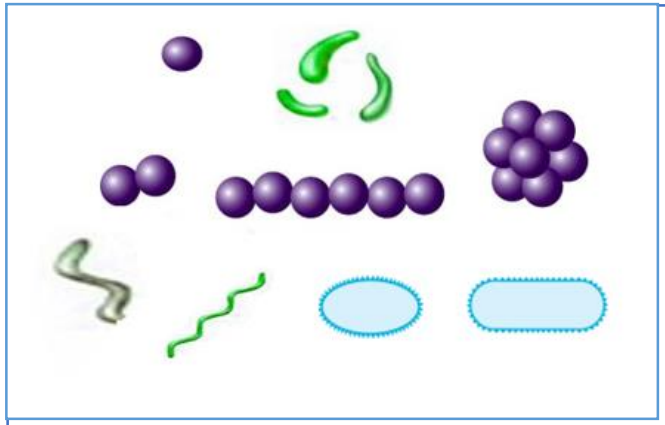
MODULE OF GENERAL MICROBIOLOGY (GMI 1522) C.U.R BLS L1



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A.Y 2018/2019

Course contents

At the end of this course students should be able to discuss:

- Classification and taxonomy of bacteria
- Bacterial anatomy & Physiology
- Bacterial genetics
- Bacterial culture media
- Staining technique in microbiology lab

Course contents…

- Pathogenicity and infection
- Bacterial Normal Flora
- Sterilization and disinfection
- Identification system of bacteria
- Biochemical & serologic tests for bacterial identification
- Sensitivity test.

What is Medical Microbiology?

- "The study of microorganisms (**including bacteria, viruses, fungi and parasites**) which are of medical importance and are capable of causing diseases in human beings"

- Microorganisms are creatures that are not directly visible to the naked eyes, with dramatically biologic diversity.

Organisms included in the study of Microbiology

- ✓**Viruses**
- ✓**Parasites**
- ✓**Bacteria**
- ✓Fungi

- ✓Virology
- ✓Parasitology
- ✓**Bacteriology**
- ✓Mycology

Introduction

- Based on the organization of their cellular structures, all living cells can be divided **into two groups**: eukaryotic and prokaryotic

  - **Eukaryotic cell types: Has true nucleus,** - Animals, plants, fungi, protozoans, and human cell

  - **Prokaryotic cell types (Bacteria)**: lack a true nucleus and don't have membrane bound organelles like mitochondria, etc.

    e.g. bacteria & blue green algae

Features	Prokaryotic cell	Eukaryotic cell
Size	small	big
Nuclear membrane	Absent	Present
Chromosome	Single	multiple
Nucleolus	Absent	Present
Histones	Absent	Present
Sexual reproduction	Absent	Present
Cytoplasmic ribosomes	70s	80s
Mitochondria	Absent	Present
Endoplasmic reticulum	Absent	Present
Lysosomes	Absent	Present
Micro filaments and tubules	Absent	Present
Site of oxidative phosphorylation	Cell membrane	Mitochondria
Site of photosynthesis	Cell membrane	Chloroplast
Peptidoglycan	Present	Absent
Cell membrane composition	Phospholipids & Proteins	Sterols
Cell wall	Usually rigid wall with peptidoglycan layer; exception: mycoplasmas	Present only in fungi: glucans,mannans, chitin,chitosan,cellulose

Discovery of Microorganisms

- The discovery of micro organisms had to wait the invention of the microscope by a scientist called Anton Van-Leeuwenhook.
- Germ theory of disease: microorganisms can cause disease and it can spread and reemerge
- Louis Pasteur (1822):
  - demonstrated that alcoholic fermentations were the result of microbial activity,
  - that some fermentations were aerobic and some anaerobic;
  - he also developed the process of pasteurization to preserve wine during storage

Cont…

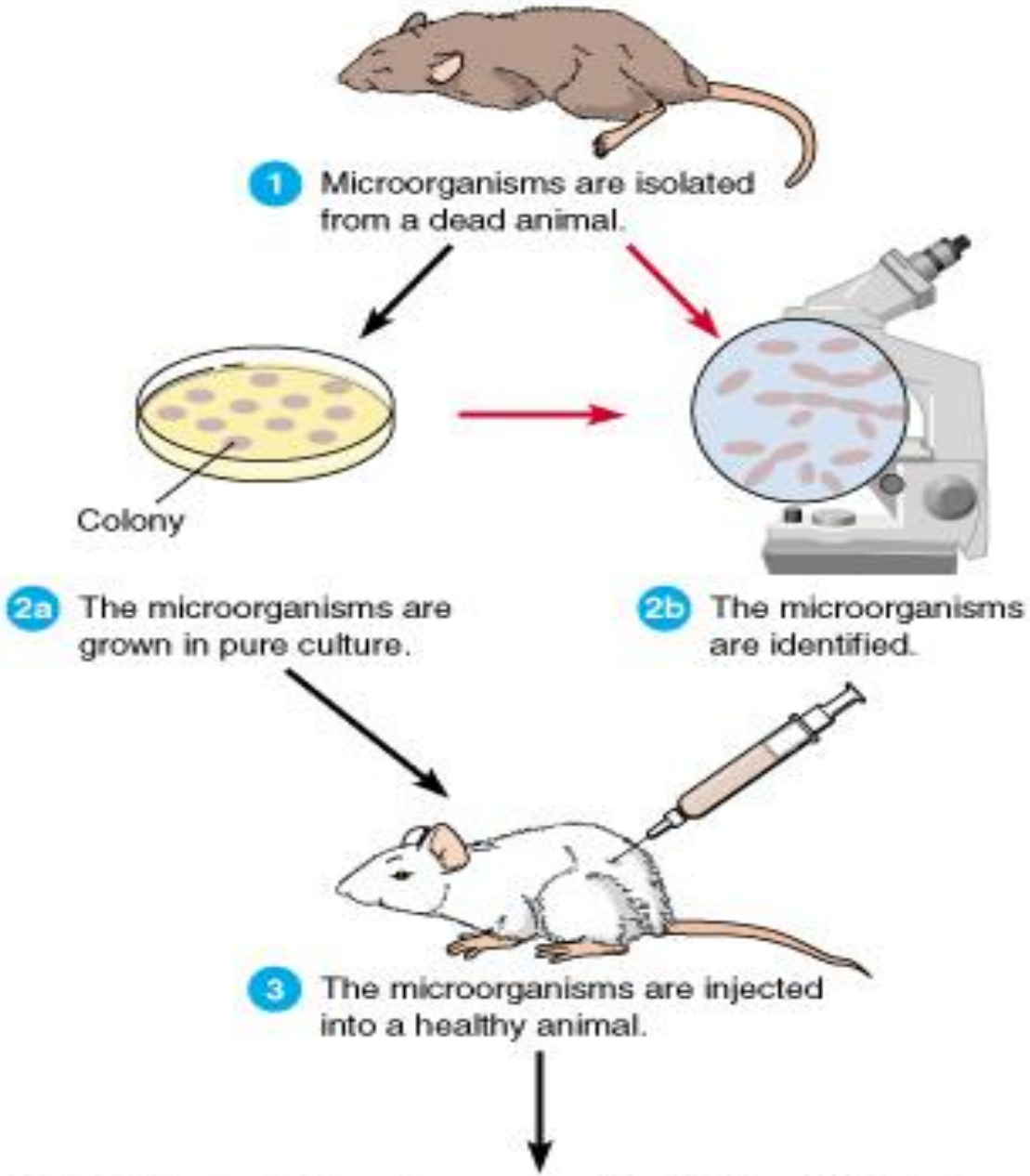
Robert Koch (1876):

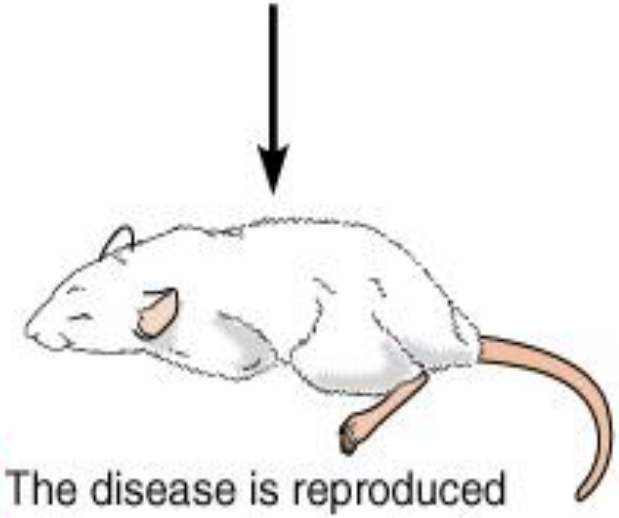
- He perfected in bacteriological techniques during his studies on the culture of anthrax bacillus.
- He introduced staining techniques and also methods of obtaining bacteria on solid media in pure culture.

- He demonstrated Koch's phenomenon which is the expression of hypersensitivity phenomena of mycobacterium tuberculosis
- He also suggested criteria, before blaming the organisms responsible for diseases.
- It goes by the name of Koch's postulates:

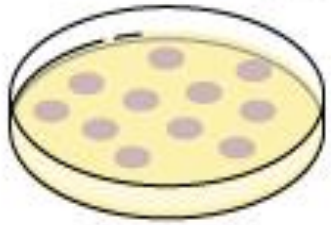
Koch’s postulates:

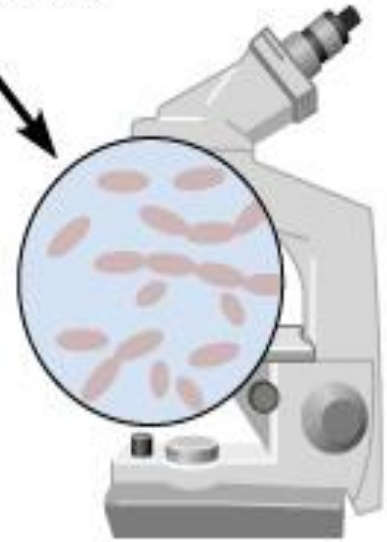
1. Microorganism must be present in every case of the disease
2. Organism must be grown in pure culture from the diseased host
3. Same disease must be produced when a pure culture of organism is injected into another host
4. Same organism must be recovered from the experimentally infected host





4 The disease is reproduced in the second animal; microorganisms are isolated from this animal.





5a Pathogenic microorganisms are grown in pure culture.

5b Identical microorganisms are identified.

Exceptions to Koch's postulates

1. Microbes that **can't be grown** on artificial media

   examples: virus, rickettsia, treponema

2. One microbe that causes **multiple diseases**

   examples: *Streptococcus pyogenes* → Strep throat, Scarlet fever

3. More than one microbe produces the **same disease**

   examples: Pneumomia, meningitis

4. Strictly human diseases with **no animal model**

   examples: Rubella

Size of Bacteria

- Unit of measurement in bacteriology is the micron (micrometer, µm)

- Bacteria of medical importance
  - 0.2 – 1.5 µm in diameter
  - 3 – 5 µm in length

Bacterial classification

WHY Classify?

- Establish criteria for Identification
- Arrange related organisms into groups
- Provide information about evolution of organisms

- Bacteria can be classified based on different aspect namely:
  1. **Shape**
  2. **Cell wall reaction to Gram stain**
  3. **Ability to form spores**
  4. **Morphology and Ziehl- Neelsen stain**
  5. **Physiologic requirements**
  6. **Ability to cause disease**

Bacteria are classified based on:

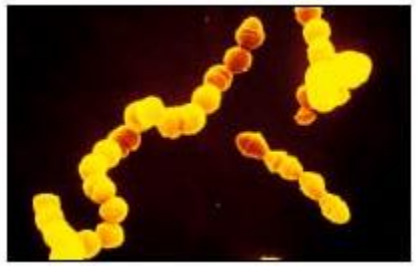
1. **Based on Shape: we have different shape of bacteria:**

- **Cocci** – spherical/ oval shaped major groups
- **Bacilli** – rod shaped,
- **Coccobacilli:** short rod
- **Comma shaped** –Vibrios
- **Rigid spiral forms** – Spirilla
- **Flexible spiral forms** – Spirochetes
- **Branching filamentous bacteria** – Actinomycetes
- **Mycoplasmas** – lack cell wall
- **Pleomorphic**: variable in shape

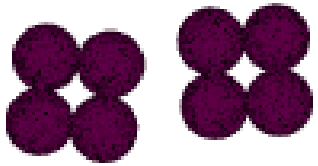
Arrangement of bacteria: Cocci

Coccus

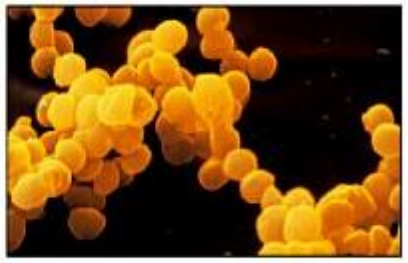
Cocci in pair –Diplococcus



Cocci in chain - Streptococci



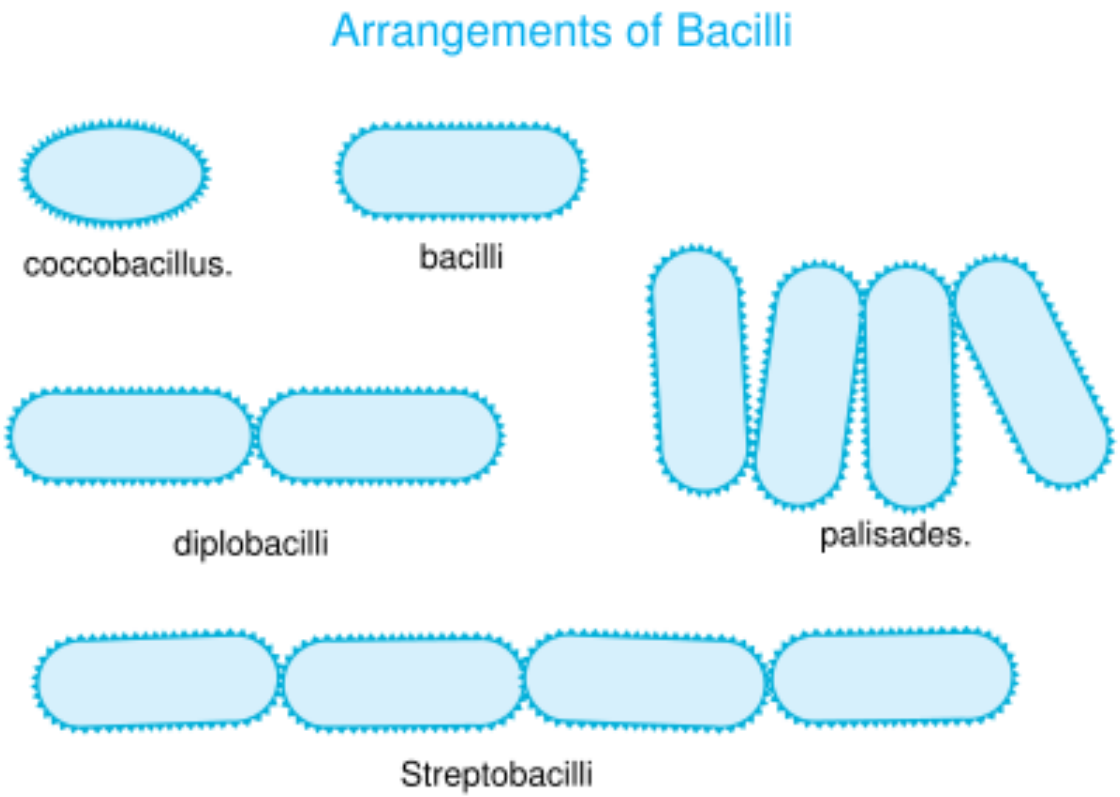
Tetrad – groups of four



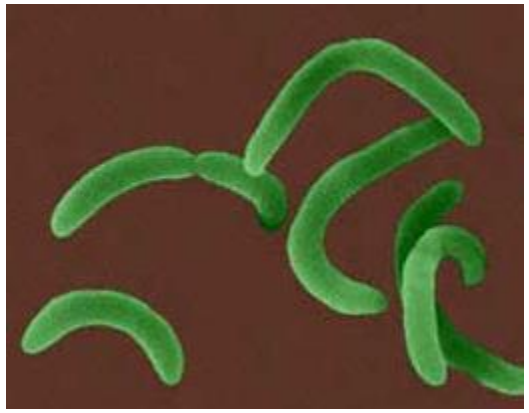
Cocci in cluster - Staphylococci

Sarcina – groups of eight

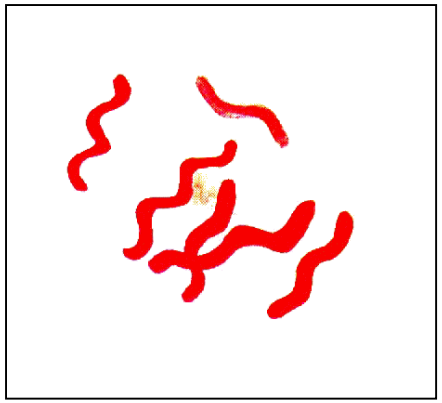
Arrangement of bacteria: Bacilli



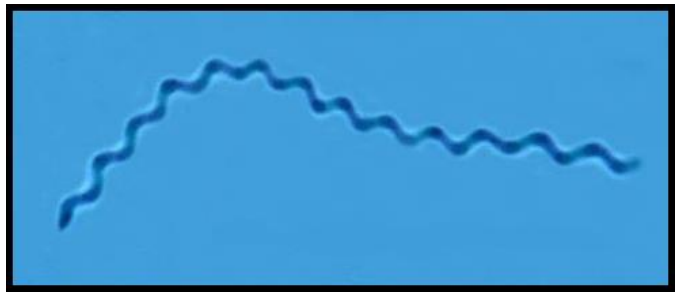
Other shapes of bacteria



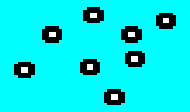
Comma shaped



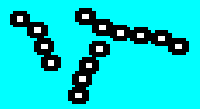
Spirilla



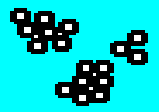
Spirochetes



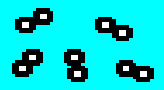
Coccus



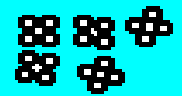
Streptococcus



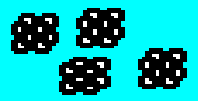
Staphylococcus



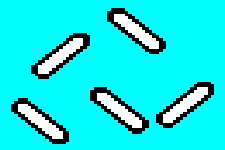
Diplococcus



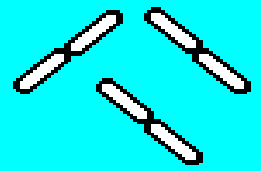
Tetrad



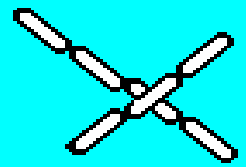
Sarcina



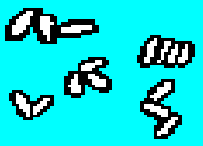
Bacillus



Diplobacillus

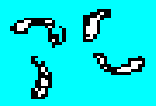


Streptobacillus

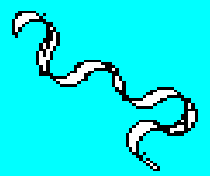


Coryneform Bacillus

Spirillum



Vibrio



Spirochete

Bacteria are classified based on…

2. Based on Cell wall Reaction to the Gram stain:

-Gram (+) bacteria

-Gram (-) bacteria

-Not Stainable with Gram stain Bacteria or stained irregularly with Gram stain

Bacteria are classified based on…

3.Based on Ability to form spores : **Spore forming and non spore forming bacteria**

4.Based on morphology and Ziehl- Neelsen stain : Acid fast bacilli and non acid fast bacilli

5. Based on Physiologic requirements:

-O2: strict aerobic, strict anaerobic & facultative anaerobic

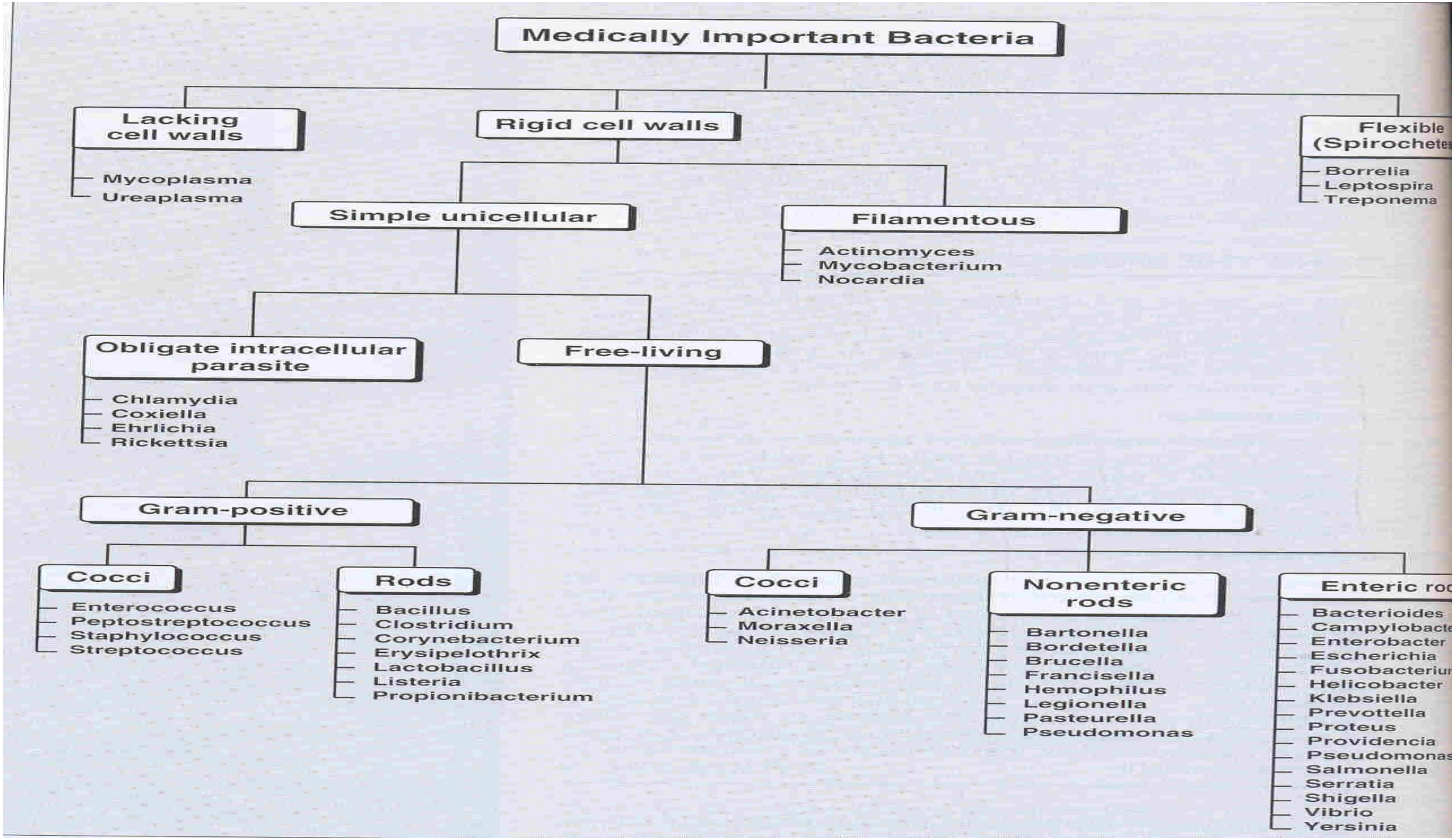
- temperature: Psychlophile, Mesophile & Thermophile

- PH: Neutrophile, Acidophile & Alkaliphile

-source of carbon: Chemoautotroph & Heterotroph

-salt loving: Halophile

6. Based on the ability to cause disease : Pathogenic & non pathogenic


Medically Important Bacteria
Lacking cell walls
Mycoplasma
Ureaplasma
Rigid cell walls
Flexible (Spirochetes
Borrelia
Leptospira
Treponema
Simple unicellular
Filamentous
Actinomyces
Mycobacterium
Nocardia
Obligate intracellular parasite
Chlamydia
Coxiella
Ehrlichia
Rickettsia
Free-living
Gram-positive
Gram-negative
Cocci
Enterococcus
Peptostreptococcus
Staphylococcus
Streptococcus
Rods
Bacillus
Clostridium
Corynebacterium
Erysipelothrix
Lactobacillus
Listeria
Propionibacterium
Cocci
Acinetobacter
Moraxella
Neisseria
Nonenteric rods
Bartonella
Bordetella
Brucella
Francisella
Hemophilus
Legionella
Pasteurella
Pseudomonas
Enteric roc
Bacterioides
Campylobacte
Enterobacter
Escherichia
Fusobacterium
Helicobacter
Klebsiella
Prevottella
Proteus
Providencia
Pseudomonas
Salmonella
Serratia
Shigella
Vibrio
Yersinia

Bacterial Taxonomy: Nomenclature

- Two kind of names are given to bacteria
  - **Casual / common name** – for local use, varies from country to country
    e.g. “typhoid bacillus”

  - **Scientific / International Name** – same all over the world, consists of two names (in Italics)

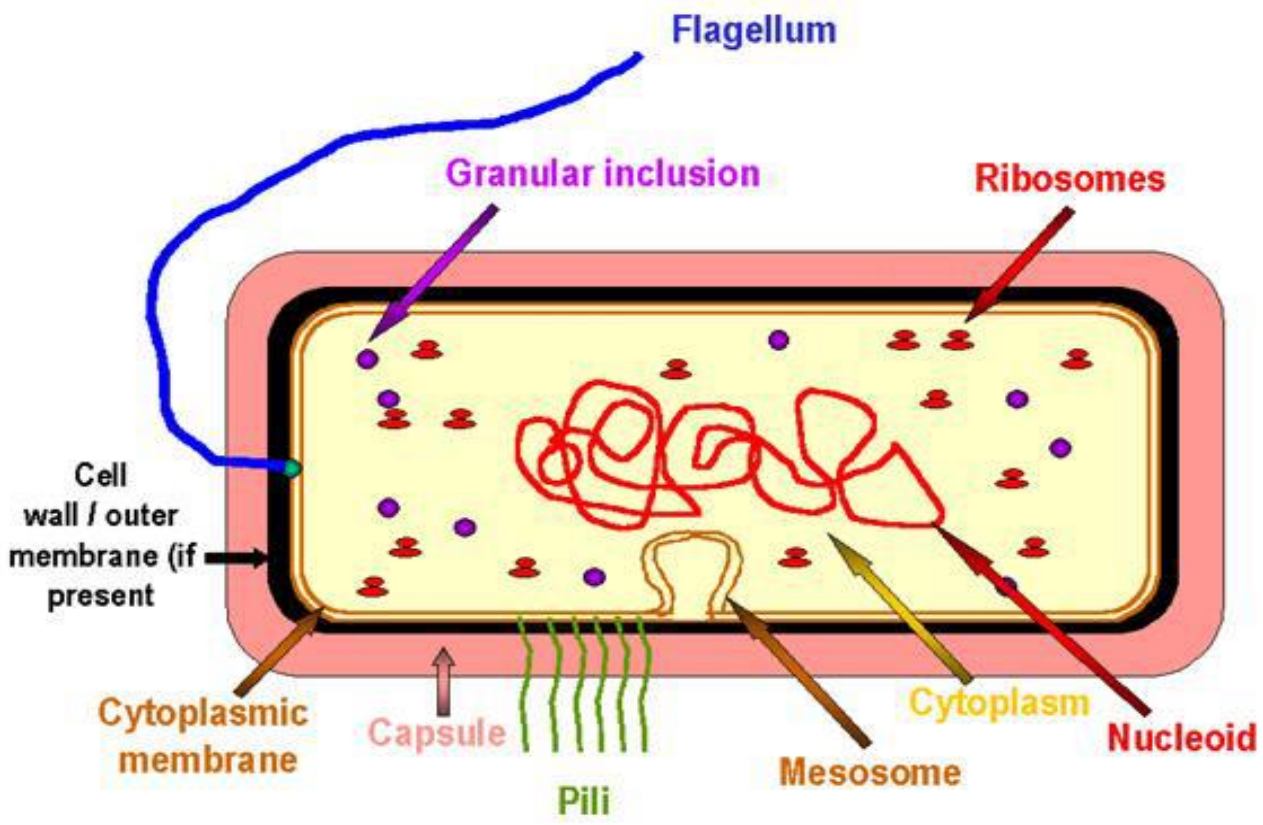
    Genus - noun, always capitalized

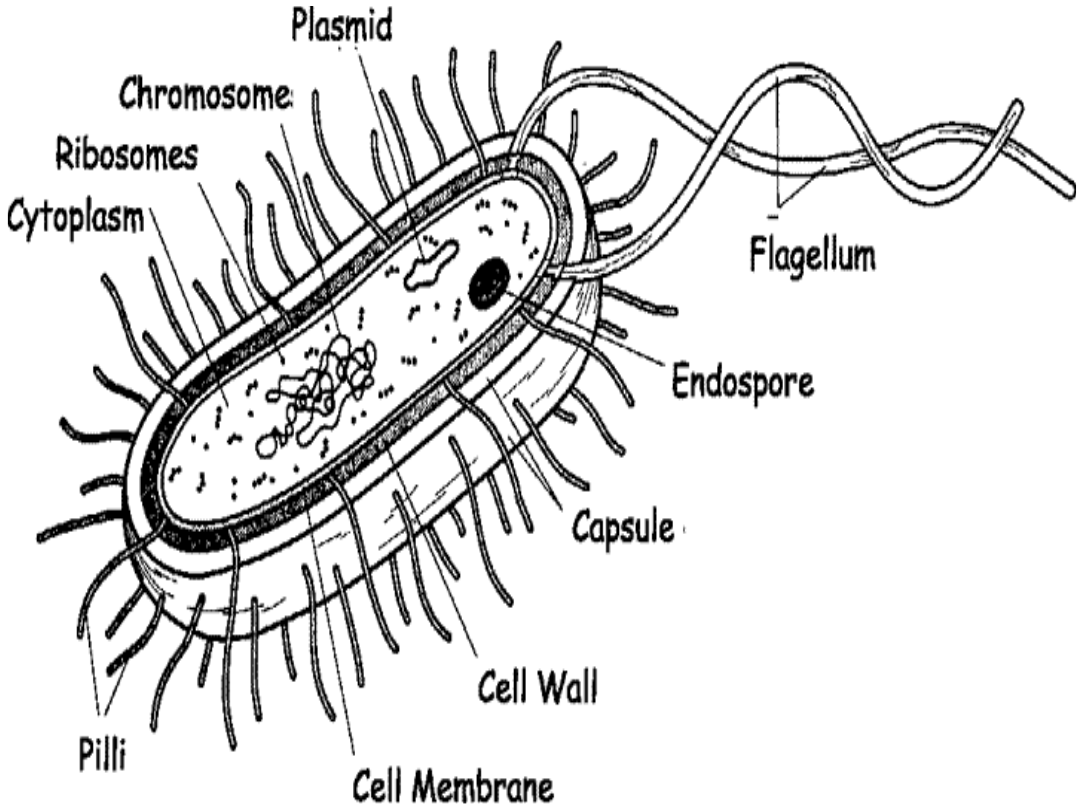
    species - adjective, lowercase

  - *E.g. Staphylococcus aureus (S. aureus)*
  - *Bacillus cereus (B. cereus)*
  - *Escherichia coli (E. coli)*

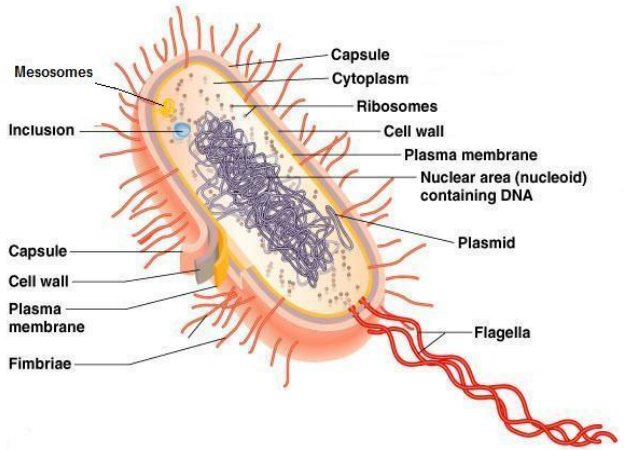
BACTERIAL ANATOMY

ANATOMY OF BACTERIAL CELL





Anatomy of a Bacterial Cell



- Outer layer – two components:
  1. Rigid cell wall
  2. Cytoplasmic (Cell/ Plasma) membrane – present beneath cell wall

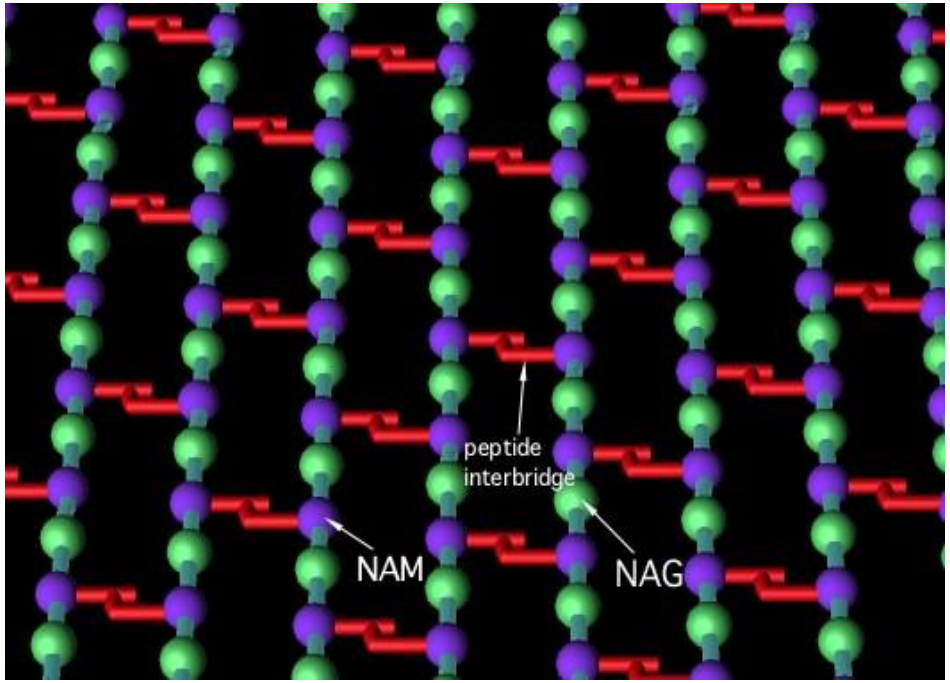
- Cytoplasm – cytoplasmic inclusions, ribosomes, mesosomes and nucleus

- Additional structures – plasmid, slime layer, capsule, flagella, Fimbriae (pili), spores

Structure & Function of bacteria Cell Components

CELL WALL

- Outermost layer, encloses cytoplasm
  1. Confers shape and rigidity
  2. 10 - 25 nm thick



3. Composed of complex polysaccharides (peptidoglycan/ mucopeptide) - formed by N acetyl glucosamine (NAG) & N acetyl muramic acid (NAM) alternating in chains, held by peptide chains.

Cell Wall

- Cell wall

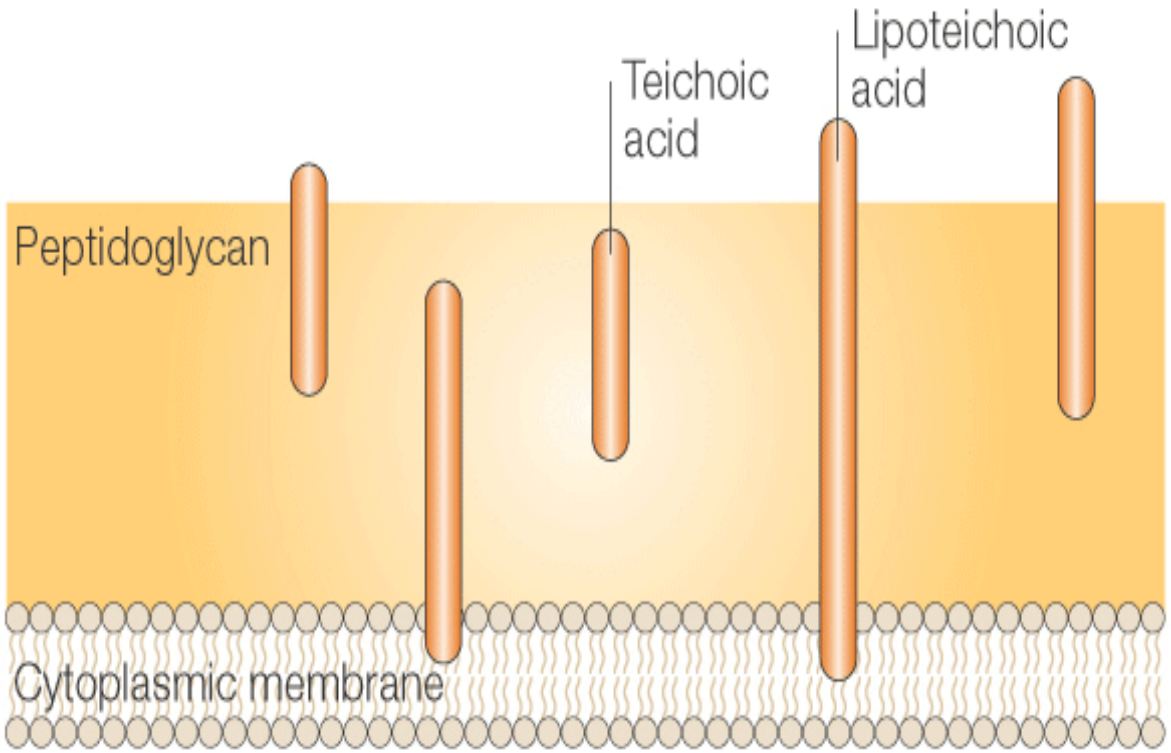
4. Carries bacterial antigens – important in virulence & immunity

5. Chemical nature of the cell wall helps to divide bacteria into two broad groups – **Gram positive & Gram negative**

4. **Gram +ve bacteria** have **simpler chemical nature** than **Gram –ve bacteria.**

5. Several antibiotics may interfere with cell wall synthesis e.g. Penicillin, Cephalosporins

Gram positive cell wall



G + bacteia cell wall is composed of:

a) Thick peptidoglycan layer (50-90% of cell wall material)

b)Teichoic acid

- mediate attachment to mucosal membranes, Induce septic shock in certain G+ve bacteria

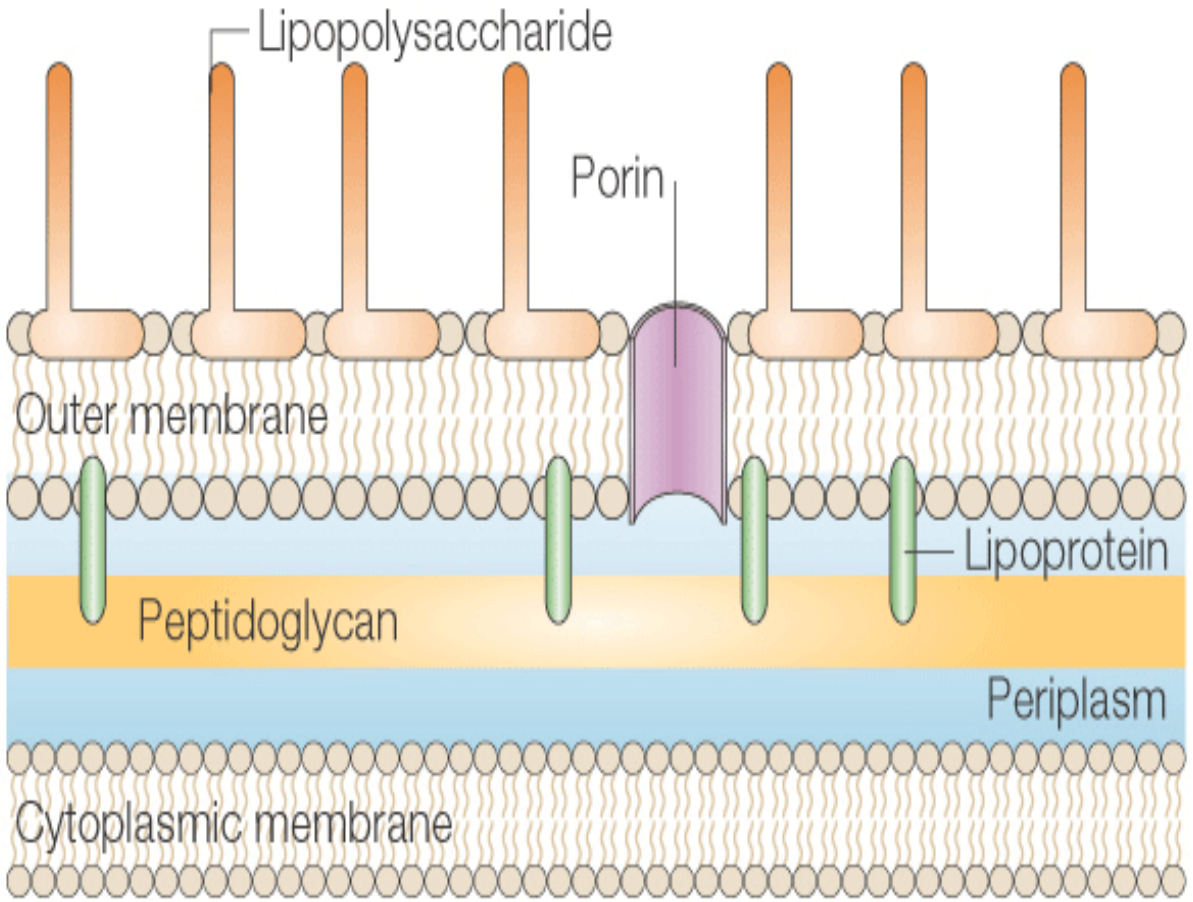
c) Lipoteichoic acid

- Anchor cell wall to cell membrane for epithelial cell adhesion

d) Polysaccharides and proteins

- protect peptidoglycan layer from action of agents such enzymes
- promote colonization by sticking the bacteria to the surface of host cells

Gram negative cell wall



a) Thin peptidoglycan layer 5-10% of cell wall material.

b) Lipoprotein anchors the outer membrane to peptidoglycan.

c) Outer membrane protects the cell from proteolytic enzymes.

d) Lipopolysaccharide (LPS)

•Major component is **Lipid A-** endotoxin

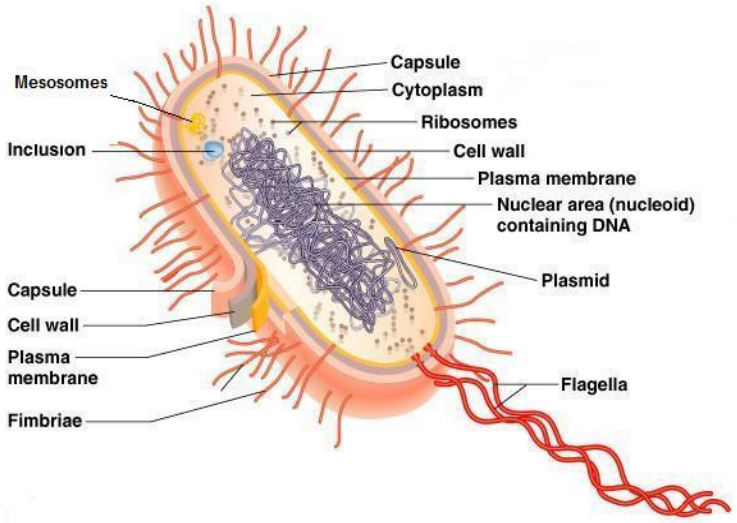
e)periplasmic space-contains digestive enzymes and other transport proteins.

f) **porin protein-** allow transport of solutes in and out of the cell

Summary of the differences between cell wall of Gram positive & Gram negative bacteria

Components	Gram positive	Gram negative
Peptidoglycan	Thicker; multilayer	Thinner; single layer
Teichoic acids	Yes	No
Lipopolysaccharide (endotoxin)	No	Yes
Outer membrane	No	Yes
Periplasmic space	No	Yes
Protein content	0%	9% e.g. Porin protein

Cytoplasmic (Plasma) membrane

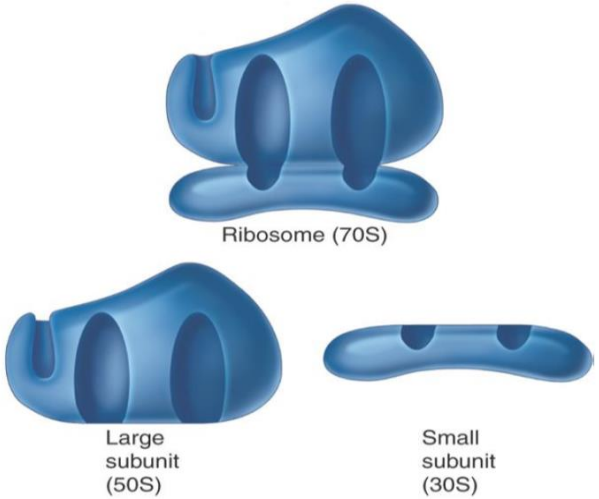


- Thin layer 5-10 nm, separates cell wall from cytoplasm
- Acts as a semi permeable membrane: controls the inflow and outflow of metabolites
- Composed of lipoproteins with small amounts of carbohydrates

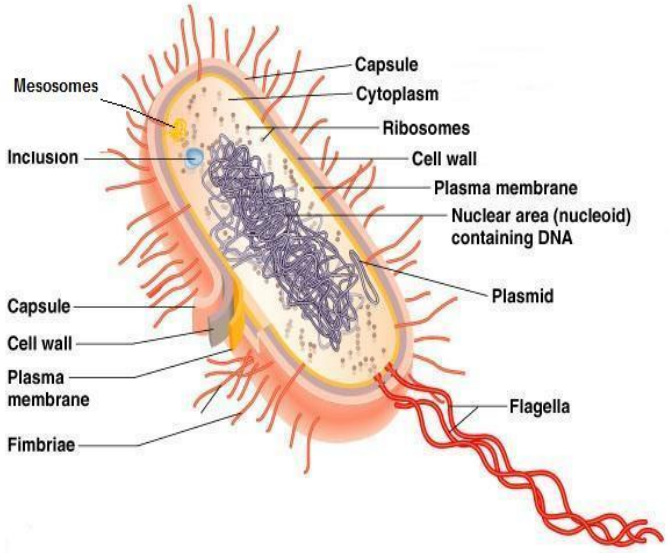
Other Cytoplasmic Components

Ribosomes –

- They are composed of **RNA and proteins.**
- Site of protein synthesis
- **Site of activity of antimicrobials** that disrupt protein synthesis
- **70S** in size with 50S and 30S subunits



Other Cytoplasmic Components..



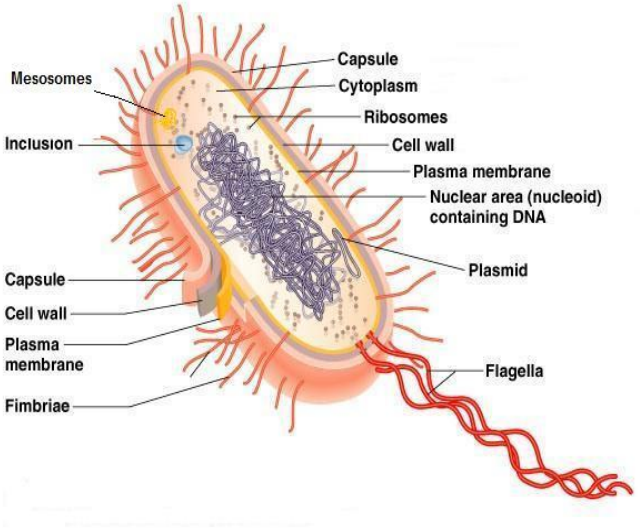
Mesosomes

- Multilaminated structures formed as invaginations
- of plasma membrane
- Principal sites of respiratory enzymes
- Coordinate nuclear & cytoplasmic division during binary fission
- More prominent in Gram +ve bacteria

cytoplasmic inclusions/nutrient granules

- **Function**; Serve as Storage for nutrients and energy for cell metabolism
- Composedof **volutin, lipid and polysaccharide**.
- Stain characteristically with certain dyes
- **Example;Volutin granules** are seen in ***Corynebacterium*** spp

Nucleoid



- Area of cytoplasm in which **DNA is located**
- Bacterial DNA consists of a single, circular double-stranded DNA, divides by binary fission.
- Lacks nuclear membrane (called nucleoid)
- Contains genetic material that codes for all genetic information expressed by the cell

Additional Organelles: Not all bacteria has them

1. **Plasmid** :
   - **Extra-nuclear DNA**
   - Transmitted to daughter cells during binary fission
   - May be transferred from one bacterium to another
   - Not essential for life of the cell
   - Confer certain properties e.g. drug resistance, toxicity
2. **Capsule & Slime layer**

a) Capsule – sharply defined structure surrounding the outside of the cell wall.

- antigenic in nature
- Protects bacteria from lytic enzymes
- **Inhibits phagocytosis**
- Stained by **negative staining** using **India Ink**

b) Slime layer – gelatinous polysaccharide attached loosely to the cell wall.

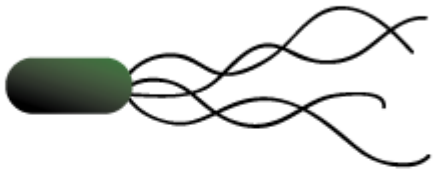
Additional Organelles

3. Flagella

- Long (3 to 12 µm), filamentous surface appendages
- **Organs of locomotion**
- Chemically, composed of proteins called flagellins
- The number and distribution of flagella on the bacterial surface are characteristic for a given species - hence are useful in identifying and classifying bacteria
- Flagella may serve as antigenic determinants (e.g. the H antigens of Gram-negative enteric bacteria)
- Presence shown by motility e.g. hanging drop preparation

Types of Flagellar arrangement

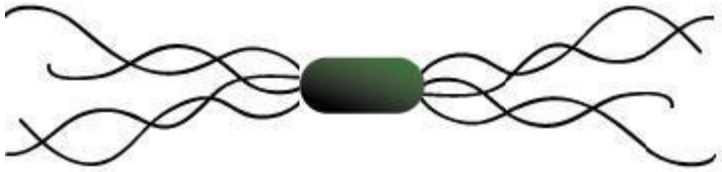
Polar/ Monotrichous – single flagellum at one pole



Lophotrichous – tuft of flagella at one pole

Amphitrichous – a single flagella at both poles

Peritrichous – flagella all over



Amphilophotrichous – tuft of flagella at both ends

Additional Organelles

4. Fimbriae/ Pili

- Thin, hairlike appendages on the surface of many Gram-negative bacteria
- 10-20µ long,
- organs of adhesion (attachment) - allowing bacteria to colonize environmental surfaces or cells and resist flushing
- Made up of proteins called pilins.

Pili can be of two types –

- Common pili – short & abundant
- **Sex pili** - small number (one to six), very long pili, helps in conjugation (process of transfer of DNA)

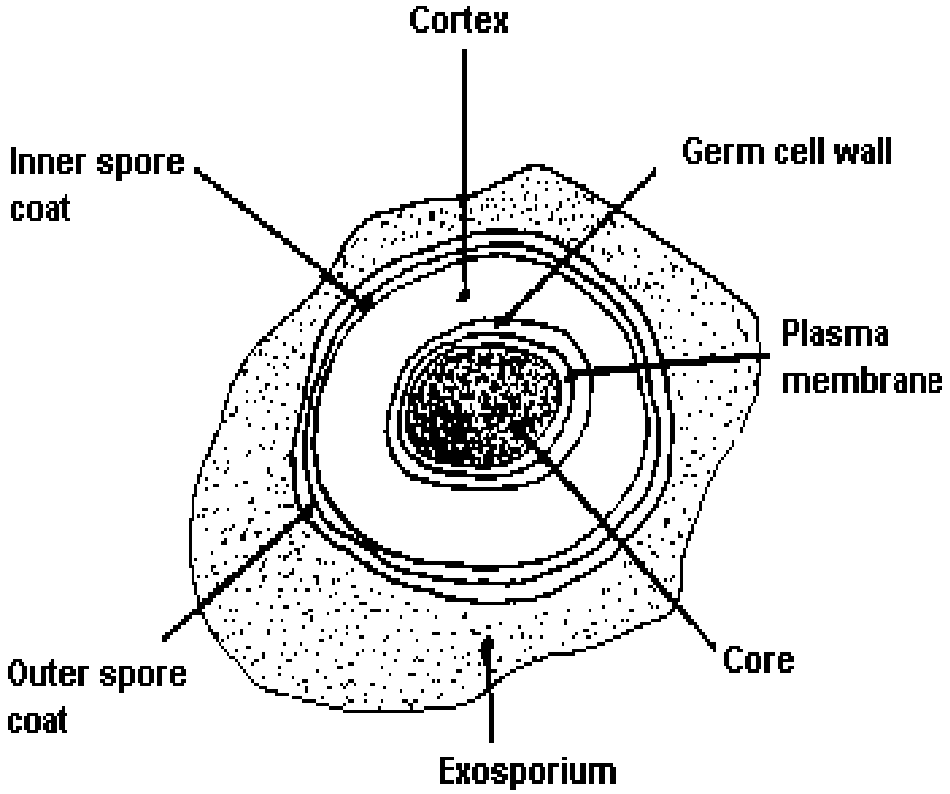
Additional Organelles

5.Spores

- Highly resistant stages formed during adverse environment (depletion of nutrients)

- They exhibit **no metabolic activity**
- **Formed inside the parent** cell, hence called Endospores

- Very resistant to heat, radiation and drying and can remain dormant for hundreds of years.
- Formed by bacteria like **Clostridia, bacillus**



BACTERIAL CELL GENETICS

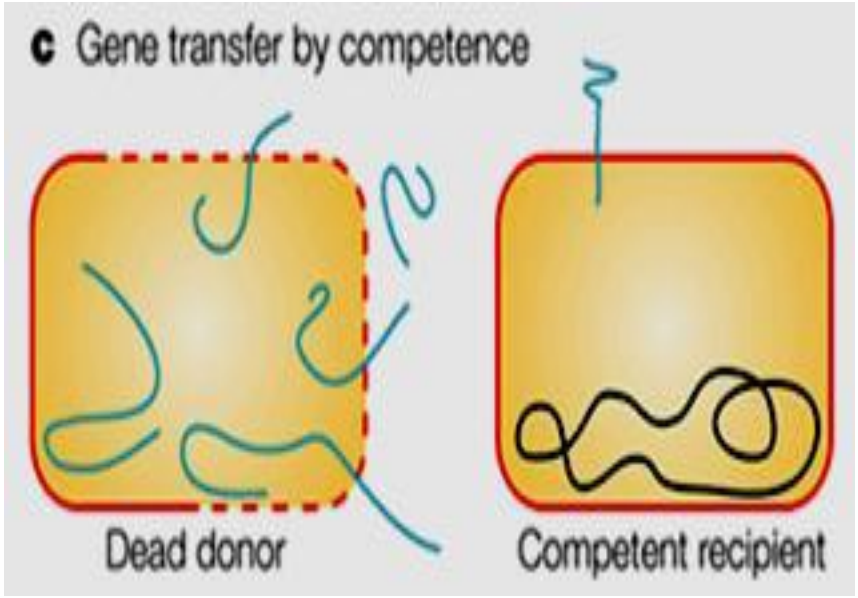
Bacteria Gene Transfer

- There are three types of gene transfer from one bacterium to the other leading to bacterial variation.
  - **Transformation**
  - **Transduction**
  - **Conjugation**.

Transformation

- This occurs when a recipient cell takes up a fragment of bacteria DNA present, free in the surrounding medium.
- This DNA fragment recombines with bacterial chromosomes which is transformed and the new genes are expressed leading **to change in the character of the recipient cell.**
- In transformation with "plasmids" DNA plasmid becomes re-established in the recipient cell and autonomously replicates

Transformation

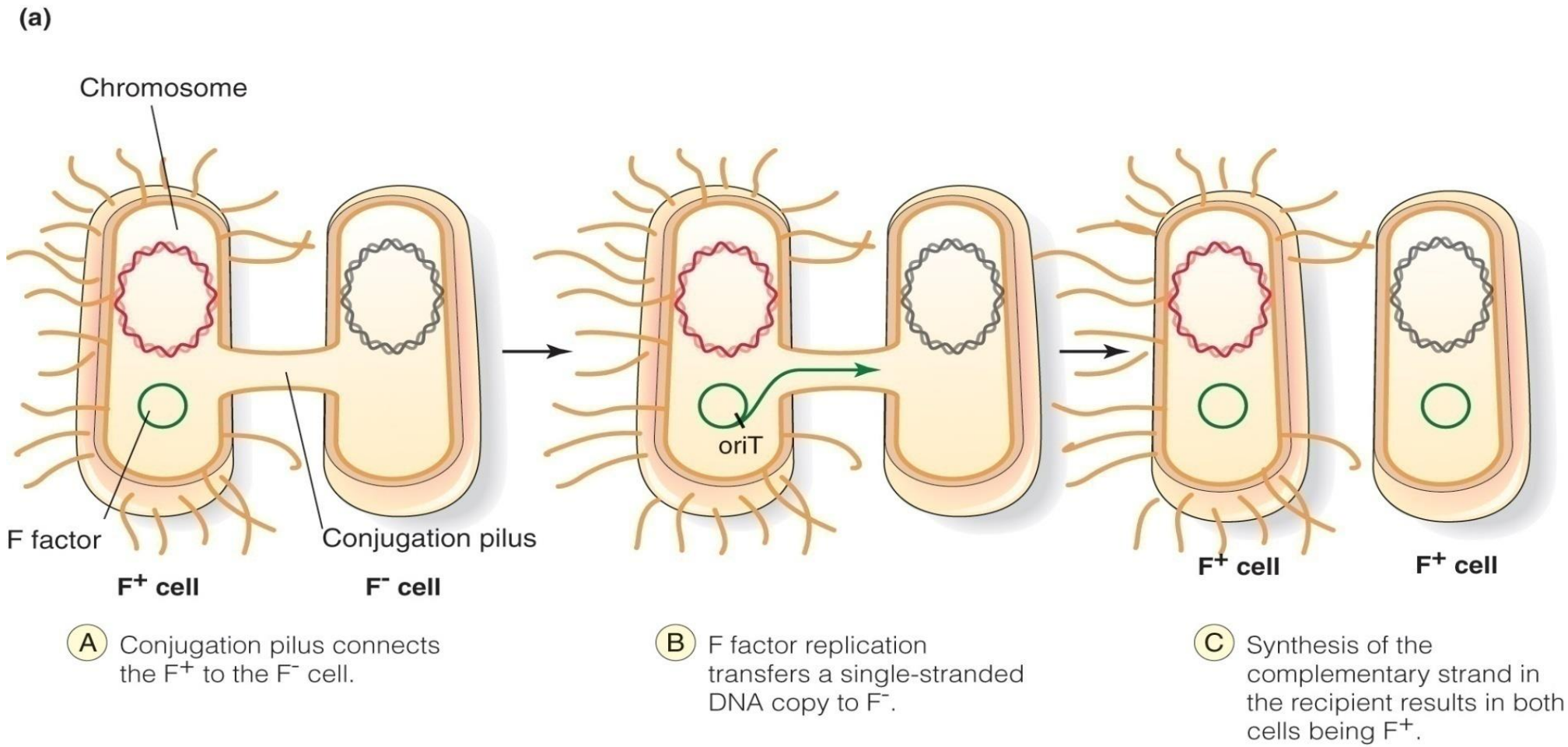


- **Significance:**
  - Transformation occurs in nature and it can lead to increased virulence.

Conjugation

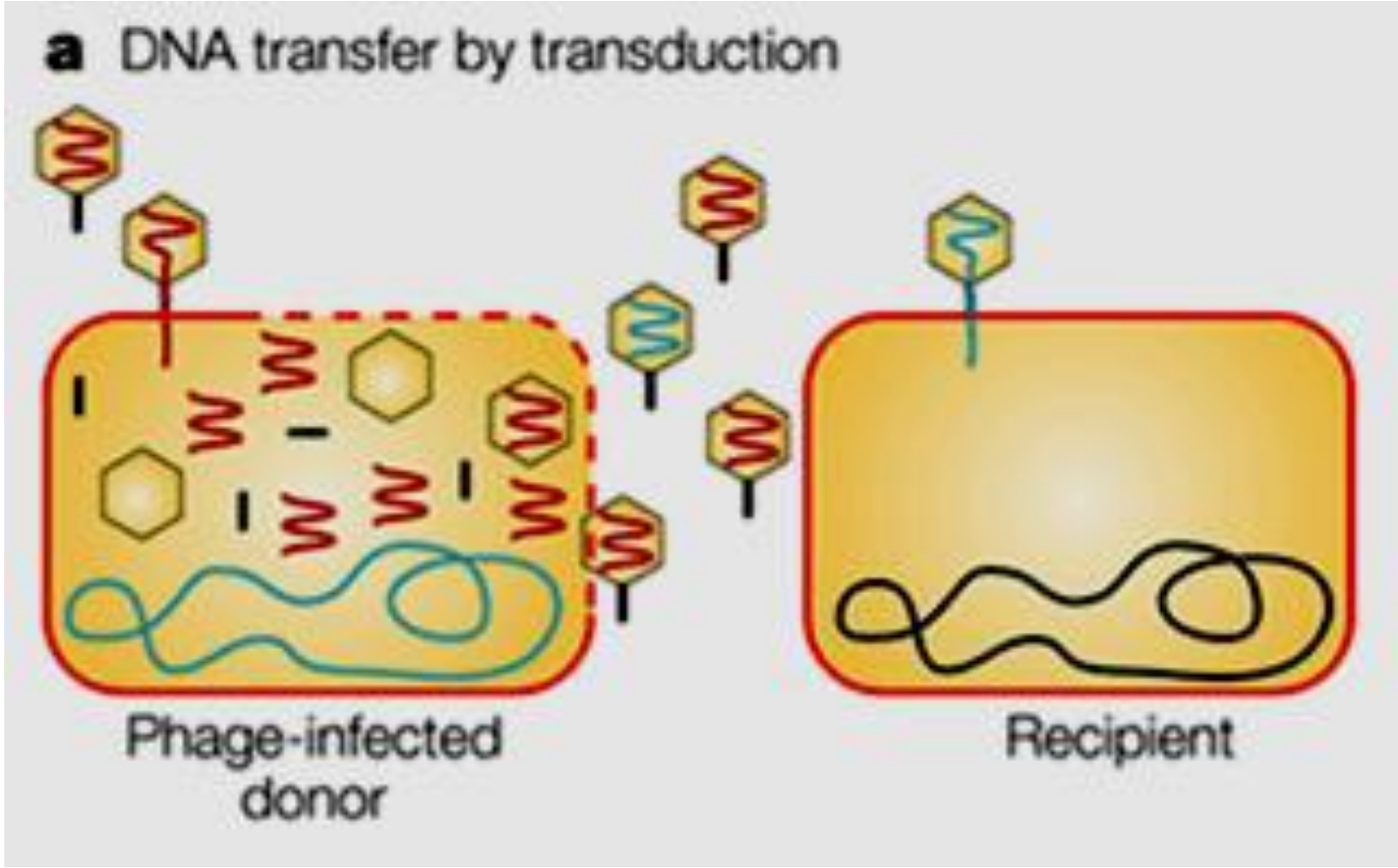
- This is the mating of two bacterial cells of the same or different species. e.g E.coli & P. aeruginosa during which DNA is transferred from the donor to the recipient cell via sex pilli.
- The process is controlled by an F (fertility factor) plasmid which carries the genes that code for formation of sex pilli which bridges between the 2 cells.
- As the two come in contact one strand of the plasmid separates and passes from F+ (donor) to F- (recipient cell which does not contain F plasmid.
- The process ends with an F+ recipient cell that acquired a plasmid copy

Conjugation



Transduction :

- Fragments of chromosome DNA can be transferred or transduced into the second bacterium by the “Bacteriophage”.
- **Transduction**: During the lytic cycle of phage replication, a piece of bacterial DNA becomes **accidentally enclosed within a phage** particle instead of a normal phage DNA.
- When this phage infects a second bacterium, the DNA from the first bacterium is released and recombines with the chromosome of the second bacterium.


a DNA transfer by transduction
Phage-infected donor
Recipient

Effect of Bacterial gene transfer

- Gene transfer can result in:
  - Antibiotic Resistance and/or
  - Increased virulence.

BACTERIAL PHYSIOLOGY

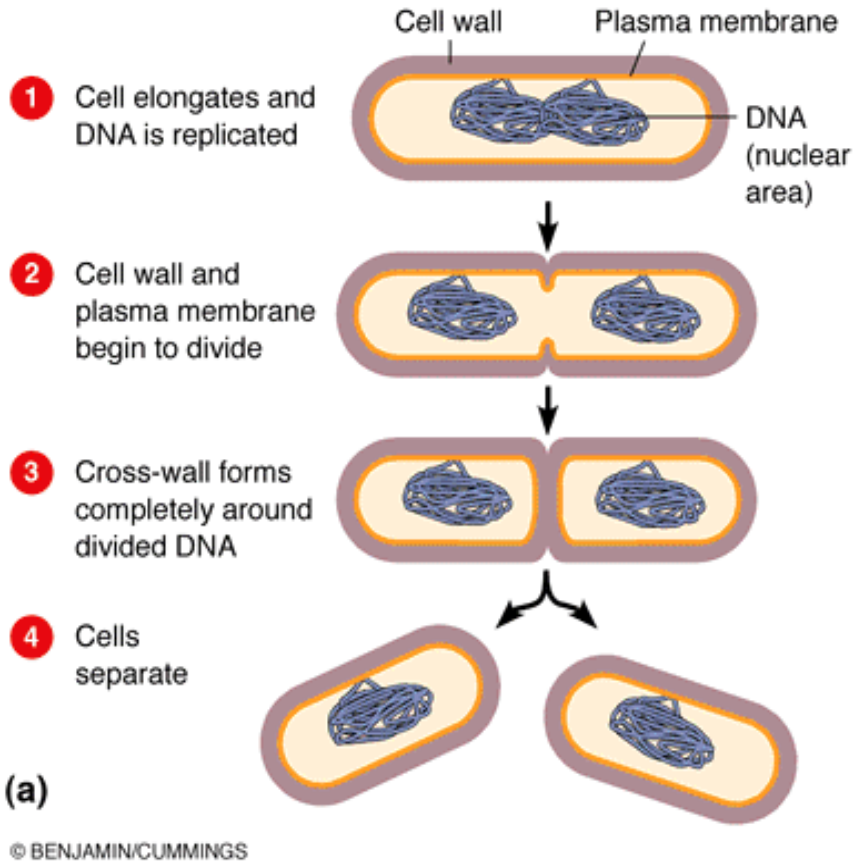
BACTERIAL PHYSIOLOGY

Definition:

- Study of how bacteria function including such processes as **nutrition, growth, reproduction and locomotion**

Growth and Proliferation of bacteria

- Bacteria growth, refers to an increase in **cell number**, **not in cell size**.
- Bacteria grow and divide by **binary fission**, a rapid and relatively simple process.



Growth of bacteria

Bacterial need some physical and chemical requirements for growth:

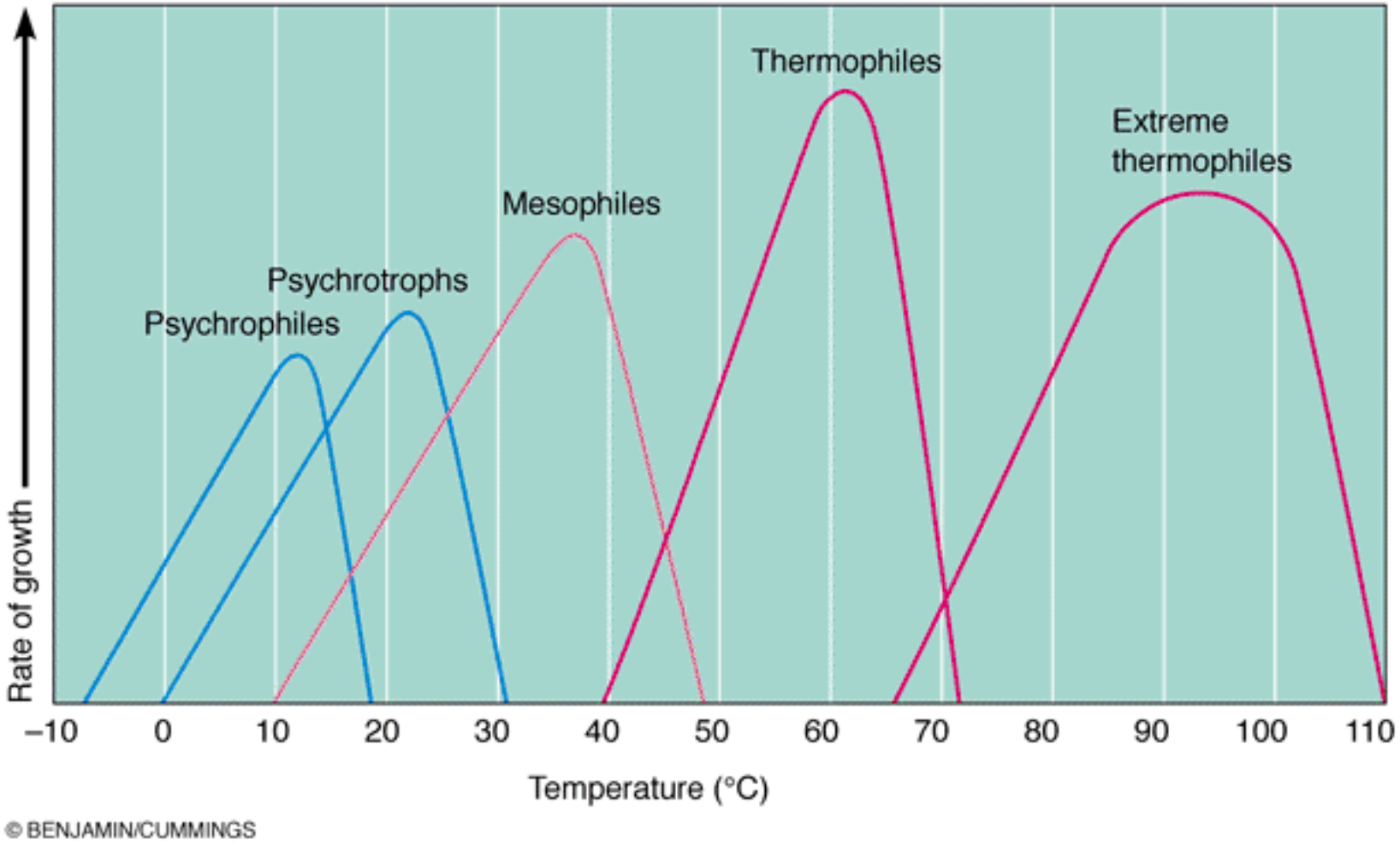
1. **Temperature**
2. **Oxygen**
3. **Nutrients**
4. **pH**
5. **moisture**

1.Temperature

Temperature – Classification

- Psychrophiles –low temp10-20°C
- Mesophiles –20-40°C
- Thermophiles –temp. > 40C

Growth Rates of Bacterial Groups at Different Temperatures



2.Bacterial O2 use

- O2 is reduced to H2O2 by enzymes;
- Bacteria require **two enzymes to utilize O2**
- **Superoxide dismutase** to catalyze the reaction: $2O_2^- + 2H \rightarrow H_2O_2 + O_2$
- **Catalase** to catalyze the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$
- **Strict anaerobes** lack one or both enzymes

O2 utilization

- **Strict (obligate) aerobes** -require O2 for growth e.g. *Pseudomonas aeruginosa*
- **Strict (obligate) anaerobes** -grow in the absence of O2 e.g. *Bacteroides fragilis*
- **Facultative anaerobes** –do not require O2 for growth but grow better in its presence e.g. *Staphylococcus* species

O2 utilization . . ..

–**Aerotolerant Anaerobes**: Can't use oxygen, but tolerate its presence. Can break down toxic forms of oxygen.

Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

- **Microaerophiles:** Require oxygen, but at low concentrations. Sensitive to toxic forms of oxygen.

  Example: *Campylobacter*.

Osmotic Pressure

- **Halophiles**: Require moderate to large salt concentrations. Ocean water contains 3.5% salt.
  - Most bacteria in oceans.

- **Extreme or Obligate Halophiles**: Require very high salt concentrations (20 to 30%).
  - Bacteria in Dead Sea, brine vats.

- **Facultative Halophiles**: Do not require high salt concentrations for growth, but tolerate 2% salt or more.

Carbon: Makes up 50% of dry weight of cell.

- Structural backbone of all organic compounds.
- **Chemoheterotrophs**: unable to synthesise their own metabolites & depend on preformed organic compounds. Obtain carbon from their energy source: lipids, proteins, and carbohydrates.
- **Chemoautotrophs and Photoautotrophs**: can synthesise all their organic compounds, able to utilise atmospheric CO_2 & N_2. No medical importance.

3.Other Requirements for growth

- **Macronutrients:** carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, iron
- **Micronutrients:** manganese, zinc, cobalt, nickel, copper
- **Growth factors:** Amino acids,Purines and pyrimidines, Vitamins

4.pH

- Neutrophiles (5.5 to 8） ex: Includes most human pathogens
- Acidophiles (below 5.5 ex: Lactobacillus
- Alkaliphiles (above 8.5 ex: *Vibrio cholerae*

Generation time

- The time required for a bacterium to give rise to 2 daughter cells under optimum conditions.

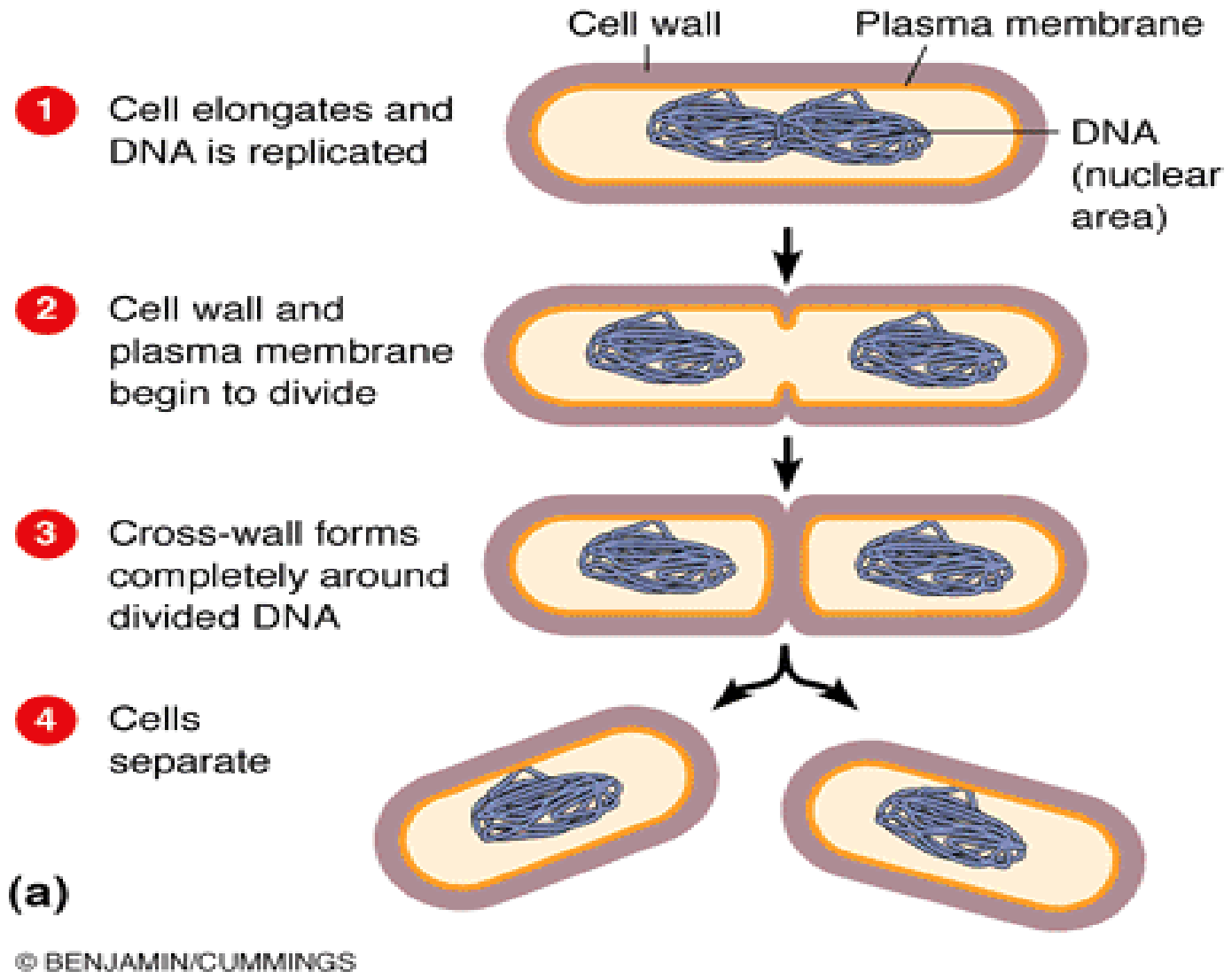
Examples

–*Escherichia coli*& other medically important bacteria –20 mins

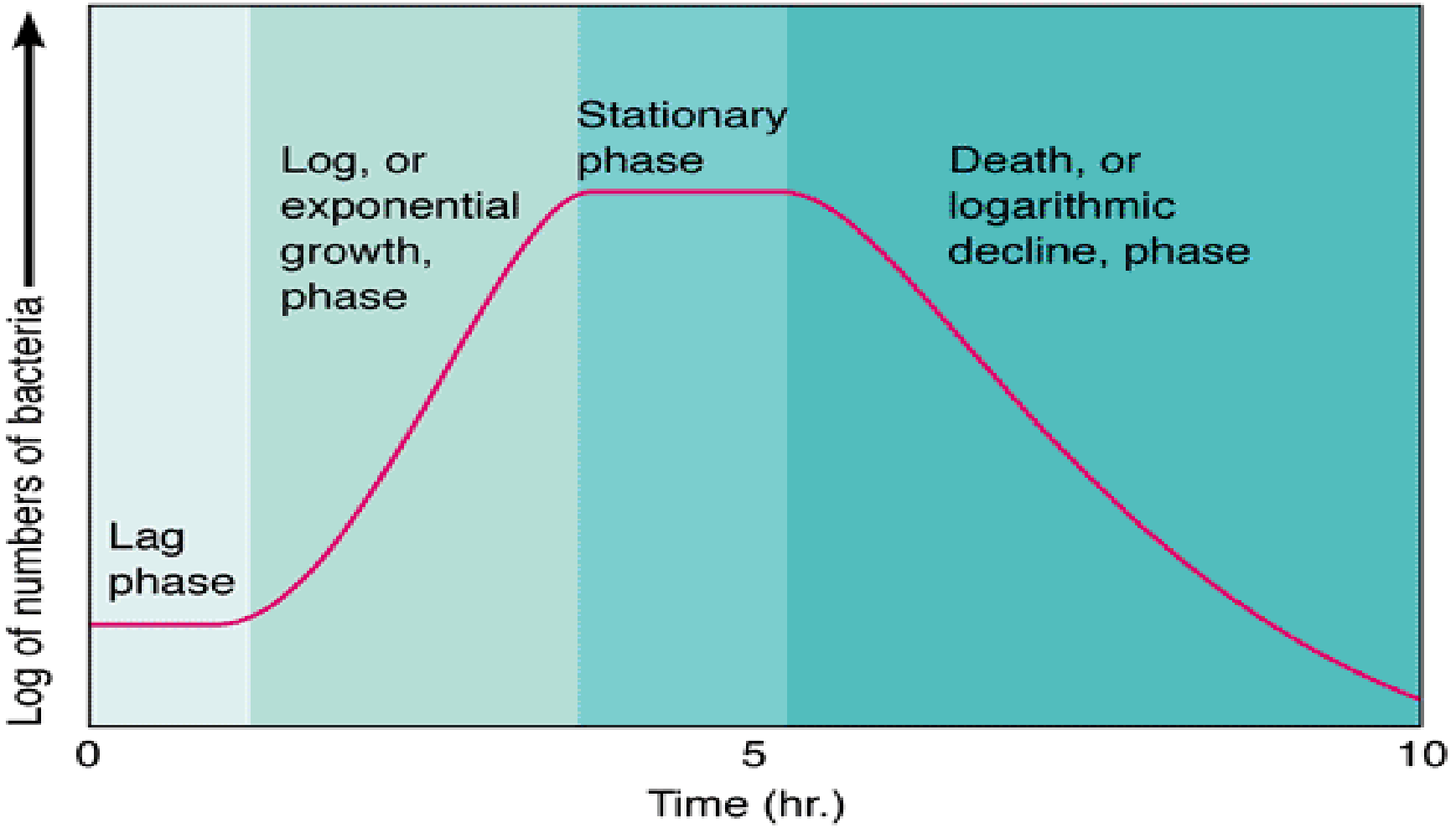
–Tubercle bacilli –20 hrs

–Leprae bacilli –20 days

Bacterial Growth: Binary Fission



Four Phases of Bacterial Growth Curve



Bacterial growth curve…

Lag phase

- The bacteria are adapting to the new environment
- No cell division
- Vigorous metabolic activity
- cells may increase in size during this time, but simply do not undergo binary fission

Bacterial growth curve…

Exponential/ Logarithmic phase

- Cells start dividing and their number increases exponentially
- Constant, maximal growth rate
- Increased rate of metabolism
- **Number of cells produced > Number of cells dying**

Bacterial growth curve…

Stationary phase

- Population size begins to stabilize.
- the death rate equals the growth rate

  Number of cells produced = Number of cells dying
- cell division stops due to depletion of nutrients & accumulation of toxic products
- Spore formation

Bacterial growth curve…

Decline/ Death phase

- Population size begins to decrease.

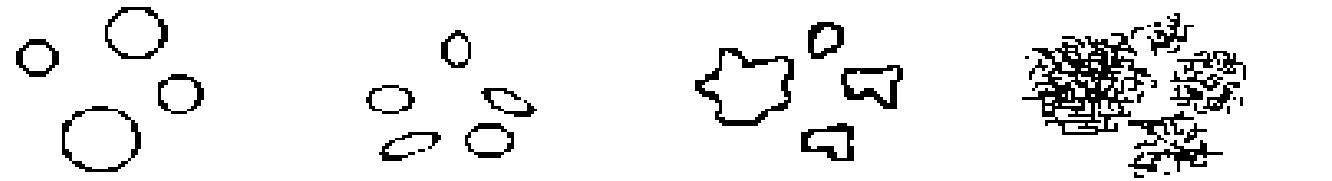
  Number of cells dying > Number of cells produced
- Loss of viability–cells die due to toxic products
- loss of selective permeability. Fluid gets into the cells causing cell lysis

How do we observe bacterial growth in lab?

- By observing colonial morphology on a solid media
- Or Turbidity in liquid media

Colonial morphology

Shape: refers to the overall shape of the colony



Margin: refers to the edge of the colony

Elevation: refers to the way the colony rises above the agar

Bacterial growth media

Why do we culture bacteria?

Bacterial Cultivation is usually required for a definitive identification and characterization of the etiologic agent (Gold standard)

- Isolate bacteria in pure cultures
- Demonstrate their properties
- Typing bacterial isolates
- Antibiotic sensitivity
- Maintain stock cultures,…

Con't..

Culture Medium: Nutrient material prepared for microbial growth in the laboratory.

- Primary ingredients required by all living organisms include:

  a **carbon source**, **water, minerals, and a nitrogen source**.

A culture medium:

- **Must be sterile**
- **Contain appropriate nutrients**
- **Must be incubated at appropriate temperature**

Classification of Media

- Culture media may be classified as:
  - **Synthetic** media (**Defined**)
  - **Complex** (Non-synthetic) media

- **Synthetic media:** contain only ingredients for which a complete **chemical formula is known**.

- **Complex media :**contain at least one ingredient for which a **chemical formula is not known** (such as milk, egg, malt, animal tissues)

Classification of Media - Consistency

- Solid media – contains solidifying agent 2% agar
- Colony morphology, pigmentation, hemolysis can be seen
- Eg: Blood agar

- Semi solid medium – 0.5% agar.
- Eg: Motility medium

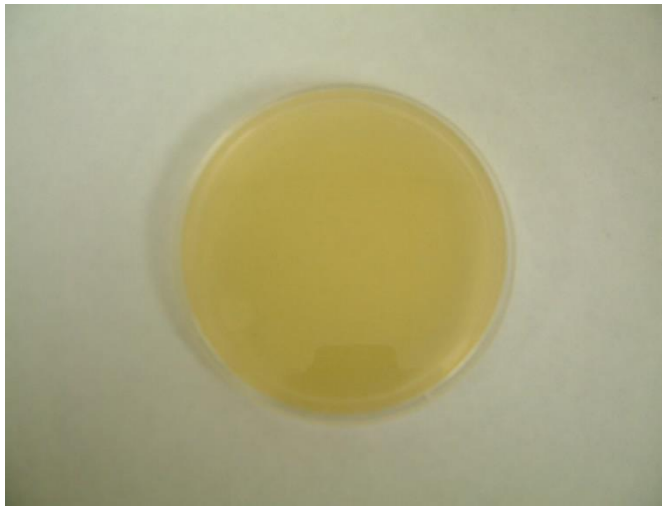
- Liquid media – no agar.
- For inoculum preparation, blood culture, for the isolation of pathogens from a mixture.Eg: Nutrient broth

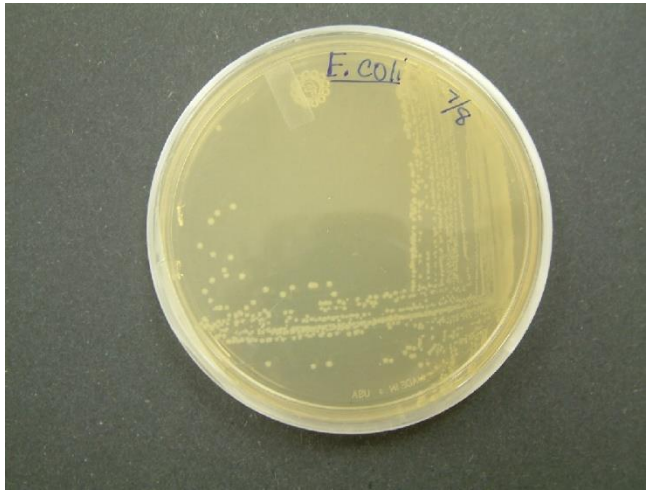
Classification of media – Function or property

- Simple media
- Enriched media
- Enrichment broth
- Selective media
- Differential media
- Transport media
- Storage media

Simple Media

- Simple or Basal media are those that may be used for growth of bacteria that do not need enrichment of the media.
- Examples: nutrient agar and peptone water.
- Staphylococcus and Enterobacteriaceae grow in these media.





Enriched Media

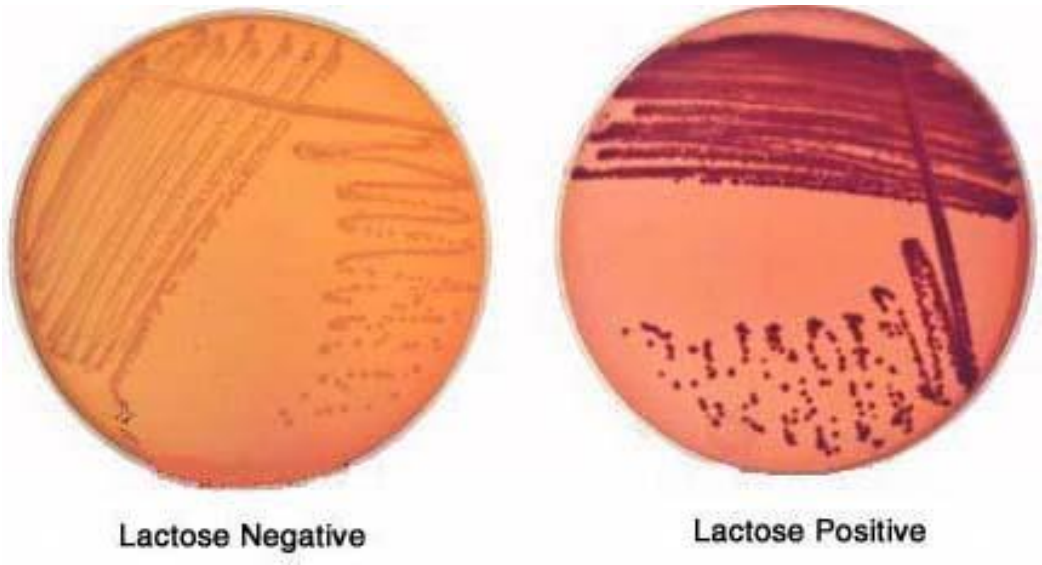
- The media are **enriched usually by adding blood, serum or egg to the basal medium.**
- Examples: blood agar
- **BA** :
  - is the most commonly used and support the growth of most fastidious organism,
  - used to determine haemolytic pattern

Enrichment Broth

- Contains substances which stimulates wanted bacteria & inhibits unwanted bacteria.
- For mixed cultures or materials containing more than one bacterium.
- e.g. **Selenite broth** : a medium for selective enrichment of salmonella from feces, food and sewage.

Differential Media

- To bring out differing characteristics of bacteria.
- e.g. Mac Conkey's agar: differentiate lactose fermenter from non lactose fermenter bacteria.

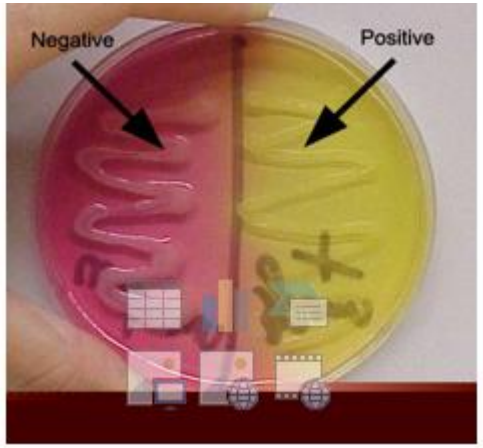


Selective Media

- These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria.

Examples:

- Mac Conkey agar: select enterobacteriaceae
- Mannital salt agar: select staphylococci,



Mannital positive on MSA

Transport Media

- These media are used when specimen cannot be cultured soon after collection.

Examples:

- Cary-Blair medium: **for stool ,**
- Stuart medium: **for gonococci samples.**

Storage Media

- Media used for storing the bacteria for a long period of time.
- Examples: Egg saline medium, chalk cooked meat broth.

Anaerobic Culture media

Using Culture Media containing reducing agent

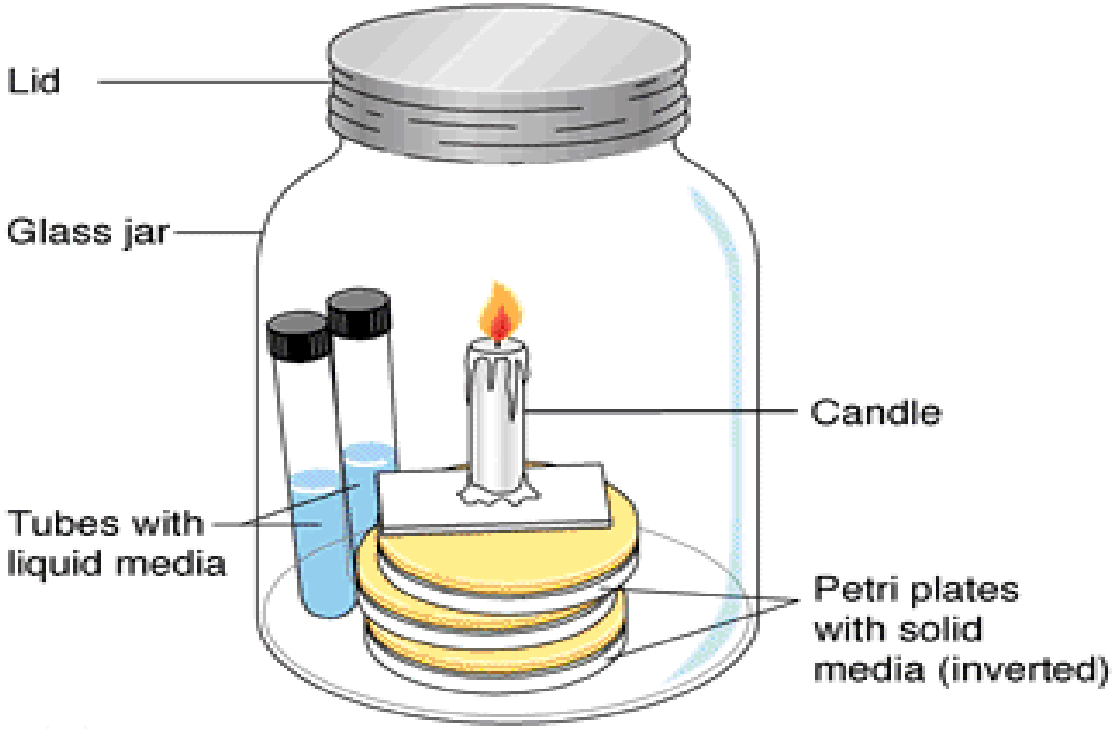
1.Thioglycollate broth

- It contains
  - Sodium thioglycollate (Reducing agent)
  - Rezazurin (redox indicator)
  - Low percentage of Agar-Agar to increase viscosity of medium

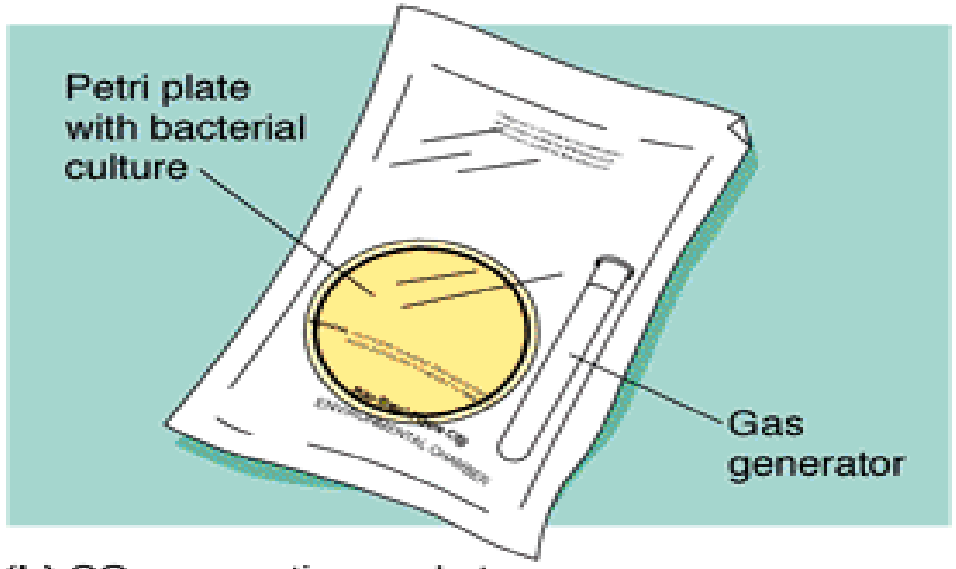
2.Robertson Cooked Meat Medium

- It contains
  - **Meat particles** (prepared from heart muscles) which contain hematin & glutathione that act as reducing agent

Equipment for Producing CO_2 Rich Environments



(a) Candle jar



(b) CO_2-generating packet

Media preparation - Agar

- Used for preparing solid medium
- No nutritive value and is not affected by the growth of the bacteria.
- Melts at 98^{o}C & sets at 42^{o}C

- The basic ingredients of media include **carbon source, nitrogen source, inorganic salt, growth factor and distilled water.**

Media preparation

1. Weigh the required amount of powder needed to dissolve in distilled water
2. Dissolve the powder using the stirring rod,

3. To dissolve the powder completely, (heat mixing is required)
   e.g. place in water bath

4. Sterilize in autoclave at 121^{o}C for 15 minutes
5. Let the media cool and dispenser in petri dish

STAINING TECHNIQUES IN BACTERIOLOGY

WHAT IS A STAIN

- **A stain** is dye that binds to a cellular structure and gives it color.
- **Staining** is an auxiliary technique used in microscopy to enhance contrast in the microscopic image.

WHY DO WE STAIN BACTERIA

- We need to stain bacteria for better contrast,
- Unstained bacterial cell are refractive and difficult to visualize clearly under the light microscopy.
- To observe the morphology, size, and arrangement of bacteria.
- To differentiate one group of bacteria from the other group.

Bacterial staining

- **Bacterial staining** is the process of coloring bacterial structural components using stains (dyes).
- Individual variation in the cell wall constituents among different groups of bacteria will consequently produce variations in colors during microscopic examination.
- **Nucleus is acidic in character** and hence, it has greater affinity for basic dyes. Whereas,
- **cytoplasm is basic in character** and has greater affinity for acidic dyes.

Types of microbiological stains

1. Basic stains
2. Acidic stains
3. Neutral stains

- **Basic stains** are stains in which the coloring substance is contained in the base part of the stain. The acidic part is colorless. **Eg. hematoxyline**

- **Acidic stains** are stains in which the coloring substance is contained in the acidic part of the stain. The base part is colorless. It is not commonly used in microbiology laboratory.

  Eg. Eosin stain

- **Neutral stains** are stains in which the acidic and basic components of stain are colored. Neutral dyes stain both nucleic acid and cytoplasm. **Eg. Giemsa stain**

NB: This classification is not based on PH of stains.

STAINING PROCEDURE

- **Making smear**: on slide and let it air dry
- **Fixation**: by heat to kill,preserve the shape of the cells, adhere on slides.
- **Staining**.

Types of staining methods

1. Simple staining method

2. Differential staining method

3. Special staining method

1. Simple staining method

1. Simple staining: utilize one type of stain which react with all microbes in identical fashion.

- They are useful solely for increasing contrast so that morphology, size and arrangement of organisms can be determined.

Two kinds of simple stains:

- **Positive staining**: The bacteria or its parts are stained by the dye.

  e.g. Carbol fuchsin stain, Methylene blue stain, Crystal violet stain.

- **Negative staining :**The dye stains the background and the bacteria remain unstained **e.g india ink**

1. Simple staining method..

Procedure of Simple staining :

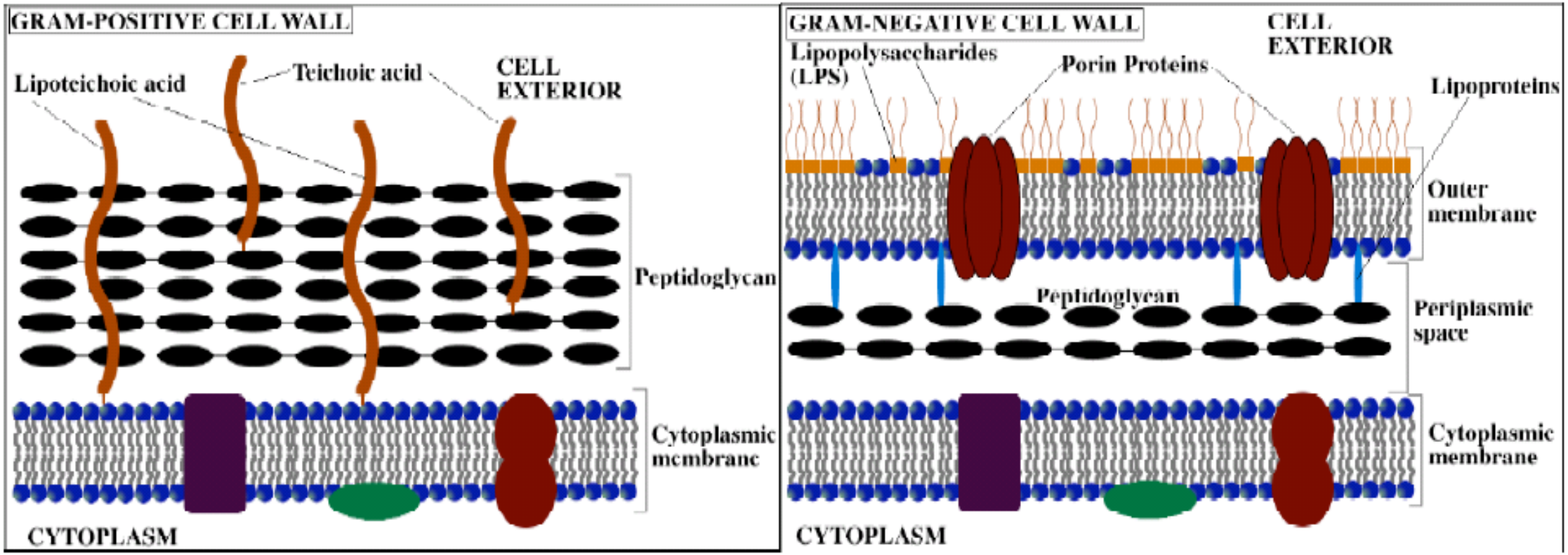
- Make a smear and label it.
- Allow the smear to dry in air.
- Fix the smear over a flame.
- Apply a few drops of positive simple stain like
  - 1% methylene blue,
  - 1% carbolfuchsin or
  - 1% gentian violet for 1 minute.
- Wash off the stain with water.
- Air-dry and examine under the oil immersion objective.

2. Differential staining method

➢ **Differential staining :** utilize more than one reagent and involve permeability of the cell wall and entry of the stain into the cell

- Grams staining
- Ziehl- Neelsen staining
- Auramine o stain

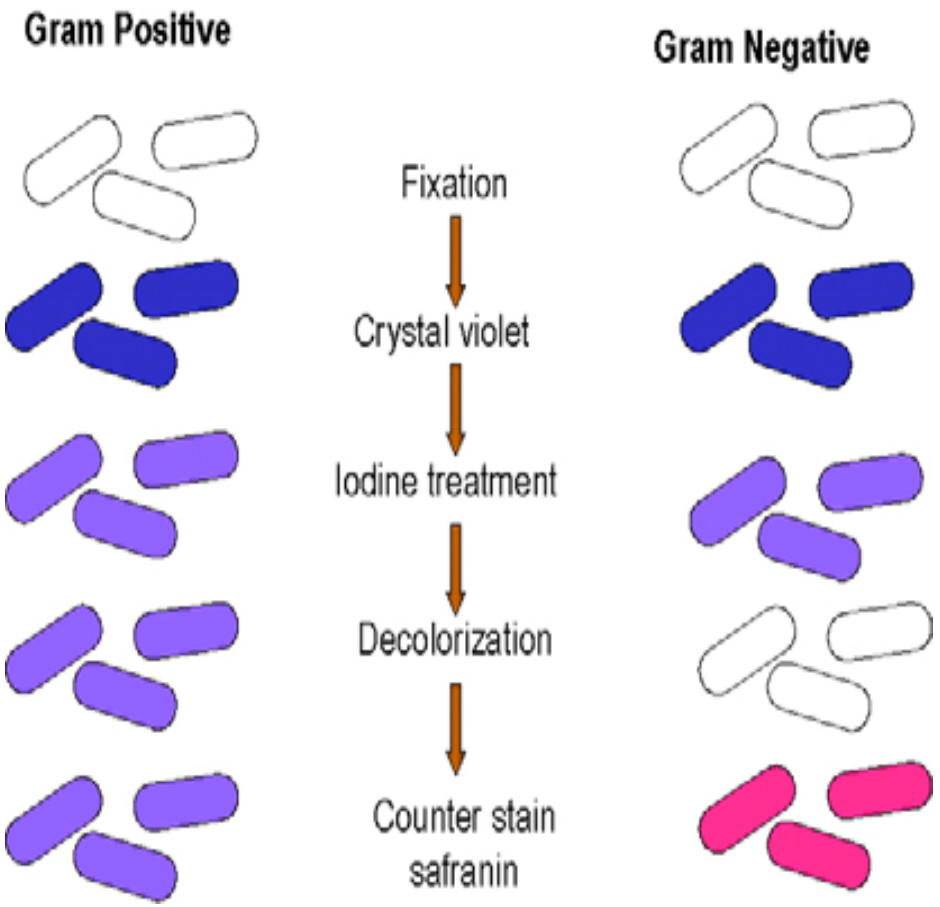
CELL WALL STRUCTURE



GRAM STAINING

- Introduced by Hans Christian Gram
- Used to classify bacteria on the basis of their cellular **morphologies, sizes and forms.**
- Permits the separation of all bacteria into two large groups, bacteria that retain the primary stain (Gram positive) and those that take the counter stain (Gram negative).

PROCEDURES & REAGENTS



Reagents:

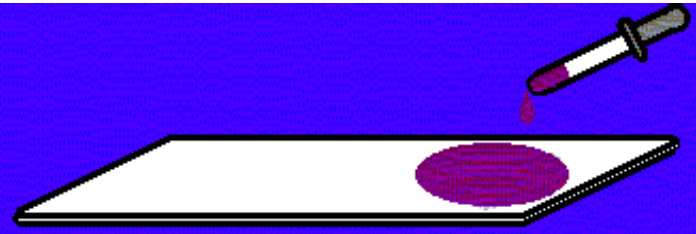
- **Primary Stain:** Crystal Violet 1min
- **Mordant:** Gram Iodine 1min
- **Decolorizer**: Ethyl Alcohol 30 sec
- **Counterstain:** Safranin Red 1min

Interpretation:

- Gram-positive bacterium …**Purple**
- Gram-negative bacterium ...**Pink**

- **Shape: Cocci or bacilli**

1. Prepare a heat-fixed bacterial smear.



2. Cover the smear with crystal violet for 60 seconds, then rinse with water.

3. Cover the smear with Gram's iodine for 30 seconds, then rinse with water.

4. Briefly decolorize the smear with acetone-alcohol, then rinse with water.

5. Cover the smear with saffranin for 60 seconds, then rinse with water and dry.

6. Observe the dried smear with the microscope, using oil-immersion.

➢PRINCIPLE

- Gram positive cell wall takes up the crystal violet and when followed by a mordant (iodine), it forms a crystal violet complex which is not removed by decolorization step.

- Gram negative since their cell wall is inside the outer membrane, it cannot form a complex with the crystal violet even when there is a mordant, the primary stain stays on the outer membrane which is then washed by the alcohol allowing the safranin red to counter stain it.

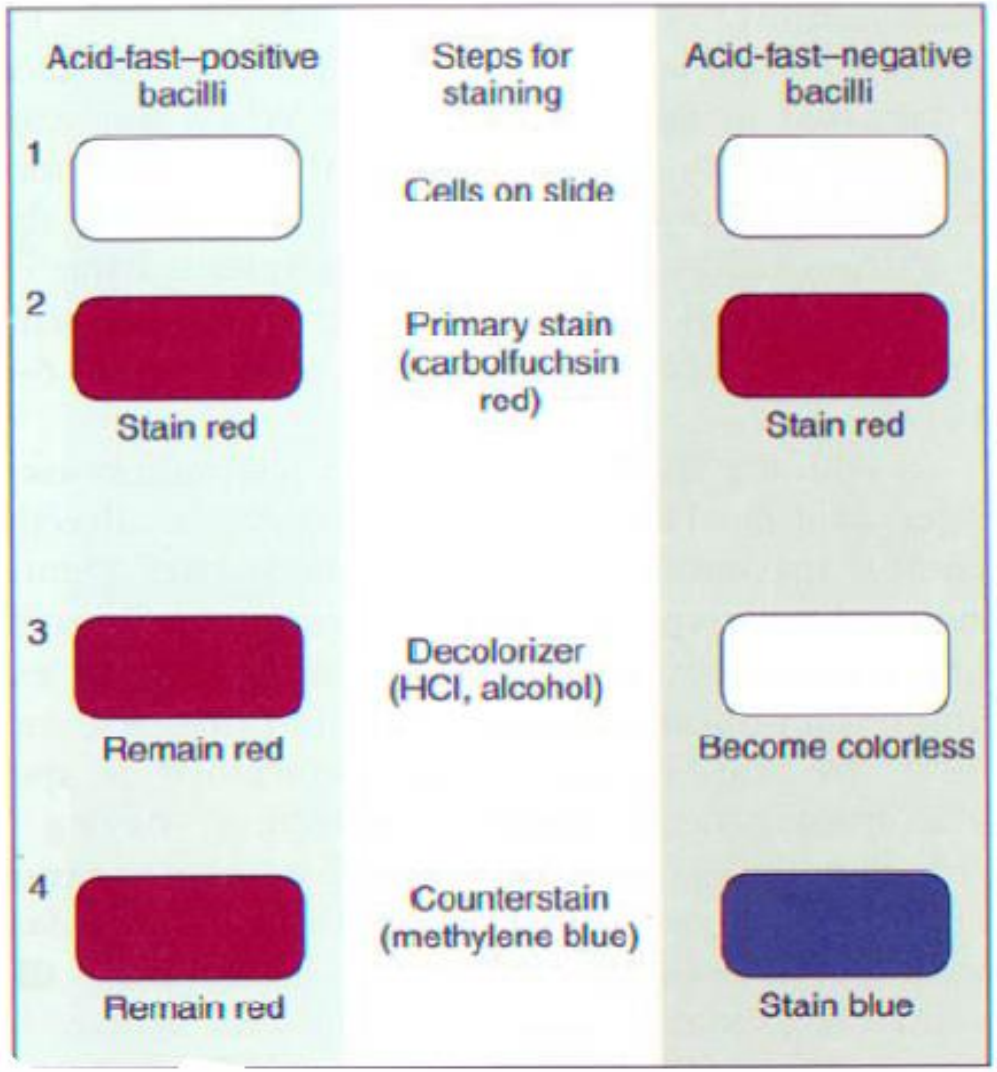
ACID FAST STAINING : ZN

Principle

- The lipid capsule of the **acid fast bacteria** contains mycolic acid (long chain fatty acid) which is responsible for its waxy characteristic that resists the penetration of an aqueous based solution (i.e. Crystal violet), instead it takes up the carbolfuchsin and resists decolorization with an acid alcohol rinse.

- Acid fast bacteria are usually members of the genus **Mycobacterium** or **Nocardia** and are stained by **ZN**.

PROCEDURES



1.Make a smear. Air Dry. Heat Fix.

2.Flood smear with **Carbol Fuchsin stain**

3.Steam for 10 minutes. Add more Carbol Fuchsin stain as needed

4.Cool slide and rinse with water

5.Flood slide with **acid alcohol 15 seconds**.

6.Rinse with water

7.counter stain with **Methylene Blue/malachite green stain** for 1 minute

8.Rinse slide. Blot dry.

9.Use oil immersion objective to view.

Interpretation:

- Acid fast bacilli……..Red
- Back ground……Blue/green

- **Reporting system**
- 0 AFB/100 field ……No AFB seen
- 1-10 AFB/100 field……1+
- 11-100AFB/100 field……2+
- 1-10 AFB/field…… ……3+

NB: AFB means number of acid fast bacilli

Fluorochrome acid-fast stain : AURAMINE O STAIN

This fluorochrome staining method is used to enhance the detection of mycobacteria using fluorescent microscopy.

PRINCIPLE

- Acid-fast mycobacteria **resist decolorization by acid-alcohol** after primary staining owing to the high lipid (mycolic acid) content in their cell walls.

- The identification of mycobacteria with a uramine O is due to the affinity of the mycolic acid in the cell walls for the fluorochromes.
- The dye will bind to the mycobacteria, which appear as bright yellow, luminous rods against a dark background.

Fluorochrome acid-fast stain

Reagents:

- **i. Auramine O**
- **ii. 0.5% Acid-alcohol**
- **iii. Counterstain (potassium permanganate or acridine orange)**

PROCEDURE

1. Flood the slide with fluorochromes stain Auramine O for 15 min.
2. Flood with **0.5% acid-alcohol Decolorizer for 30-60 s**. (some protocols call for 2 min.)
3. Flood the slide with Counterstain potassium permanganate or acridine orange for 2 min
4. Air dry and Examine the smear with a fluorescent microscope

NOTE: Timing is critical during the counterstaining step with potassium permanganate. Counterstaining for a longer time may quench the fluorescence of acid fast organisms.

3. Special stains method

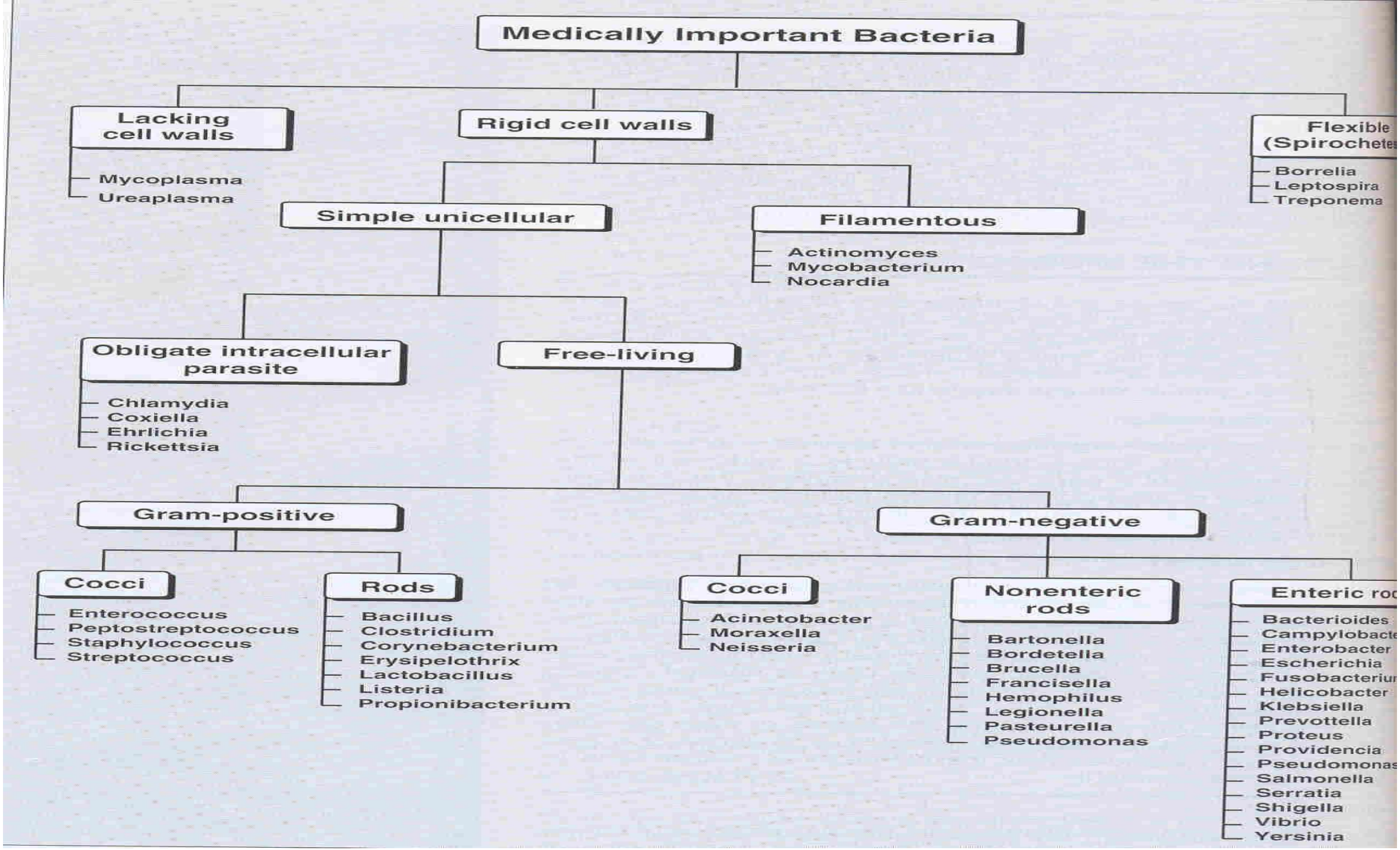
- **Cytoplasmic inclusion stains**: identifies intracellular deposits of starch, glycogen, polyphosphates.

Eg; **Albert staining** is used to stain metachromatic granules of *Corynebacterium diphteria*.

(bacillus stains green with Albert's stain whereas the granules stain bluish black)

- **Spore staining method**

- **Capsule staining method**


Medically Important Bacteria
Lacking cell walls
Mycoplasma
Ureaplasma
Rigid cell walls
Flexible (Spirochetes
Borrelia
Leptospira
Treponema
Simple unicellular
Filamentous
Actinomyces
Mycobacterium
Nocardia
Obligate intracellular parasite
Chlamydia
Coxiella
Ehrlichia
Rickettsia
Free-living
Gram-positive
Gram-negative
Cocci
Enterococcus
Peptostreptococcus
Staphylococcus
Streptococcus
Rods
Bacillus
Clostridium
Corynebacterium
Erysipelothrix
Lactobacillus
Listeria
Propionibacterium
Cocci
Acinetobacter
Moraxella
Neisseria
Nonenteric rods
Bartonella
Bordetella
Brucella
Francisella
Hemophilus
Legionella
Pasteurella
Pseudomonas
Enteric roc
Bacterioides
Campylobacte
Enterobacter
Escherichia
Fusobacterium
Helicobacter
Klebsiella
Prevottella
Proteus
Providencia
Pseudomonas
Salmonella
Serratia
Shigella
Vibrio
Yersinia

Bacterial pathogenicity and infection

Few Concepts

- **Contamination**
- **Colonisation**
- **Infection**
- **Virulence**
- **Invasiveness**
- **Pathogen**

Few Concepts

✓**Contamination**

- Presence of an organism in a culture that was not in the sample when taken.

e.g. a culture of blood contaminated with an organism from the skin

✓**Colonisation**

- Presence of an organism at a site but not causing a tissue reaction (inflammation), symptoms or disease.
- Could be normal flora
- Could be abnormal flora-such as after the patient has received antibiotics.

Few Concepts…

✓**Infection:** multiplication of pathogenic microrganisms in the host with or without the manifestation of disease

✓**Virulence** – degree or intensity of pathogenicity. Measured by;

-**Infectious dose(ID)-** no. of microbes required to cause an infection. The smaller the ID, the more virulent the organism

-**Lethal dose**-the number of microorganisms required to kill 50% of the test host

- **Virulence factor-** product of pathogen that contributes to virulence e.g: capsule

✓**Invasiveness** – the ability of an organism to spread to adjacent tissues

- **Less invasive** organisms cause localised lesions e.g; Staphylococcus abscess, whereas
- **Highly invasive** organisms cause generalised infections e.g Streptococcal septicemia.

Few Concepts…

✓**Pathogen** - any organism that produces a disease; The harmless micro-organisms are called **non pathogens**

- **Pathogenicity**–ability of an organism to cause disease
- **True pathogen:** any microorganism capable of causing disease;an infecting agent
- **Opportunistic pathogen :** a harmless microorganism that becomes pathogenic in immunocompromised people causing an opportunistic infection

Characteristics of Pathogenic Bacteria

1. **Transmissibility**
2. **Adherence to host cells**
3. **Invasion of host cells and tissue**
4. **Evasion of the host immune system**
5. **Toxigenicity**

A bacterium may cause diseases by:

1. **Destroying tissue (invasiveness)**
2. **Producing toxins (toxigenicity)**
3. **Stimulating overwhelming host immune responses**

Sources of infection

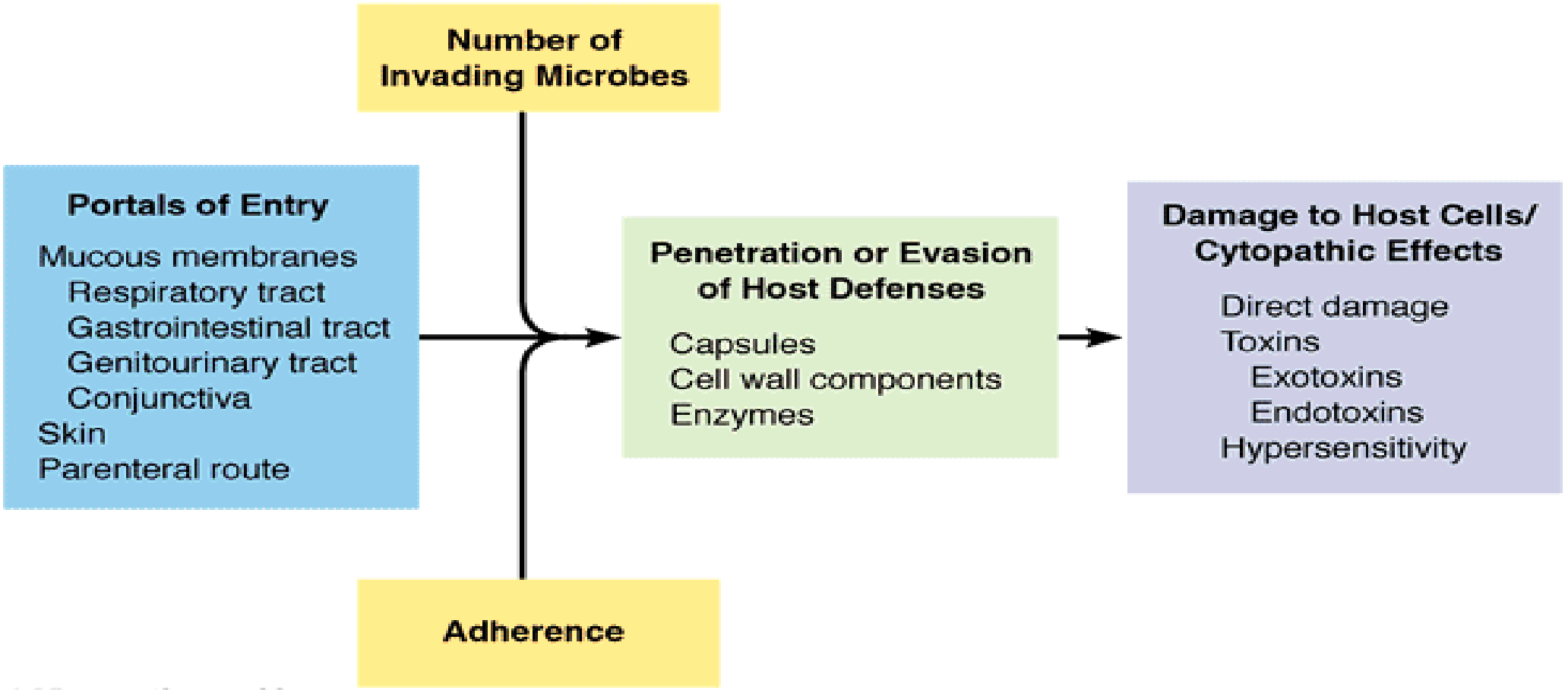
The following are the sources of infection:

- Human beings; patients or carriers
- From animals e.g rabies, brucellosis
- Insects , as vectors
- Soil and water
- Contaminated food.

Modes of transmission of infection

- ✓Contact
- ✓Inhalation
- ✓Ingestion
- ✓Inoculation: either as object or sucking insects
- ✓congenital

Mechanisms of bacterial pathogenesis



Mechanisms of bacterial pathogenesis

✓**Entry Into Host**

- through skin –usually via cut or lesion or bite
- through mucus membranes that lines the GIT, respiratory, genitourinary tract, and conjunctiva

✓**Adherence**

- Attachment to the host by the microbe at the portal of entry
- It is accomplished through surface molecules –**adhesions or ligands** that bind specifically to the complementary host surface receptors
- most microbial adhesions are glycoproteins or lipoproteins located on the **glycocalyx, capsule, pili/fimbriae**

✓Penetration

- pathogens invade and penetrate deeper into tissue to cause an infection.

- This is accomplished by:
  a)avoiding destruction by a host cell
  b)producing factors that allow for invasion of the host.

- Virulence factors that allow for bacterial **invasion and penetration** include;
  a) Possession of a capsule-antiphagocytic.
  b) Cell wall components can contribute to virulence;
  Ex: **Mycolic acid** of *M.Tuberculosis* resist digestion by Phagocytes.
  c) production of extracellular enzymes: e.g Coagulases,Hyaluronidase, Collagenase,Streptokinase, staphylokinase,…..

✓Damage to Host Cells

- Occurs through following mechanisms;

A)Direct Damage to colonized tissue/organ

–growth and replication in host cells which results in host cell lysis

–Microbial metabolism and multiplication kills host cells

–Production of components to aid in the acquisition of nutrients

a)**siderophores**–for high affinity up take of iron

b)**hemolysins**–lyse RBC

B)Hypersensitivity reaction;

- An immune response that is excessive beyond the normal, to a point where it leads to damage

C)Production of Toxins

- Are poisonous substance produced by microbes that cause wide spread damage/disease in host
- Toxins are categorized into two:
    - **exotoxins**
    - **endotoxins**


Cell wall
Exotoxin
Endotoxin

Exotoxin

- Are protein in nature
- Are heat-labile
- **Produced mostly by gram-positive bacteria** and **some gram-negative bacteria**
- produced inside of cells and cause damage upon release from the cell.
- Are extremely lethal.
- They are immunogenic -Vaccines can be produced by inactivating the exotoxin molecule (**toxoid**).

- Have **A-B subunit structure**-A is the active (toxic) and B is the binding unit.
- Have specific targets or sites of action.

- Thus there exist: –**Cytotoxins;** *diphtheria toxin*
  –**Enterotoxins**; cholera toxin
  –**Neurotoxins***; botulinum toxin,tetanus toxin*

Endotoxins

- Form part of the outer membrane portion of the cell wall of gram negative bacteria (Lipopolysaccharide (LPS))
- It is released upon lysis of the bacterial cell
- heat stable
- less potent
- weakly immunogenic-do not form toxoids
- If released in large amounts, endotoxins can lead to *septic* shock that can result to death

ENDOTOXINS	EXOTOXINS
1. Integral part of cell wall	1. Released from the cell before or after lysis
2. Endotoxin is LPS; lipid A is toxic	2. Protein
3. Heat stable	3. Heat labile
4. Antigenic; questionable immunogenicity	4. Antigenic and immunogenic
5. Toxoids not be produced	5. Toxoids can be produced
6. Many effects on host	6. Specific in effect on host
7. Produced only by gram-negative organisms	7. Produced by gram-positive & gram-negative organisms

Body response to bacteria invasion

- The discrimination between self and non-self, & the subsequent destruction and removal of foreign material is accomplished by two types of immune systems:

a. **The innate (“natural”) immune system**
b. **Adaptive (“acquired”) specific immune system**

BACTERIAL NORMAL FLORA

BACTERIAL NORMAL FLORA

- **Normal flora** or **normal microbiota** or **indigenous microbiota**
- **Normal flora:** Organisms that are always present at any anatomical site without causing disease to the host.
- Includes bacteria, fungi, protozoa,..
- **Establishment of normal flora**: Acquisition of normal flora begins at birth and the flora changes in response to the changing condition

Normal microbial flora

- Only one out of 10 cells in our bodies is human
- Colonization occurs after birth, stabilizes by 2-3 years,
- Foetal skin sterile in utero

- There are **two groups of normal flora**.

1. Resident normal flora

2. Transient normal flora

- **Resident normal floras** are relatively fixed microorganisms regularly inhabiting the skin and mucus membrane of the normal host.
- **Transient normal floras** are non-pathogenic microorganisms that inhabit the skin and mucus membrane for a short period of time like hours, days and weeks.

Roles of (Resident) normal flora

1. Prevent colonization by pathogenic micro-organisms and possible disease through "bacterial interference".

a. Competition for nutrition with pathogenic bacteria

b. Competition for binding sites with pathogenic bacteria.

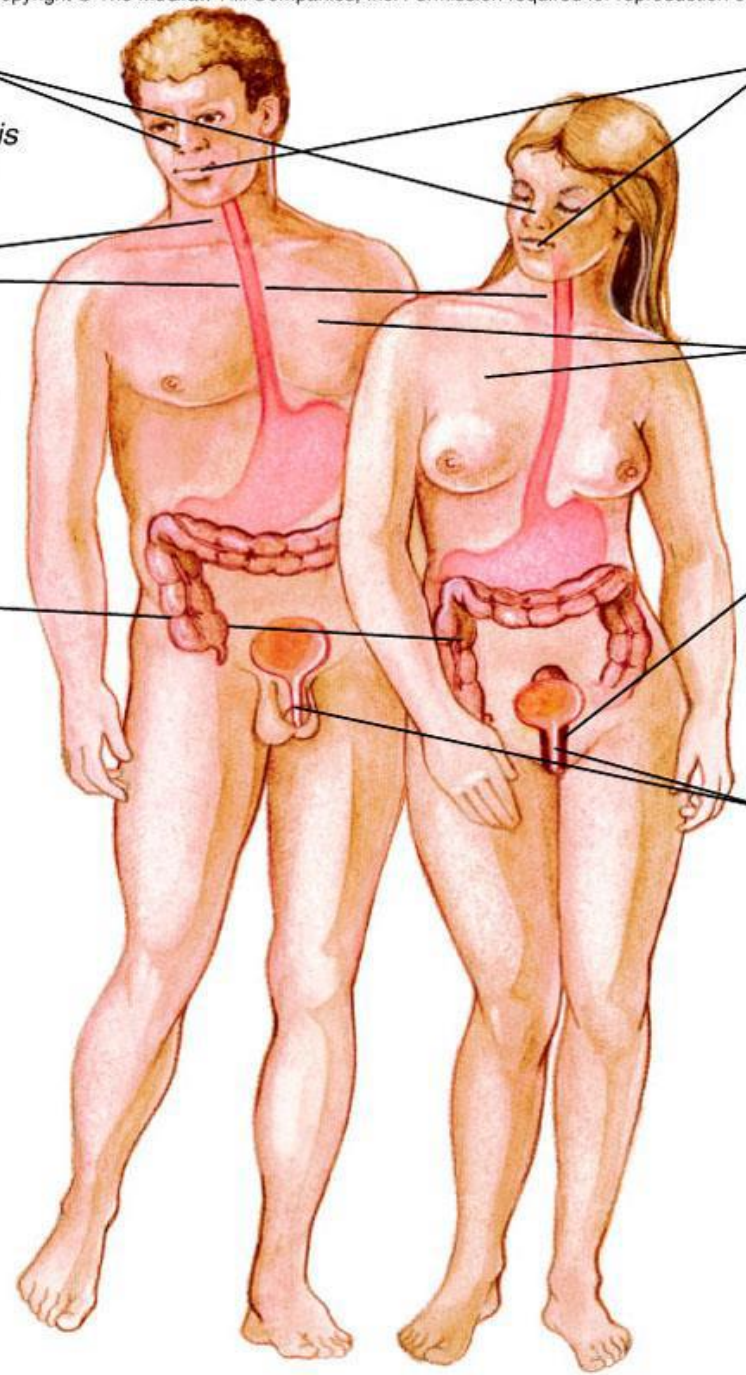
c. Mutual inhibition by their toxic metabolites.

d. Mutual inhibition by bacteriocins

2. Synthesis of vitamin K in the gastrointestinal tract

3. Aid in the absorption of nutrients in the small intestine.

NB: Normal flora can cause disease when the defense mechanisms of the body is breached or when the micro-organism is placed in the abnormal body site.



Nose
Staphylococcus aureus
Staphylococcus epidermidis
Corynebacterium species

Throat
Streptococcus species
Branhamella catarrhalis
Corynebacterium species
Haemophilus species
Neisseria species
Mycoplasma species

Large intestine
Bacteroides fragilis
Escherichia coli
Proteus mirabilis
Klebsiella species
Lactobacillus species
Streptococcus species
Candida albicans
Clostridium species
Pseudomonas species
Enterococcus species

Mouth
Streptococcus species
Fusobacterium species
Actinomyces species
Leptotrichia species
Veillonella species

Skin
Staphylococcus epidermidis
Propionibacterium acnes
Pityrosporum ovale

Vagina
Lactobacillus species
Streptococcus species
Candida albicans
Gardnerella vaginalis

Urethra
Streptococcus species
Mycobacterium species
Escherichia coli
Bacteroides species

Harmful effects of normal flora

1. Competition of nutrients with the host
2. Induction of low grade toxaemia
3. Endogenous infection
4. Transfer to susceptible host thus causing disease

STERILIZATION AND DISINFECTION

DEFINITION OF TERMS

✓Cleaning

- general removal of debris (dirt, food, feces…)
- reduces amount of organic matter that contributes to proliferation of bacteria and viruses

✓Disinfection : Reducing the number of contaminating organisms to a level which is deemed no longer harmful to health.

✓Antiseptic- a disinfectant applied to living tissue such as a wound, skin.

✓Sterilization : the killing or removal of all organisms

MEASUREMENT OF MICROBIAL DEATH

- Validation of all methods to demonstrate the required degree of microbial kill.
- **Two variables:**
    - the concentration of the killing agent and
    - the length of time the agent is applied.
- The rate of killing is defined by the relationship:
- N = 1/ CT
    - N- number of survivors
    - C- concentration, T- time
    - CT- dose

Factors influencing resistance

- Species or strain of the microorganism
- Physiological state
- Ability to form spores
- Number of microorganisms

STERILIZATION

- All items introduced into normally sterile tissue cavities or bloodstream must be sterile

4 Main sterilization methods

1. Heat
2. Ionizing radiation
3. Filtration
4. Sterilant chemicals e.g gases

1. Heat method

✓**Dry heat**

a)Hot air oven sterilizer for materials that can resist high temperature but affected by contact with steam e.g. lab glassware

b)Flaming: bunen burner flame e.g. wire loop

c)High vacuum infra red sterilizer- industries

✓**Moist heat**

- Done using an autoclave
- High temperature conditions attained by increasing the pressure of steam in a high pressure vessel

Process	Temperature	Hold time-minutes
Dry heat	160	120
”	170	60
”	180	30
Moist heat	121	15
”	126	10
”	134	3

2. Radiation

1. UV lighT

- Used in irradiation of air in or and TB labs
- Inhibit DNA replication

2. Ionizing radiation

- Gamma irradiation
- Disposable plastic syringes, gloves, specimen containers, petri dishes

3. Gaseous Chemicals

Mainly used in industries

1. Ethylene Oxide:
   - Highly penetrative
   - Useful for sterilization of heat sensitive materials e.g plastics, surgical instruments
2. Formaldehyde: at low t^o gives an effective sporicidal process

3. Glutaraldehyde: more effective than formaldehyde.
   - are used are used to sterilize respiratory therapy equipment

4. Filtration

- Remove most bacteria but viruses and some small bacteria e.g chlamydia and mycoplasma may pass through
- Made from nitrocellulose
- Filter of pore size: 0.22µm
- Useful in sterilizing fluids e.g antibiotic solutions, blood product

Monitoring of Sterilization

Mechanical Indicators

- Gauges, displays, printouts
- Indicates if device working properly
- Not indicator of sterility

Chemical Indicators

- Change color with timed exposure to heat, steam
- Used to show items have gone through sterilization process
- Not indicator of sterility

Biological Indicators

- Indicator of sterility
- Demonstrates bacterial spores on test strips or in vials/containers have all been killed

DESINFECTION

- Cleaning is a prerequisite for successful disinfection

- **Method used:**
  1. UV radiation
  2. Gases
  3. Filtration
  4. Chemicals

Disinfection by Ultraviolet Radiation:

- effective radiation of 240-280 nm can be produced by mercury lamps
- used to reduce the number of bacteria in air inside operation rooms, laboratory safety cabinet.

Disinfection by Gases:

- Formaldehyde gas – 5%,
- Paraformaldehyde-
- to disinfect complex heat sensitive equipment such as baby incubators, anaesthetic machines, disinfect rooms (smallpox..)

Disinfection by Filtration:

- A properly installed HEPA (high efficiency particulate air) filter achieves 99.997% arrestance to particles of 0.5um.
- operating theatres, ventilation systems, pharmaceutical clean rooms etc.

Disinfection by Chemicals:

- Alcohols
- Aldehydes
- Biguanides
- Halogens
- Phenolics
- Surface acting agents
- Oxidizing agents

✓Alcohols

- - Isopropyl alcohol, ethyl alcohol.
- - Optimal bactericidal activity at 70%.
- -Useful for skin disinfection.

✓Aldehydes-

- Glutaraldehyde, Formaldehyde.
- Used in adequately ventilated areas.
- Irritant to eyes, skin, respiratory mucosa so staff need protective clothing.
- Useful for disinfecting heat sensitive equipment eg endoscopes.

✓**Biguanides-**

- Chlorhexidine eg hibitane.
- Disinfection of skin and mucous membranes.
- Can be combined with a compatible detergent for hand washing or with alcohol as a hand rub.
- Has low irritancy and toxicity so can be used on exposed healing surfaces.

✓Halogens-

- **Hypochlorites** eg hypochlorite/ bleach…'JIK'.

  - Inexpensive, bleach is stable in concentrated form, stored in a cool dark place once diluted.

  -Lab disinfectant for bench surfaces.

- **Iodine-**

  -used as surgical scrubs, skin disinfectants.

  -Inactivated by organic matter

  -can cause staining.

✓**Phenolics-**

-Includes Lysol, Cresol, Hexachlorophene,..

-general purpose environmental disinfectant

-broad spectrum activity

-relatively cheap.

✓Surface active agents

- Includes quaternary ammonium compounds like Cetrimide.
- for cleaning trauma wounds.

✓Oxidizing agents

- Includes chlorine dioxide, hydrogen peroxide
- Corrosive to skin and metals.

DESINFECTANT

Disinfectant use- considerations

- Follow manufacturer's recommendations to achieve disinfection and to avoid medical device damage method
- Use correct dilution – more is not better!

- Use correct contact time
- Use correct temperature

- Understand employee and environmental safety issues
- Assess compatibility with gloves, basins, other products

Properties of an ideal disinfectant

- Broad antimicrobial spectrum
- Fast acting
- Non-toxic
- Surface compatibility…non-corrosive
- Easy to use
- Odourless
- Stable
- Environment friendly- not damage the environment on disposal
- Economical

DESINFECTANT TESTING

- need to monitor the performance of disinfectants over time.

-Dilutions of disinfectants are taken to determine the survival and multiplication of contaminating pathogens.

-**Include:**

- screening tests of the rate of kill,
- in-use tests on equipment and solutions

IDENTIFICATION SYSTEM OF BACTERIA

Identification System of bacteria

1. Microscopic morphology after Gram stain
2. Culture on media
3. Macroscopic morphology – colony appearance
4. Physiological / biochemical characteristics
5. Biochemical test
6. Serological analysis
7. Genetic and molecular analysis
8. Sensitivity test

OUTLINE OF LABORATORY ISOLATION AND IDENTIFICATION

Specimen collection and transport

↓

Gram stain from specimen

↓

Culture in media

↓

Colonial morphology

↓

Gram stain of colony

↓

Biochemical tests

↓

Antibiotic susceptibility test

1. Possible specimen in bacteriology lab include:

- Blood
- Csf
- Urine
- Stool
- Pus
- Vaginal, uretral, wound or oral swab
- Body fluid aspirate
- Sputum,….

➢Should be collected appropriately and transported to the lab without harm

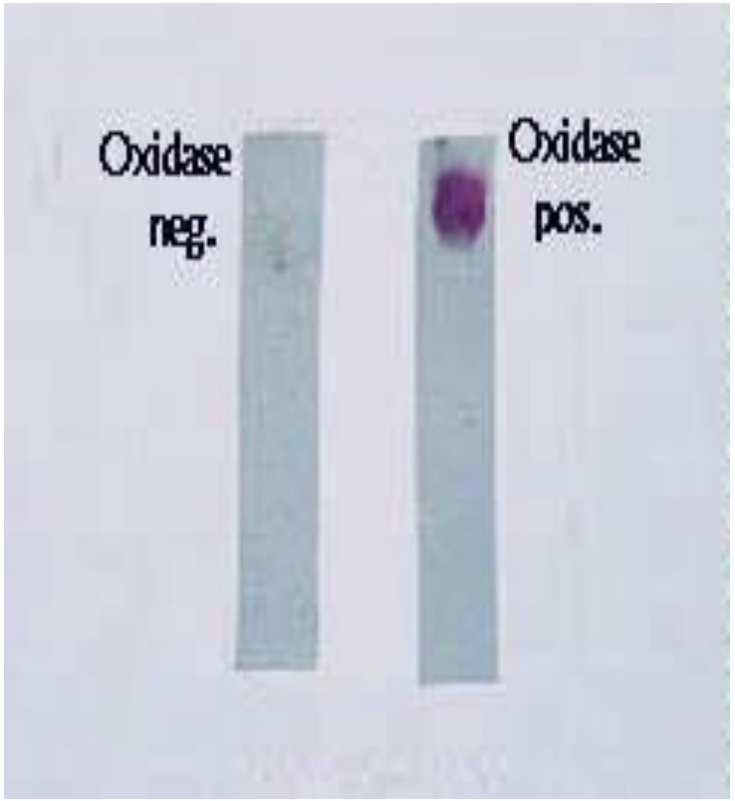
➢Transport media can be used if distance is too long

BIOCHEMICAL TESTS

Biochemical Tests for identification of Bacteria

- **Oxidase Test-**
- **Principle** -Oxidase test is used to deter-mine the ability of certain bacterial species to produce cytochrome oxidase.
- If the cytochrome oxidase is produced, the colonies will turn into blue or black colo-ration 10-15 seconds after the addition of p-amino dimethyl aniline oxalate reagent.

Oxidase test



Biochemical Tests for identification of Bacteria…

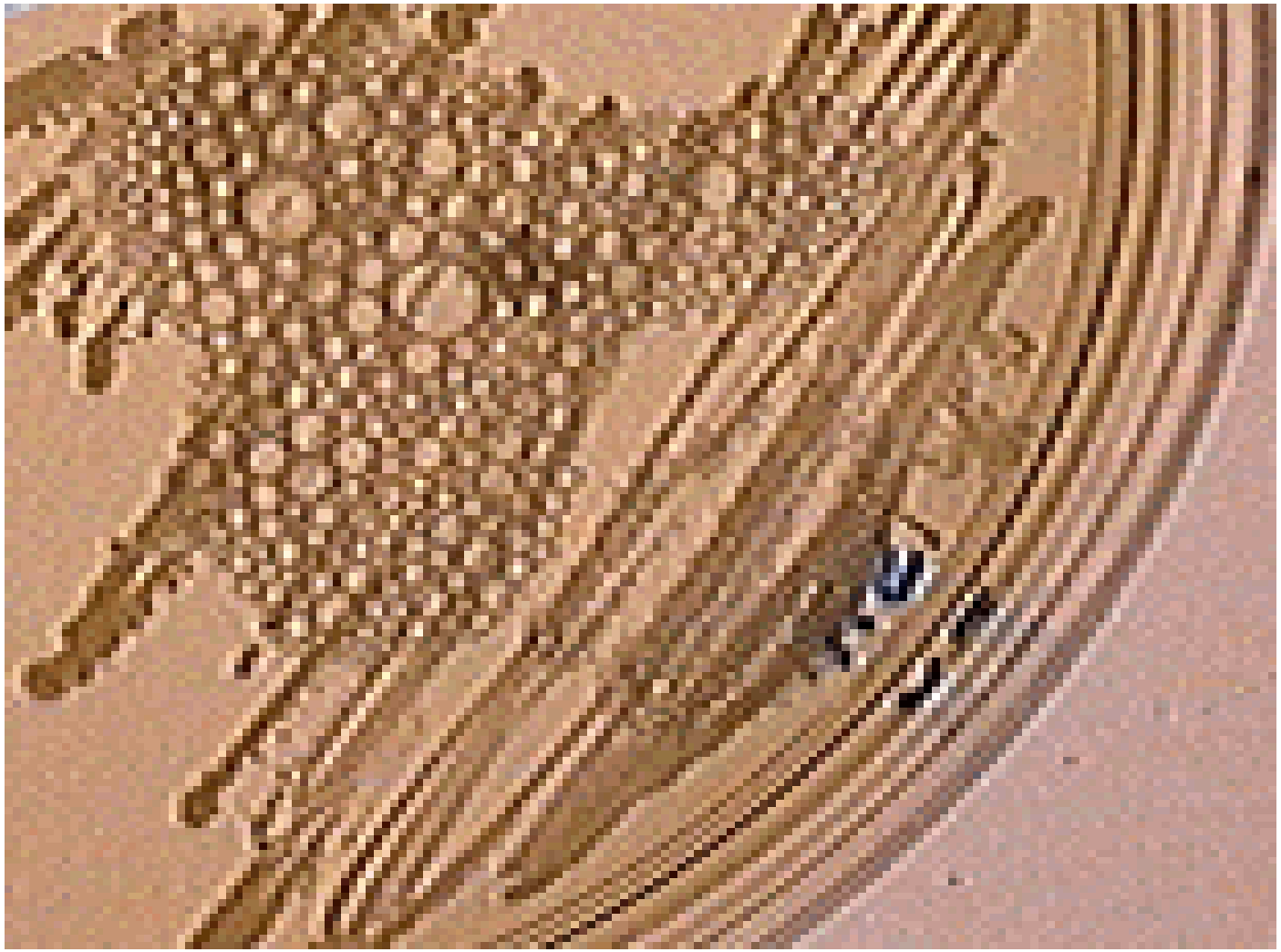
- **Catalase Test**

- **Principle-**Catalase test is used to test the ability of bacteria to degrade hydrogen peroxide by using the enzyme catalase.

- The production of bubbles after addition of hydrogen peroxide (H_20_2) shows that the catalase is present. The culture should not be more than 24 hours old.

- **POS. CONTROL**: *E. coli* ,**NEG. CONTROL**: *Streptococcus sp*

Catalase Production



Biochemical Tests for identification of Bacteria…

- **Urease Test**
- **Principle-** It is used to test for urea catabolism by the enzyme urease. As the substrate urea is degraded into ammonia and carbon dioxide, the alkaline environment will cause the phenol red to change into a bright pink color.

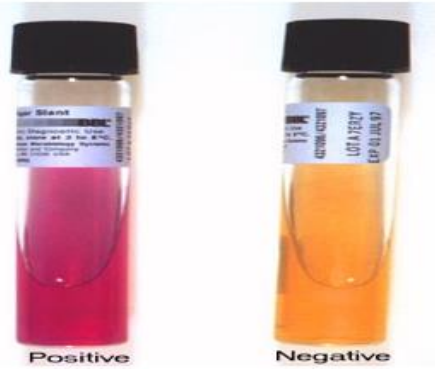
- This test is important in differentiating enterobacteria. Urea agar or broth is the medium used for the urease tests.

Urease Test

- **Urea media:--**Inoculate heavily the test organism in a bottle containing 3ml sterile Christensen's modified urea broth.

-Incubate at 35-37ºC for 3-12 hours.

-Look for a pink colour in the medium.



- **Results**:- Pink colour = Pos. test,
- No pink colour = Neg. test.

Biochemical Tests for identification of Bacteria

- **Bacitracin Test**- Bacitracin test is used to identify and classify **Group A *Streptococcus.*** In bacitracin test, the inoculums will be streaked on the blood agar plate.
- Then, using an alcohol dipped and flamed forceps, add a single bacitracin disc on the surface of the heavy inocu-lation.
- Incubate the plate in the inverted position at 37°C for 24 hours.
- Observe for appearance of a zone of growth inhibition surrounding the disc, indicative of **Group A *Streptococcus*.**

Optochin Test

- **Opthocin Test**-Opthocin test is used to identify *Streptococcus pneumonia.*
- Non pneumococcal α-hemolytic strepto-cocci are resistant to opthochin and does not show any zone of inhibition or produce a zone less than 15 mm.

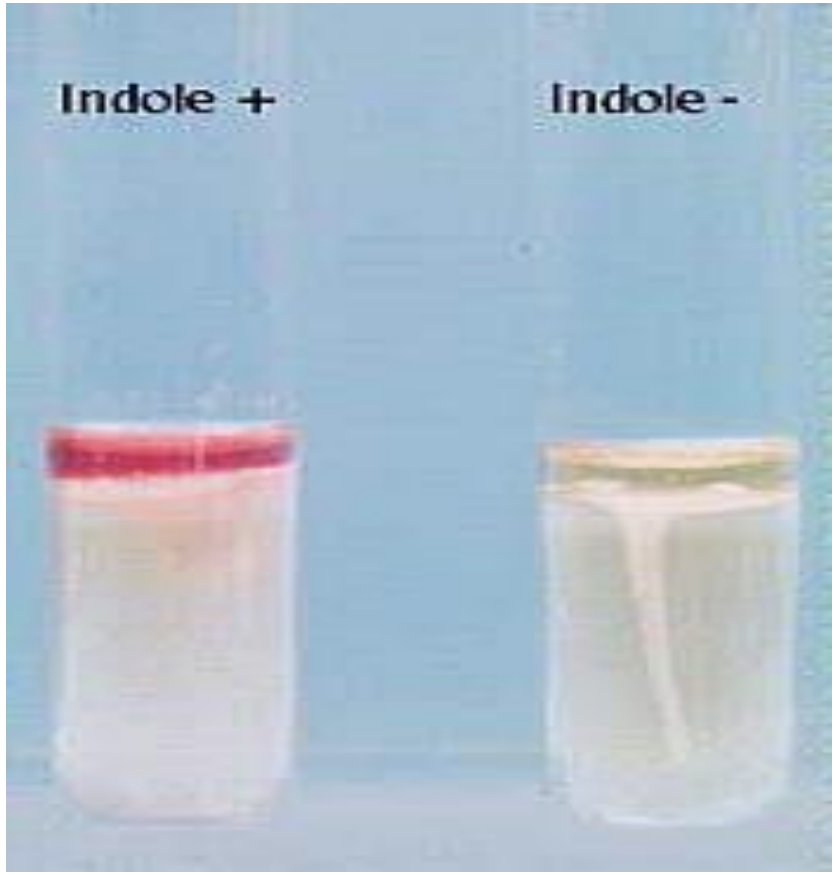
Indole Test

- **Principle-** Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase.
- When tryptophan is broken down, the presence of indole can be detected through the use of Kovacs' reagent, which contains 4(P)-dimethyl aminobenzaldehyde.
- Kovac's reagent, which is yellow, reacts with indole and produces a red colour on the surface of the test tube.
- This test is used in the identification of enterobacteria.

Indole Test

- **Detecting indole using trypton water**
- Inoculate the test organism in a bijou bottle containing 3ml of sterile peptone water.
- Incubate at 35-37ºC for up to 48 hours.
- Test for indole by adding 0.5ml of Kovac's reagent. Shake gently. Examine for a red colour in the surface layer within 10 mins.

- **Results**
- Red surface layer = Positive indole test
- No red surface layer = Negative indole test.



Coagulase Test

- **Coagulase Test**-This test is used to identify *S. aureus* which produces the enzyme coagulase.
- **Principle-**Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus.*

Citrate Utilization Test

- **Citrate Utilization Test:-**This is one of several techniques used occasionally to assist in the identification of enterobacteria.
- The test is based on the ability of an organism to use citrate as its only source of carbon.

- Prepare slopes of the medium.
- Using sterile wire, first streak the slope with a saline suspension of the test organism and then stab the butt.
- Incubate at 37C for 48 hours. Look for a bright blue colour in the medium.

Results

- **-Bright blue = Pos. test,**
- **no change in colour of medium = Neg. test.**

Serologic test

- In serological diagnosis of disease, a blood sample is scanned for the **presence of antibody using an antigen of known specificity** or vice versa.

- **I. Direct slide and tube agglutination tests** in which a bacterial antigen reagent is used to agglutinate antibody in serum.
- Bacterial suspension + Antibody in patient's serum = AGGLUTINATION

- **II**. **Latex agglutination tests** in which latex particles are coated with antigen to detect antibody in patient's serum.
- Latex antigen reagent + Antibody in patient's serum Latex particles = AGGLUTINATED

- III. ELISA

Genetic and Molecular Analysis

ANTIBIOTICS SUSCEPTIBILITY TEST

Antibiotics sensitivity testing

- Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro*.
- Used to select effective antimicrobial drugs

Various methods of antibiotic susceptibility testing are:

1. **Qualitative Methods: e.g. disc diffusion technique**
2. Quantitative Methods: e.g. dilution technique
3. Automated Susceptibility Tests
4. Newer Non-Automated Susceptibility Tests: e.g E-test
5. Molecular Techniques

Qualitative Methods:

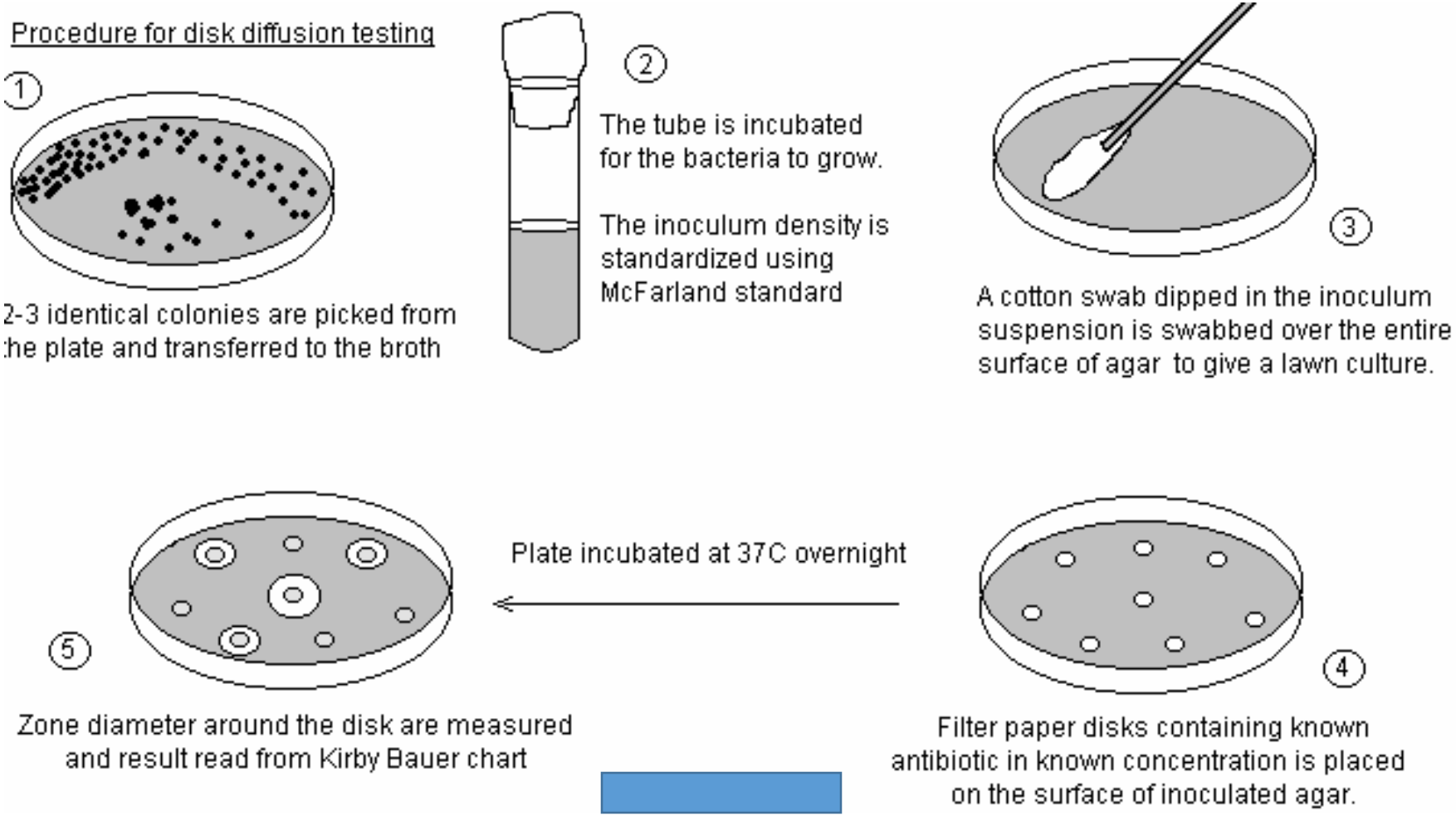
- These tests categorize a bacterial isolate as sensitive, intermediate or resistant to a particular antibiotic.

- **What is the Kirby Bauer disk diffusion test?**
- The Kirby Bauer test is a qualitative assay whereby discs of paper are impregnated with a single concentration of different antibiotics.

- The discs are placed on the surface of an agar plate that has been inoculated with test bacteria.
- During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient.

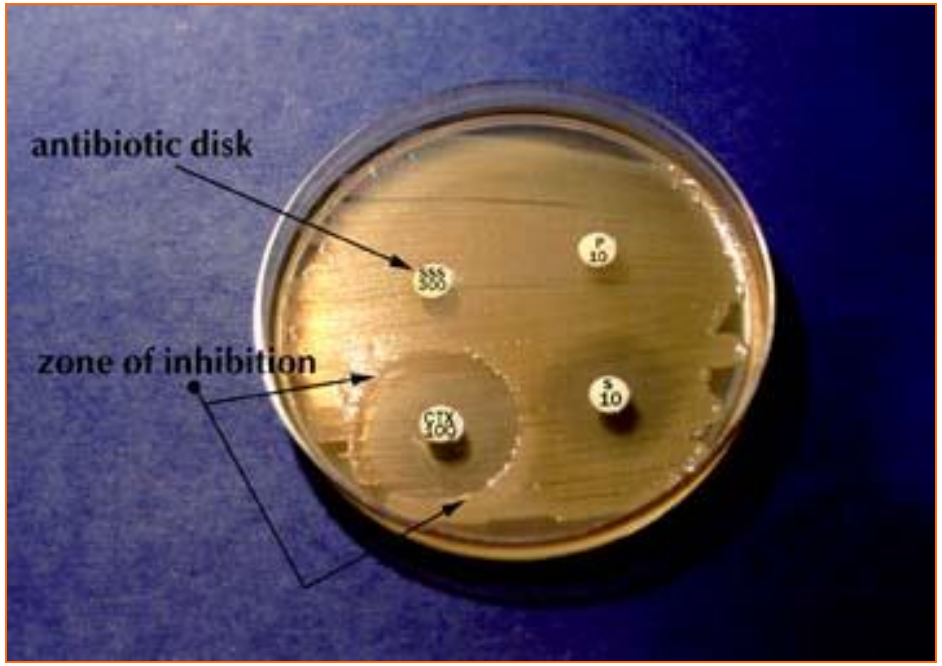
- After 18-24 hours, the zone diameter (zone of inhibition) is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs.

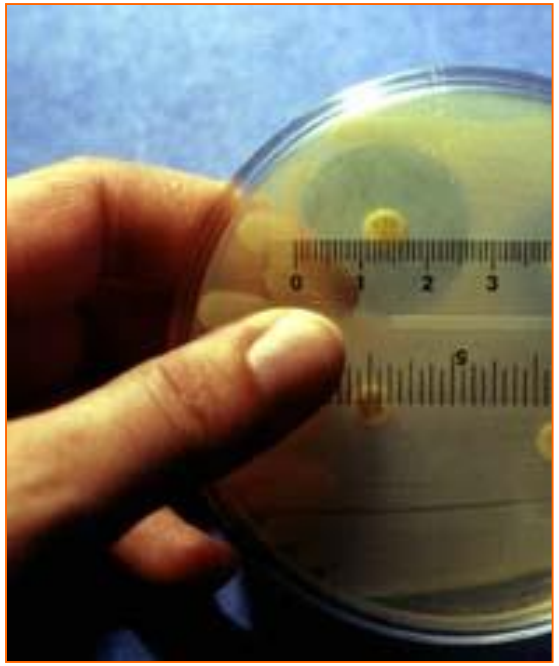
PROCEDURE FOR KIRBY BAUER DISC DIFFUSION METHOD

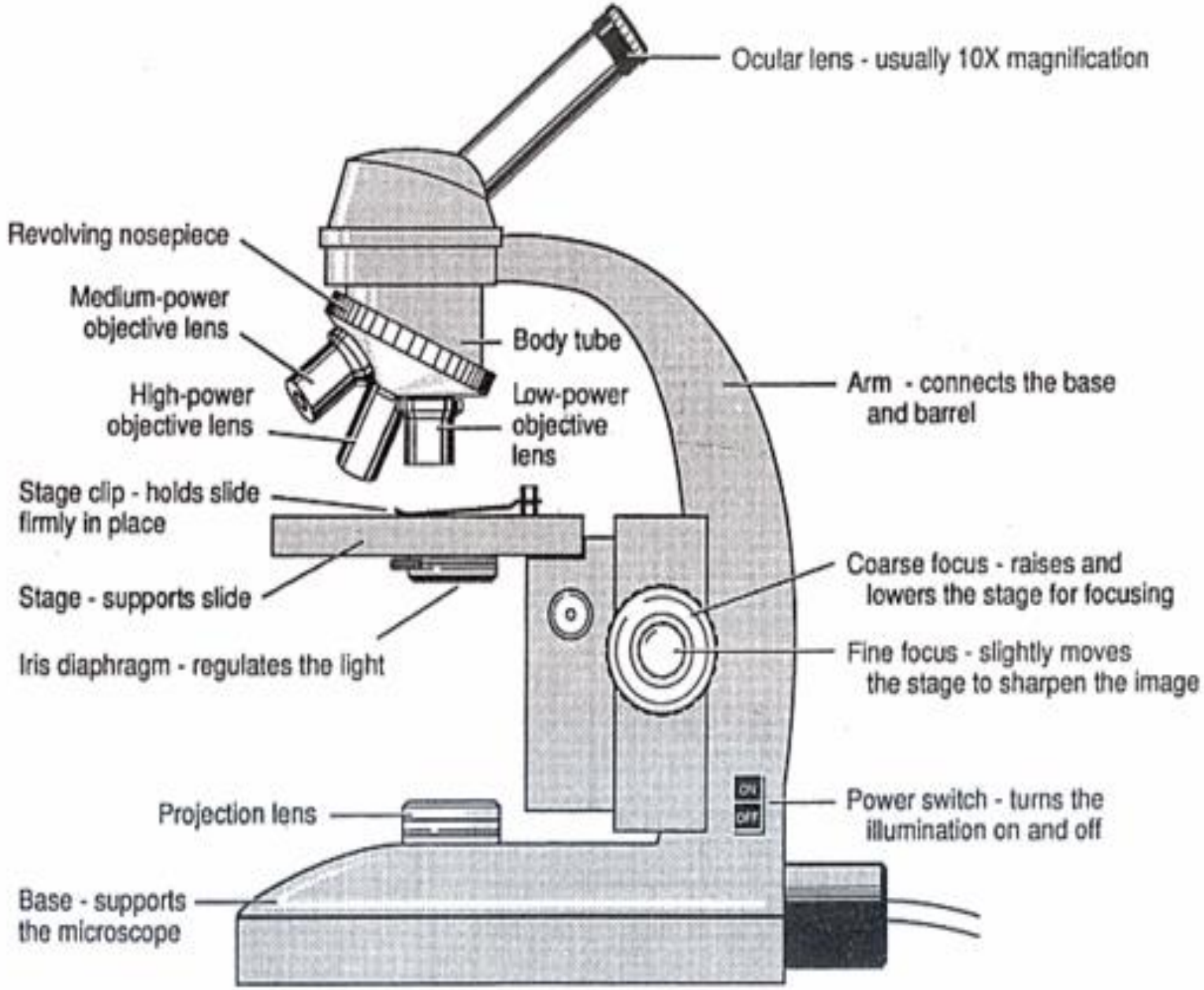


Antibiotic Sensitivity Test

Kirby-Bauer Test (disc diffusion test)






Ocular lens - usually 10X magnification
Revolving nosepiece
Medium-power objective lens
Body tube
High-power objective lens
Low-power objective lens
Arm - connects the base and barrel
Stage clip - holds slide firmly in place
Coarse focus - raises and lowers the stage for focusing
Stage - supports slide
Iris diaphragm - regulates the light
Fine focus - slightly moves the stage to sharpen the image
Projection lens
ON
OFF
Power switch - turns the illumination on and off
Base - supports the microscope

REFERENCES

- Modern Medical Microbiology: The Fundamentals by Stuart Clarke (Editor) Arnold Publishers (2004).

- Medical microbiology by Mims, C. A., Playfair, J. H. L. et al., Mosby Publishers.

- Designing a Modern Microbiological/Biomedical Laboratory by Jonathan Y. Richmond (Editor).