

Introduction To General Microbiology

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The Microbial World

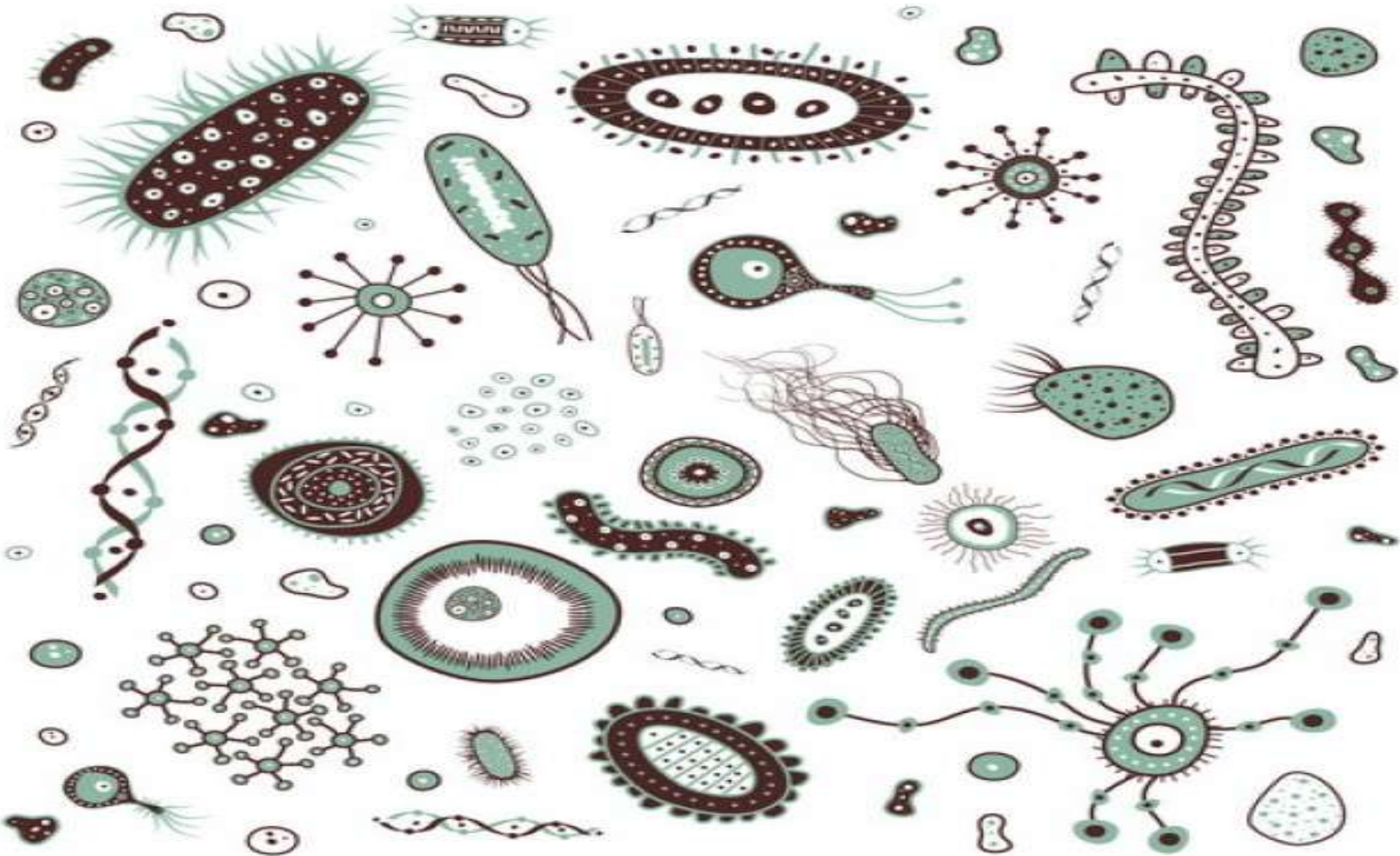
- The microbial world is composed of commensally and pathogenic Microbes/ Microorganisms.. Bacteria, Fungi (Yeast/ Moulds), Algae, Protozoa/ Parasites and viruses.
- **Microbiology** is concerned with the study of these microbes.. Mostly are beneficial.. Few types cause harmful effects ..Disease in human & animals.
- **Microorganisms** are unicellular cell.. too small to be seen with the naked eye(0,1-10um).. recognized by light microscope.. Most microbes capable of grow & existence as single organism.. Widely distributed in Human, Animal, Nature.

Microbiology

- Viruses sizes **< 0.01um** Composed of only **DNA** or **RNA**.. grow only in living cells/tissue culture.. are non independent cellular entities..can't be considered true microorganisms..Their presence structures can be seen only with electron microscope.
- **Microbiology** has many areas of specialization including **Bacteriology**, **Mycology (fungi)**, **Virology**, Medical microbiology, Immunology, Food microbiology, Biotechnology, Microbial genetics ..Industry.. Agriculture Veterinary.

Classification of Microorganisms

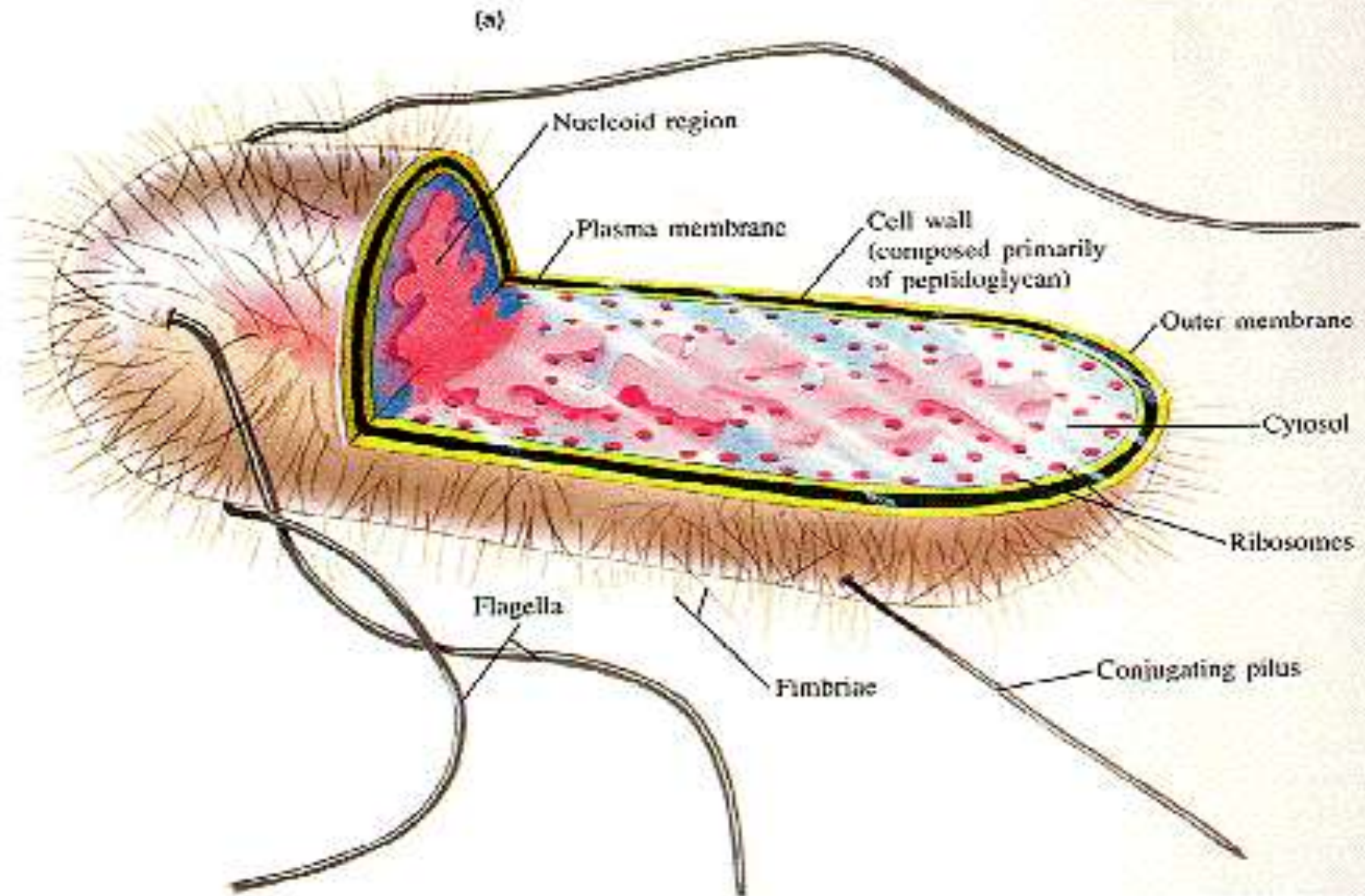
- Two fundamentally different types of cells are classified in the microbial world, **Prokaryotic .. Eukaryotic cells**.
- Eukaryotic cells have a "**true**" **nucleus**.. Prokaryotic cells have a **naked nucleus**/ composed of a **single DNA Chromosome**.. not enclosed within a nuclear membrane.
- The shape and composed of **Prokaryotic cells**.. Bacterial, Fungi, Parasites cells are of fundamental importance in the classification, identification & pathogenicity of these microbes in Labs.



Bacteria

- **Bacteria** are unicellular microorganisms.. Size range (0.2umDiameter, 0.2-10um Length) having a variety of shapes ..Growth patterns & metabolic characteristics allowing their classification.
- Major bacteria cell shapes are arranged: **Coccus/cocci, Bacillus/bacilli or Rods, Coccobacilli, Spiral forms-spirochetes, Vibrios**
- Individual cells may be arranged in pairs or clusters or chains.. Their morphologies are useful for the identification & classification of bacterial **Genera and Species**.. colored by Gram-stain or other stains (Fig-1)

Figure -2 Bacteria Cell structure



Bacterial Cell structures-1

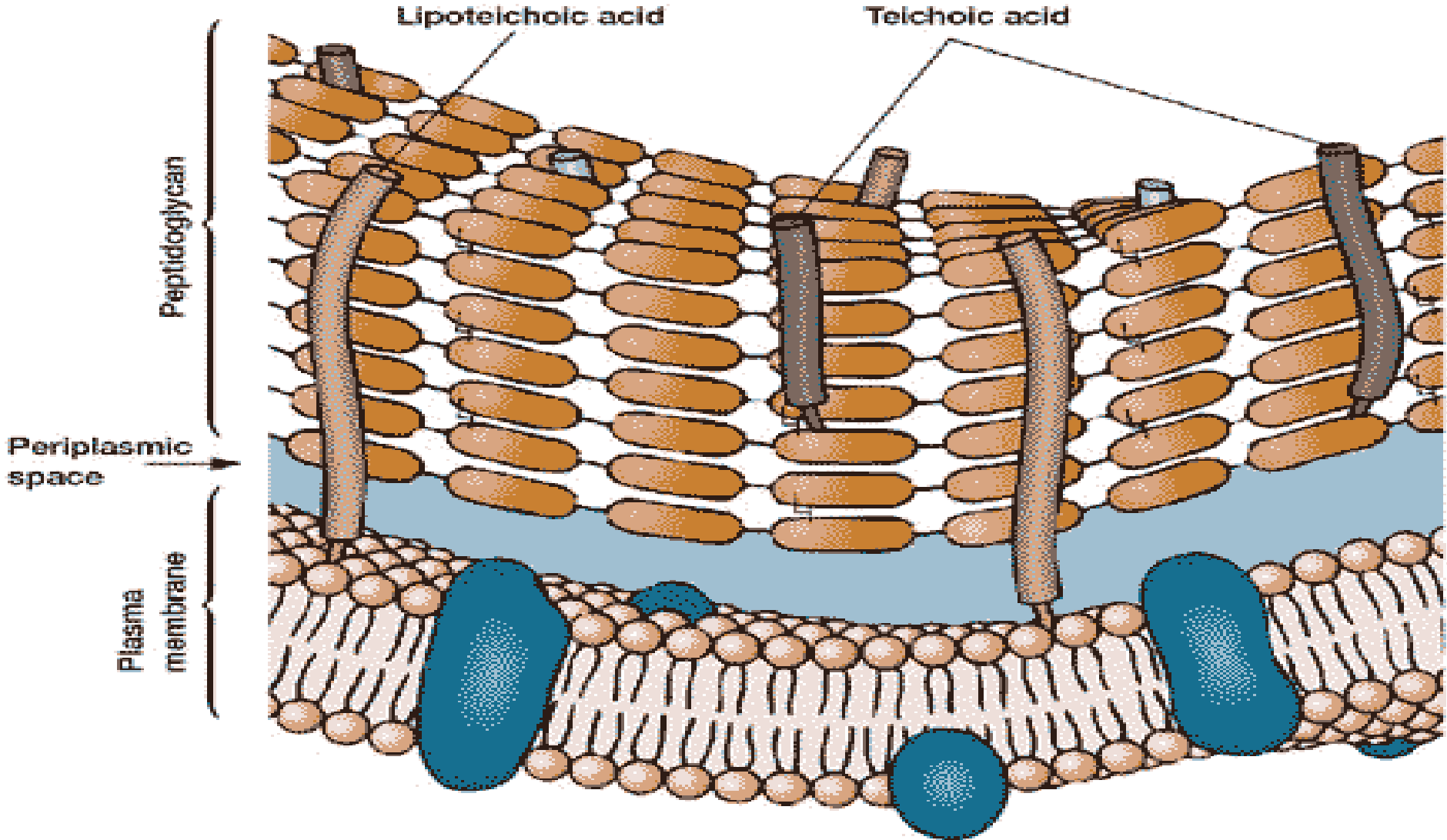
- **Cell structures:** A rigid cell wall, composed many peptidoglycan layers .. Outer membrane, Periplasmic space, Cytoplasmic membrane lacking sterols, Cytoplasm .. **70S Ribosomes**, Mesosomes, Storage granules (Lipids, glycogen, polysaccharides, sulfur, phosphate .. others storage compounds).
- **Bacterial genome**.. One single super coiled DNA chromosome, DNA plasmids(≥ 1).
- **Flagella:** Organs of motility, composed of flagellins (polymer proteins) long filament.. length up to 20 μm (Figure 2).. Attachment.. Nutrition..Single polar flagellum (**monotrichous**).. Several polar flagella at one, each end of the cell or covering the entire cell surface (**peritrichous**).. **Antigenic determinants** (H-antigen)..observed during bacterial infection..

Bacterial Cell structures-2

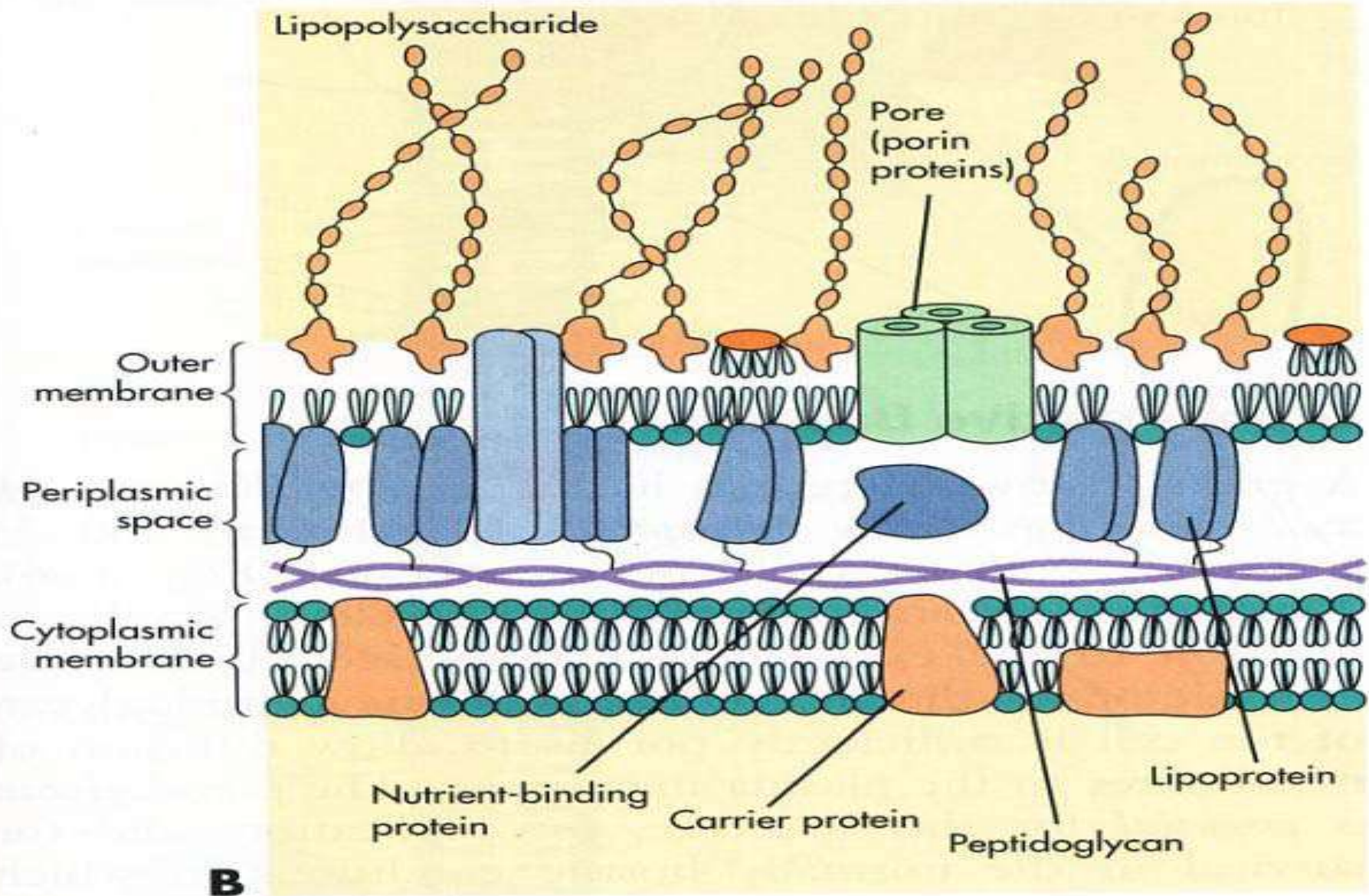
- **Fimbriae.. Pili:** Small Surface appendages (proteins).. Few numbers Pili.. Sex function /Large Numbers fimbriae..specific functions .. Attachment/Adhesion to host epithelial cells/colonization & antigenic determinants.
- **Capsules:** surface layer of cell wall.. a slime layer composed mostly of high molecular weight polysaccharides.. provide resistance to phagocytosis.. avoid the killing effects of lysosomal enzymes, and serve as antigenic determinants.. (K-antigen).. Major virulence factor in certain bacteria
- **Virulence factor**.. Any bacterial part/product Associated with pathogenic potential.. causing human/animal infection/disease.

Cell wall Gram-positive bacteria-3

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Cell Wall Gram-negative bacteria-4



Bacterial Cell wall Structures-1

- Bacterial cell wall contains a special polymer called **Peptidoglycan**.. Its basic structure is a carbohydrate backbone of alternating units of N-acetyl glucosamine and N-acetyl muramic acid.
- These are cross-linked with oligopeptides.. contain both D- and L-amino acids.
- **Teichoic acid-Lipoteichoic acids:** Both are found only in Gram-positive bacteria.
- **Lipopolysaccharides:** Lipopolysaccharides (LPS) found only in Gram-negative bacteria..part of somatic antigen (O-Antigen).

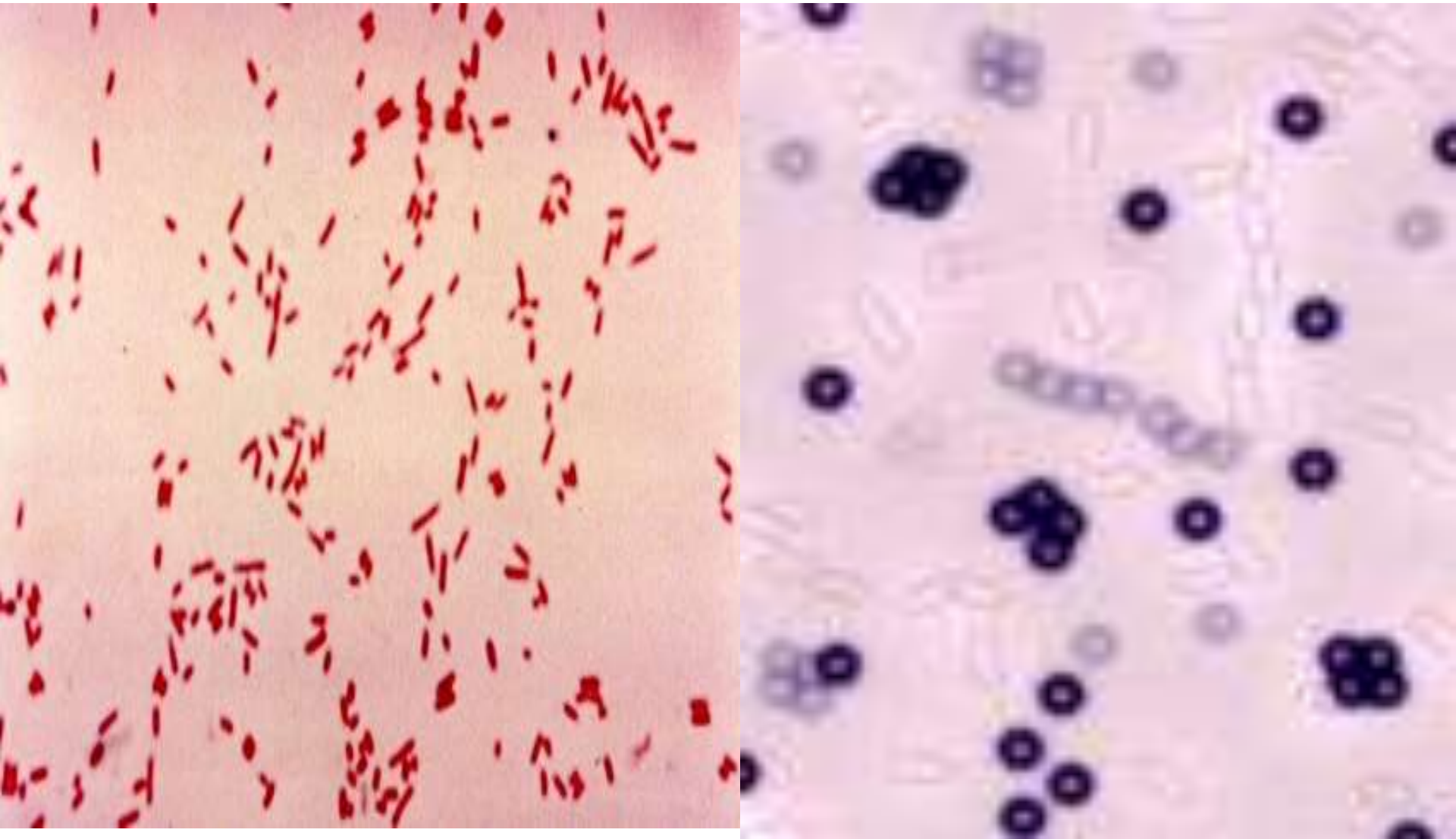
Bacterial Cell wall Structures-2

- (**O-Antigen**) developed during Infection..
- **LPS** structures are composed of **lipid A**, which binds to the outer membrane.. **Endotoxic portion** of the molecule.. Causing Toxic Shock.. High Fever, Sepsis during infection
- The polysaccharide moiety appears on the cell surface, serving as an antigenic determinant
- Cell wall is the basis for classification of bacteria into Gram-positive & Gram-negative by Gram-stain
- Cell membrane: **A phospholipid bilayer** responsible for transport of ions, nutrients, water, drugs and waste across the membrane.. Control the cell plasma contents

Gram-Stain

- A- Gram-positive bacteria cell wall contains a thick layer of peptidoglycan, Many sheets.. external to the cytoplasmic membrane.. Lipoteichoic acids.. stained **Blue**.. Example: *Staphylococcus, Streptococci, Bacillus*..
- Bacterial cell Protoplasts..L-form..observed in G+ve Staphylococci..Loss most cell wall structure.. by using antibiotics.. by body Lysozyme effect or Blood serum complements during infection..may result in chronic infection .
- B- Gram-negative bacteria cell wall contains lipopolysaccharide (LPS) attached to the outer membrane... source of the O-antigen and endotoxin reaction.. Stained **Purpel/Red**.. Example:Enteric bacteria group.. *Esch. coli, Klebsiella, Salmmonella Pseudomonas* ..

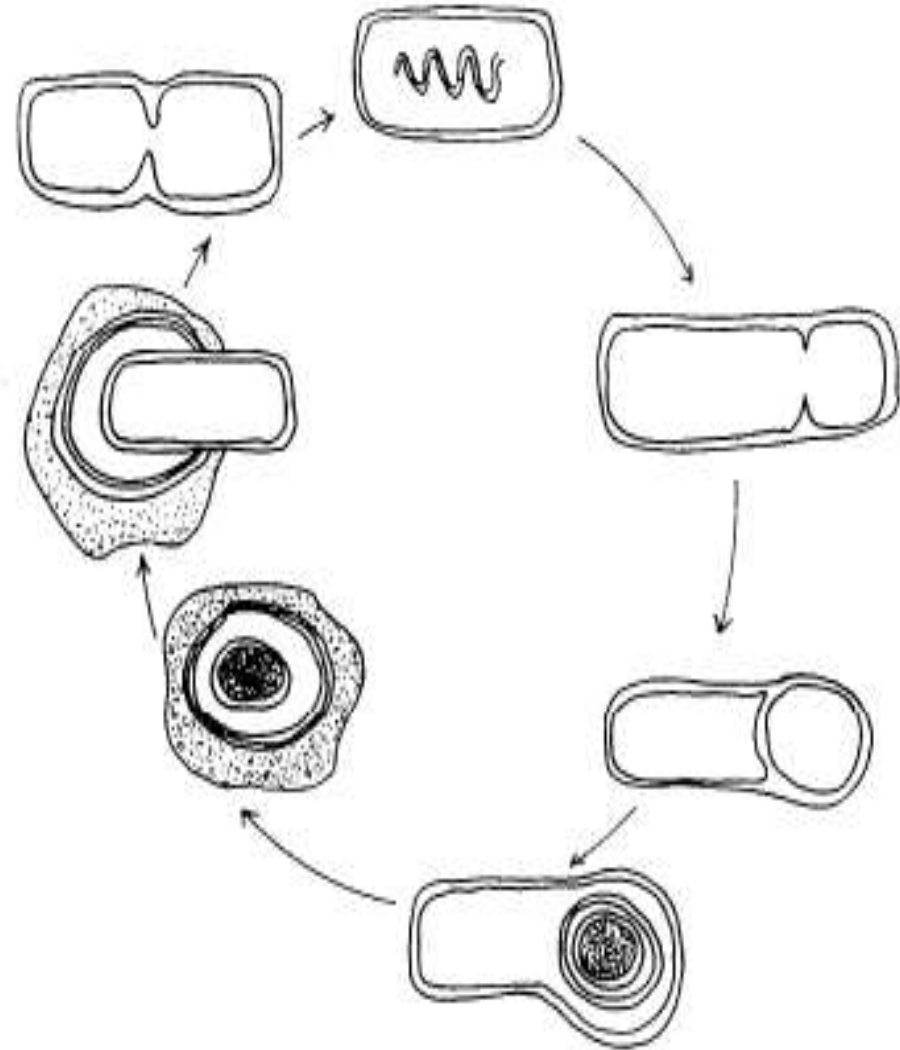
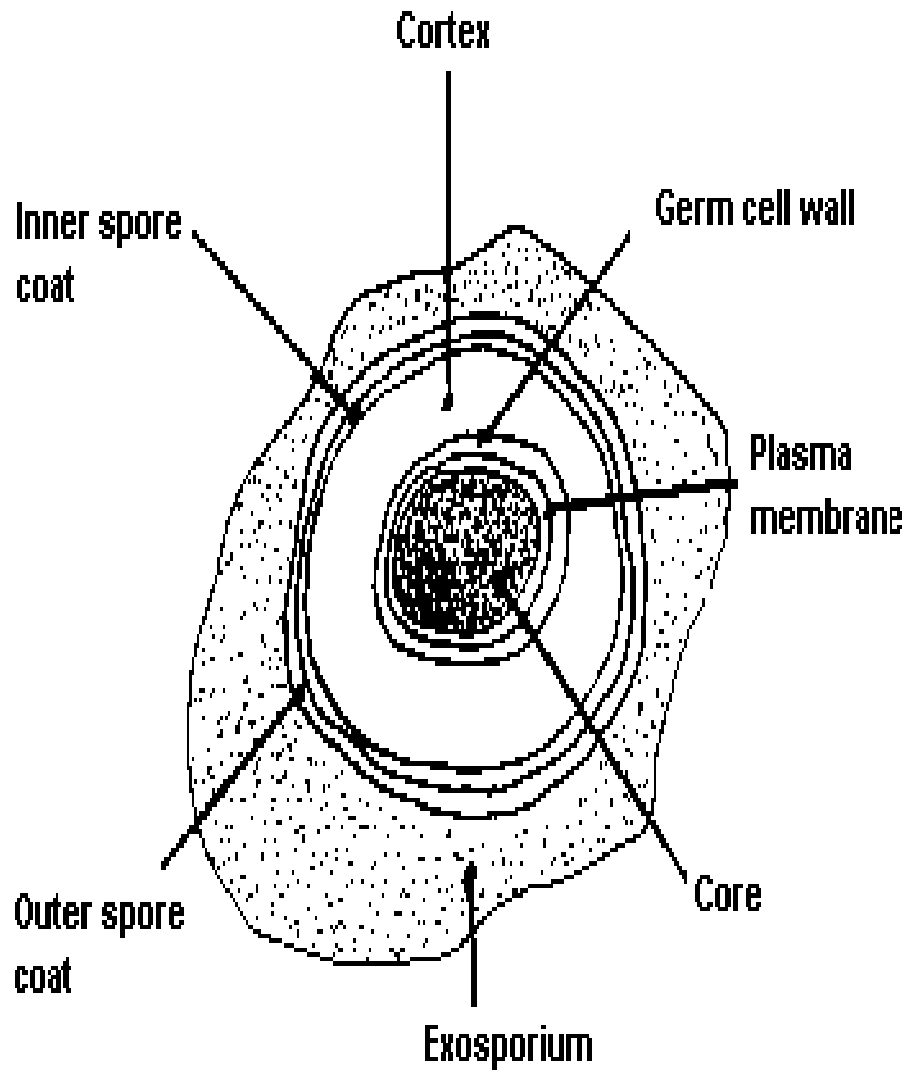
Fig-1: Gram-Stain:Negative/positive



Spore-Forming Bacteria

- ENDOSPORE FORMATION: The process of sporulation begins when vegetative (actively growing cells) exhaust their source of nutrients .. begin of forming endospores.. Common in nature (Figure 4).
- Spore forming Bacteria are **Gram-positive** ..very resistant to lysozyme, heat, radiation, drying and can remain dormant for hundreds of years in nature.. Once conditions are again favorable for growth, the spores can germinate and return to the vegetative state.
- Aerobic Bacillus group & Anaerobic Clostridium.. develop Endospore formation.. Both are widely distributed in nature ..intestinal -human and animals.

Bacterial Spore - Fig-5



Bacterial Growth & Nutrition-1

- Requirements for growth: Oxygen, water, pH, temperature, source of carbon, nitrogen (organic compounds), inorganic salts.. Na, K, S, P, Ca, Mg, Cl, Fe, vitamins, etc.
- Obligate Aerobic bacteria ..*M. tuberculosis*, *P.aeruginosa* grow using atmospheric oxygen by oxidation process .. recipient Oxygen..
- Aerobic bacteria encounter the oxygen.. damaging effect during their growth by producing oxidizing enzymes:
- Superoxidase dismutase: Reduce O_2^- into $H_2O_2 + O_2$..
- Catalase: Reduce H_2O_2 into $2H_2O + O_2$.
- Peroxidase: Oxidize H_2O_2 into $2H_2O + NAD$

Growth & Nutrition-2

- Certain Pathogens grow with reduced level of oxygen..
Microaerophilic bacteria..Neisseria
- Facultative anaerobes.. prefer growing in the presence of oxygen, but can continue to grow without it.. Most human pathogens & part normal flora.. G+ve Staphylococci, streptococci, G-Ve Enteric bacteria ..E.coli
- Obligate Anaerobic bacteria grow by absence of oxygen..
using recipient inorganic molecule.. Glucose fermentation process.. Mostly found in intestinal tract (95-99%), Mouth & Vagina(90%)
- Anaerobes: Gram-ve Bacteriodes fragillis, G+ve Clostridia, Gram+ve Cocci

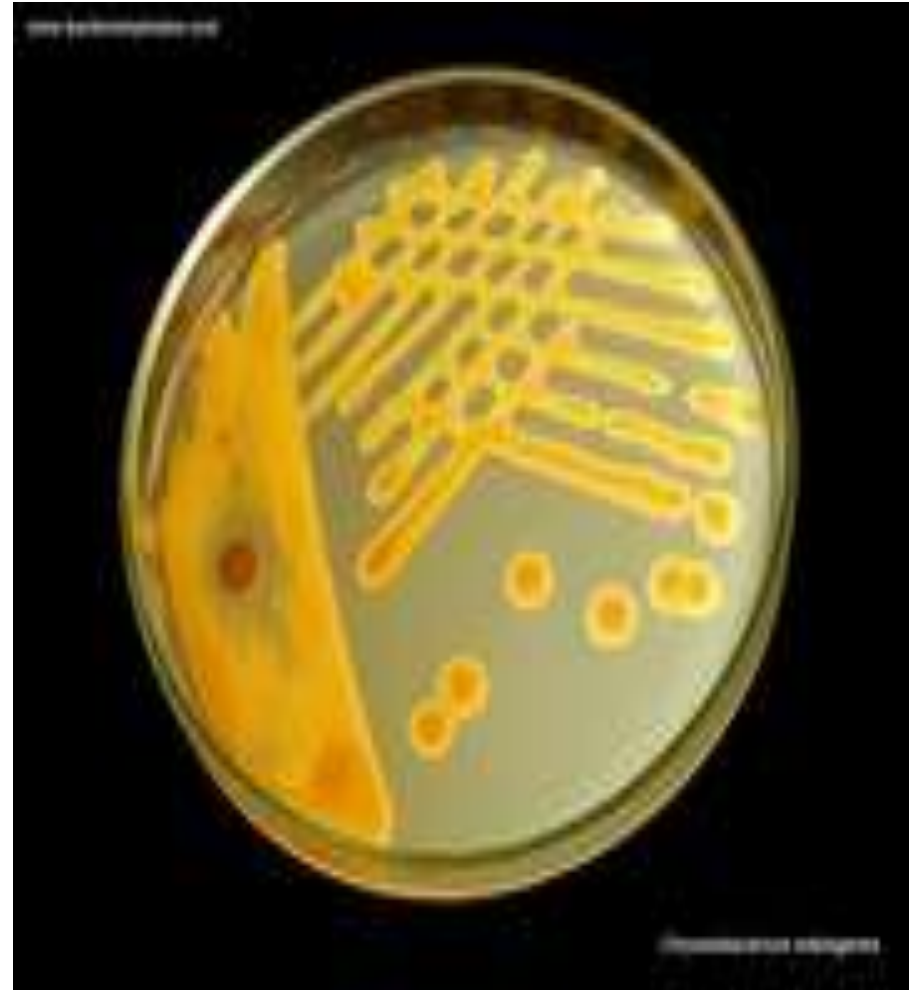
Growth & Nutrition-3

- Bacteria classified by the source of their energy oxidation-reduction process into two groups:
- Heterotrophs: derive energy from breaking down complex organic compounds.. protein, sugar, fats.. human tissues.. All human commensals-pathogens
- Autotrophs: fix carbon dioxide to make their own food source.. using light energy photoautotrophic, or oxidation of nitrogen, sulfur, other elements chemoautotrophic.. sulfur & nitrogen fixing bacteria.. Widely present in environment.
- Saprophytic bacteria/ Nonpathogenic.. take energy by fermentation/respiration.. found in nature.. in decaying material.. soil, water..vegetations.. Important for circulation of minerals in nature.

4/

- Culture Media: Nutrients (carbohydrates & proteins, blood, minerals) Source.. Water..Broth medium, Solid medium/
Blood agar, Petri dishes/Plate, Growth/Culture (Fig 5)
- Neutrophilic bacteria.. Grow best (pH 7-7.2) Most human-animal commensales & pathogens
- Acidophilic Bacteria (< 5 pH) Lactobacilli.
- Mesophilic Bacteria (20-40C)..Most human commensal & pathogens
- Psychrophilic bacteria(<10C) see water. Thermophiles bacteria (> 60C) Common in hot spring water
- Counting bacteria growth: Colonies plate counts, Turbidity, Dry weight using solid culture agar

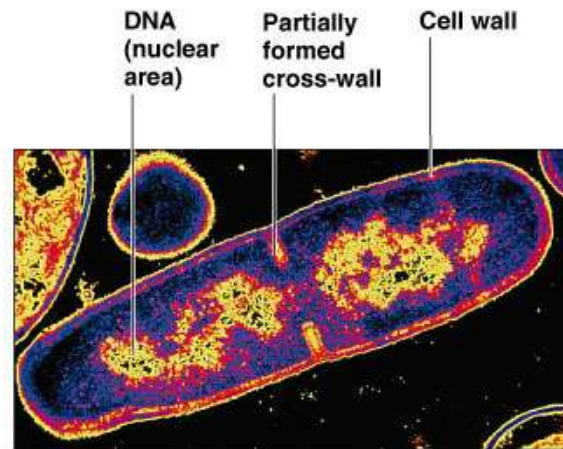
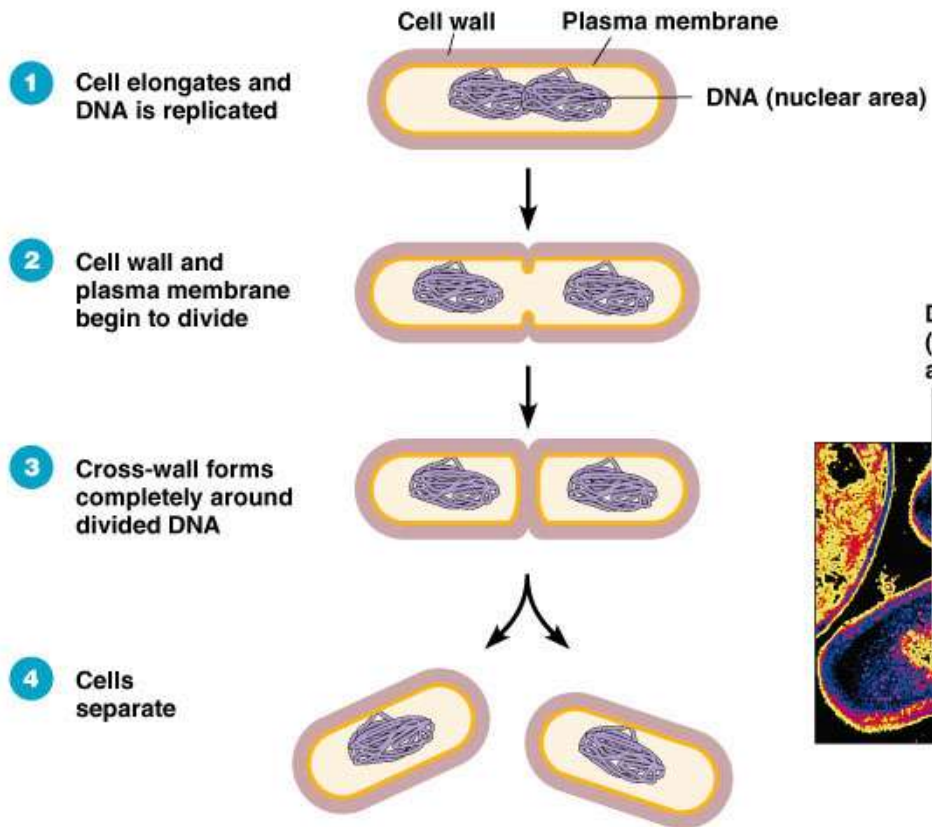
Bacterial Growth-Colonies & Culture



Bacterial growth-1

- **Bacterial growth** is the division of one bacterial cell into 2 identical daughter cells..4,8.16.. binary fission..Generation time (15-25 min), most human commensal & pathogens.. Each produce one colony contains 10^3 - 10^9 cells (Fig-4).
- Bacterial Strain originated from a single cell.
- Bacterial Growth Curve: 4 phases of visible growth...Lag, Log, Stationary, death/ decline.
- Measurement of bacterial growth followed by:
- A) Growth/enumeration of cells by direct cell counting in nutrient broth.. microscopic or counting viable cells/ colony forming unit.. Plate counts/ Electronic counting..using solid culture media..nutrient agar

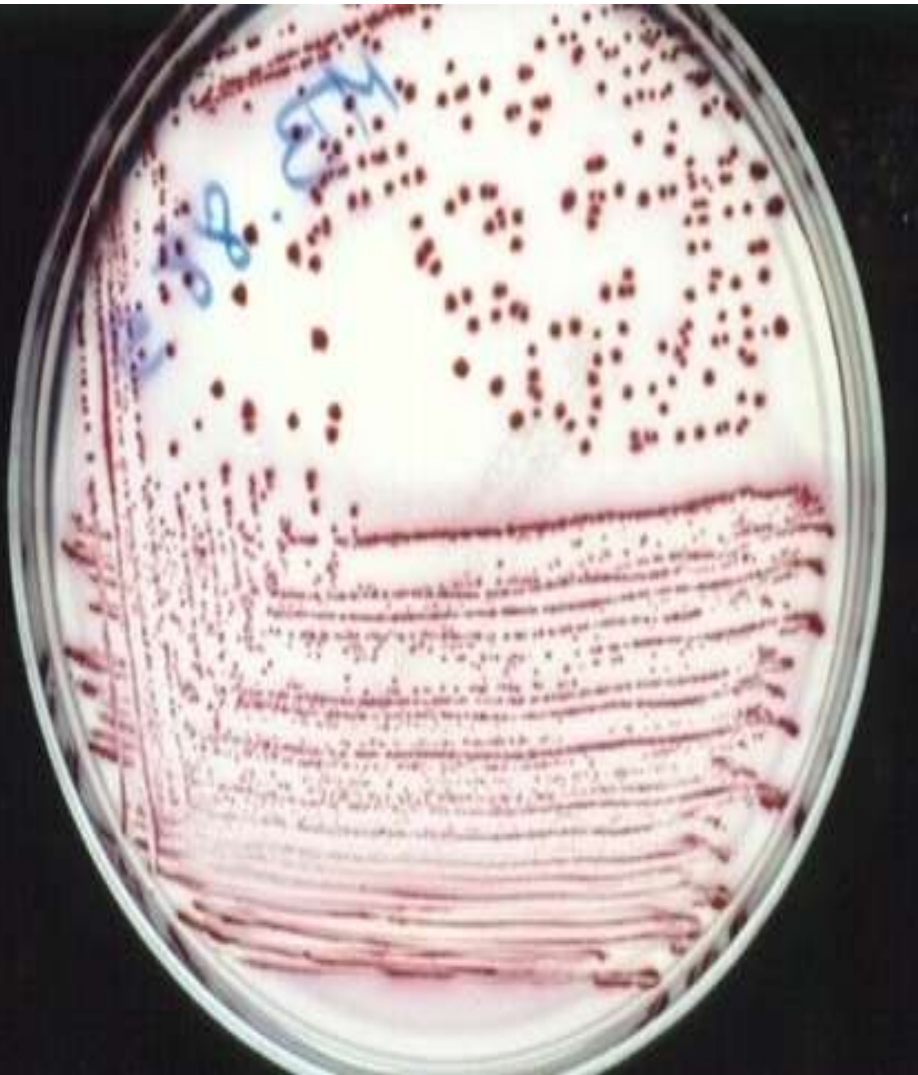
Binary fission Bacteria (Fig-6)



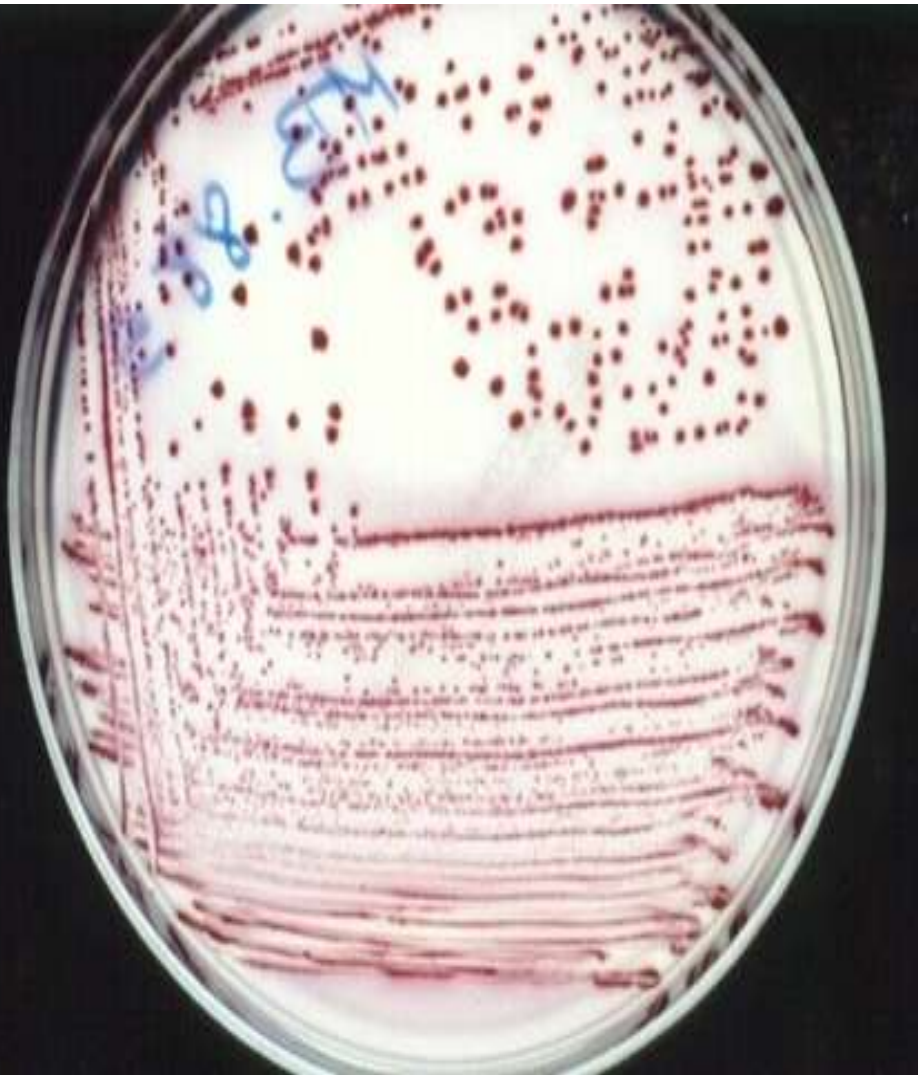
(a) A diagram of the sequence of cell division.

(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Bacterial Growth –MacConkey agar & Tube Broth (Fig-7)



Bacterial Growth –MacConkey agar & Tube Broth (Fig-7)



2/Measurement of Growth

- B) **Indirect counting of growth** in fluid medium.. most probable number by measuring turbidity, wet or dry weight.. G/ml.. Important in study research to detect antibiotics & treatment of infection.
- Types of culture media:
- **General culture media:** Nutrient agar, blood agar, chocolate agar..growth of most human pathogens.. Gram-ve & Gram-ve bacteria.
- **Selective & differential media**..MacConkey agar
Bile salts+ Lactose+neutral red dye ..Gram-ve bacteria, E.coli, other enteric bacteria
- **Selective media:** S-S agar .. For Isolation of Salmonella, Shigella , V.colerae from stool specimens.

Bacteria Growth Curve (Fig-8)

