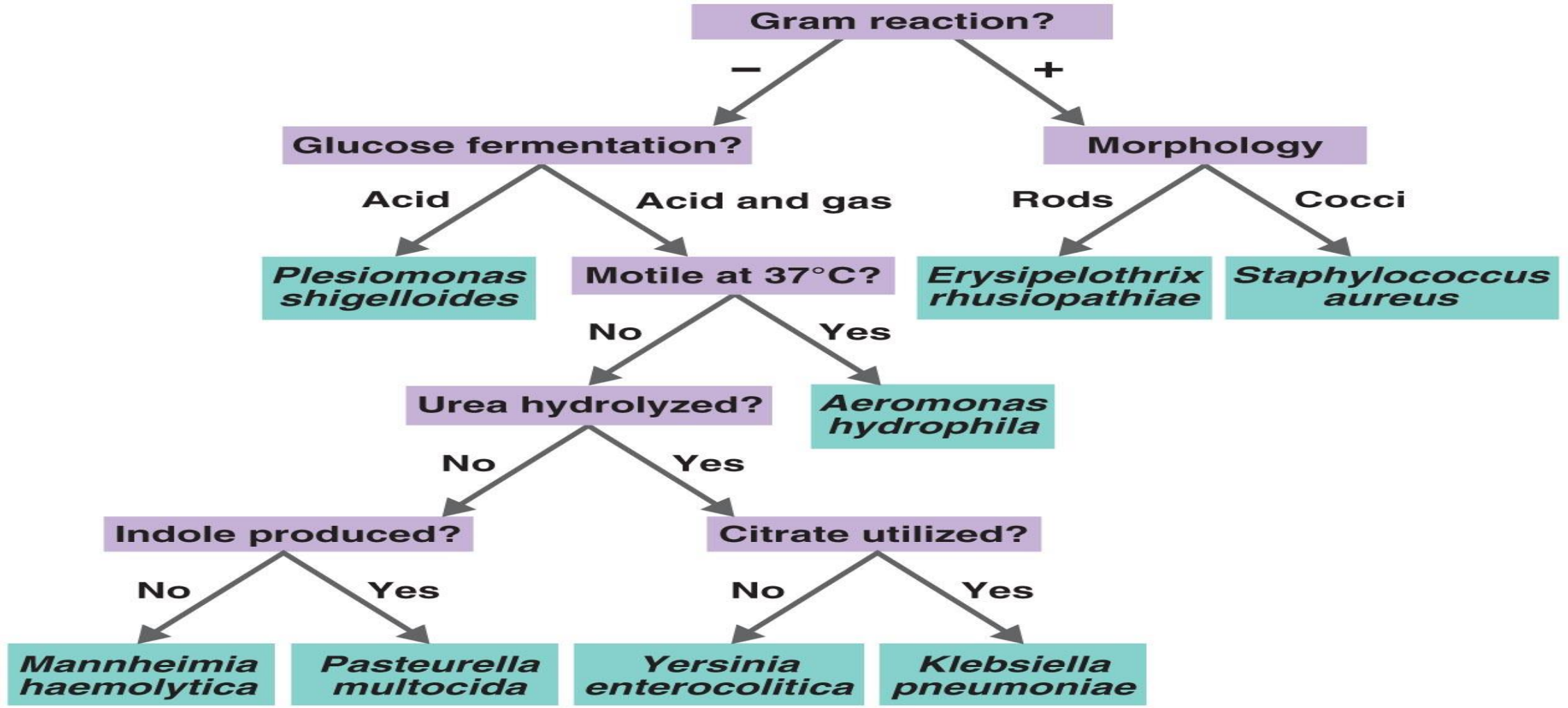
Identifying Bacteria

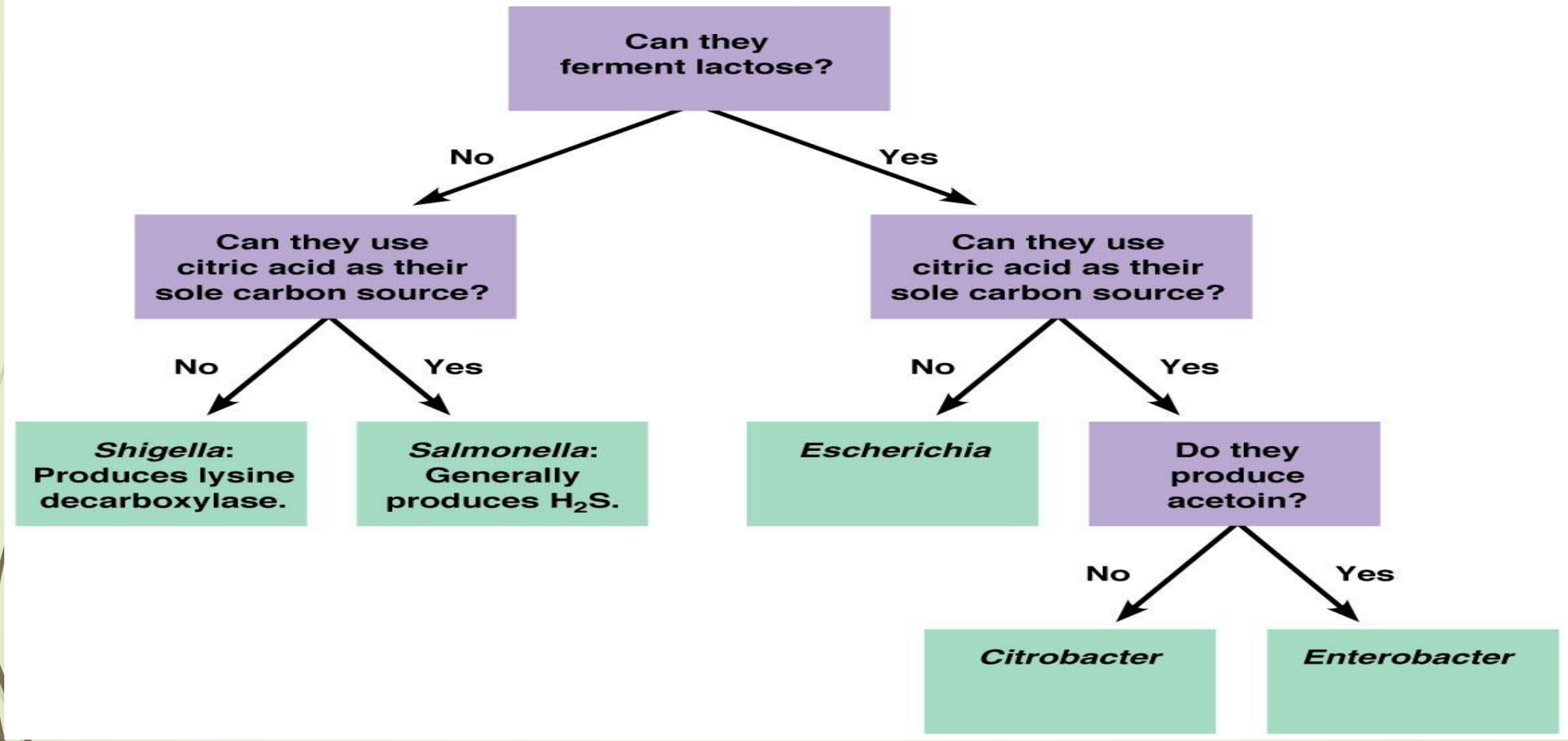


Classification and Identification

Classification: Placing organisms in groups of related species. Lists of characteristics of known organisms.

Identification: Matching characteristics of an "unknown" to lists of known organisms.

Classification and Identification



Bergey's Manual: Classifying and Identifying Prokaryotes

Bergey's Manual of Determinative Bacteriology Provides *identification* schemes for identifying bacteria and archaea	Morphology, differential staining, biochemical tests
Bergey's Manual of Systematic Bacteriology Provides *phylogenetic* information on bacteria and archaea	Based on rRNA sequencing

Eubacteria

- Prokaryotic
- Unicellular
- Shapes
- Vary in size
- Taxonomy: Bergey's Manual
  - Low G+C Gram Positive
  - High G+C Gram Positive
  - Gram Negative Proteobacteria
    - Nonpathogenic: environmental contributions
    - Pathogenic

Methods for Classifying and Identifying Bacteria

Criterion or Method	Used for	
	Classification	Identification
Morphological characteristics	No (yes for cyanobacteria)	Yes
Differential Staining	Yes (for cell wall type)	Yes
Biochemical Testing	No	Yes
Serology	No	Yes
Phage Typing	No	Yes
Fatty Acid Profiles	No	Yes
Flow Cytometry	No	Yes
DNA Base Composition	Yes	No
DNA Fingerprinting	Yes	Yes
PCR	Yes	Yes
Nucleic Acid Hybridization Techniques	Yes	Yes
rRNA Sequencing	Yes	No

Low G+C Gram Positive Bacteria

- The DNA of all living things is made up of four nucleotide bases Adenine (A), Cytosine (C), Guanine (G) and Thymidine (T). In a double helix of DNA, Adenine pairs with Thymidine and Guanine pairs with Cytosine. Therefore the number of Cytosine bases equals the number of Guanine bases and likewise A=T. The percentage of G+C is one of many general features used to characterize bacterial genomes

Low G+C Gram Positive Bacteria

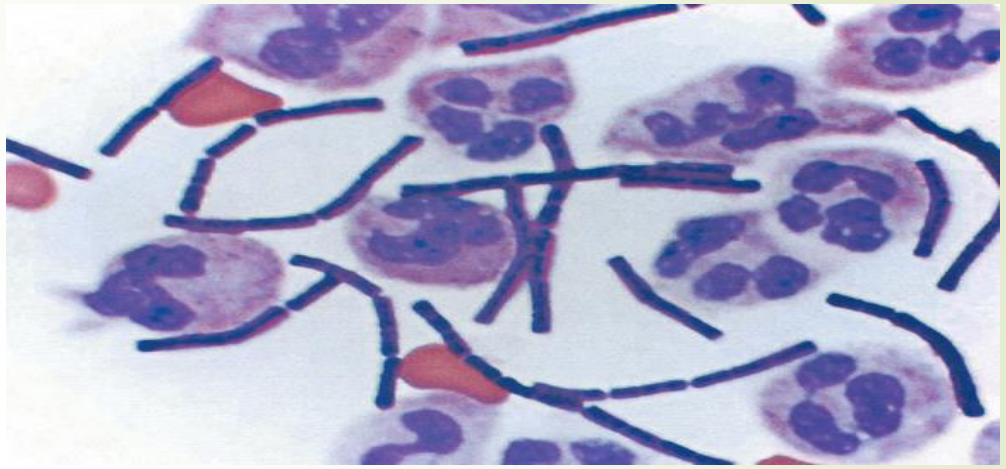
- This is an ecologically and industrially important group of microorganisms. The group name refers to a phylum of Bacteria, also known as the Firmicutes, which share a common evolutionary history. Many have certain distinct cellular characteristics. Gram-positive organisms stain purple with a differential staining procedure developed in 1884 by Christian Gram. This procedure identifies cells that have a thick cell wall of peptidoglycan. While many Firmicutes stain Gram-positive, some do not. In fact, some Firmicutes have no cell wall at all! They are called "low G+C" because their DNA typically has fewer G and C DNA bases than A and T bases as compared to other bacteria. Exceptions have been identified and some Firmicutes have G+C content as high as 55% (*Geobacillus thermocatenulatus*). Certain Firmicutes make resistant progeny called endospores, while others can only reproduce through binary fission. It is evident that Firmicutes are as diverse as they are important.
- The typical Firmicutes cell envelope consists of a layer of peptidoglycan, which is a polymer of protein and carbohydrate that gives structure and shape to the cell and protects the bacterium from osmotic stress (see Link 2). Underneath the peptidoglycan there is a phospholipid bilayer and its associated proteins that act as a selective barrier. Many members of the Firmicutes have an outermost envelope layer of protein called the S layer. The function of the S layer is not known but it is believed to prevent predation in the environment.

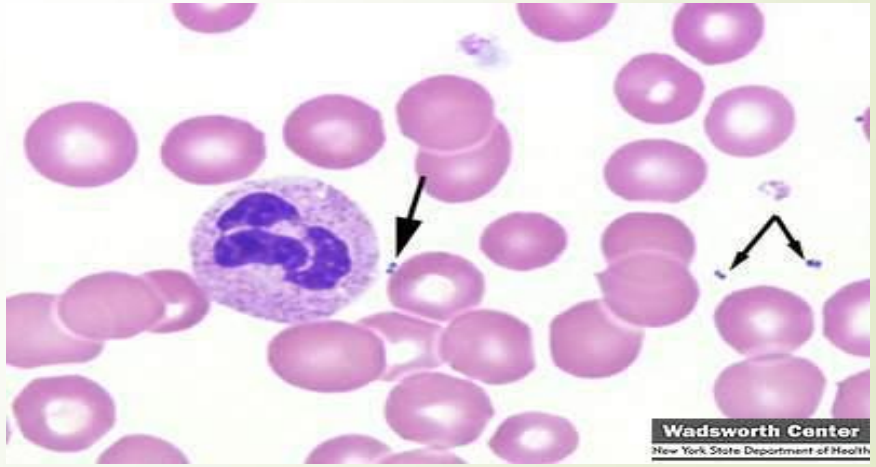
Gram Negative Proteobacteria

- The **Proteobacteria** are a major group (phylum) of gram-negative bacteria. They include a wide variety of pathogens, such as*Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, and *Yersinia*, and many other notable genera.[2] Others are free-living (nonparasitic), and include many of the bacteria responsible for nitrogen fixation.
- Carl Woese established this grouping in 1987, calling it informally the "purple bacteria and their relatives".[3] Because of the great diversity of forms found in this group, the Proteobacteria are named after Proteus, a Greek god of the sea capable of assuming many different shapes; it is not named after the genus *Proteus*.

Low G+C Gram Positive Organisms

- Rods
  - Clostridia
  - Mycoplasmas
  - Bacillus
  - Listeria
  - Lactobacillus
- Cocci
  - Streptococcus
  - Enterococcus
  - Staphylococcus

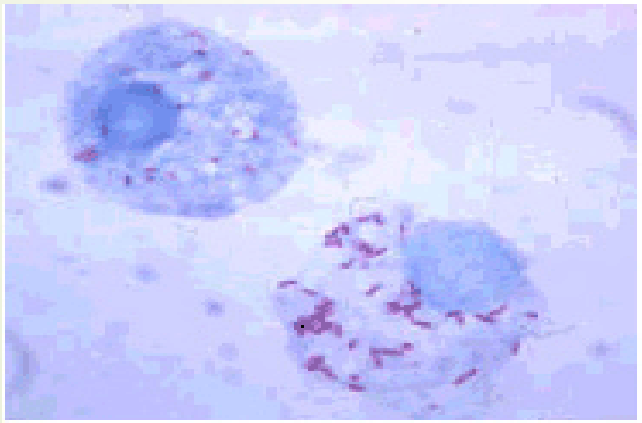




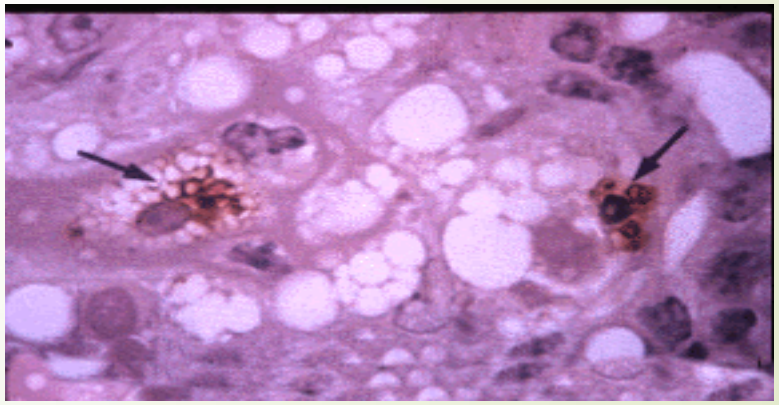
Gram Negative Alpha Proteobacteria

- Pathogenic
  - Rickettsia
  - Brucella
  - Ehrlichia

Ehrlichia

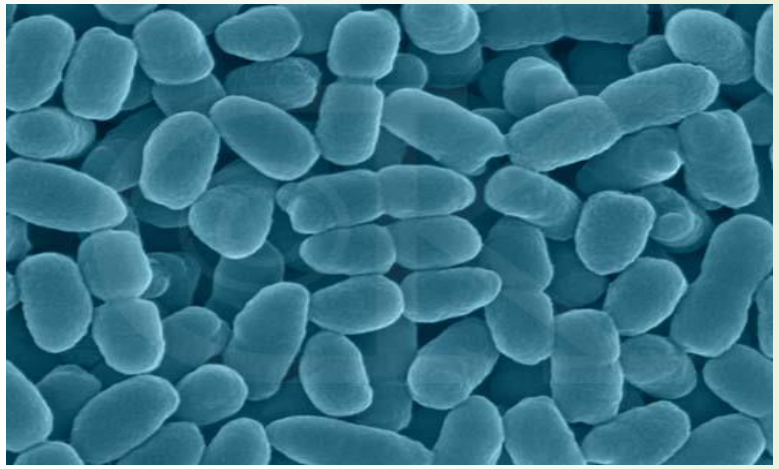


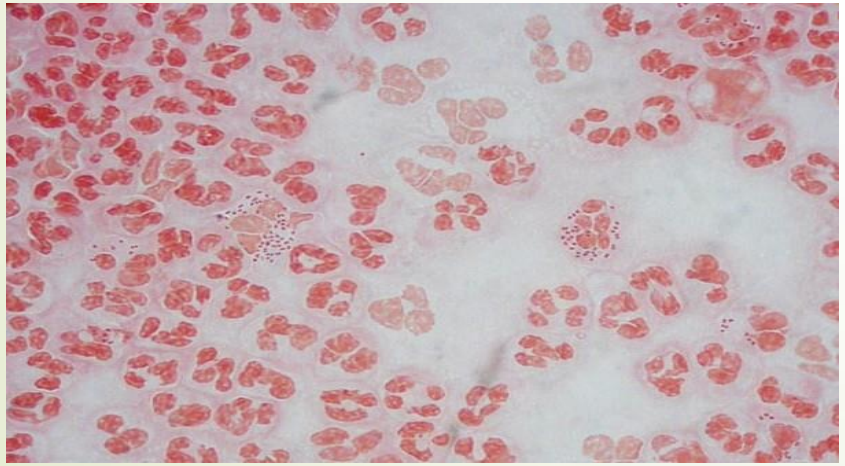
Gimenez stain of tick hemolymph cells infected with *R. rickettsii* CDC



Gram Negative Beta Proteobacteria

- Pathogenic
  - Neisseria
  - Bordetella
  - Spirillum
  - Burkholderia





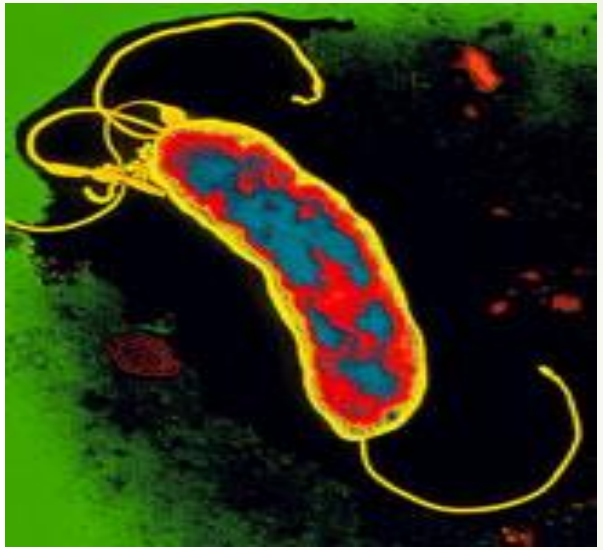
Gram Negative Gamma Proteobacteria

- Pathogenic
  - Legionella
  - Pseudomonads
  - Coxiella
  - Enterobacteriaceae
    - E. coli
    - Salmonella
    - Shigella
    - Proteus
    - Yersinia
    - Enterobacter
    - Serratia

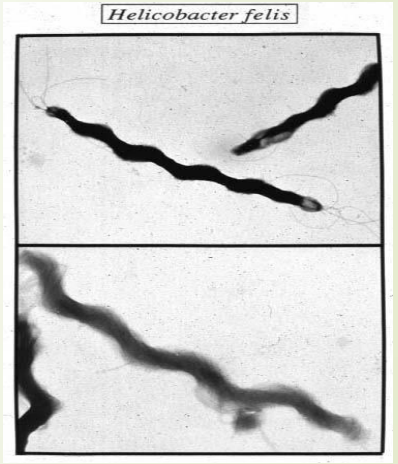
Pseudomonas

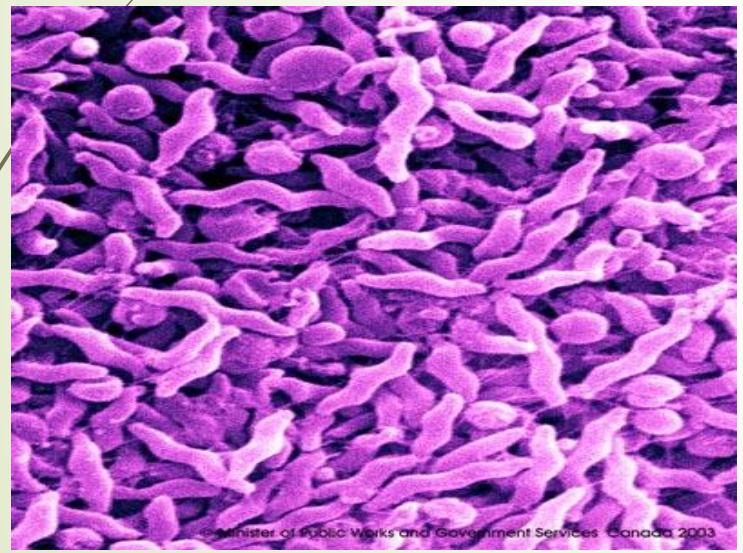
Gram Negative Epsilon Proteobacteria

- Pathogenic
  - Campylobacter
  - Helicobacter



Helicobacter



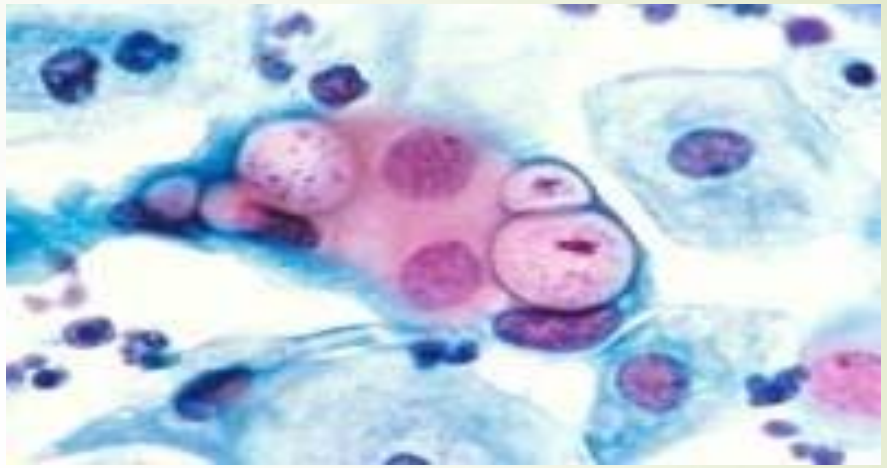


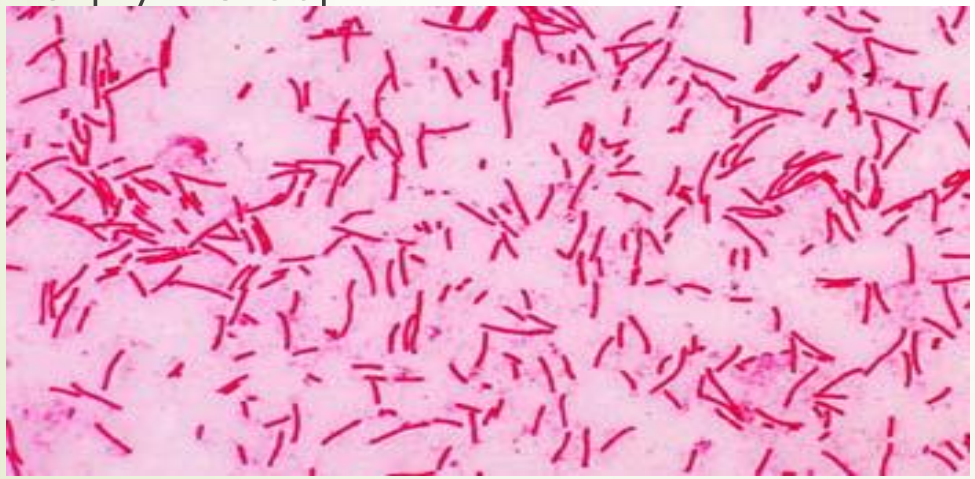
Campylobacter

Other Bacteria

- Pathogenic
  - Chlamydia
  - Spirochetes
    - Treponema [syphilis]
    - Borrelia [Lyme ds]

Chlamydia





Borrelia

Treponema

Microbes and the Immune System

Clinical Lab Identification

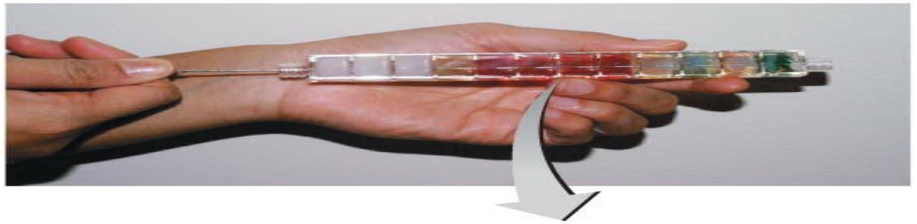
Morphological characteristics Useful for identifying eukaryotes

Differential staining Gram staining, acid-fast staining

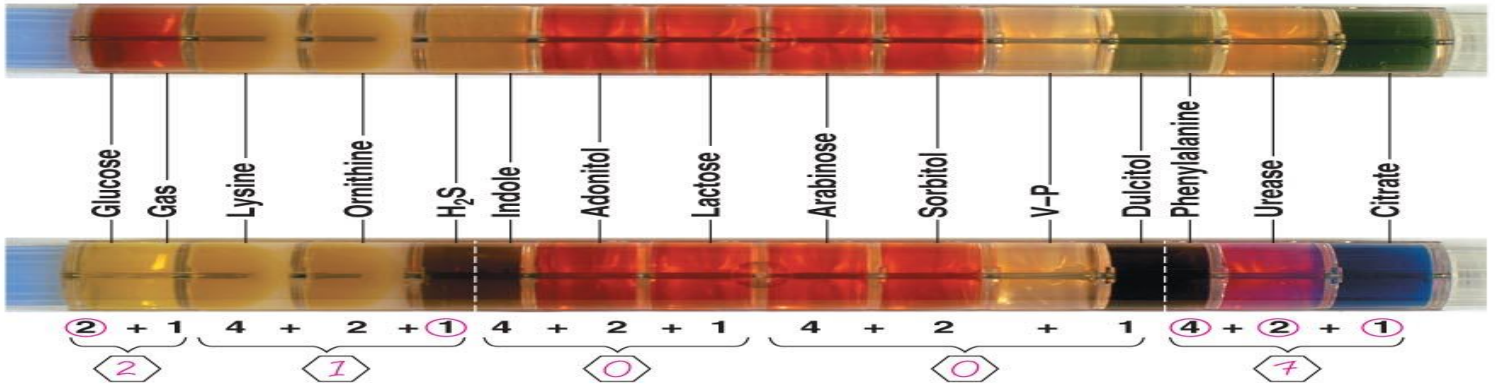
Biochemical tests Determines presence of bacterial enzymes

Numerical Rapid Identification

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	*Proteus mirabilis*	Ornithine⁻	Sucrose
21007	*Proteus mirabilis*	Ornithine⁻	
21020	*Salmonella choleraesuis*	Lysine⁻	

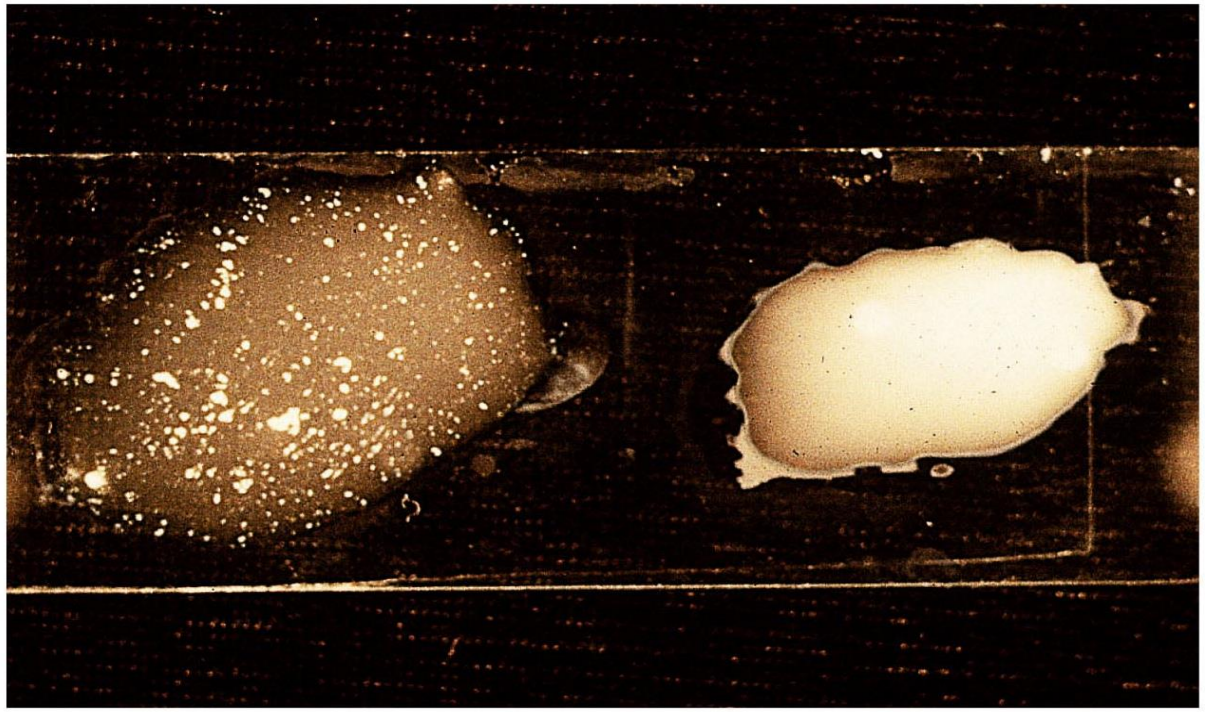
Serology

- Involves reactions of microorganisms with specific antibodies: *Combine known anti-serum with unknown bacterium*
- Useful in determining the identity of strains and species, as well as relationships among organisms.

Examples:

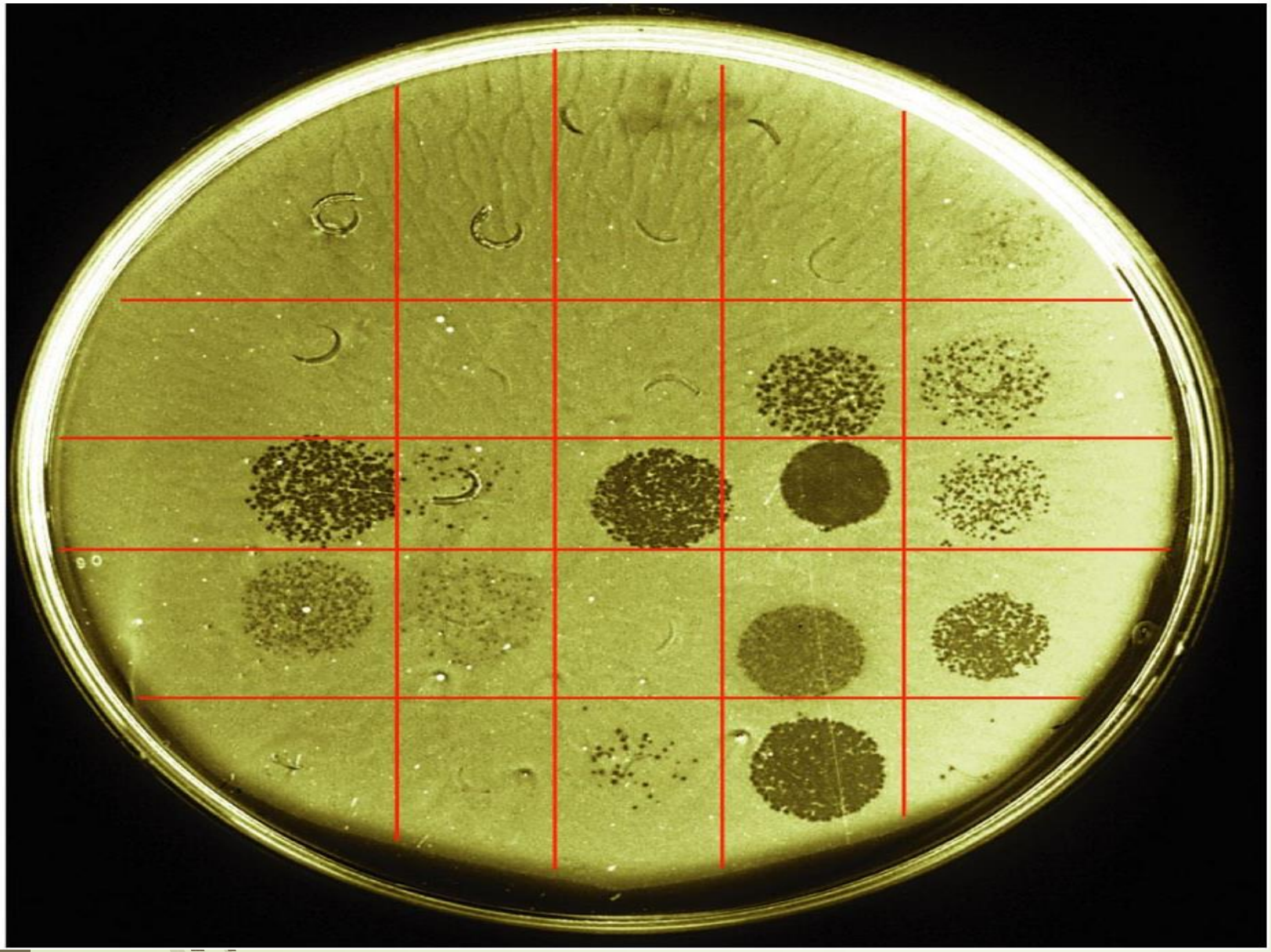
- Slide agglutination
- ELISA (see lab)
- Western blot (no details)

: Slide Agglutination



(a) Positive test **(b)** Negative test

Phage Typing

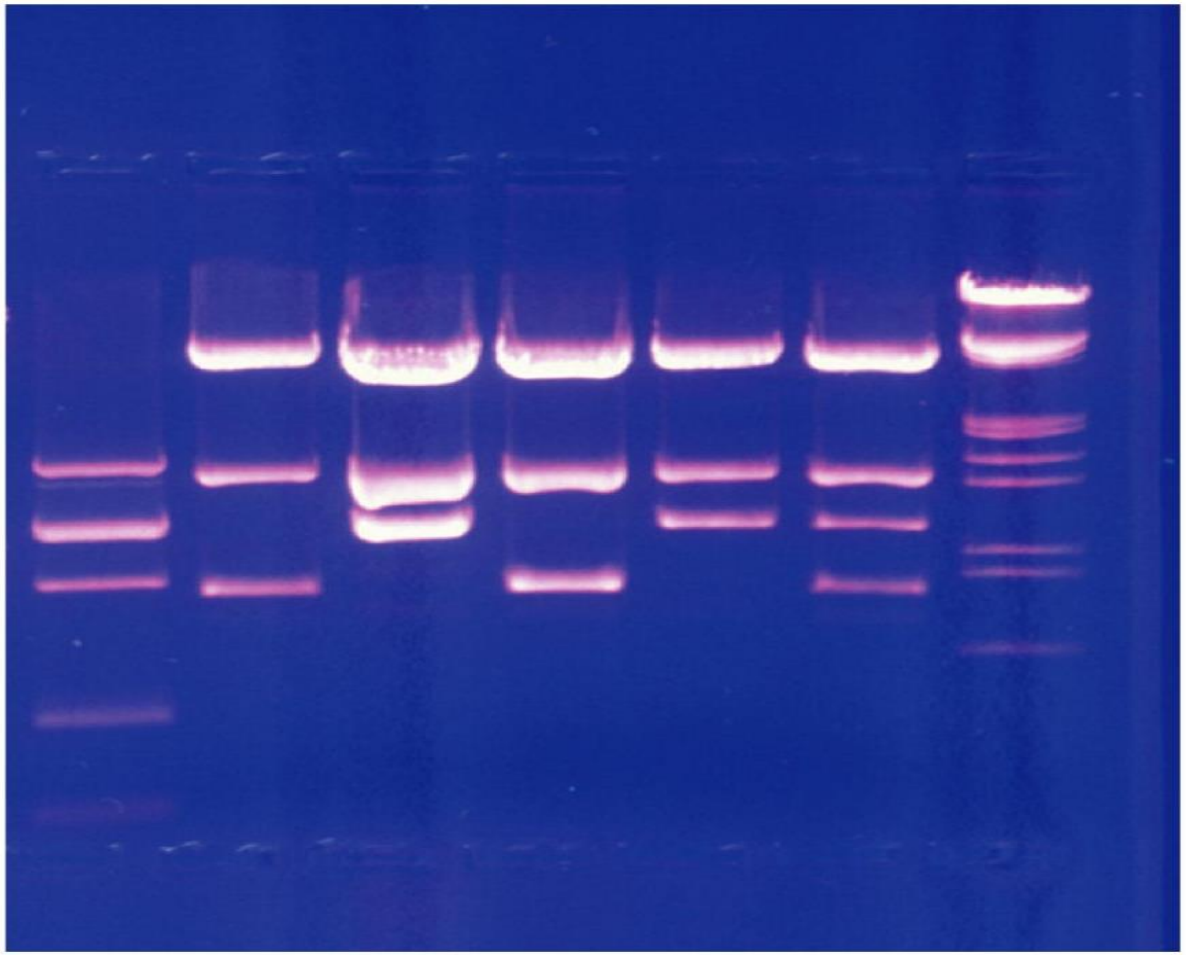


Identification of bacterial species and strains by determining their susceptibility to various phages.

More details on bacteriophages in Ch 13

Genetics

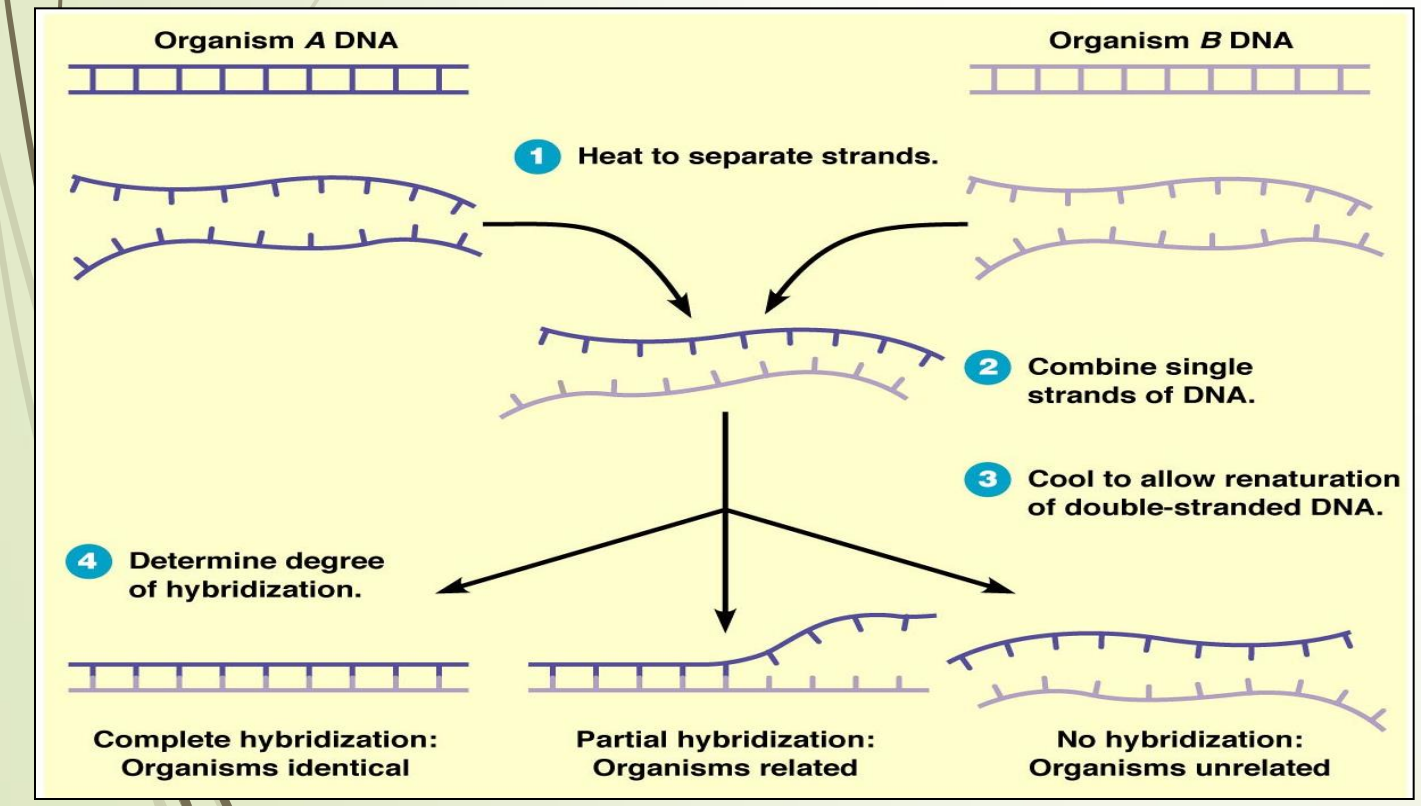
- **DNA fingerprinting:** Number and sizes of DNA fragments (fingerprints) produced by RE digests are used to determine genetic similarities.
- Ribotyping: rRNA sequencing
- Polymerase chain reaction (PCR) can be used to amplify a small amount of microbial DNA in a sample. The presence or identification of an organism is indicated by amplified DNA. *(see lab)*



Electrophoresis of RE digest of plasmid DNA

Nucleic Acid Hybridization

Single strands of DNA or RNA, from related organisms will hydrogen-bond to form a double-stranded molecule; this bonding is called nucleic acid hybridization.



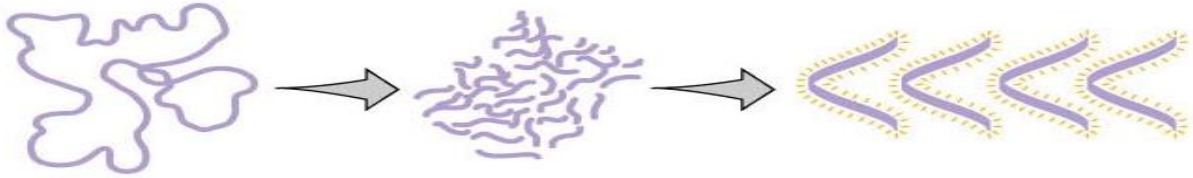
Examples of Applications:

- Southern blotting,
- DNA chips, and
- FISH

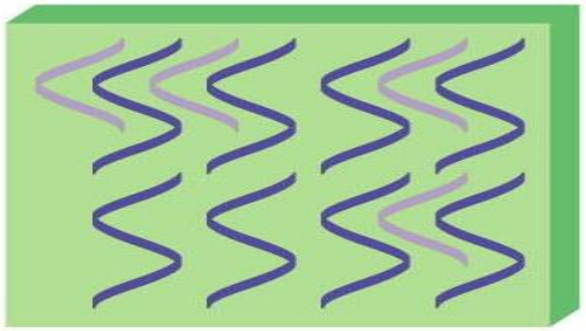
Nucleic Acid Hybridization: DNA Chip

Fig 10.17

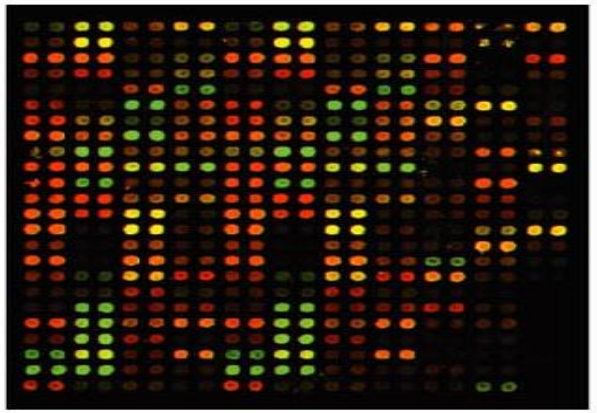
(a) A DNA chip can be manufactured to contain hundreds of thousands of synthetic single-stranded DNA sequences. Assume that each DNA sequence was unique to a different bacterial species.



(b) Unknown DNA from a patient is separated into single strands, enzymatically cut, and labeled with a fluorescent dye.



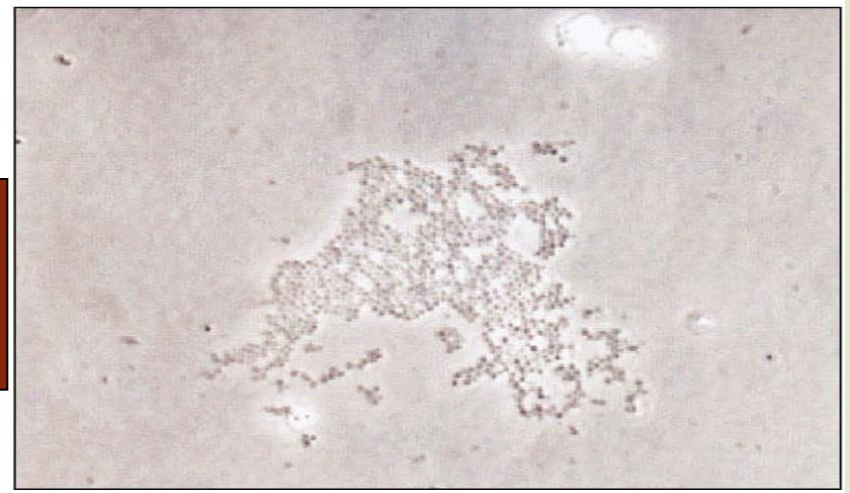
(c) The unknown DNA is inserted into the chip and allowed to hybridize with the DNA on the chip.



(d) The tagged DNA will bind only to the complementary DNA on the chip. The bound DNA will be detected by its fluorescent dye and analyzed by a computer. The red light is a gene expressed in normal cells; green is a mutated gene expressed in tumor cells; and yellow, in both cells.

Fluorescent In Situ Hybridization (FISH)

Add DNA or RNA probe attached to fluorescent dye
for S. aureus



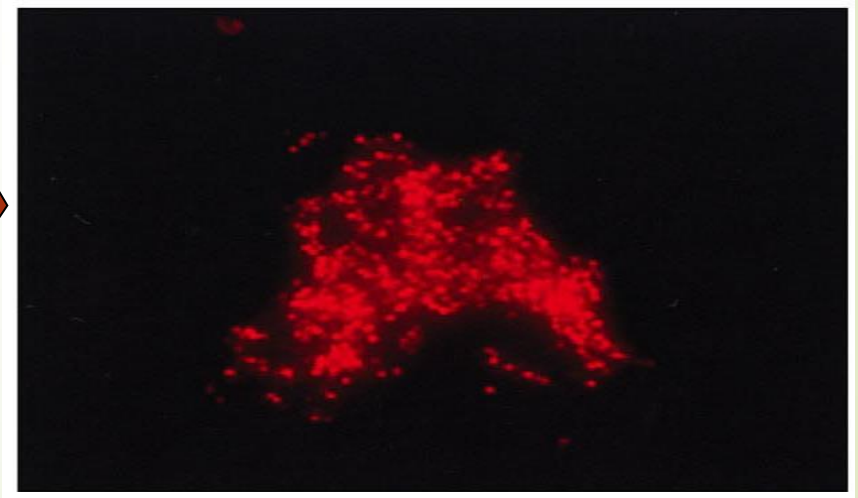


Fig 10.18a–b