CLASSIFICATION OF BACTERIA

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Objectives of today's class

- To describe the classification of bacteria

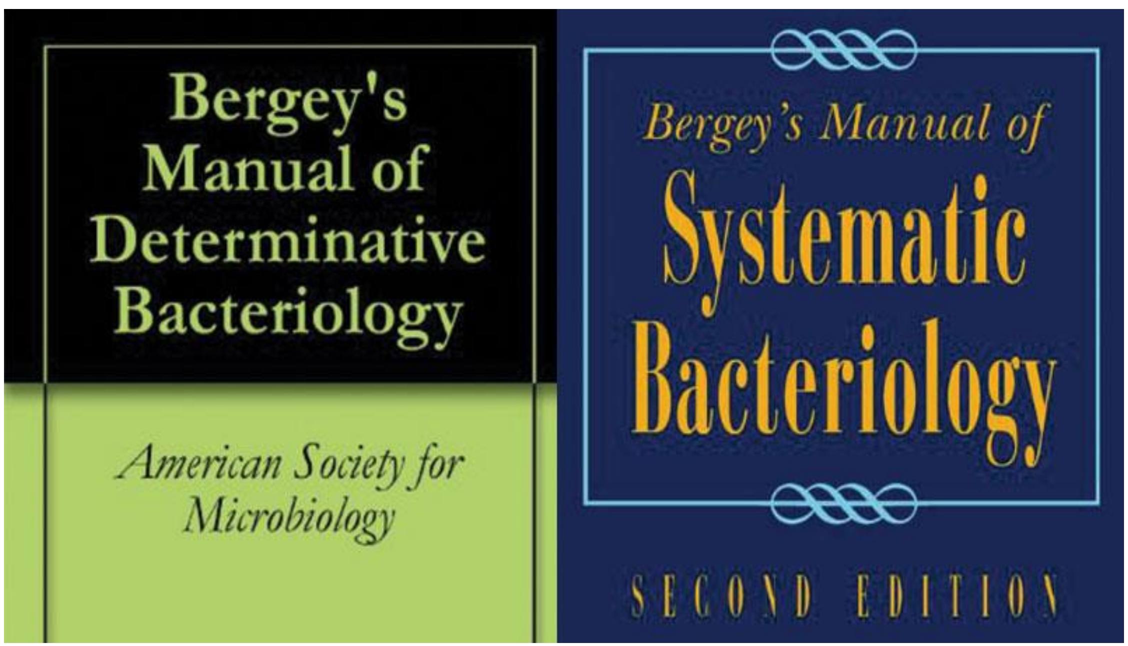
&

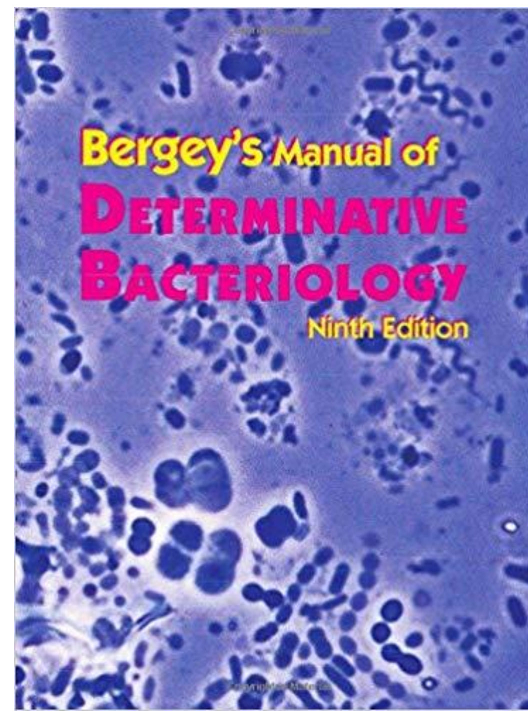
- To describe the bacterial properties used in classification

Key Points

Why do we need bacterial classification?

How do we classify the bacteria?





Time span	Classification is mainly based on
Late 19th century	Morphology, Growth requirements, Pathogenic potential
1900-1960	Morphology, Physiology, Biochemistry
1960-1980	Chemotaxonomy, Numerical taxonomy, DNA–DNA Hybridization
1980–today	Genotypic Analyses, Multi-Locus Sequence Analyses, Average Nucleotide Identity, Whole Genome Analysis

Basis of classification

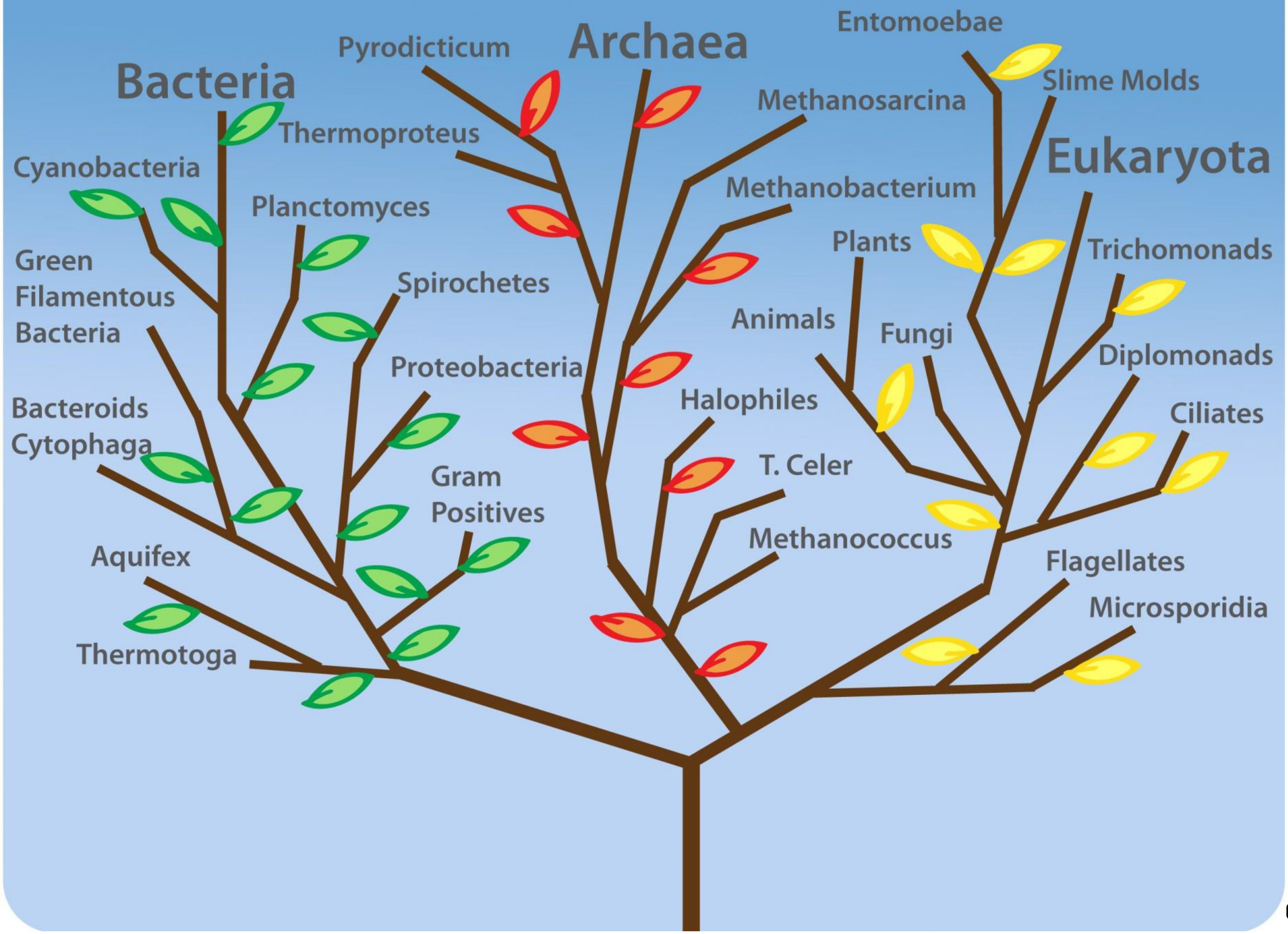
Phenotypic classification

- Morphological
- Anatomical
- Staining
- Cultural characteristics
- Nutrition / Metabolism
- Environmental factors
- Biochemical reactions
- Antigenic structure etc.

Genotypic classification

- Ribosomal RNA (rRNA) sequence analysis
- Universal phylogenetic tree
- DNA-DNA hybridization
- G+C content

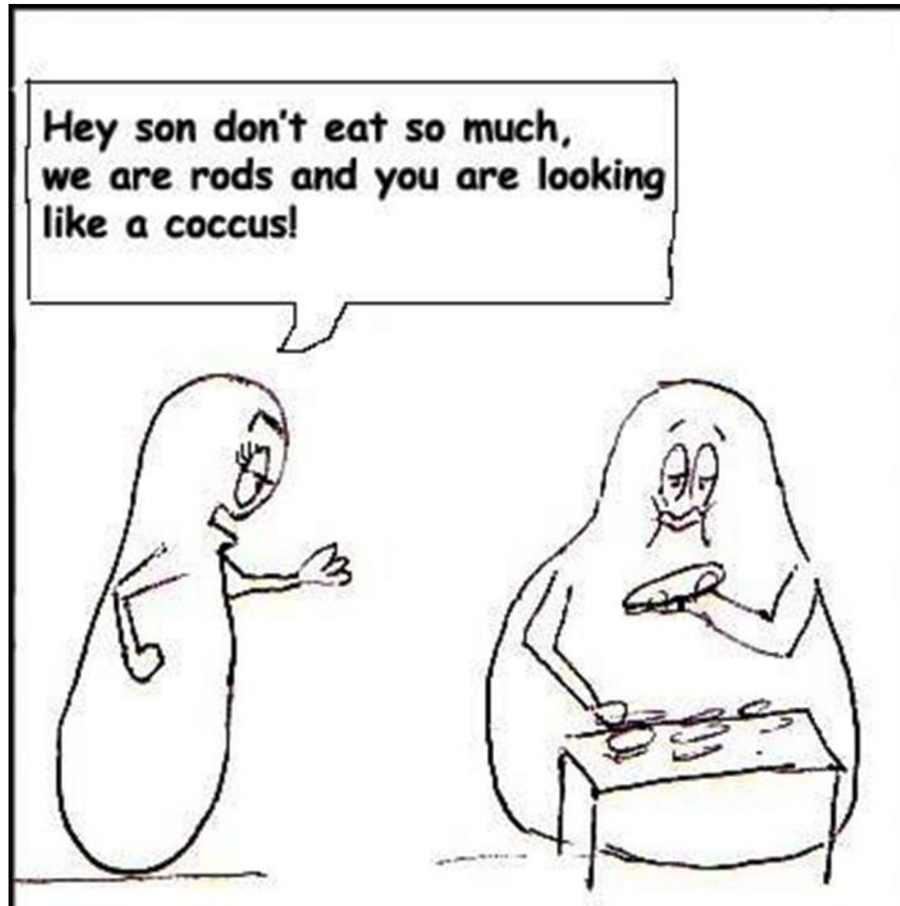
Phylogenetic Tree



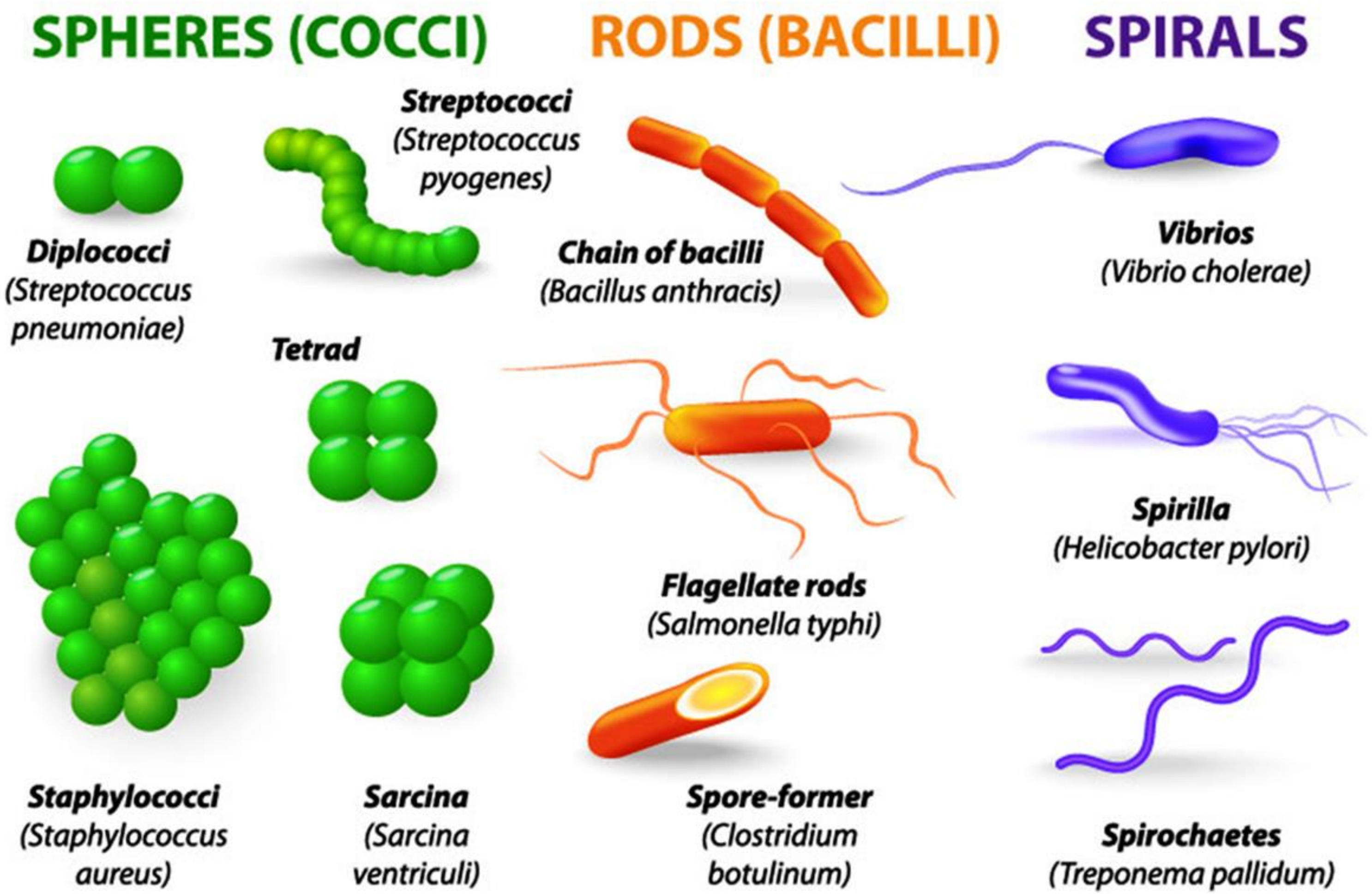
Phenotypic classification
Morphological

Bacteria can be classified into several groups on morphological basis:

1. Cocci
2. Bacilli
3. Spirals
    1. Vibrio
    2. Spirilla
    3. Spirochaetes
4. Actinomycetes
5. Mycoplasmas
6. Rickettsiae and
7. Chlamydiae



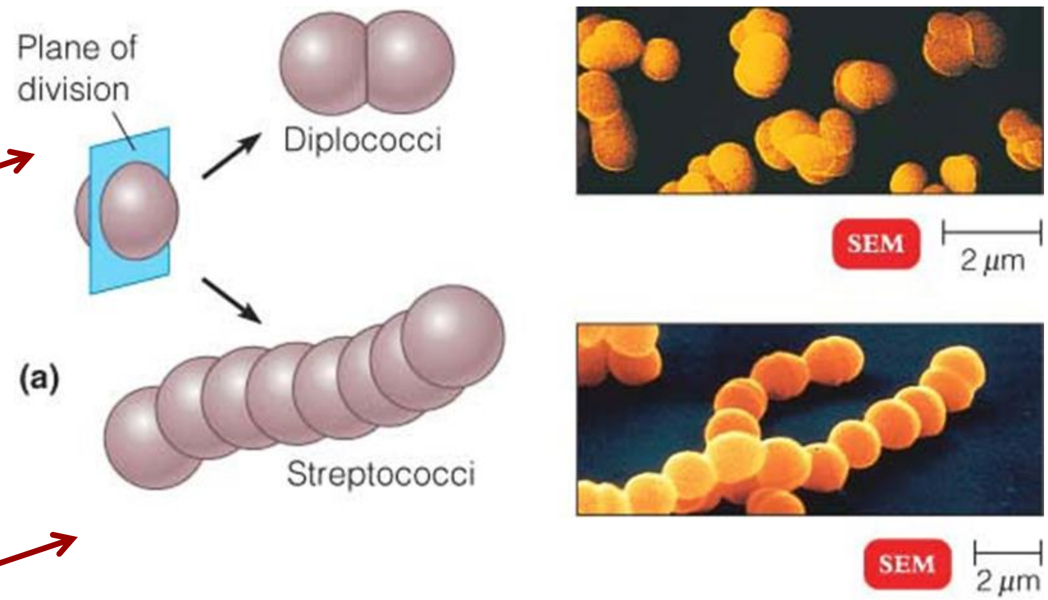
Shapes of Bacteria



Morphological classification
COCCI

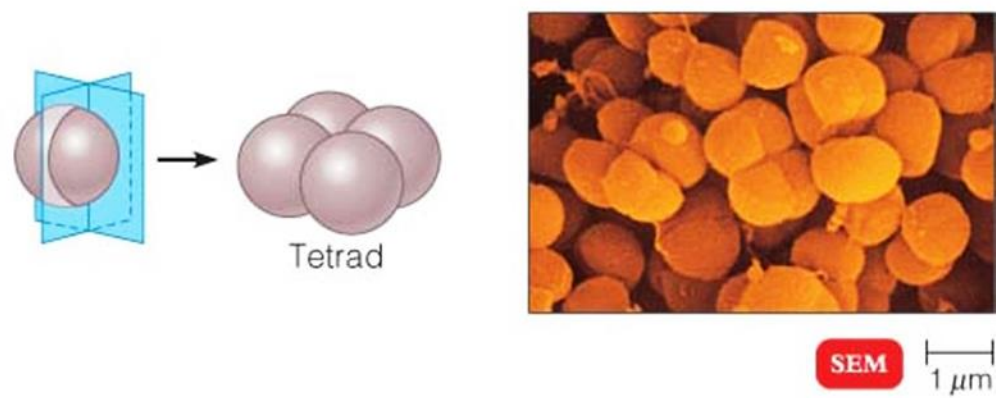
- These are spherical or oval cells. Based on the arrangement of individual organisms they can be described as:
  - **Monococci** (Cocci in singles) – *Monococcus* spp.
  - **Diplococci** (Cocci in pairs) – *Streptococcus pneumoniae*
  - **Staphylococci** (Cocci in grape-like clusters) – *Staphylococcus aureus*
  - **Streptococci** (Cocci in chains) – *Streptococcus pyogenes*
  - **Tetrad** (Cocci in group of four) - *Micrococcus* spp.
  - **Sarcina** (Cocci in group of eight)

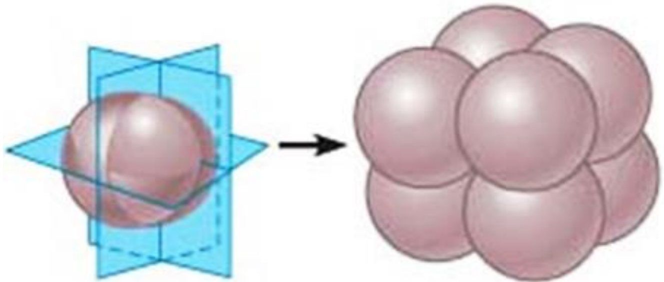
Streptococcus pneumoniae,
Moraxella catarrhalis,
Neisseria gonorrhoeae



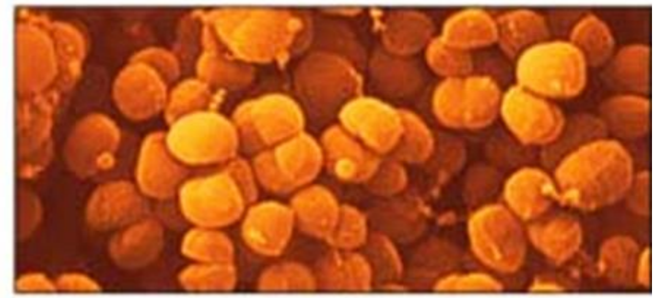
Streptococcus pyogenes,
Streptococcus agalactiae

Aerococcus,
Pediococcus,
Tetragenococcus



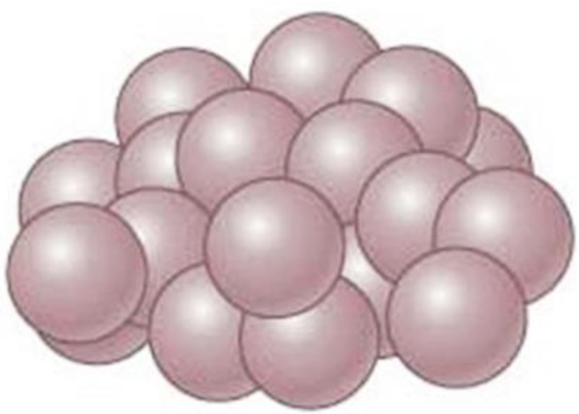


Sarcinae



SEM 2 μm

Sarcina ventriculi,
Sarcina ureae



Staphylococci

SEM 2 μm

Staphylococcus aureus

Morphological classification
BACILLI

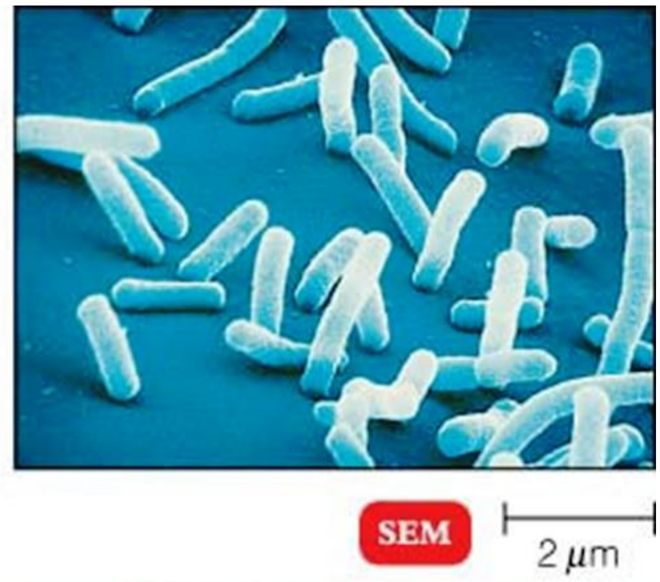
- These are rod-shaped bacteria. Based on the arrangement of organisms, they can be described as:
  - **Diplobacilli**
  - **Streptobacilli**
  - **Palisades**
    - **Chinese-letter form**
  - **Coccobacilli**

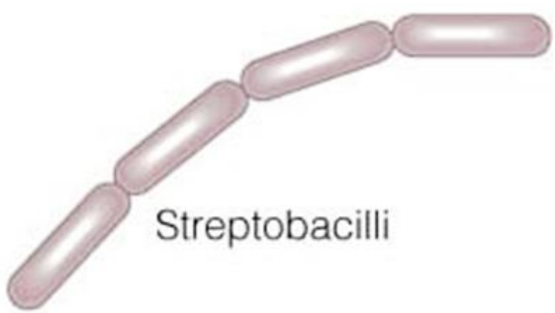
Bacillus cereus
Escherichia coli

Single bacillus

Diplobacilli

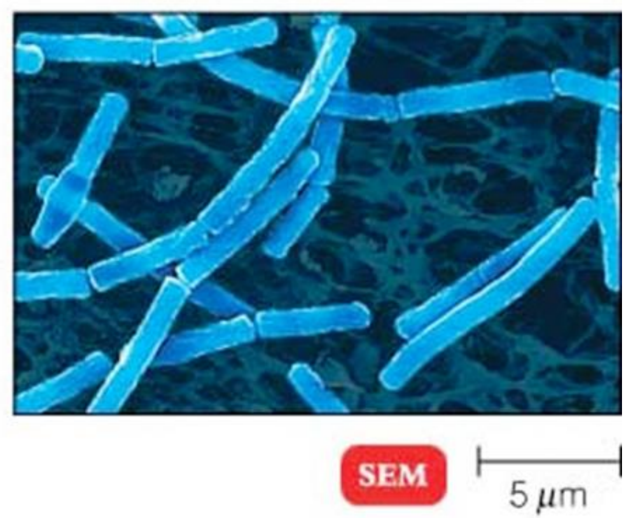
Coxiella burnetii,
Moraxella bovis,
Klebsiella rhinoscleromatis





Streptobacilli

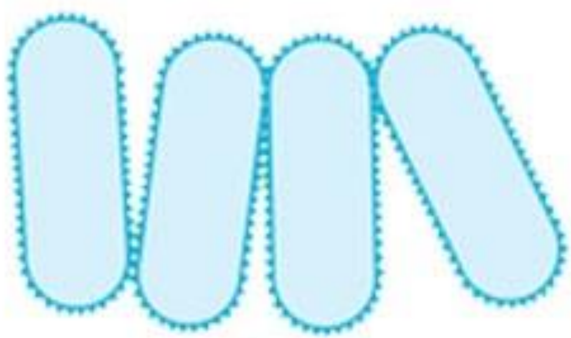
Streptobacillus moniliformis



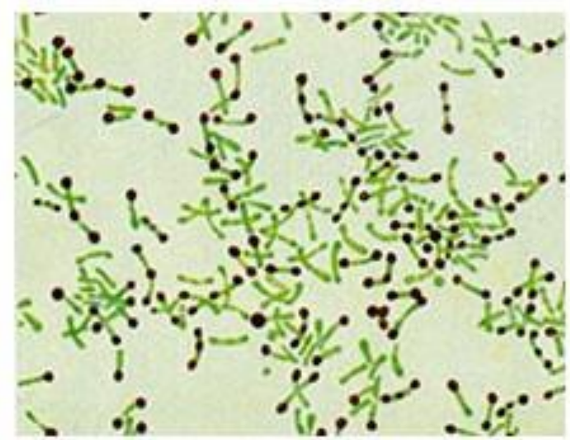
Haemophilus influenzae,
Gardnerella vaginalis,
Chlamydia trachomatis

Coccobacillus

Corynebacterium diphtheriae

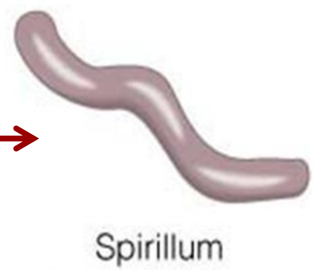


Palisades

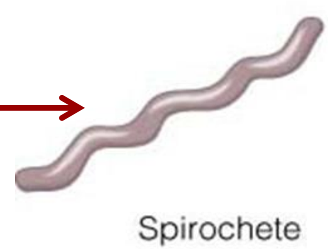


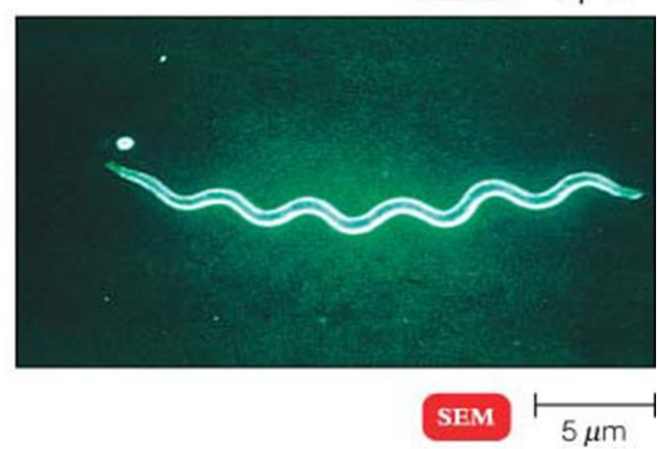
Vibrio chlorae

Helicobacter pylori



Treponema pallidum





Morphological classification

ACTINOMYCETES

- These are rigid organisms like cocci and bacilli
- The characteristic shape is due to the presence of rigid cell wall..
- They are branching filamentous bacteria.

Morphological classification

Mycoplasmas

- These bacteria lack in rigid cell wall (cell wall lacking) and are highly pleomorphic and of indefinite shape..
- They occur in round or oval bodies and in interlacing filaments..

Rickettsiae and Chlamydiae

- These are very small, obligate parasites, and at one time were considered closely related to the viruses.. Now, these are regarded as bacteria..

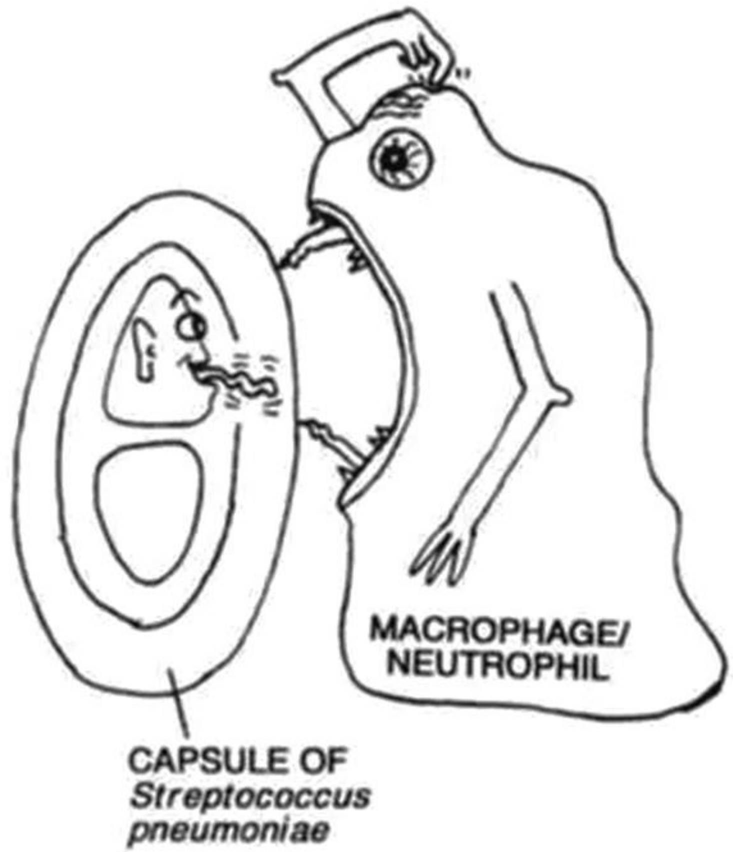
Based on anatomical features

- Capsule
  - **Capsulate**– *Streptococcus pneumoniae*
  - **Non-capsulate** – Viridans streptococci
- Flagella
  - **Flagellate** –
    - **Monotrichous**
    - **Lophotrichous**
    - **Amphitrichous**
    - **Peritrichous**
  - **Aflagellate** – *Shigella* spp.
- Spore
  - **Spore-forming** – *Bacillus* spp.
  - **Non-sporing** – *Escherichia coli*

Based on anatomical features
Capsule

Encapsulated Bacteria

- Capsules serves as anti-phagocytic virulence factor
- Positive Quellung reaction – capsule swells when specific anticapsular antisera is added
- Examples:
  - *Klebsiella pneumoniae*
  - *Salmonella*
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae type B*
  - *Neisseria meningitidis*



Based on anatomical features

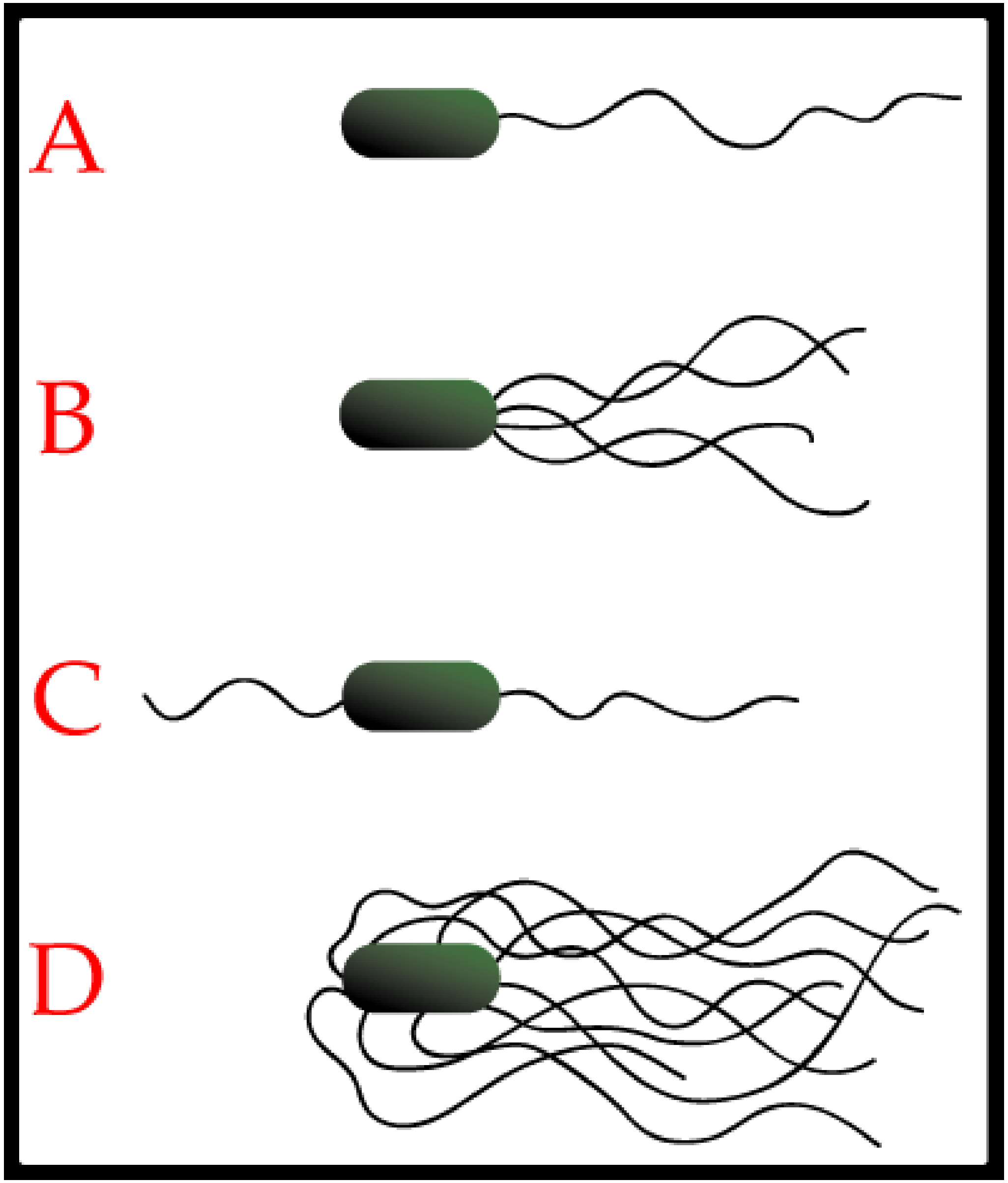
Flagella

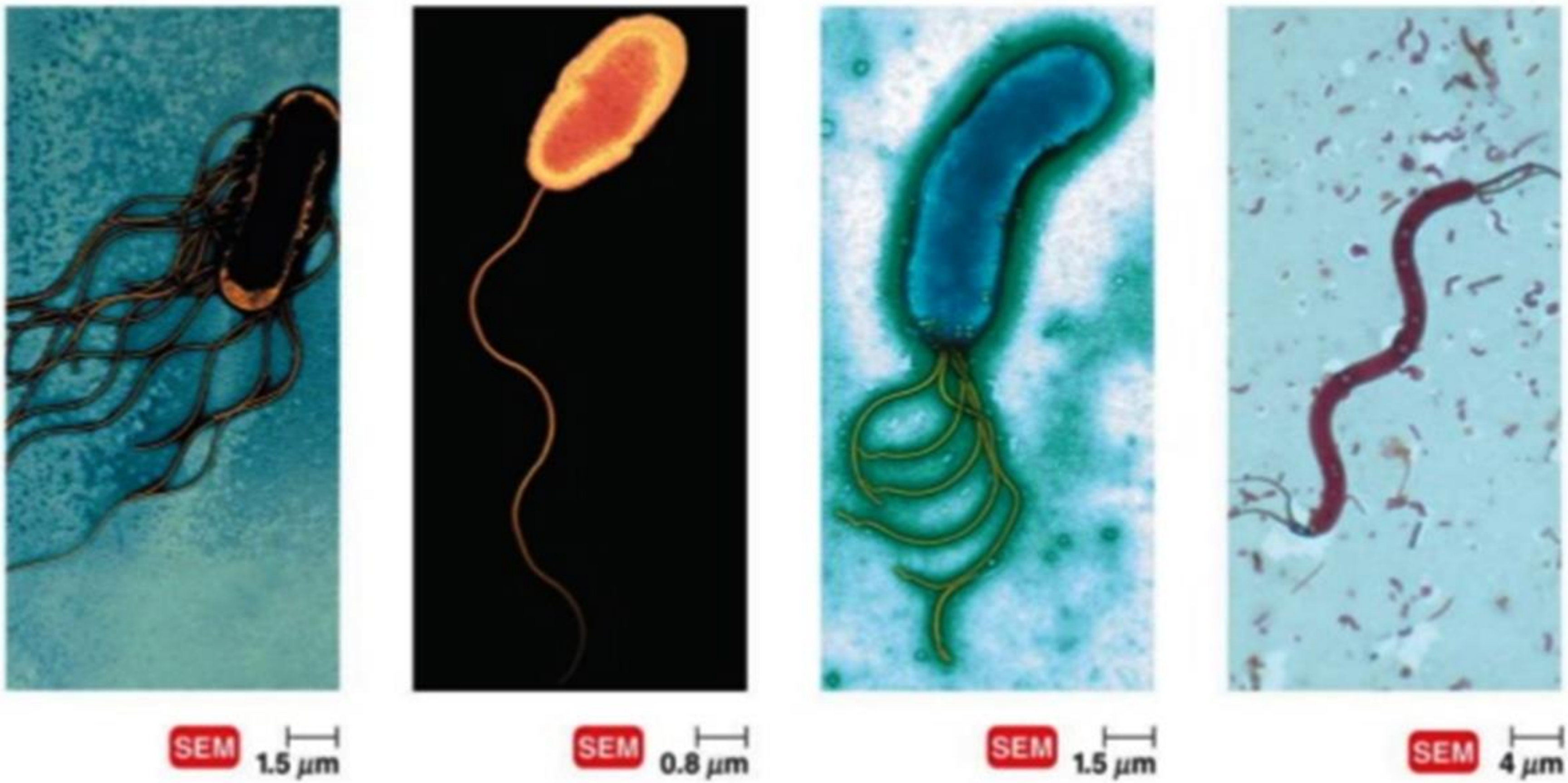
A. **With flagella**

1. Monotrichous (single polar flagellum) : *Vibrio cholerae*
2. Lophotrichous (multiple polar flagella) : *Spirilla, Barthonella baciliformis etc.*
3. Peritrichous (flagella distributed over the entire cell) : *Salmonella, E. coli etc.*
4. Amphitrichous (single flagellum at both ends) : *Spirillum minus*

B. **Without flagella**

- These are called *Atrichous bacteria.*


A
B
C
D



(a) Peritrichous

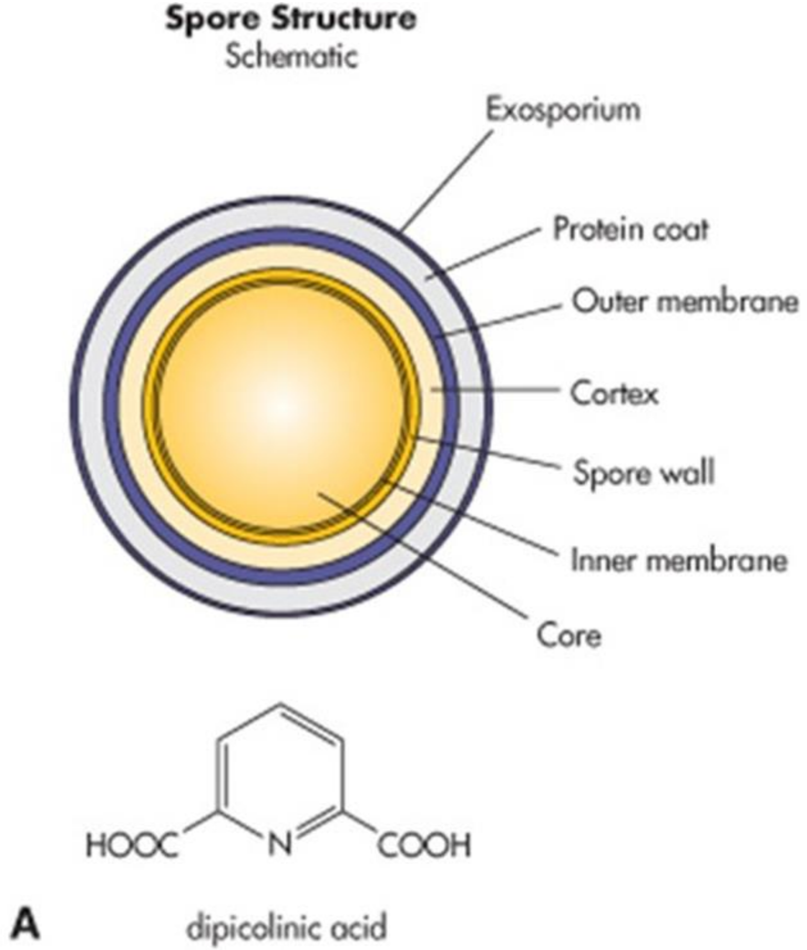
(b) Monotrichous and polar

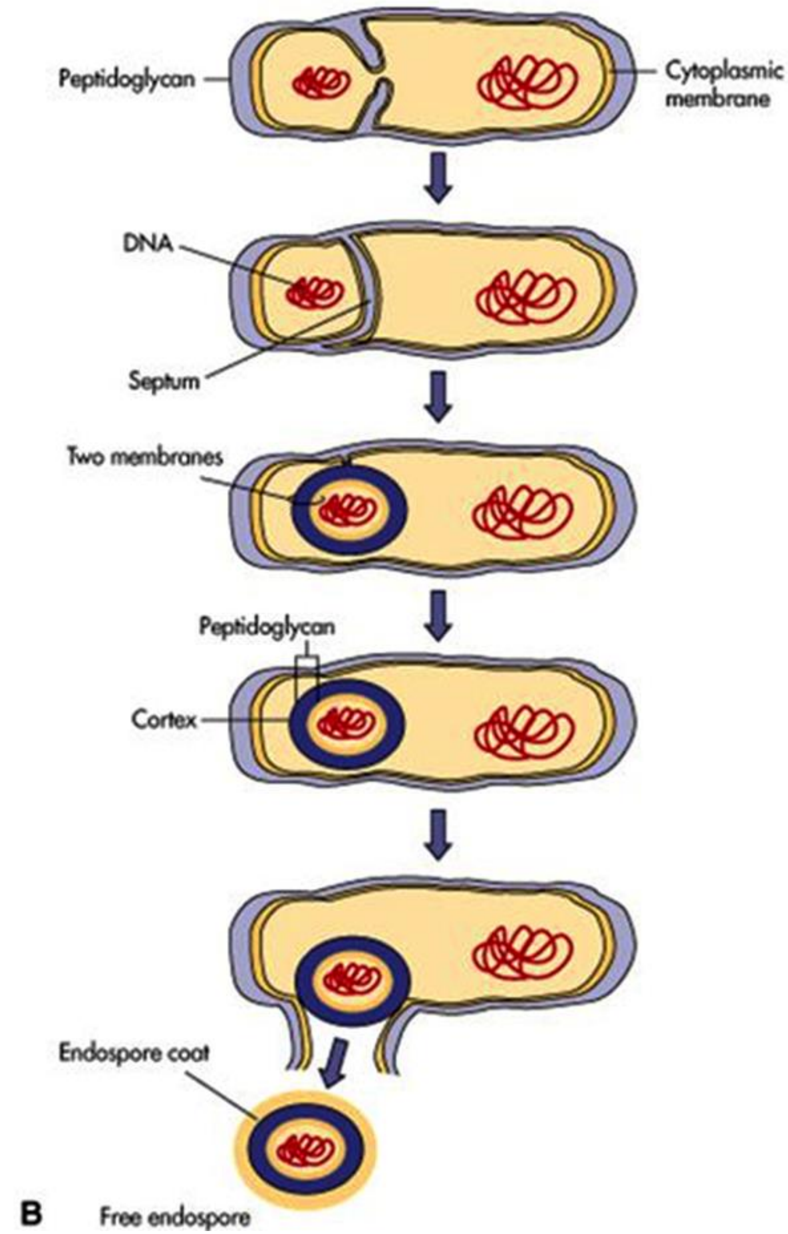
(c) Lophotrichous and polar

(d) Amphitrichous and polar

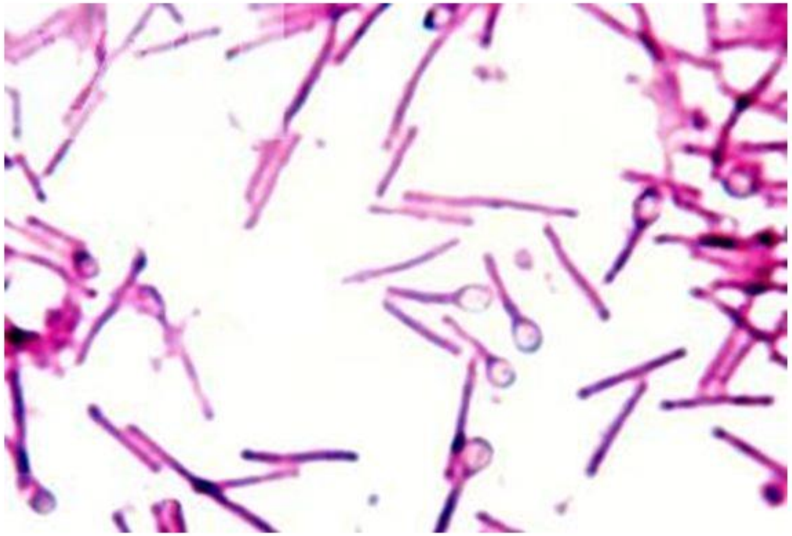
Based on anatomical features

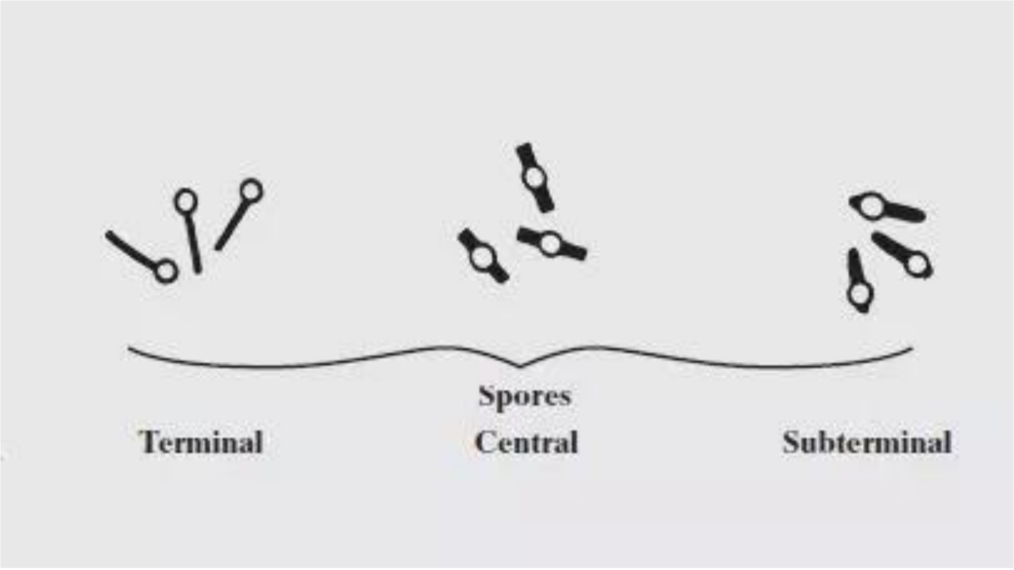
Spore

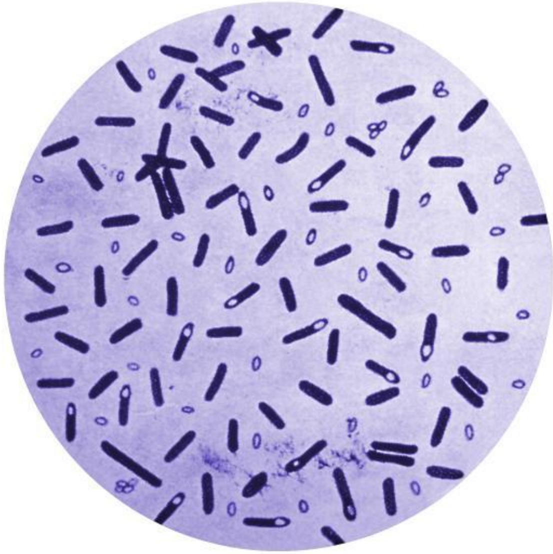




Clostridium tetani Bacteria are gram-postive rods or bacilli with terminal spores that cause tetanus in humans, spore





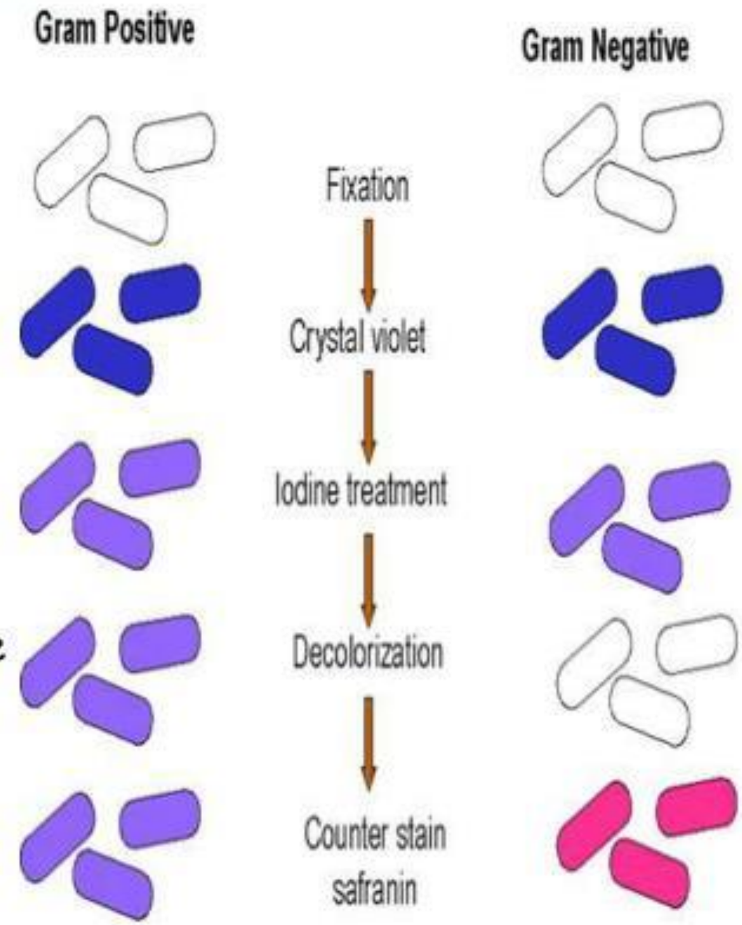


Based on staining

Gram Stain

Principle of staining technique:

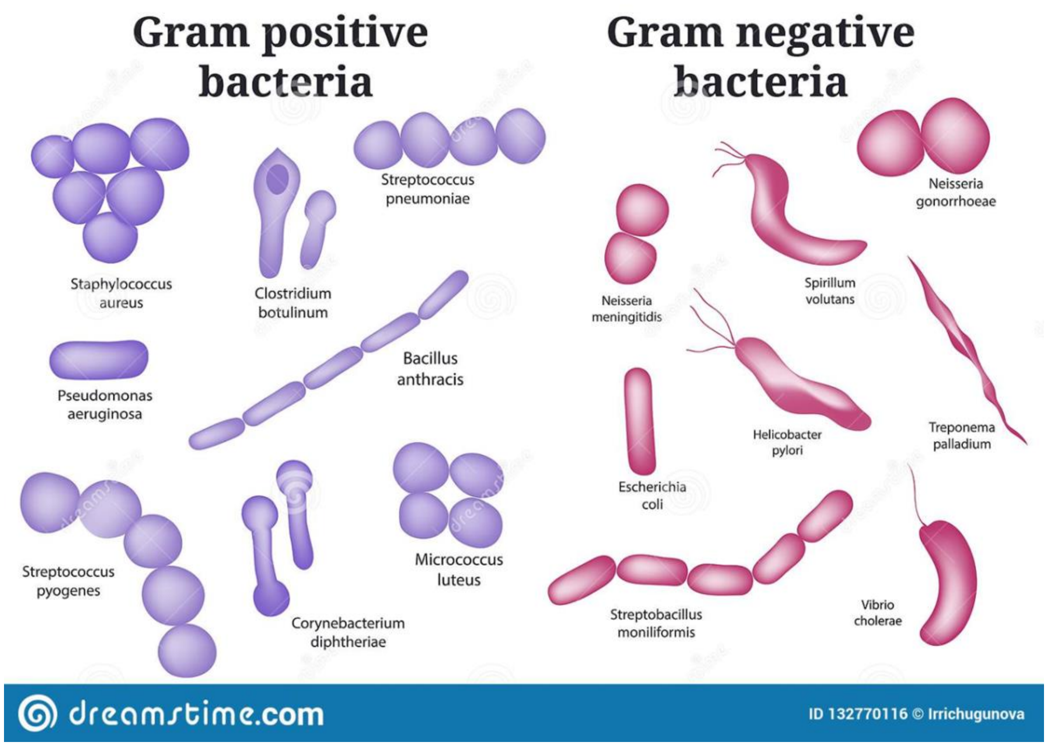
1. Primary stain:- Crystal Violet
2. Mordant(fixes the dye):- Iodine
3. Decolorizing agent:-Alcohol/Acetone
4. Counter stain;- Safranin



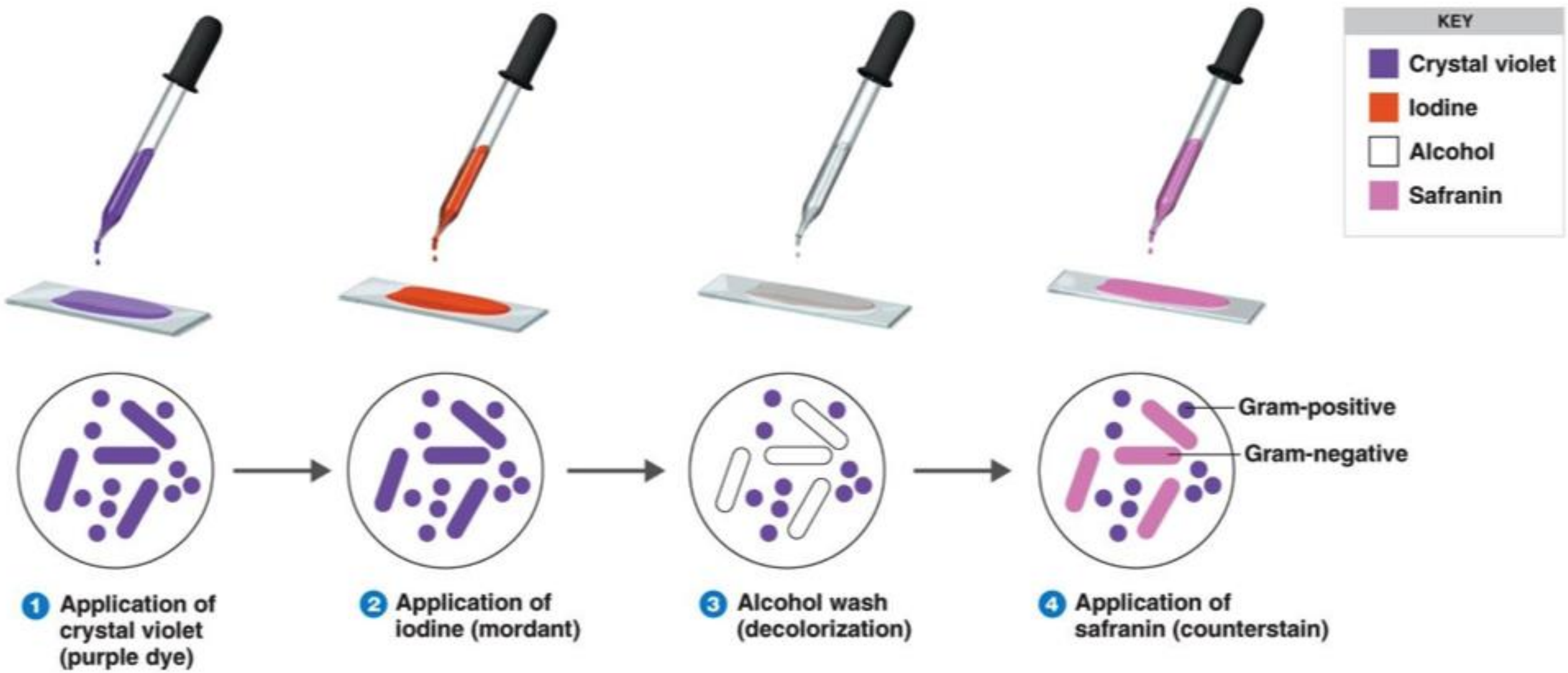
Based on staining

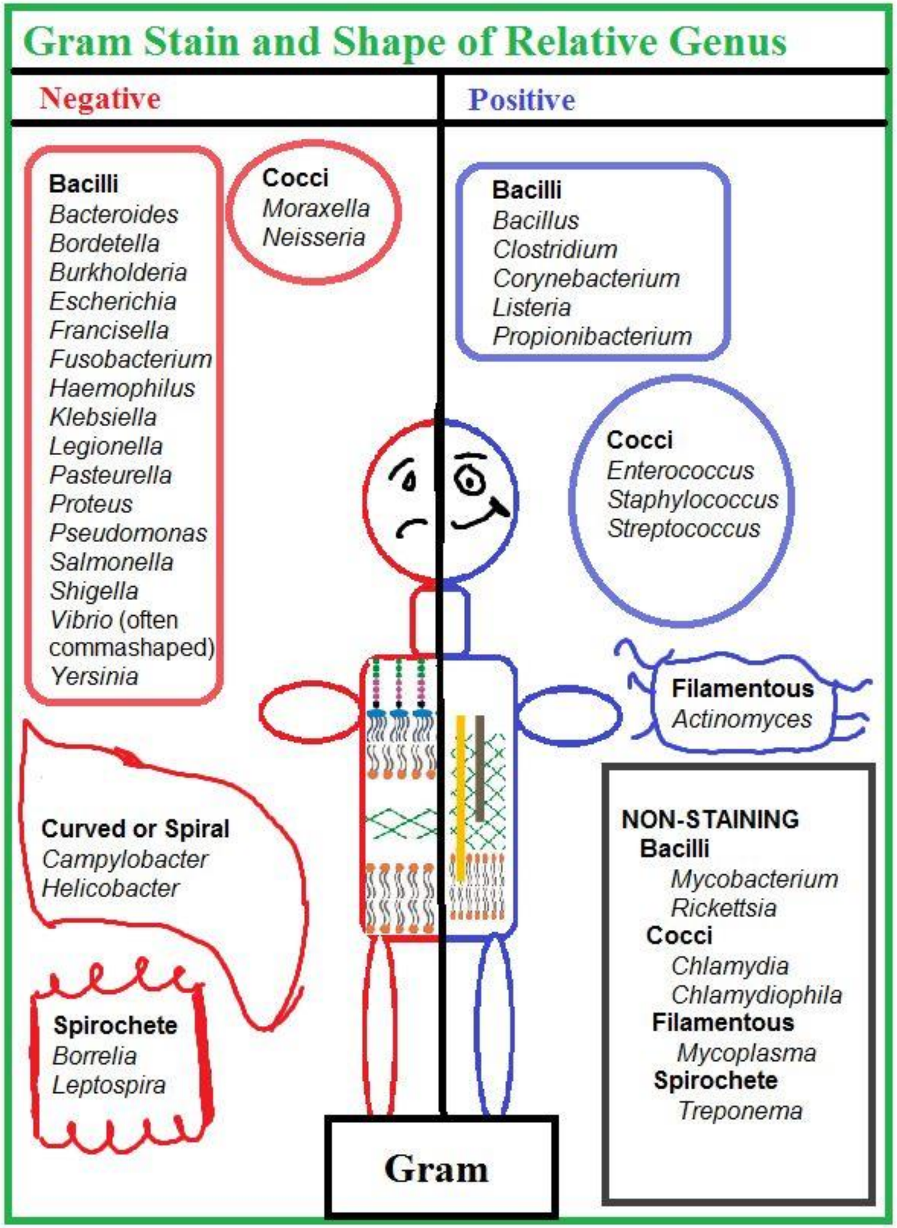
- **GRAM'S STAIN**
  - **Gram-positive cocci –** *Staphylococcus aureus*
  - **Gram-negative cocci –** *Neisseria gonorrhoeae*
  - **Gram-positive rods –** *Clostridium spp.*
  - **Gram-negative rods –** *Escherichia coli*

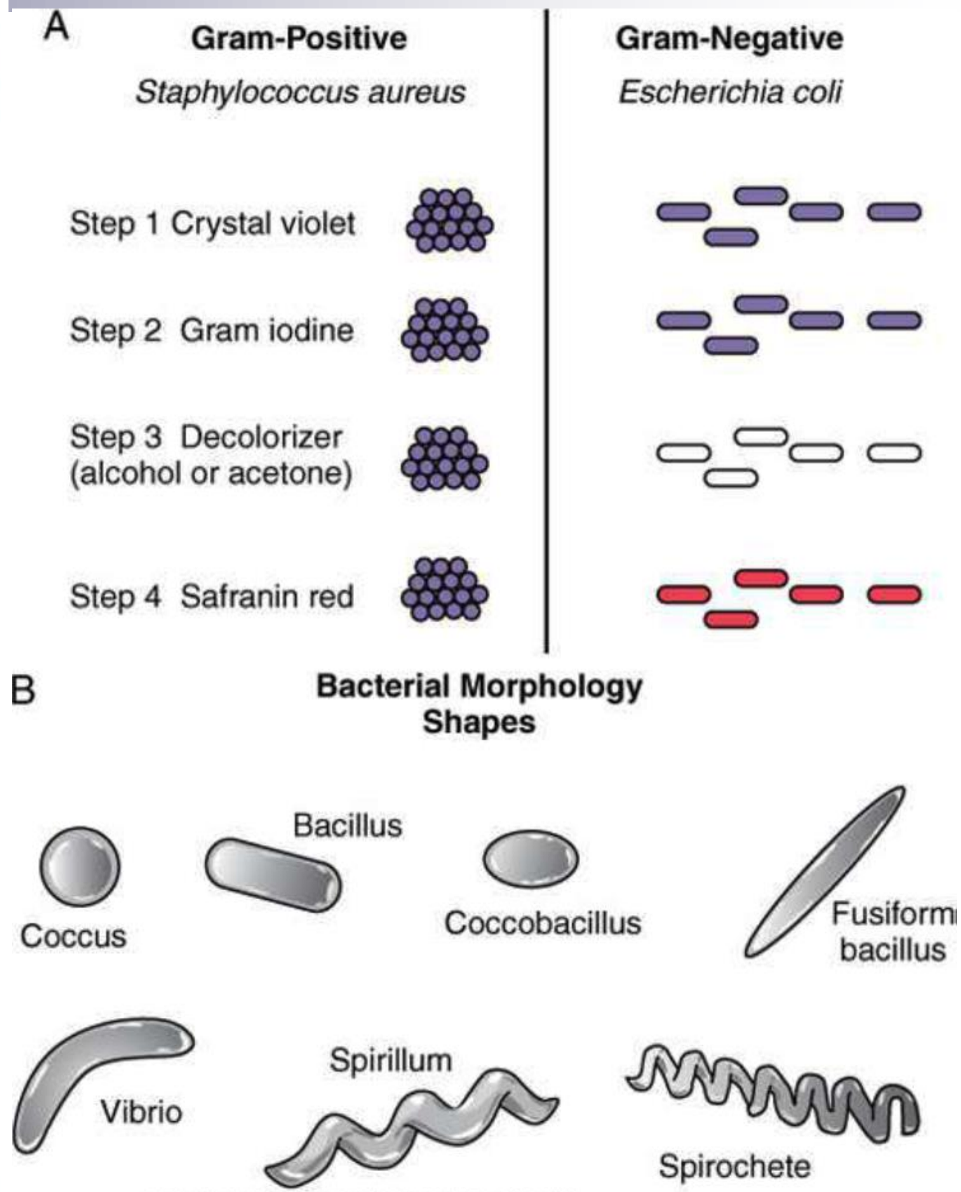
- **ACID FAST STAIN**
  - **Acid-fast bacilli –** *Mycobacterium tuberculosis*
  - **Non-acid-fast bacilli –** *Staphylococcus aureus*



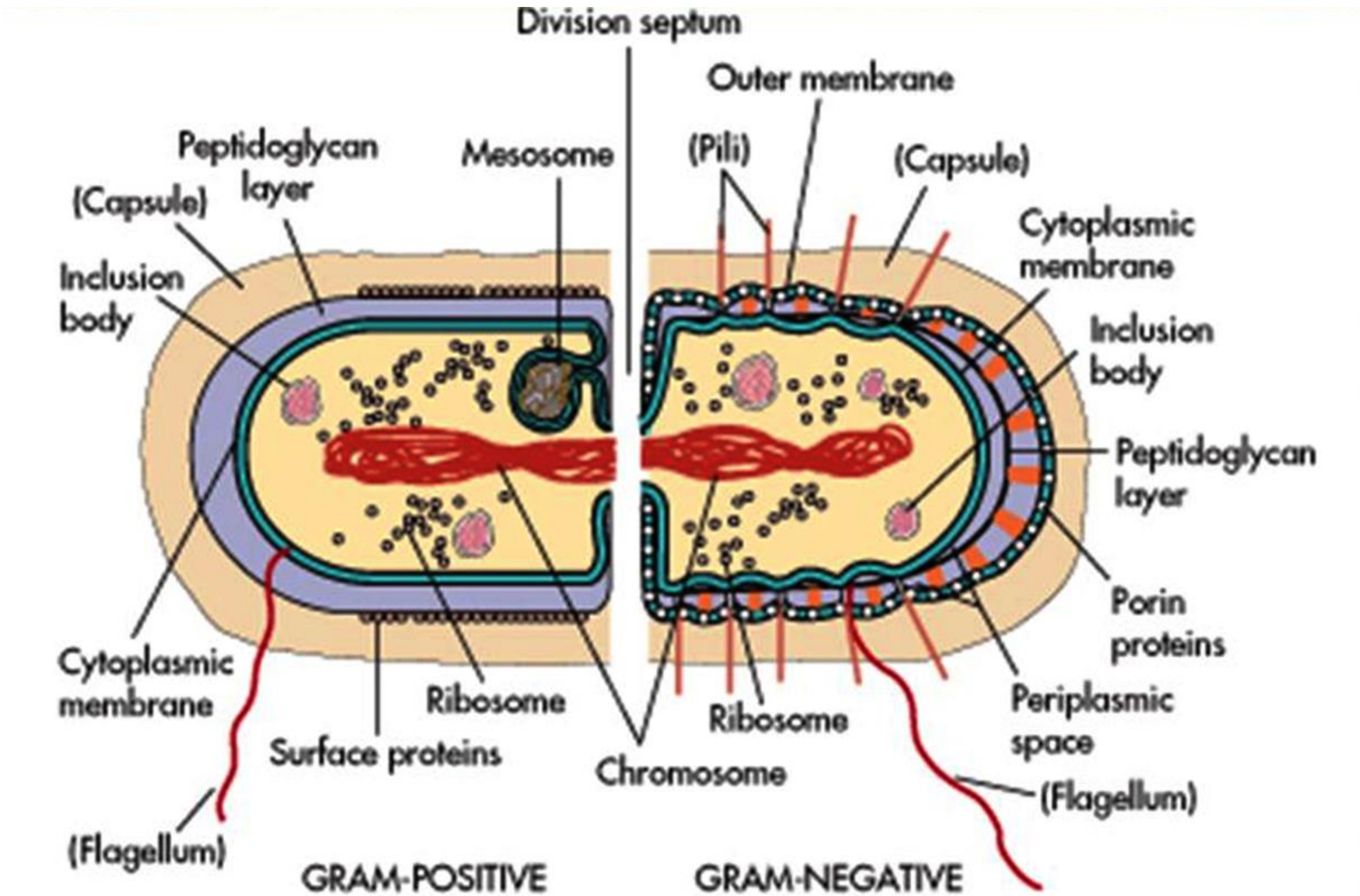
Principle of Gram staining procedure



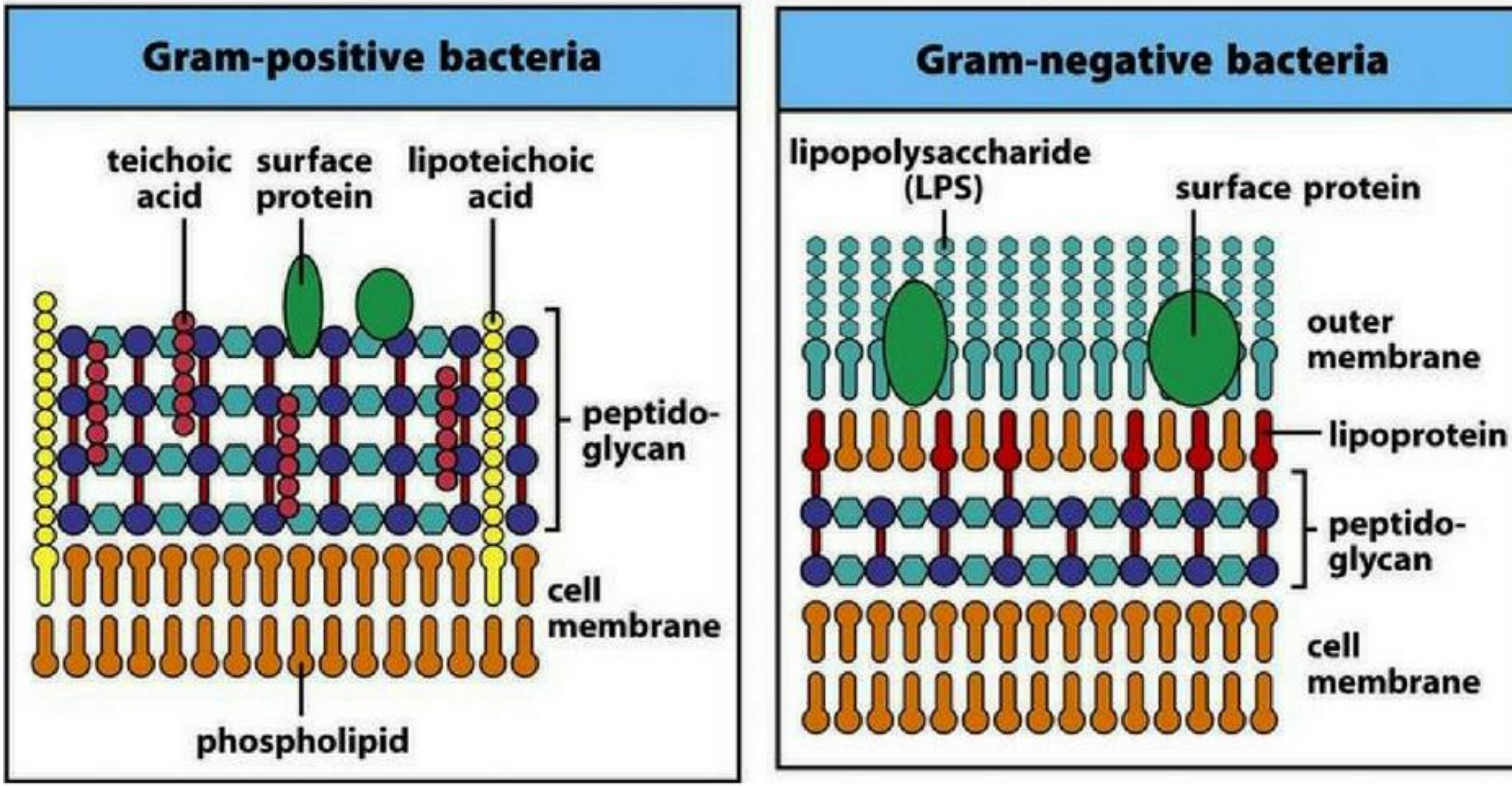




Murray et al: Medical Microbiology, 6th Edition.

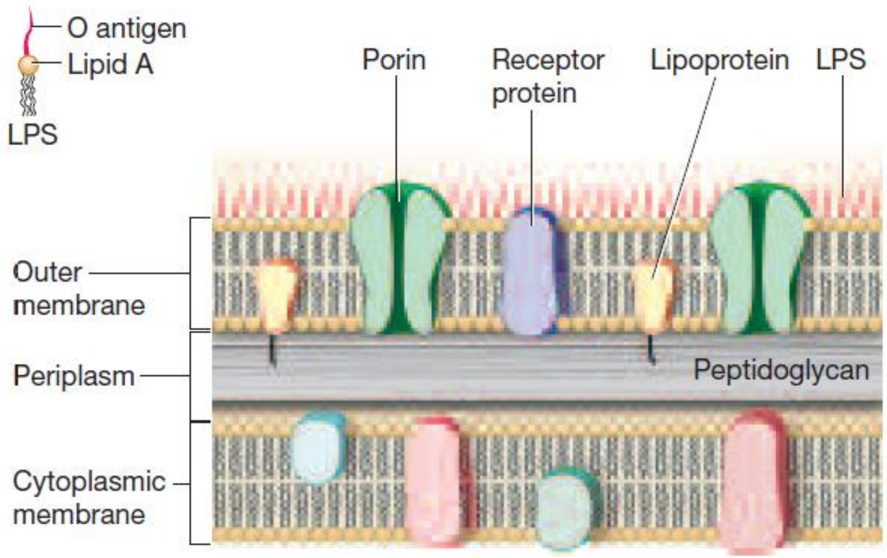

Division septum
Outer membrane
Peptidoglycan layer
Mesosome
(Pili)
(Capsule)
(Capsule)
Cytoplasmic membrane
Inclusion body
Inclusion body
Peptidoglycan layer
Porin proteins
Cytoplasmic membrane
Ribosome
Ribosome
Periplasmic space
Surface proteins
Chromosome
(Flagellum)
(Flagellum)
GRAM-POSITIVE
GRAM-NEGATIVE

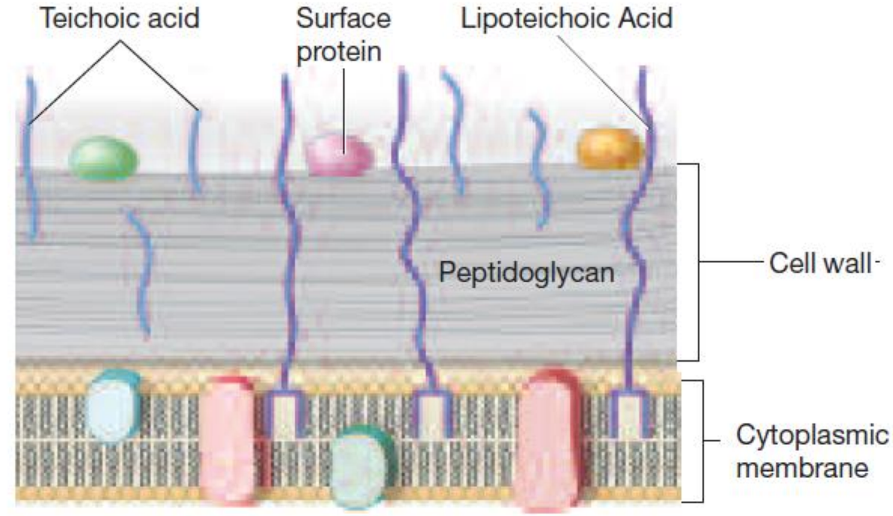
Cell wall of Gram Positive vs Gram Negative Bacteria



Membrane Characteristics of Gram-Positive and Gram-Negative Bacteria

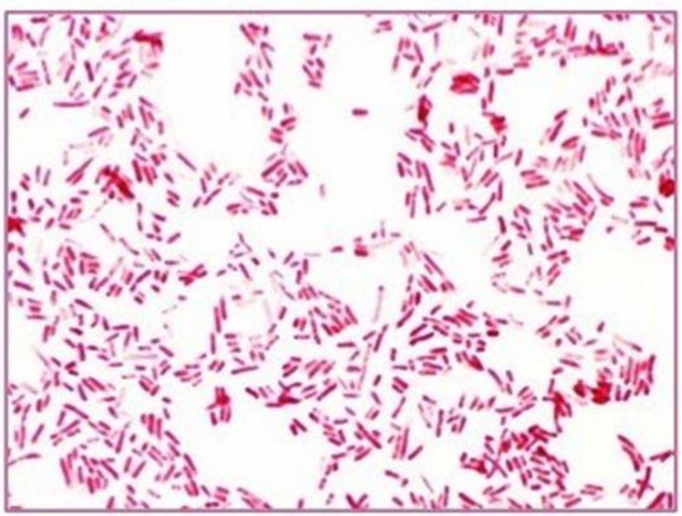
Characteristic	Gram-Positive	Gram-Negative
Outer membrane	-	+
Cell wall	Thicker	Thinner
Lipopolysaccharide	-	+
Endotoxin	-	+
Teichoic acid	Often present	-
Sporulation	Some strains	-
Capsule	Sometimes present	Sometimes present
Lysozyme	Sensitive	Resistant
Antibacterial activity of penicillin	More susceptible	More resistant
Exotoxin production	Some strains	Some strains



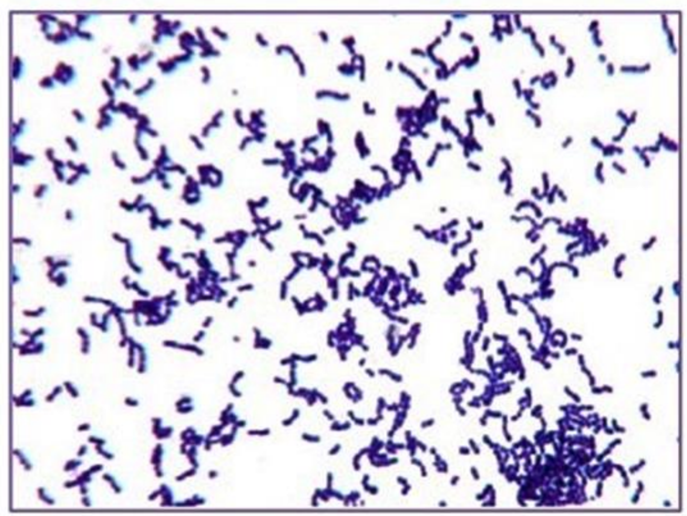


Cell Wall of Gram Negative Organism

Cell Wall of Gram Positive Organism



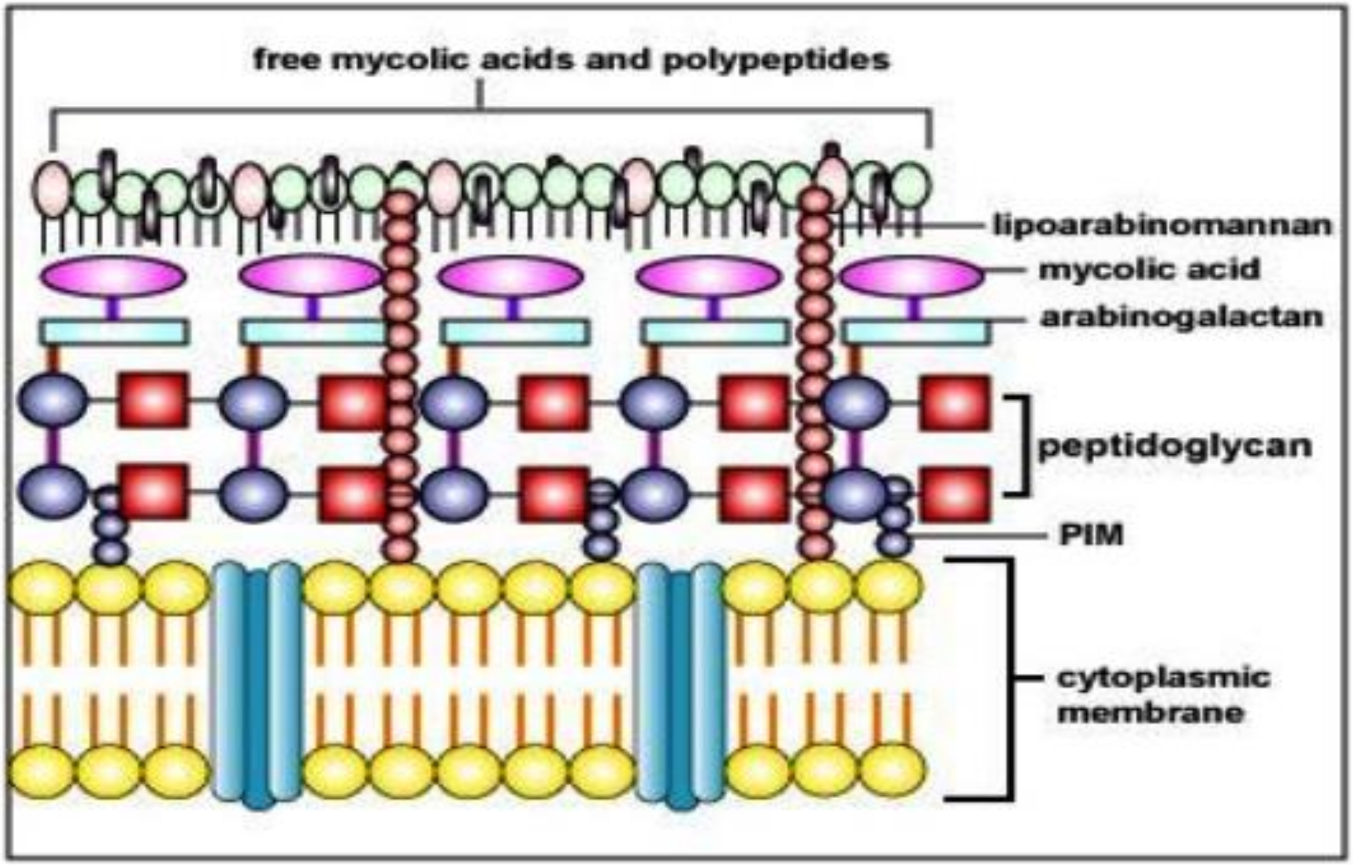
Gram-Negative Bacteria



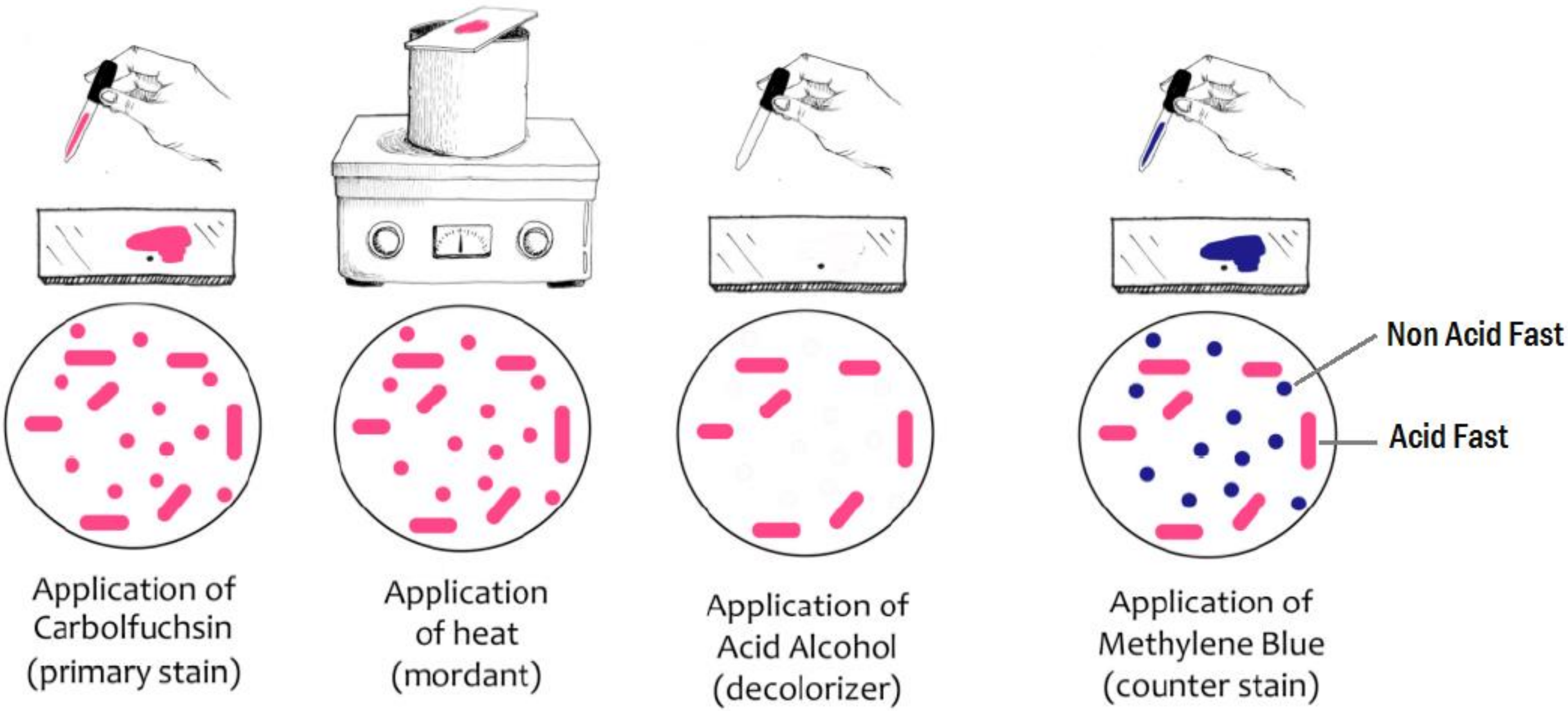
Gram-Positive Bacteria

Acid fast bacteria

CELL WALL OF ACID FAST BACTERIA



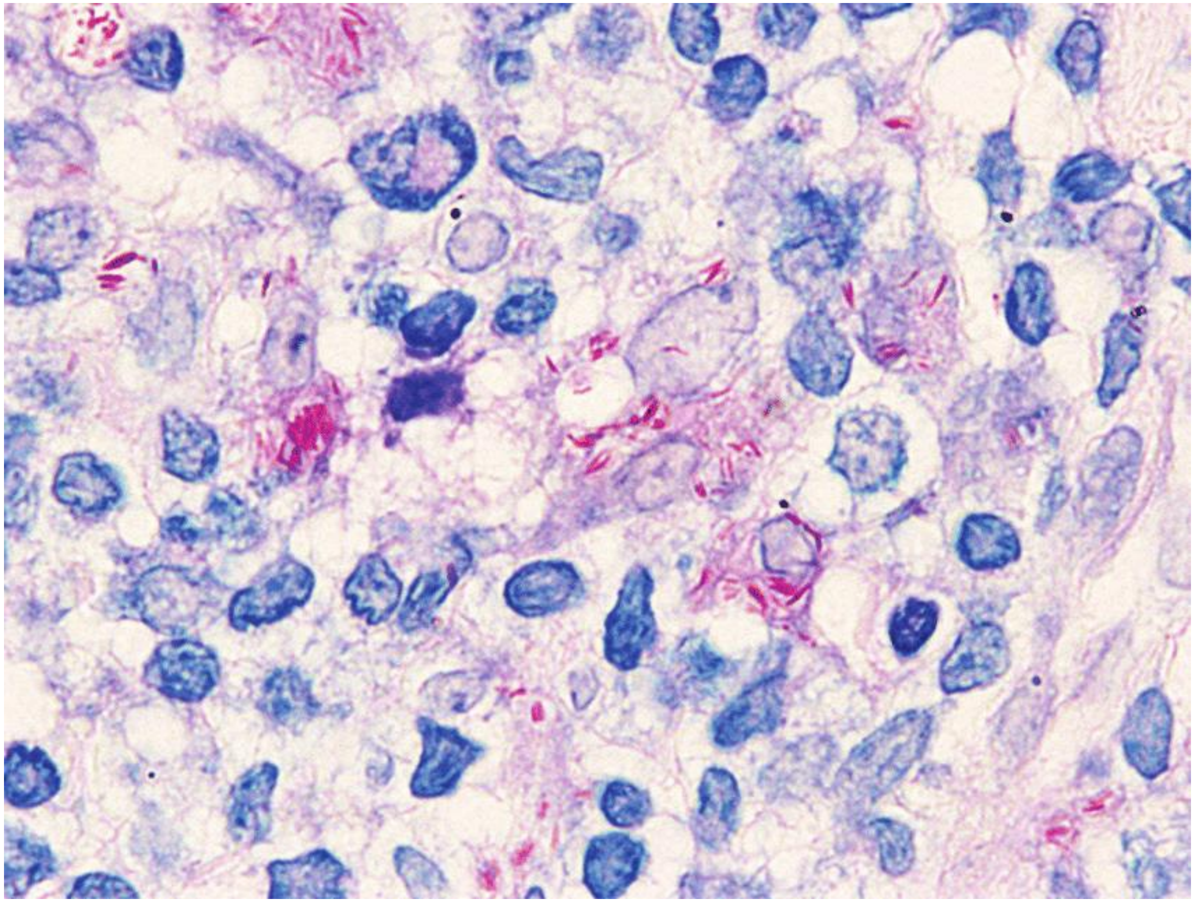
Ziehl-Neelsen Stain (ZN-Stain) Procedure



Acid fast staining

Examples of Acid-fast bacilli:

- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Nocardia asteroides*
- *Actinomycetes*



Based on Cultural Characteristics

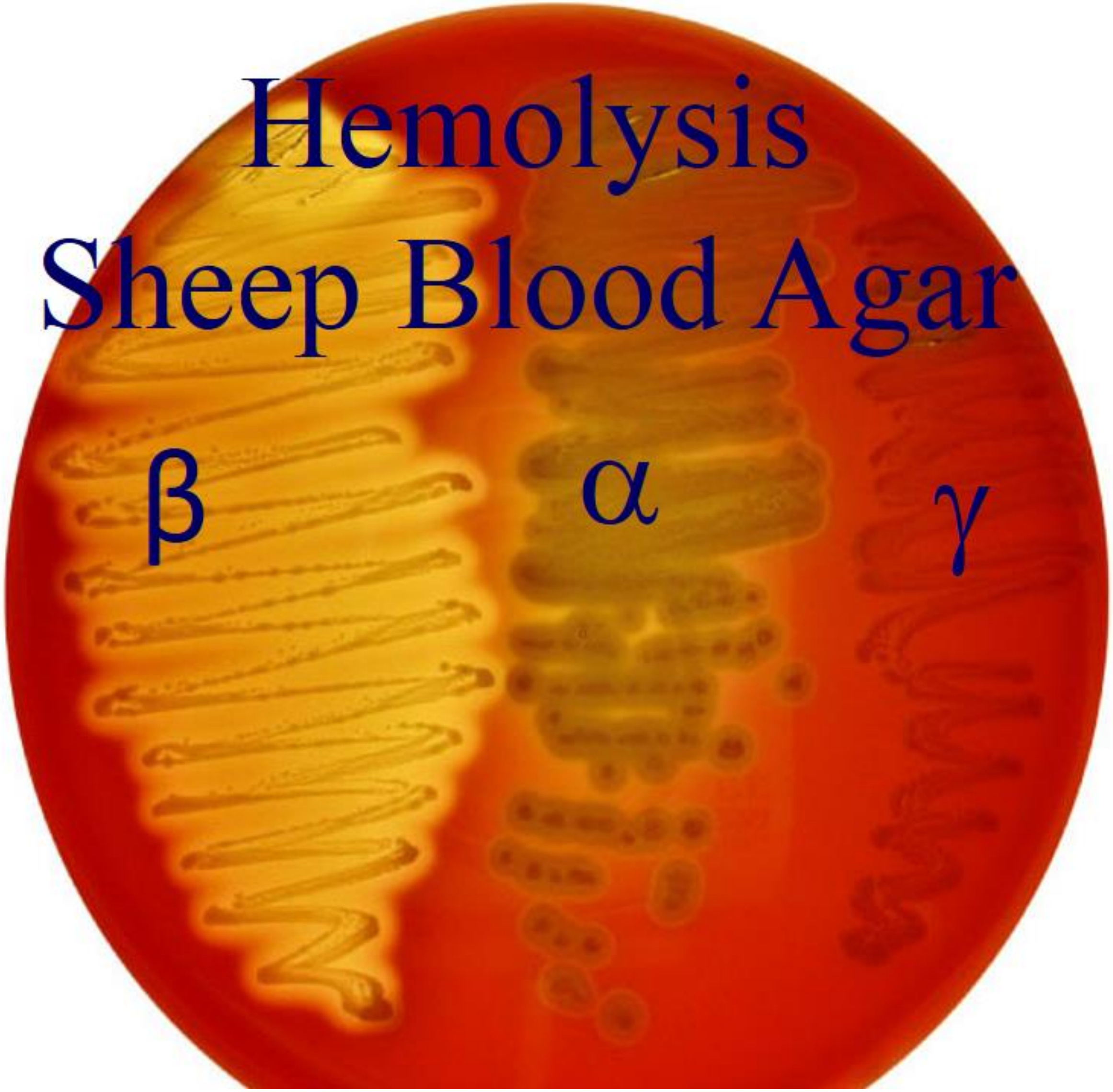
- Extra growth factor requirements
  - **Fastidious** – *Hemophilus influenzae*
  - **Non-fastidious** – *Escherichia coli*

Blood agar is an **enriched**, bacterial growth **medium. Fastidious** organisms, such as streptococci, do not grow well on ordinary growth media

- Hemolysis on Sheep Blood Agar
  - **Alpha-hemolysis** – *Streptococcus pneumoniae*
  - **Beta-hemolysis** – *Streptococcus pyogenes*

- Utilization of carbohydrates
  - **Oxidative** - *Micrococcus*
  - **Fermentative** – *Escherichia coli*

Hemolysis
Sheep Blood Agar

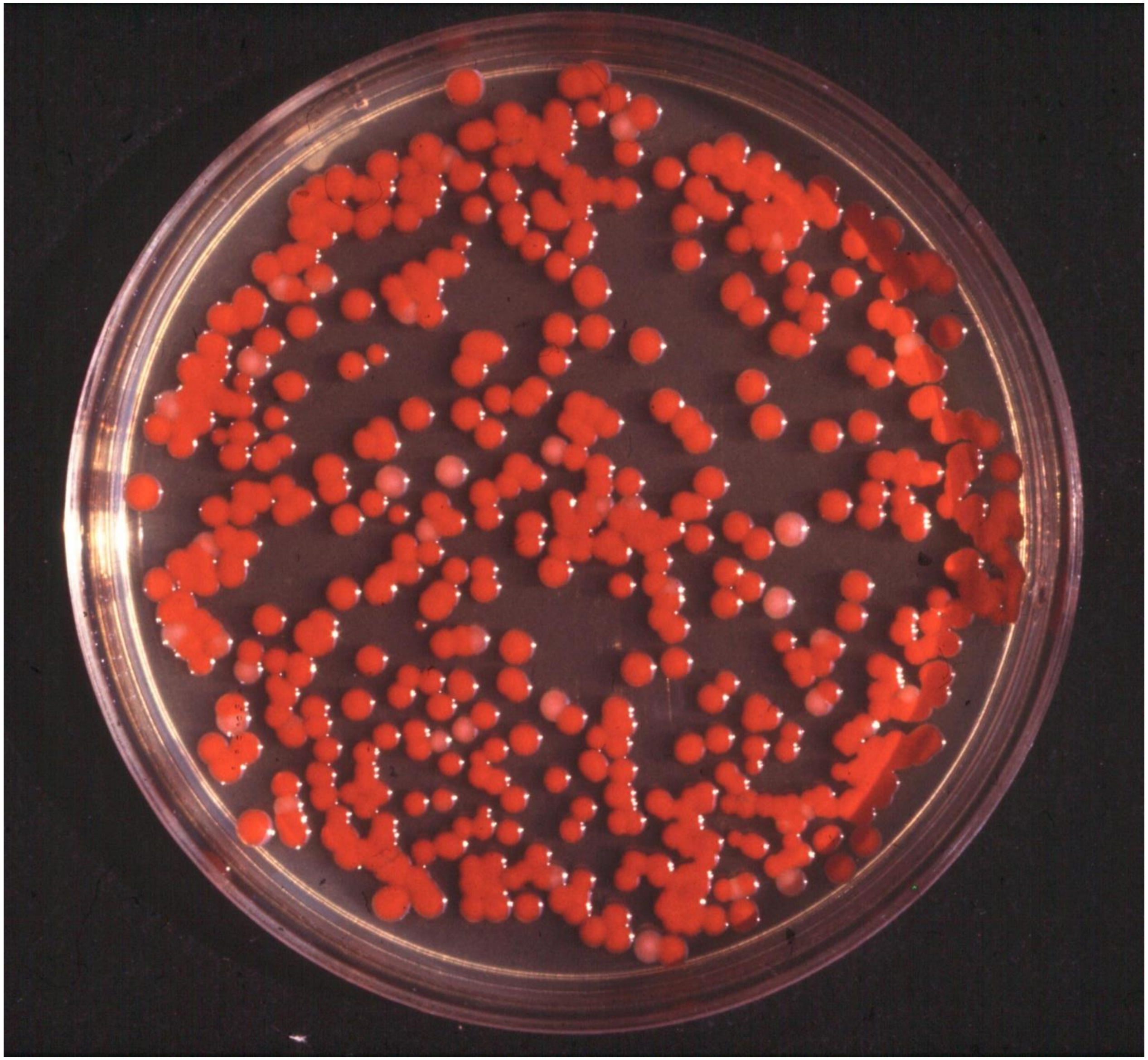


Based on Cultural Characteristics

- Growth rate
  - **Rapid growers**- *Vibrio cholerae*
  - **Slow growers** - *Mycobacterium tuberculosis*

- Pigment production
  - **Pigment producer** - *Staphylococcus aureus*
  - **Pigment non-producer** - *Escherichia coli*

Serratia marcescens



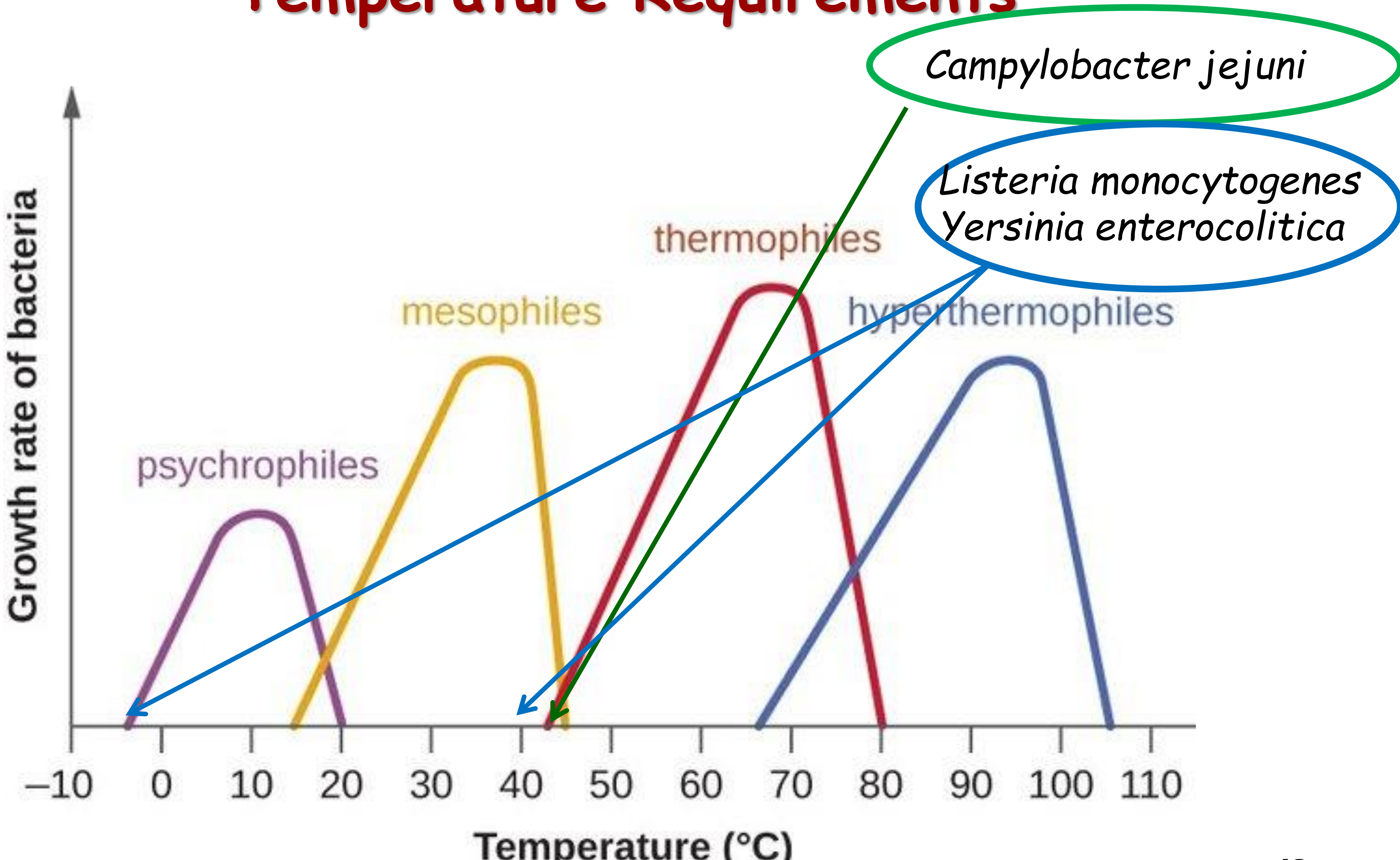
Based on environmental factors

- Temperature
- Oxygen dependence
- pH
- Salt concentration

On the basis of temperature

- **Psychrophiles** ($15-20^0C$) – *Pseudomonas fluorescens*
- **Mesophiles** ($20-40^0C$) – *Escherichia coli, Salmonella enterica, Staphylococcus aureus*
- **Thermophiles** ($50-60^0C$)- *Bacillus stearothermophilus*
- **Extremely thermophiles** (as high as 250^0C)

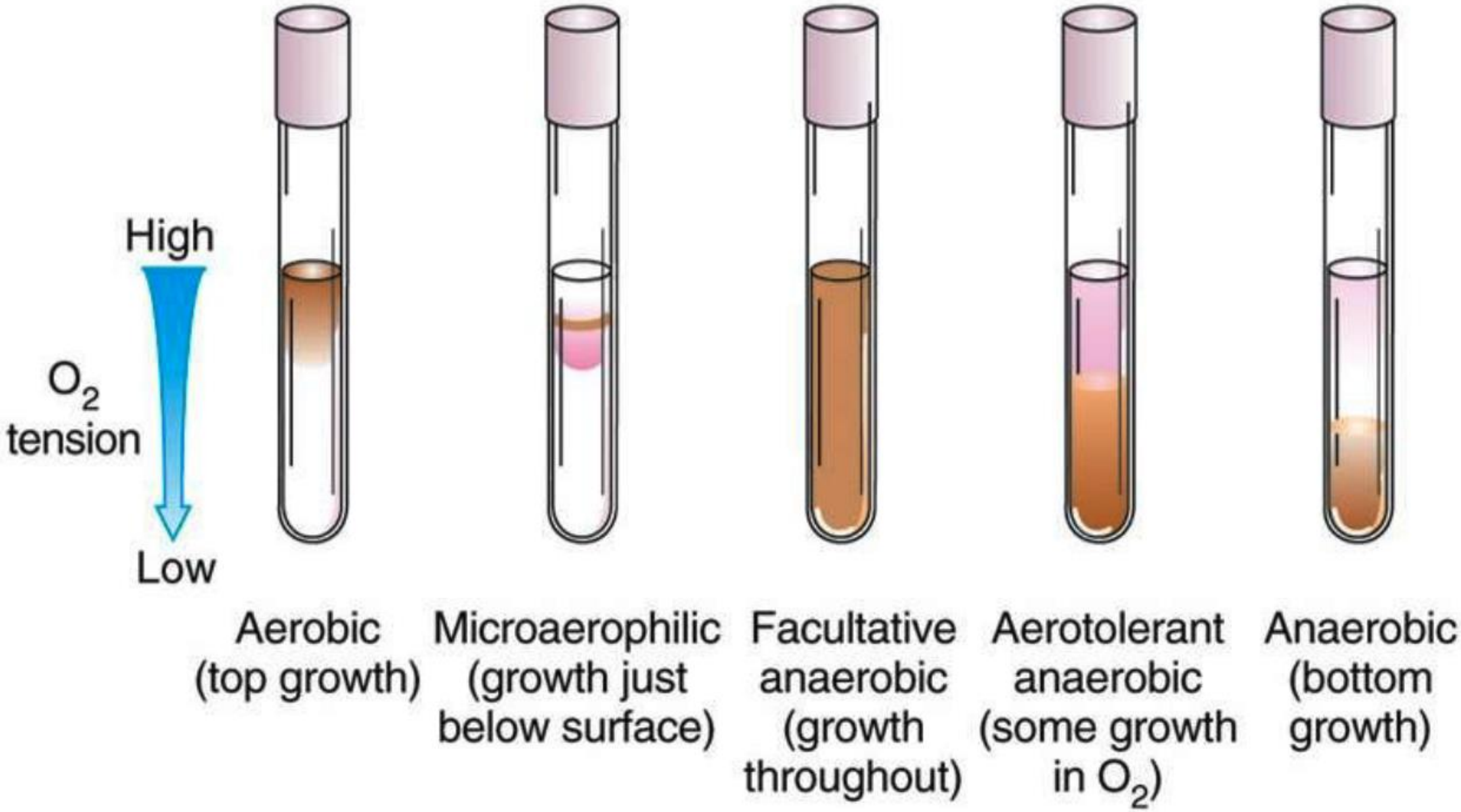
Classification of Microorganisms by Temperature Requirements



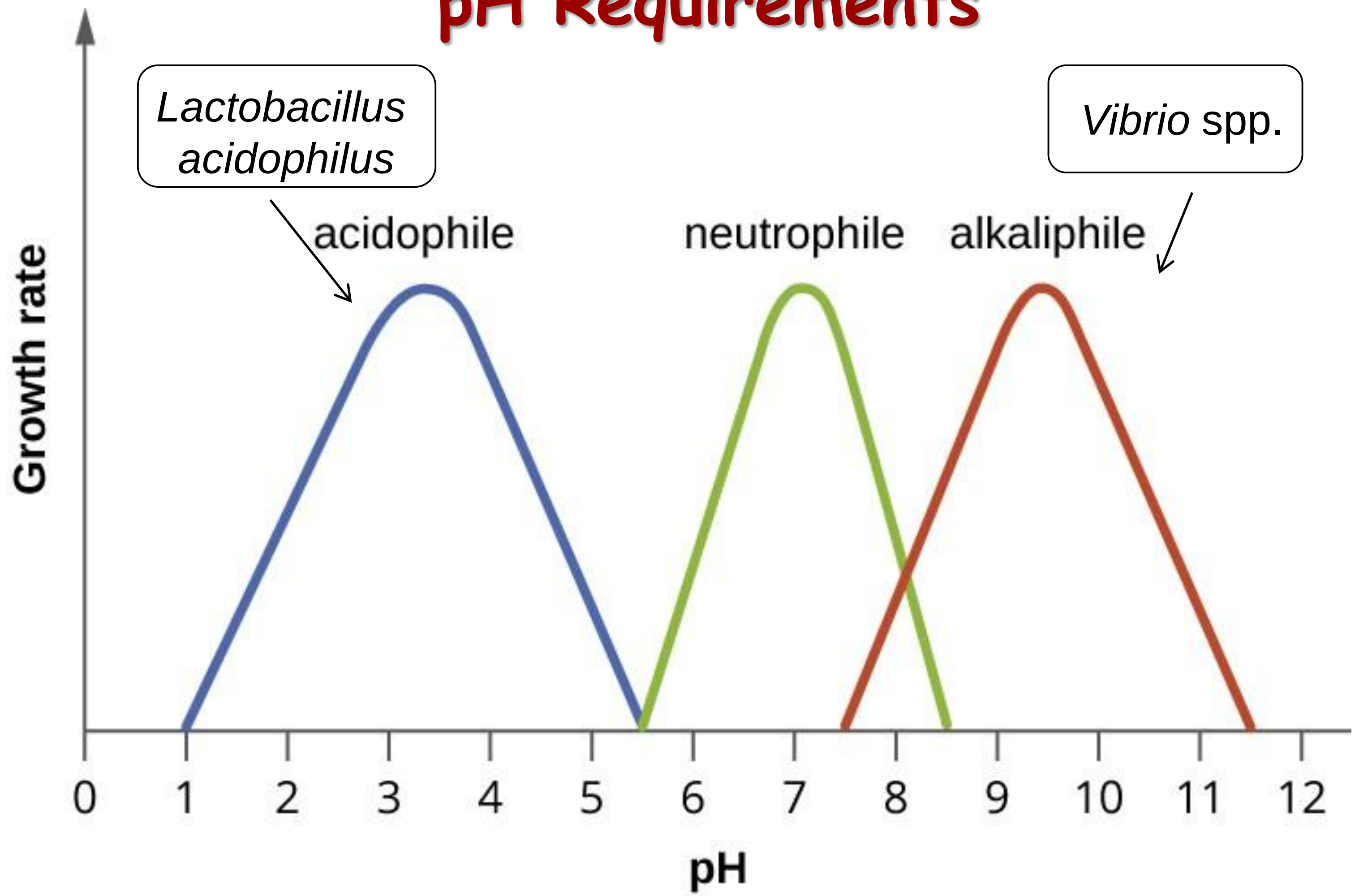
Oxygen dependence

Aerobes	Anaerobes
• Obligate • Microaerophiles • Facultative	• Aerotolerant • Obligate

Demonstration of Oxygen Requirements



Classification of Microorganisms by pH Requirements



Majority of the medically important bacteria grow best at neutral or slightly alkaline reaction (pH 7.2-7.6)

On the basis of salt concentration

- **Non-halophiles:** Unable to grow in high salt concentration (eg: *E. coli*)
- **Halotolerant**: Tolerate low level of salt concentration i.e. 8% salt
- **Halophiles:** Grow in high salt concentration i.e. salt loving
  - Slightly: Require 0.5to 3% NaCl
    - *Pseudomonas* spp., *Vibrio*, *Moraxella*, *Acinetobacter*
  - Moderately: Require 3% to 15% NaCl
    - *Bacillus* spp., *Micrococcus* spp.
  - Extremely: Require 15% to 30% salt
    - *Halobacterium* spp., *Halococcus* spp., *Natranobacterium* spp., *Natranococcus* spp., *Haloterax* spp

Other ways of classification

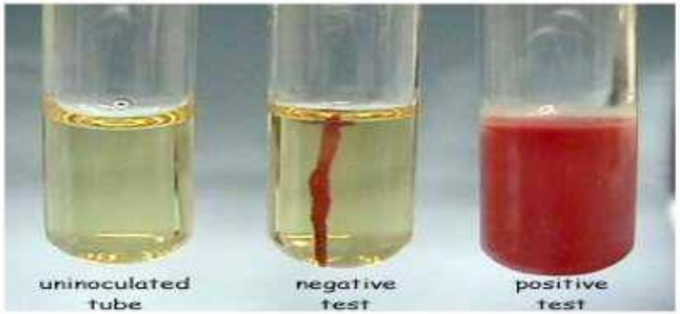
- Motile/Non-motile
- Pathogenic/Non-pathogenic
- Sensitive/Resistant (to particular antibiotic/ chemicals)
- Lactose fermenter/Lactose non-fermenter

On the basis of motility

1. **Motile :**
   - *Salmonella*
   - *Vibrio cholerae*
   - *Pseudomonas*
2. **Non motile :**
   - *Staphylococcus*
   - *Shigella*

Motility test

- to determine whether a bacterium is motile.
- Non-motile organisms which lack flagella, are usually going to form a single line of growth that does not spread into the surrounding area. While a motile bacterium will grow and make a hazy zone around the stab line.



On the basis of relationship with their host

- **Saprophytes**: Those bacteria that are free living but depend on dead and decayed organic matter for their growth

- **Parasites**: Those bacteria that can establish themselves and multiply in host tissue deriving nourishment from the host

Genotypic classification

- Ribosomal RNA (rRNA) sequence analysis
- Universal phylogenetic tree
- DNA-DNA hybridization
- G+C content

THE END

Thanks for having patience with me ☺

KEEP CALM AND Love Laboratory

References

- Medical Microbiology; Murray, Rosenthal, Pfaller; 7th Ed; Elsevier Saunders; 2013
- Jawetz, Melnick & Adelberg's Medical Microbiology; Brooks G, Carroll KC, Butel J, Morse S (Eds); 27th Ed; McGraw Hill Lange; 2016
- Sherris Medical Microbiology; 6th Ed; Ryan KJ, Ray CG; McGraw Hill Education; 2014