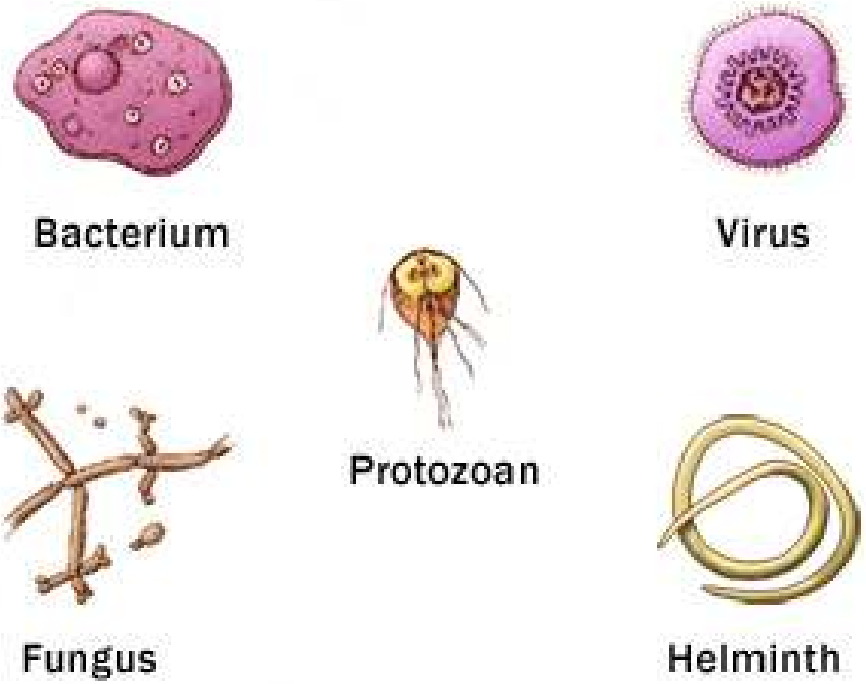
Introduction To General Microbiology

The Microbial World

- The microbial world is composed of microbes/ microorganisms
- Free living
- Commensal
- Pathogenic



- Consists of
- Bacteria
- Fungi (Yeast/ Moulds)
- Algae
- Protozoa/ Parasites and Helminths
- Viruses

- **Microbiology** is concerned with the study of these microbes.
  - **Mostly are beneficial**
  - **Few species cause harmful effects**
- **Microorganisms** are unicellular cell, too small to be seen with the naked eye, recognized by light microscope.
- Bacteria, fungi & parasites, size above **> 0.1 um**
- Most microbes capable of grow & existence as **single organism or together with others.** Widely distributed in human, animal, nature.

Microbiology

- Viruses sizes **< 0.01um**
- Composed of only **DNA** or **RNA**.
- Grow only in living cells/tissue culture.
- 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, Industrial microbiology, Agriculture Veterinary.

Bacteria

- **Bacteria**
- Unicellular microorganisms.
- Size (0.2umDiameter, 0.2-10um Length)
- Having a variety of shapes.

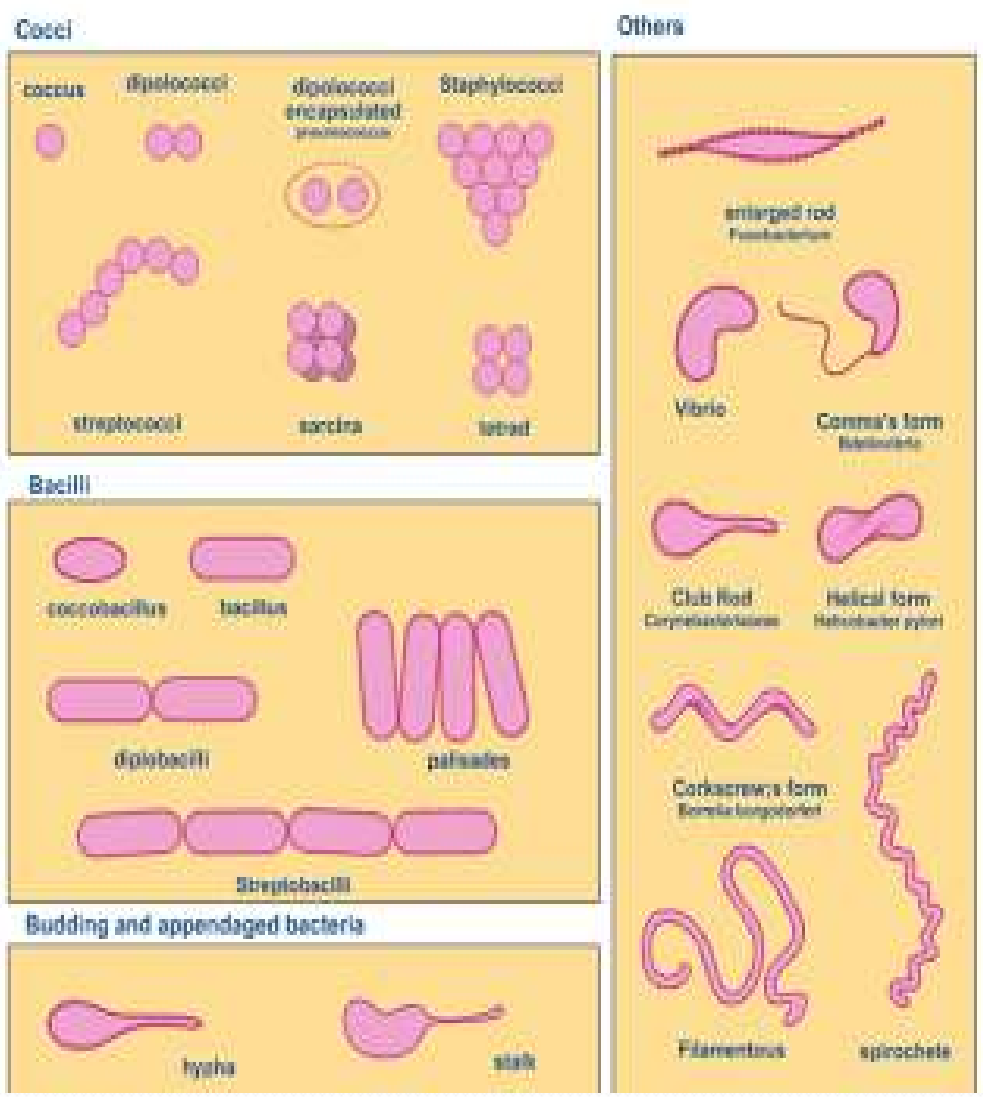
Coccus/*cocci*,

Bacillus/*bacilli* or *Rods*

Coccobacilli

***Spiral forms*- spirochetes**

Vibrios



- Growth patterns & metabolic characteristics allowing their classification.

- 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)

Fig-1: Gram-Negative/positive

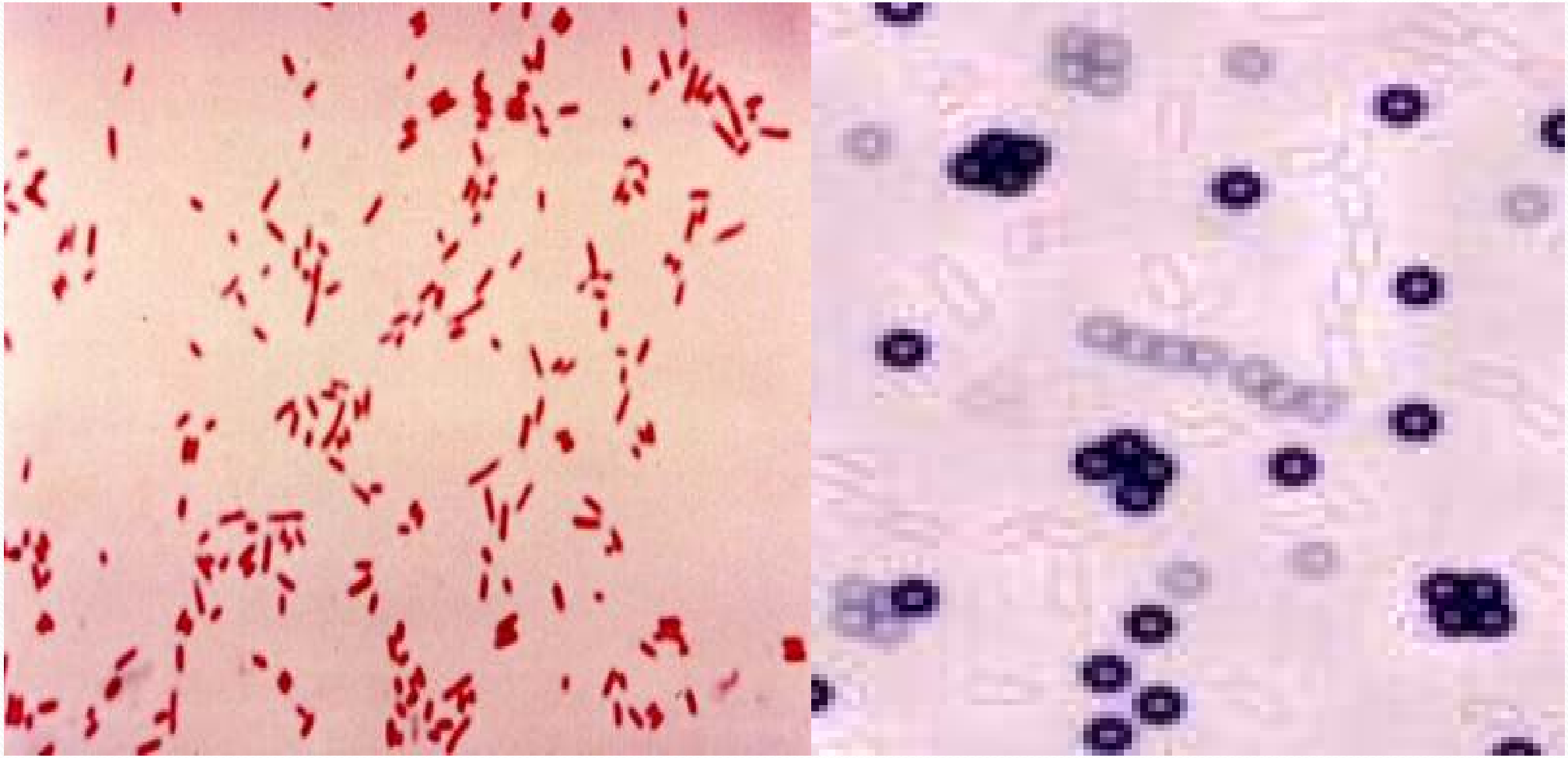
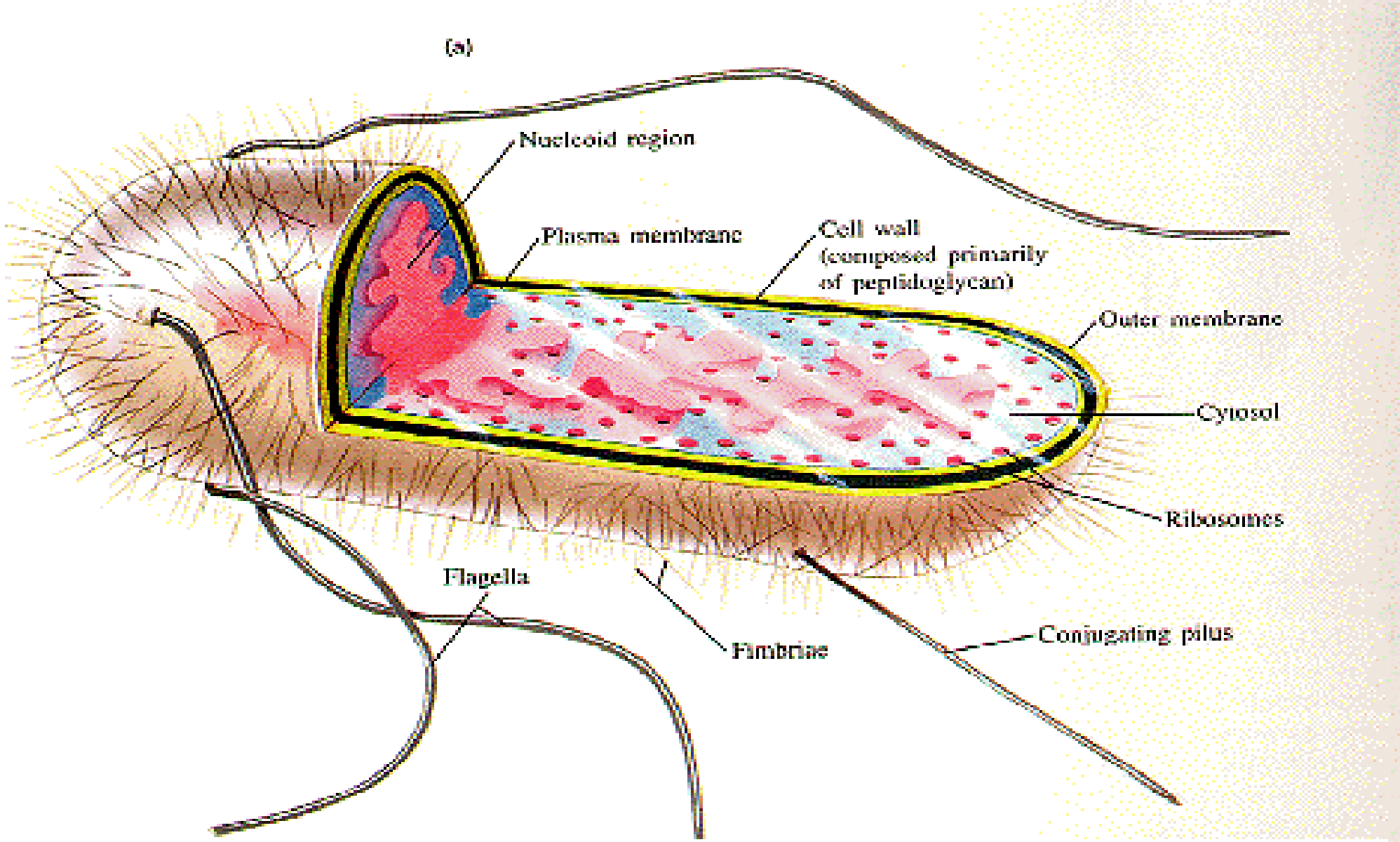


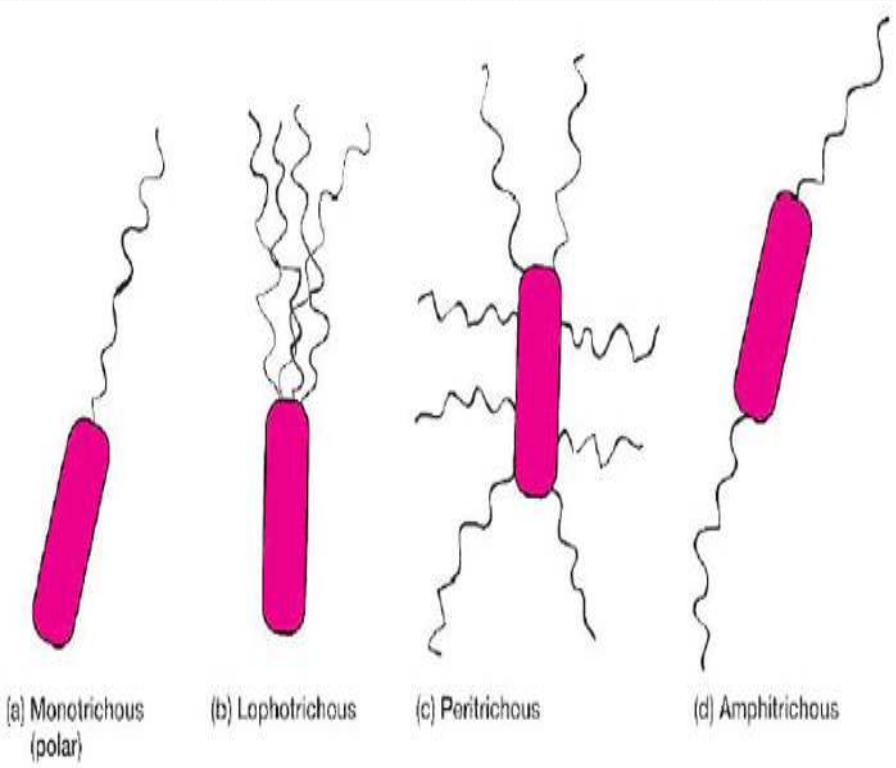
Figure -2 Bacteria Cell structure



- **Flagella:**

- Organs of motility,
- Composed of flagellins (polymer proteins) long filament
- Length up to 20 um
- Attachment..
- Nutrition..
- Single polar flagellum **(monotrichous)**
- Several polar flagella at one, each end of the cell or covering the entire cell surface **(peritrichious)**
- **Antigenic determinants**

  (H-antigen), observed during bacterial infection.



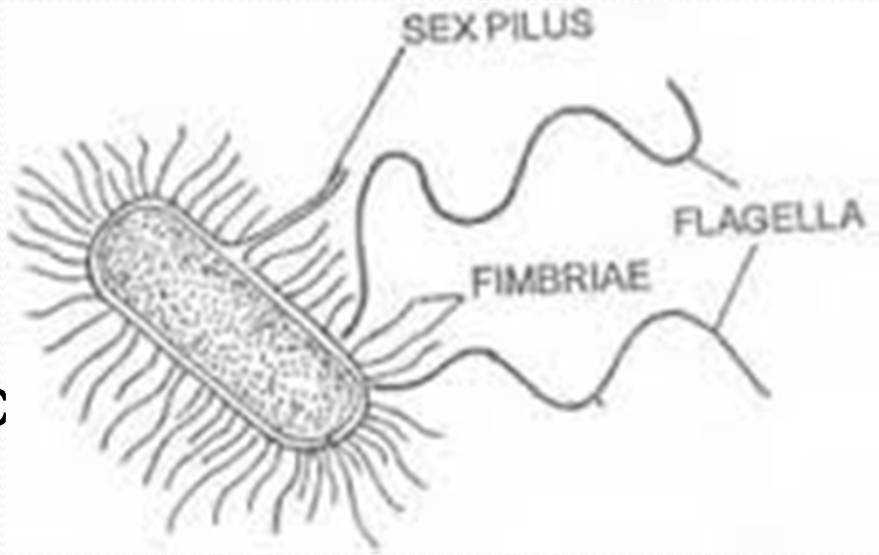
Bacterial Cell structures-2

- **Fibmriae and Pili:**

- Small surface appendages (protein)

- Few numbers Pili
  (**Sex pili in conjugation)**

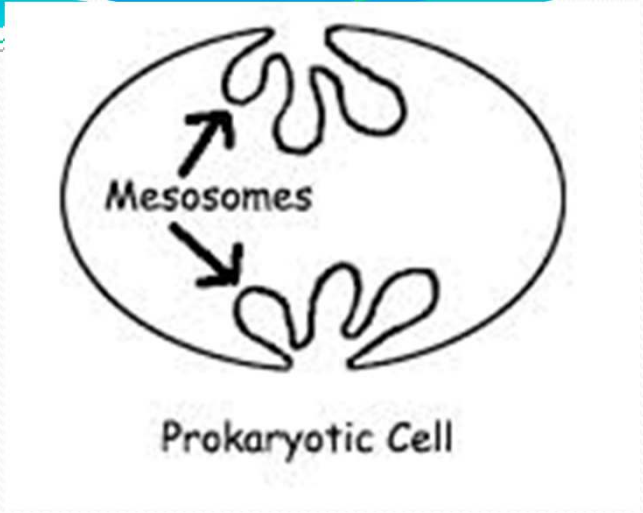
- Large Numbers fimbriae



- Both pili and fimbraie have specific functions such as
  * **Attachment/Adhesion** to host epithelial cells/colonization
  * **antigenic determinants..**
  Anti-H antibodies

Other components in bacterial cells

- 70S ribosomes



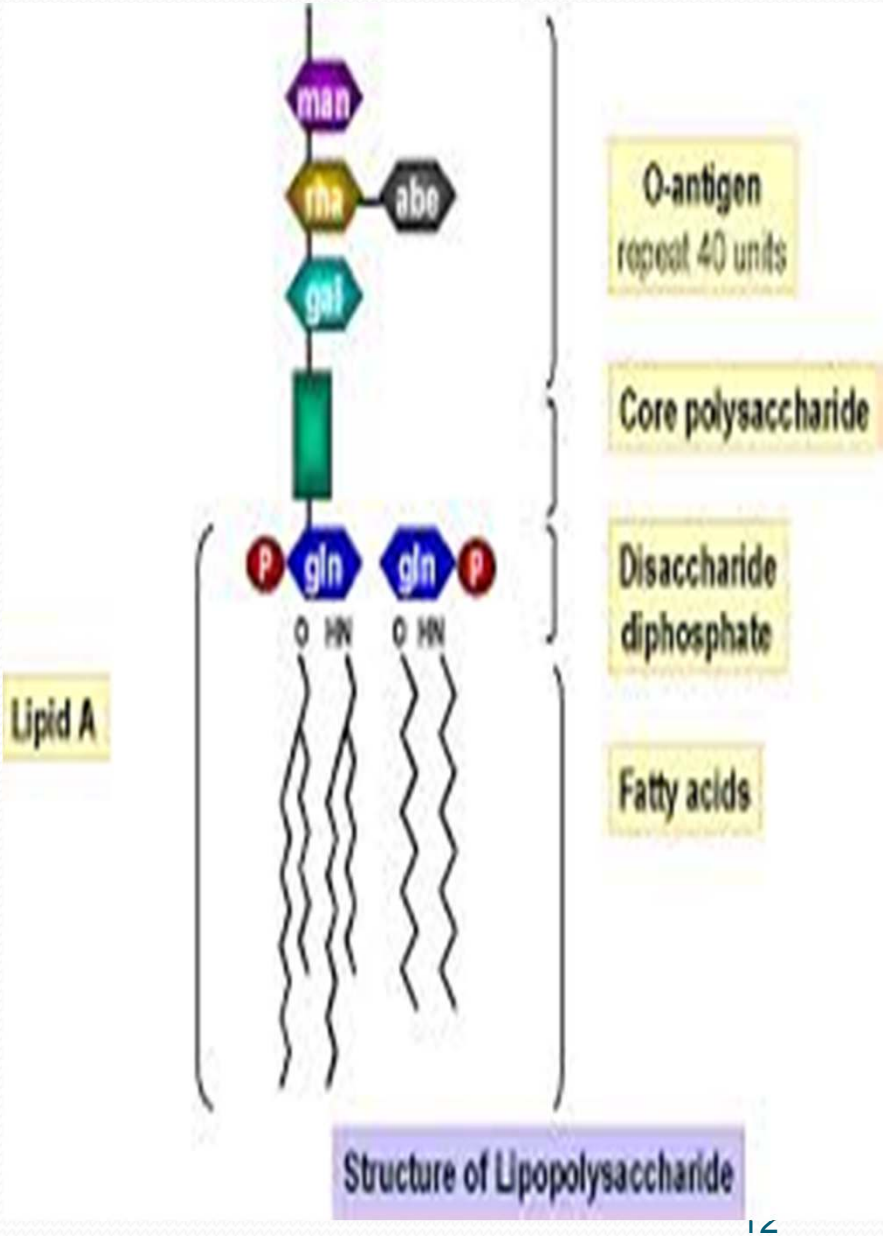
- Mesosomes

  Infoldings in the plasma membrane,these are rich in enzymes that helps to perform functions like cellular respiration, DNA and cell division (most important function)

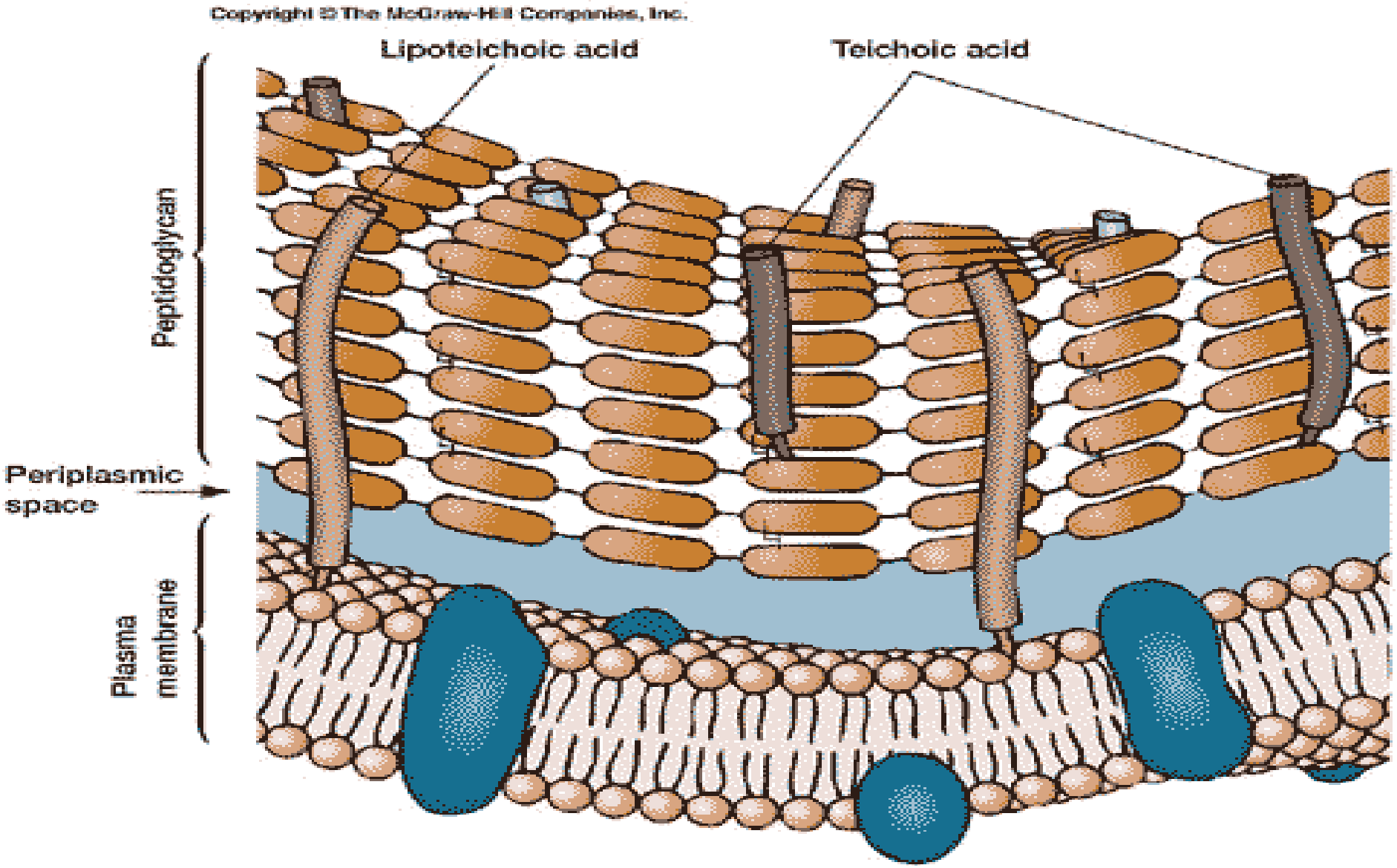
- Storage granules -Lipids, glycogen, polysaccharides, sulfar, phosphate and others storage compounds.

Cell wall

- Teichoic acid and Lipoteichoic acid in gram positive bacteria
- Lipopolysaccharide (LPS or endotoxins) in gram negative bacteria cause endotoxic shock.
  - **LPS** structures are composed of **lipid A,** which binds to the outer membrane
  - **Endotoxic portion** of the molecule, causing toxic shock, high fever, sepsis
  - The polysaccharide moiety appears on the cell surface, serving as an antigenic determinant **O antigen**-
  - Host cells develop during bacterial Infection..**Anti-O Antibodies**



Cell wall Gram-positive bacteria-3



Bacterial Cell structures-

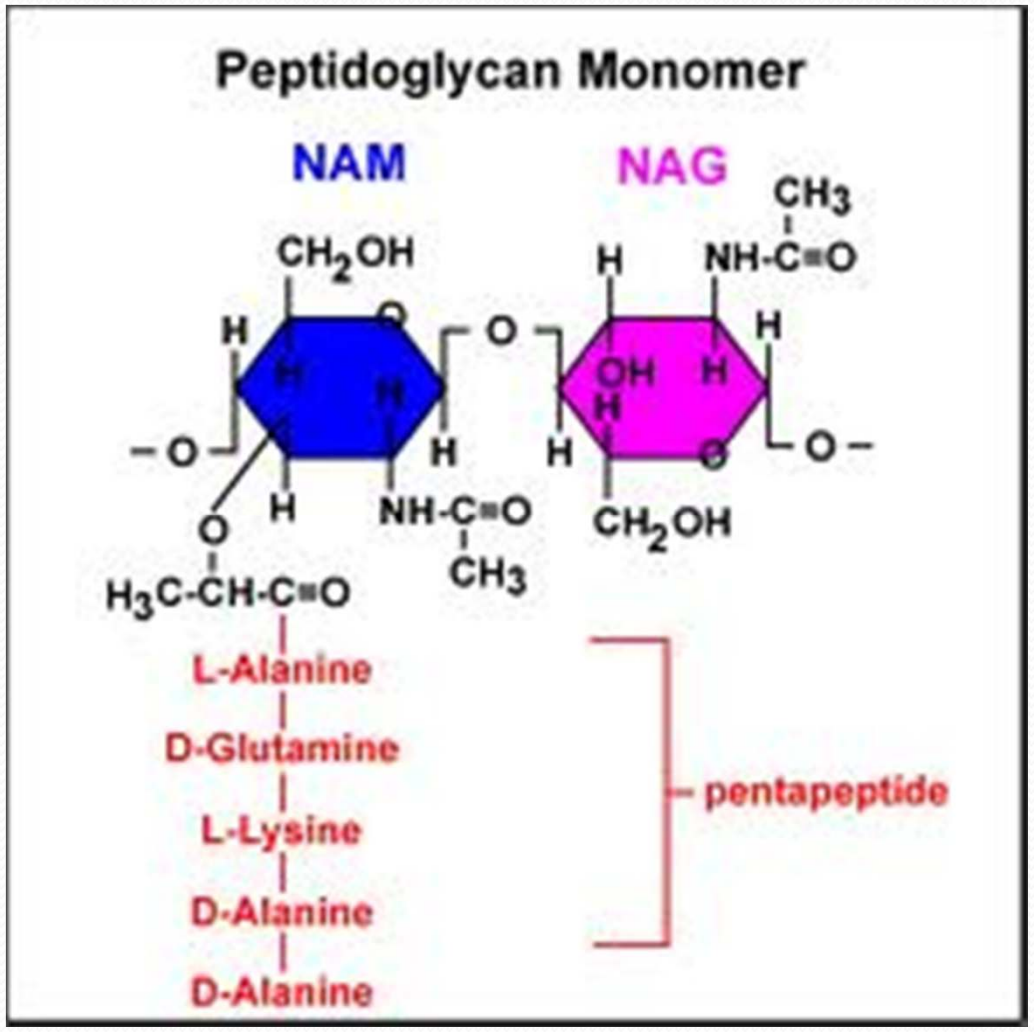
- **Cell wall structures:**

- A rigid cell wall
- Cell wall is the basis for classification of bacteria into Gram-positive & Gram-negative by Gram-stain
- Composed of many peptidoglycan layers

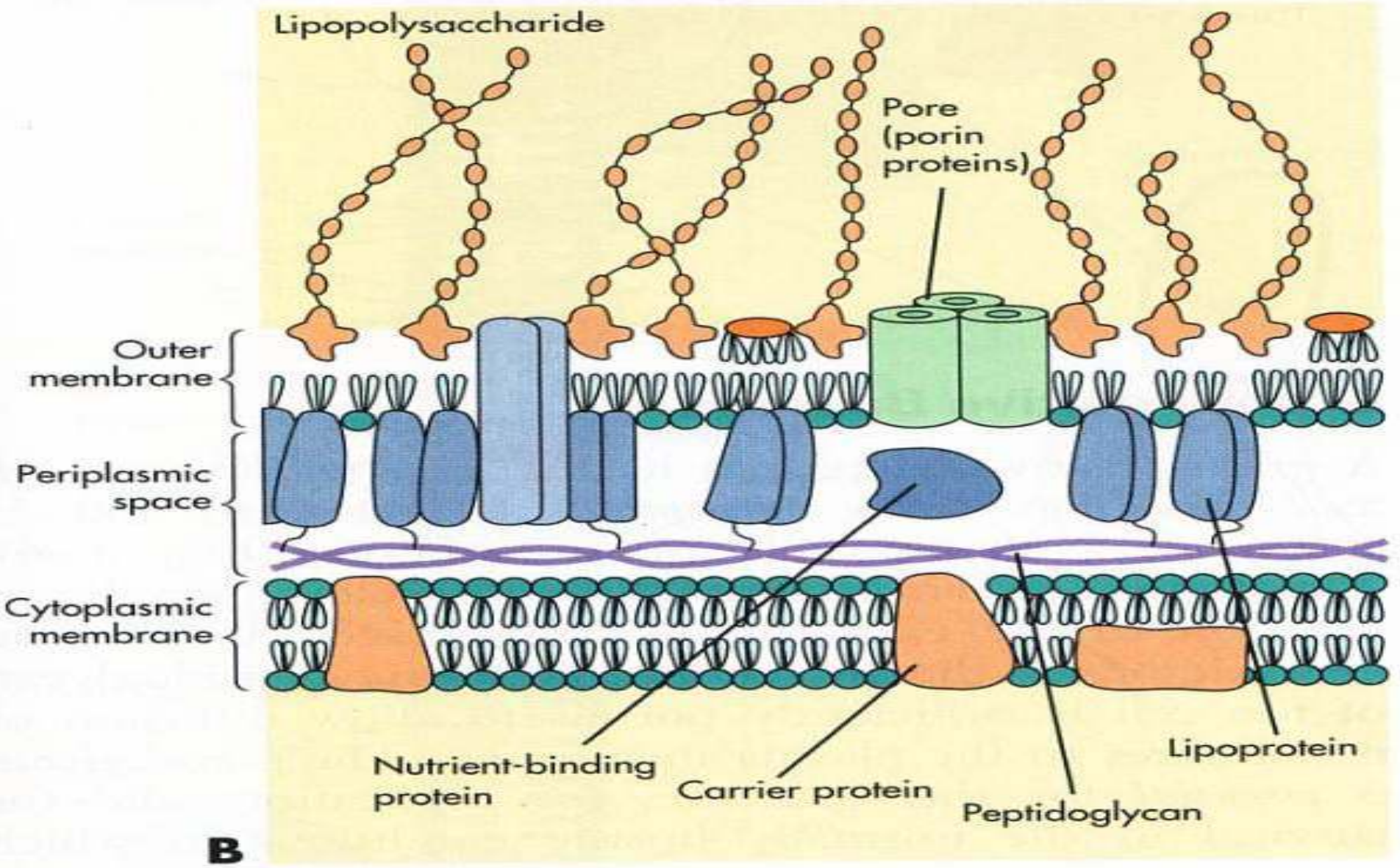
N-acetylglucosamine

N- acetylmuramic acid

Pentapeptide.

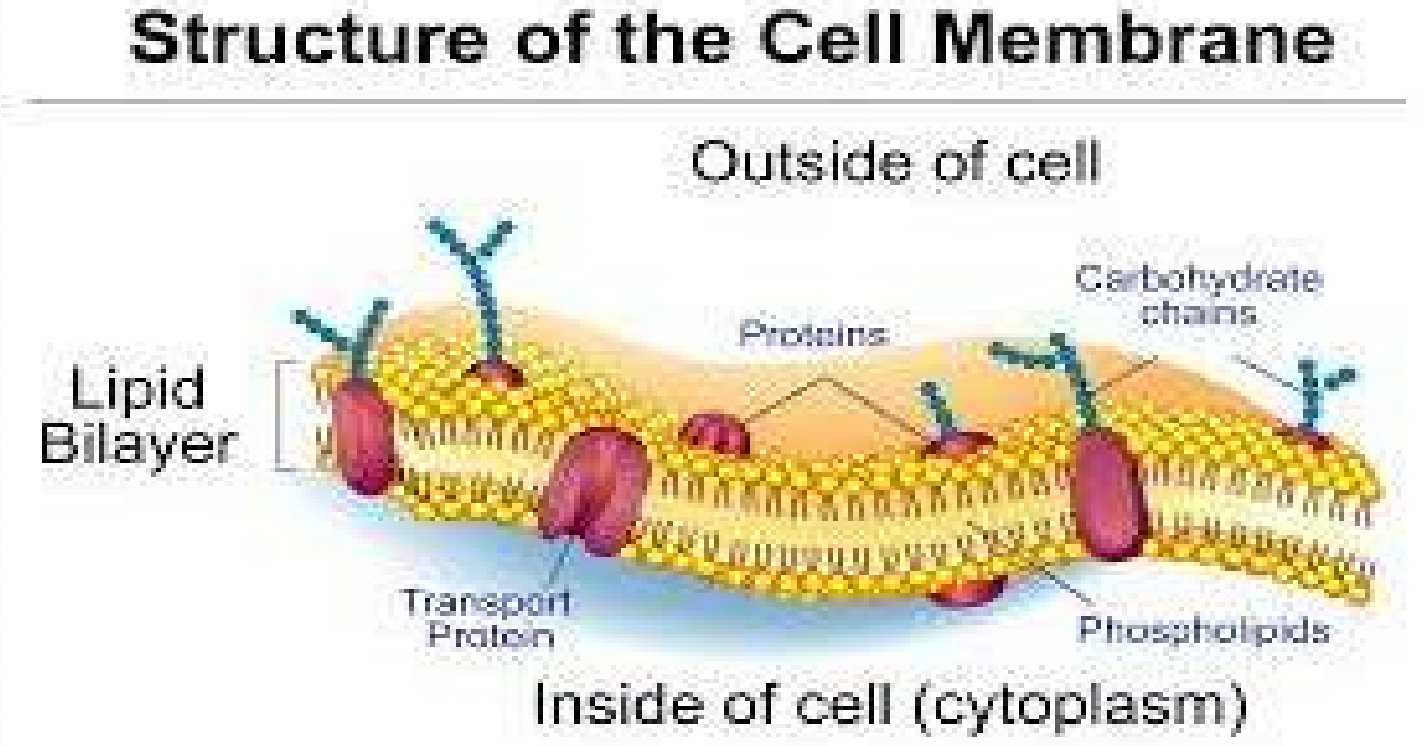


Cell Wall Gram-negative bacteria-4

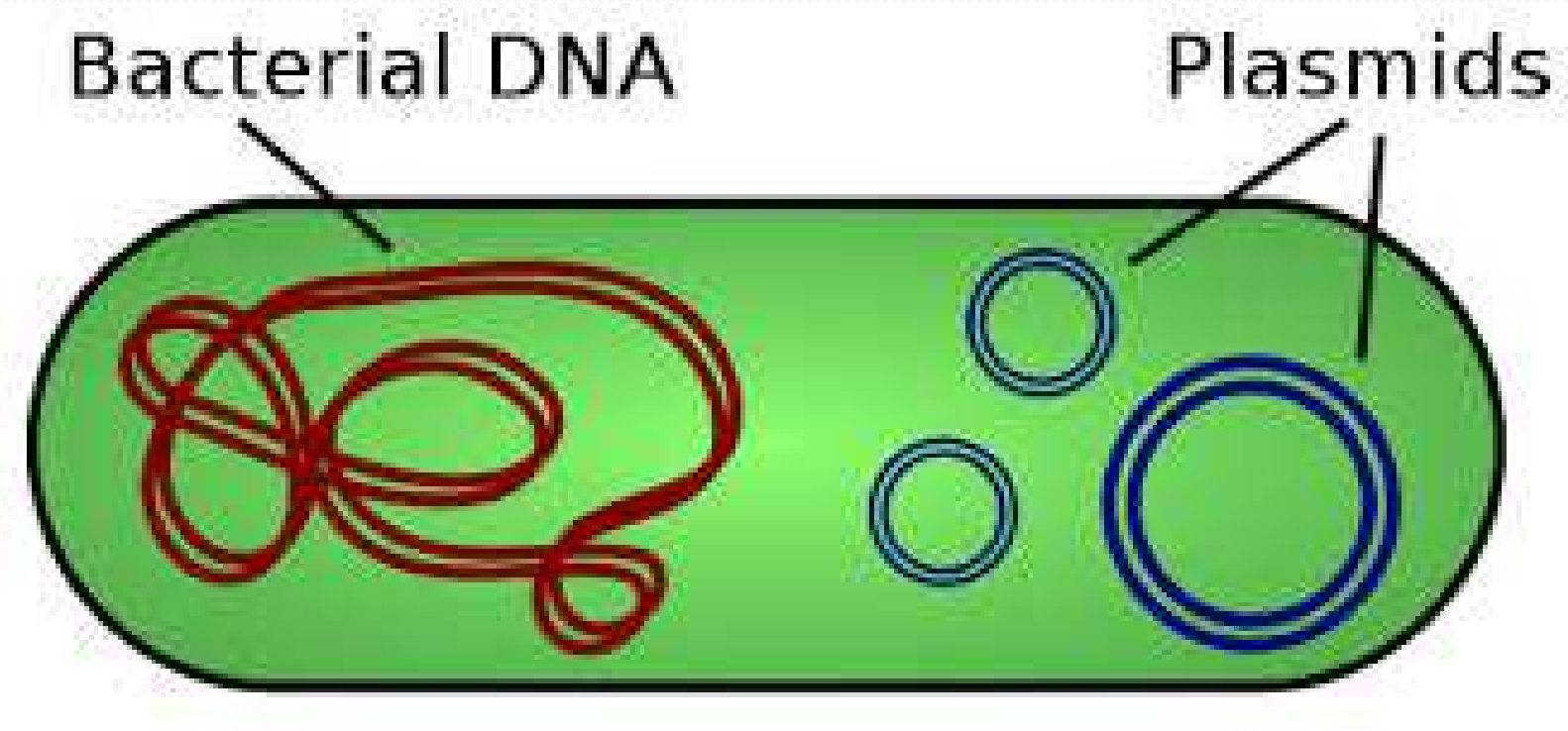


Cytoplasmic membrane

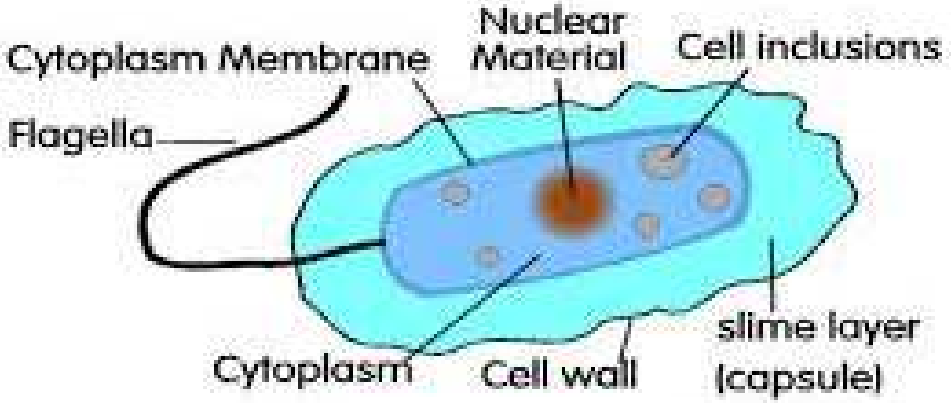
- Phospholipid bilayer
- Cytoplasmic membrane lacking sterols
- Contain various membrane proteins, enzymes and permeases
- Responsible for transport of ions, nutrients and waste across the membrane and Control the cell plasma contents



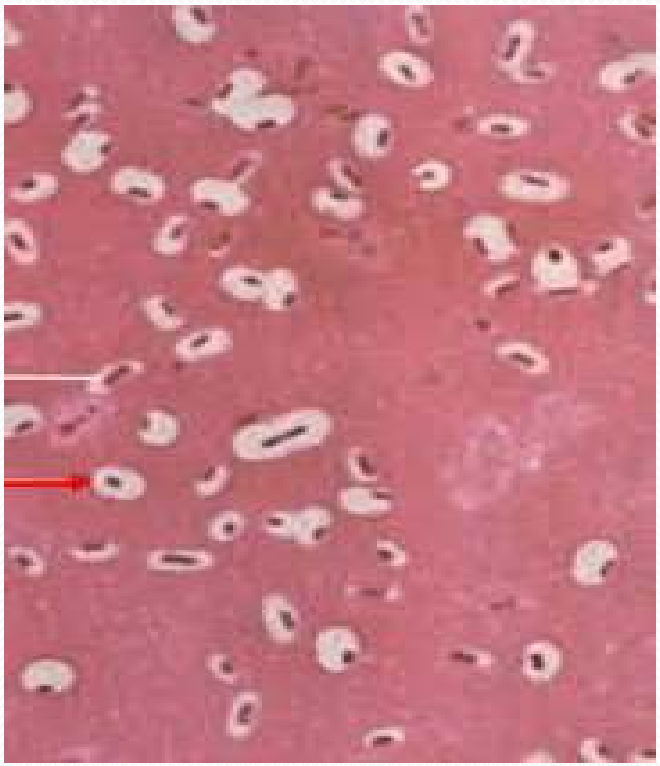
- **Bacterial genome**..
- One **single** supper coiled DNA chromosome
- Plasmids (≥).



- **Capsules:**
- Surface layer of cell wall
- Slime layer composed mostly of high molecular weight polysaccharides
- Functions
- Provide resistance to phagocytosis
- Avoid the killing effects of lysosomal enzymes, and
- Serve as antigenic determinants (**K-antigen)**
- Major virulence factor in certain bacteria
- Biofilm



Cell structure



Gram-Stain

A- **Gram-positive** Stained purple (crystal violet)

Staphyloccocus, Streptocooci, Bacillus

* Protoplasts & L-form due to lysozyme effect, loss most Cell wall, Burst +Lysis

B- **Gram-negative** Stained red (safranin)

Enteric bacteria group. *E. coli, Klebsiella, Salmmonella, Pseudomonas*

* *Spheroplasts (L-form)* in gram negative

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 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
- Application of moist heat at□00-□20°C for a period of □0-20 min may be needed to kill spores.
- **Aerobic *Bacillus*** group & **Anaerobic *Clostridium***
- Both are widely distributed in nature, intestinal -human and animals.

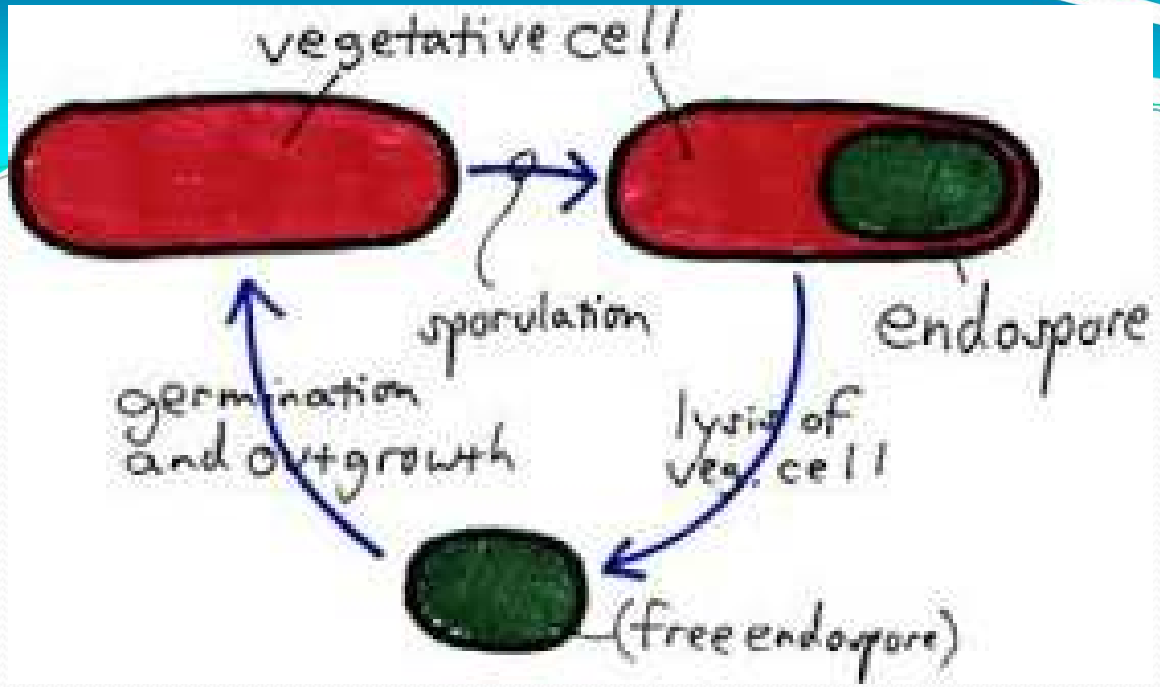
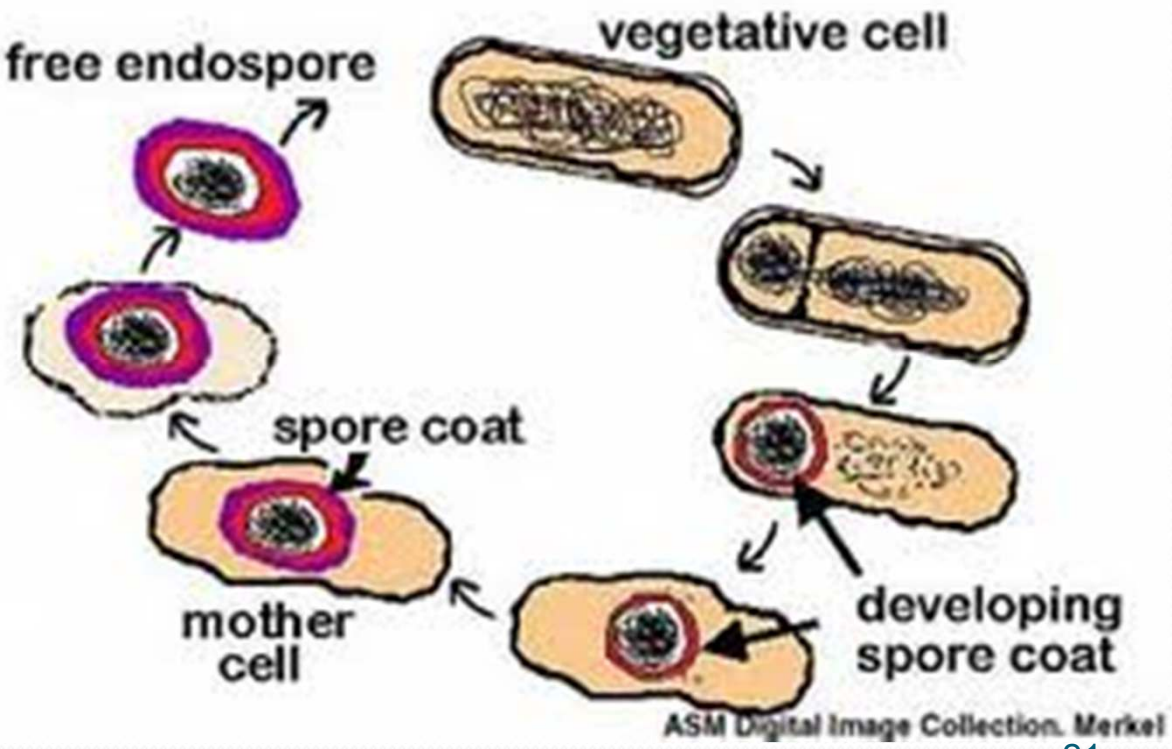

vegetative cell
sporulation
endospore
germination
and outgrowth
lysis of
veg. cell
(free endospore)

Figure 4

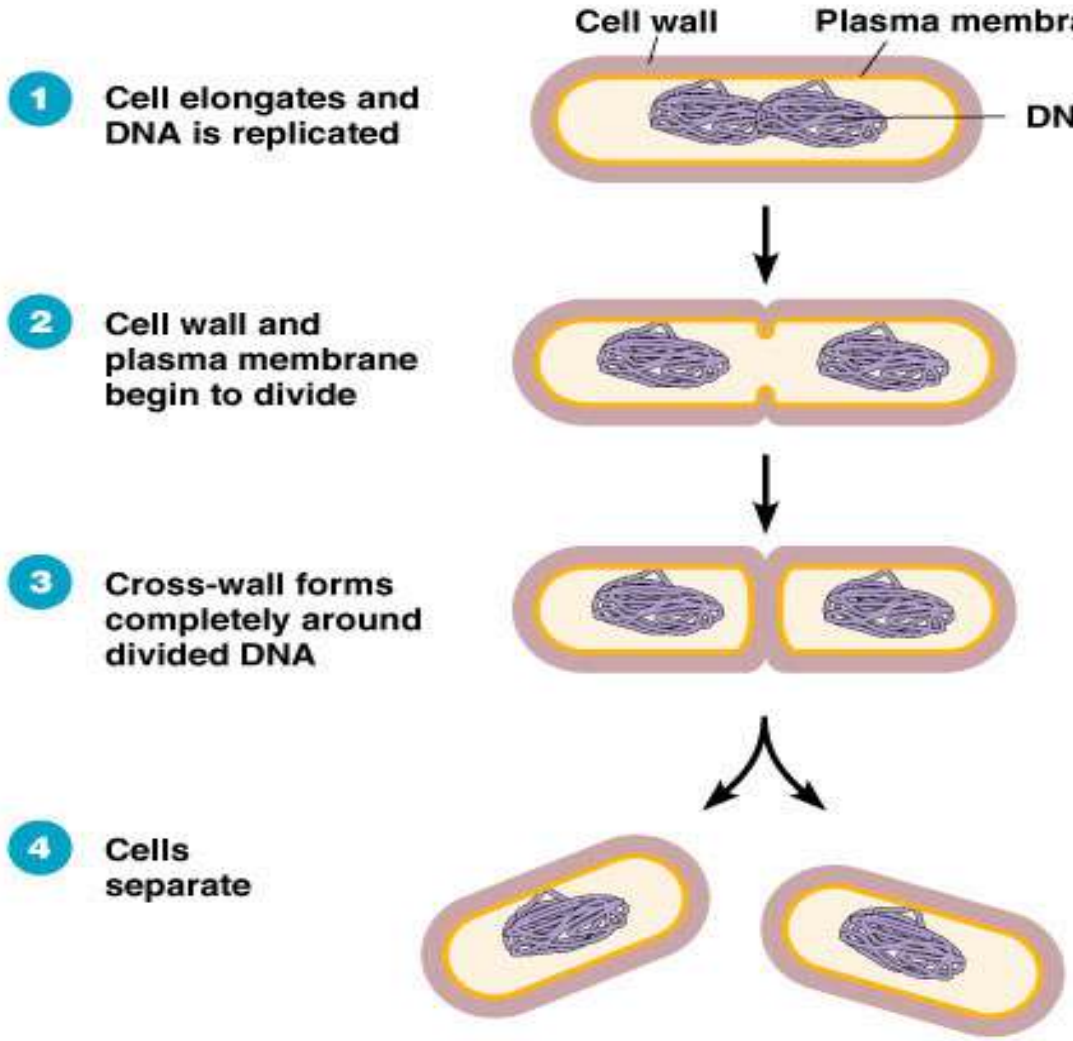

free endospore
vegetative cell
spore coat
mother
cell
developing
spore coat
ASM Digital Image Collection. Merkel

Virulence factor

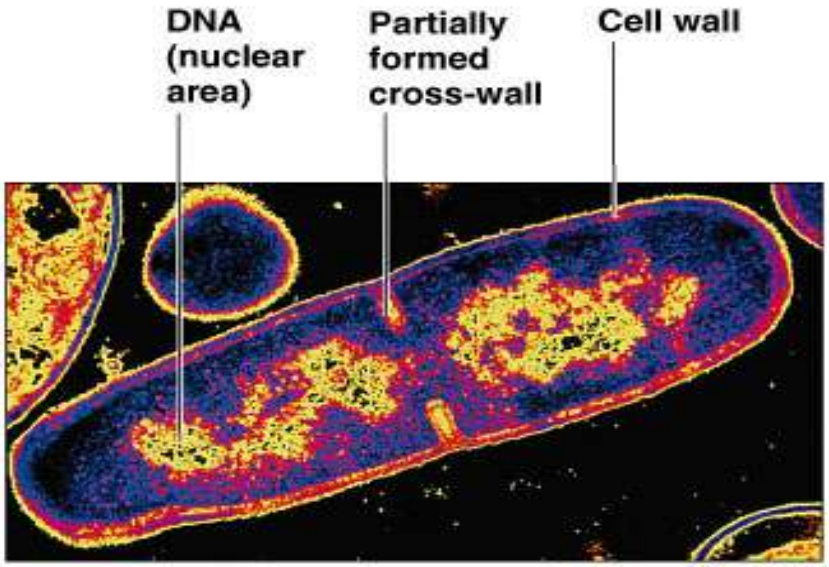
Any bacterial part/product associated with pathogenic potential cause human/animal is starting:

- colonization of a niche in the host (includes attachment to cells)
- Immunoevasion of the host's immune response
- Immunosuppresion.. inhibition of the host's immune response
- entry into and exit out of cells
- obtain nutrition from the host..causing **sepsis, septicemia**

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.

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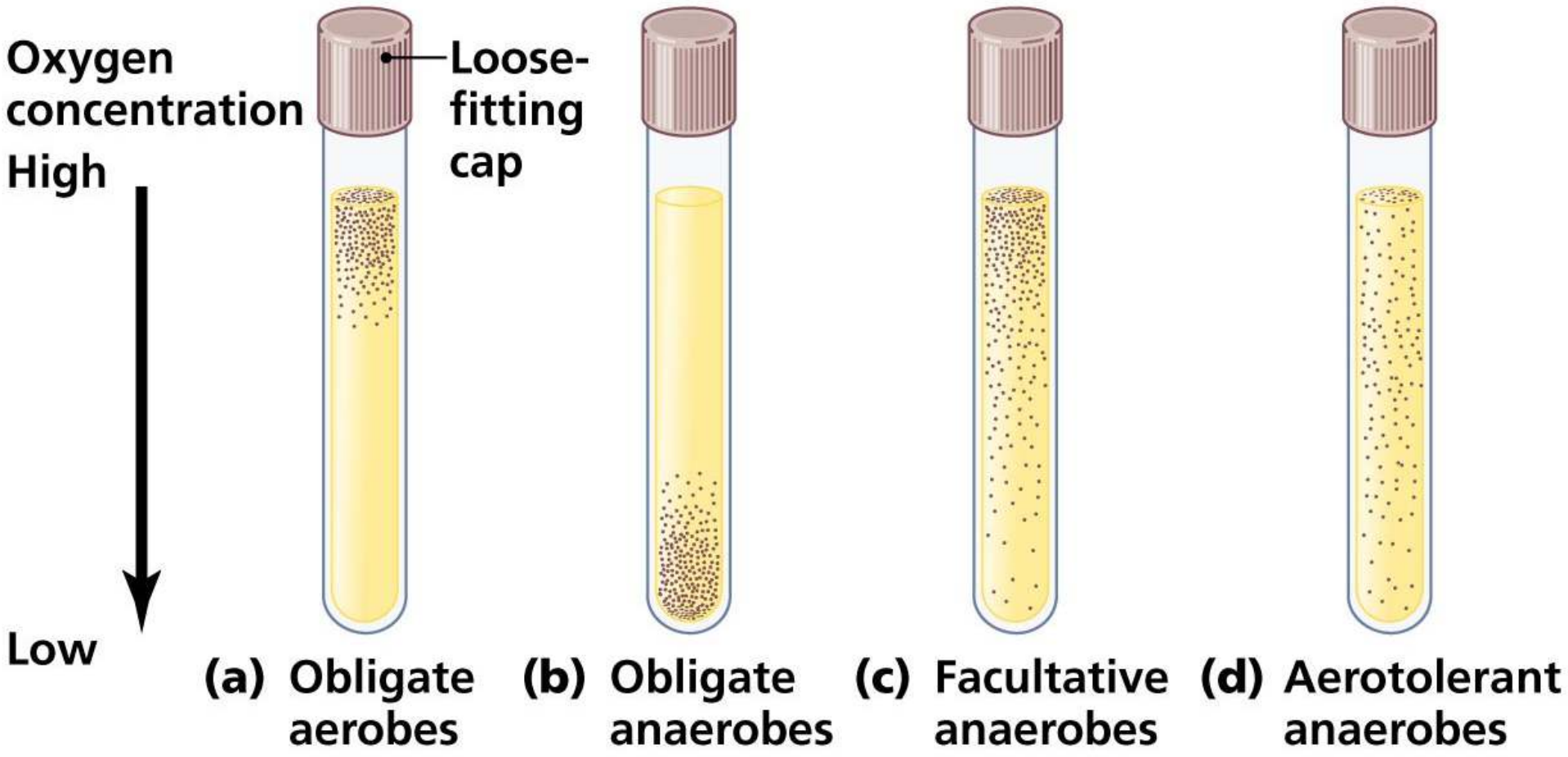
Growth & Nutrition-

- Requirements for bacterial growth: temperature, oxygen, water, pH, temperature, source of carbon, nitrogen (organic compounds), inorganic salts.. Na, K, S, P, Ca, Mg, Cl, Fe, vitamins, etc.
- **Obligate aerobic** such as *M. tuberculosis*, *P.aeruginosa* grow using aerobic respiration. Oxidation. recipient Oxygen.
- Aerobic bacteria encounter the oxygen **damage cell membrane** (superoxide O^{-2} and hydroxyl OH^{-} radicals and hydrogen peroxide H_2O_2) during their growth by producing oxidizing enzymes:
- Peroxidase: $H_2O_2 \rightarrow 2H_2O+NAD.$
- Superoxidase dismutase: $O_2- \rightarrow H_2O_2 +O2$
- Catalase: $H_2O_2 \rightarrow 2H_2O+O_2.$

Oxygen requirement by bacteria



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Growth & Nutrition-2

- Certain Pathogens grow with reduced level of oxygen.. **Microaerophilic bacteria**..Neisseria species
- **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 such as protein, sugar, fats. Human tissues. All commensals-pathogens and **Saprophytic bacteria**/ Nonpathogenic. Take energy by fermentation/respiration. Found in nature and in decaying material, soil and water, important for circulation of minerals.
- **Autotrophs**: fix carbon dioxide to make their own food source.
  - **photoautotrophic**, using light energy
  - **chemoautotrophic** oxidation of nitrogen, sulfur, other elements. Such as sulfur & nitrogen fixing bacteria found in the environment.

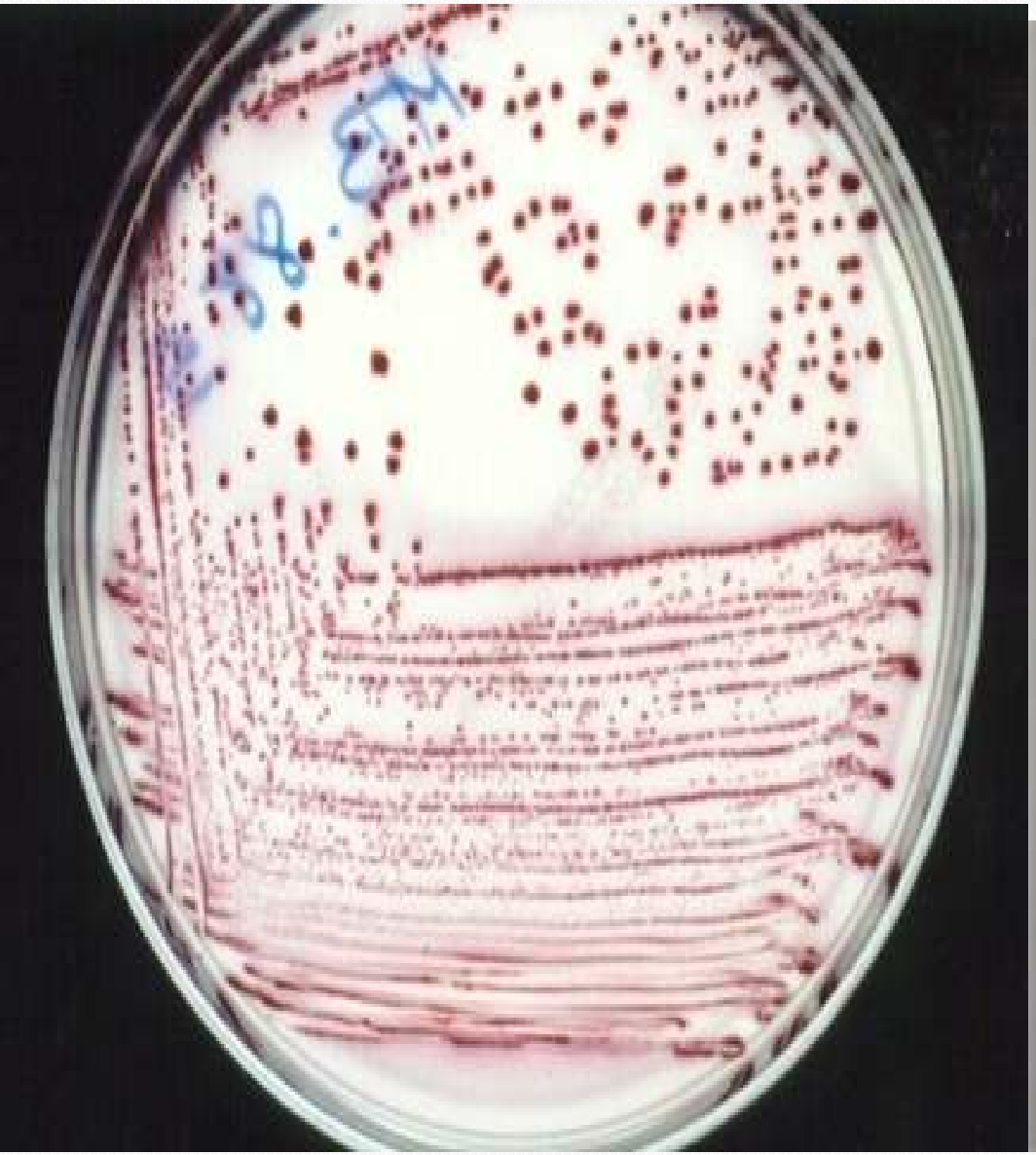
Culture Media:

- Nutrients (carbohydrates & proteins, blood)
- Minerals
- Water
- pH
- Tempertaure

- Growth cultures (Fig 7):

- Broth medium
- Solid medium such as Blood agar in Petri

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

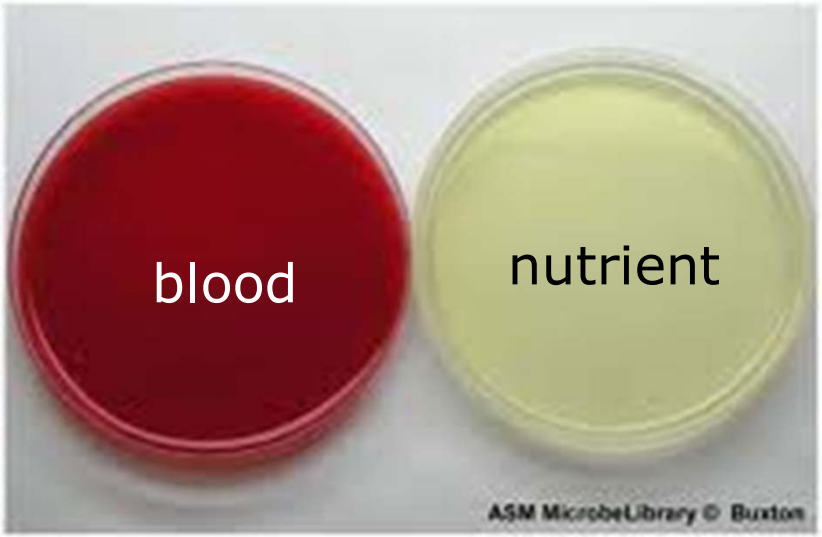


Types of culture media:

1. **General culture media:** growth of most human pathogens, Gram-ve & Gram-ve bacteria
   Nutrient agar
   Blood agar
   Chocolate agar

2. **Selective & differential media**..
   MacConkey agar (Bile salts+ Lactose+neutral red dye) Inhibits Gram-ve bacteria enhance the growth of E.coli, other enteric bacteria

3. **Other Selective media:** S-S agar .. For Isolation of Salmonella, Shigella , *V.cholerae* from stool specimens.


blood
nutrient
ASM MicrobeLibrary © Buxton


MacConkey

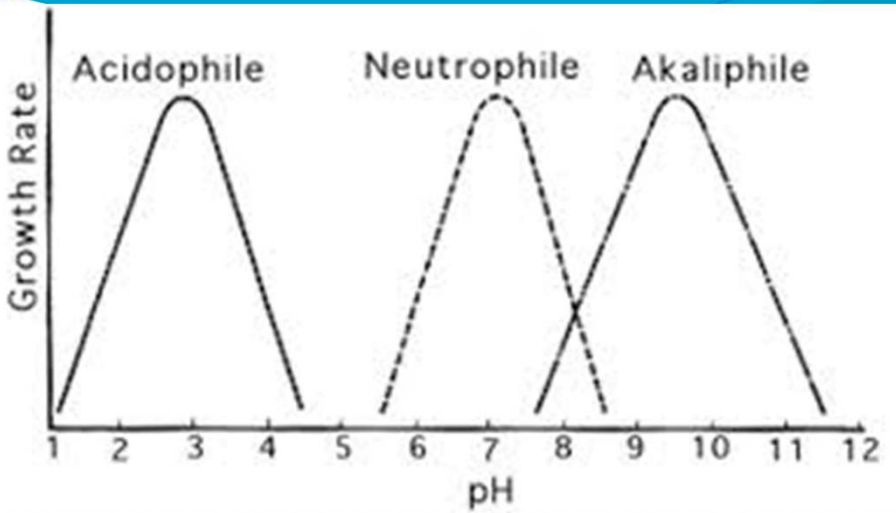

Chocolate


SS

- **Neutrophilic bacteria**

  Grow best pH (7-7.2) Most human-animal commensals & pathogens
- **Acidophilic bacteria**

  < 5 pH.. Lactobacilli

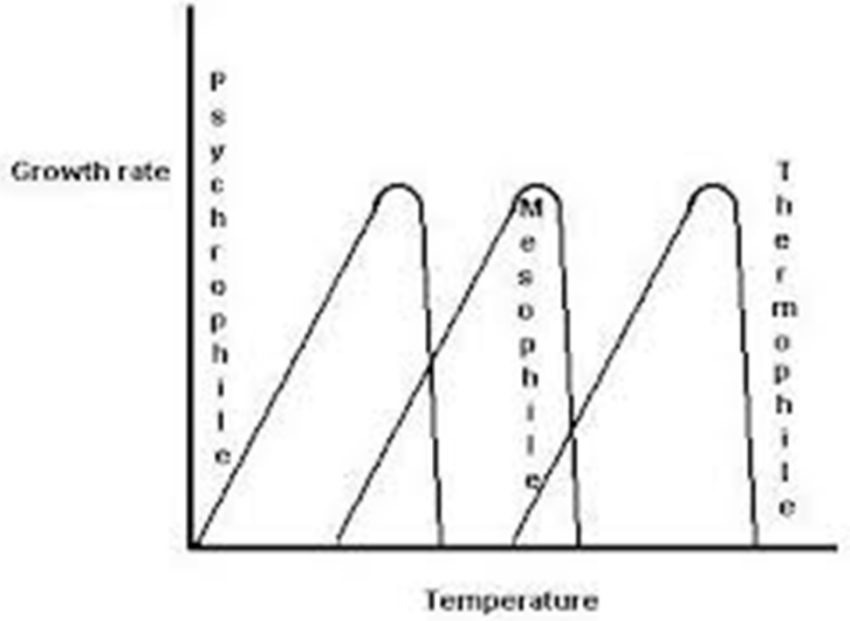


- **Mesophilic bacteria**

  Grow at (20-40C). Most human commensal & pathogens.
- **Psychrophilic bacteria**

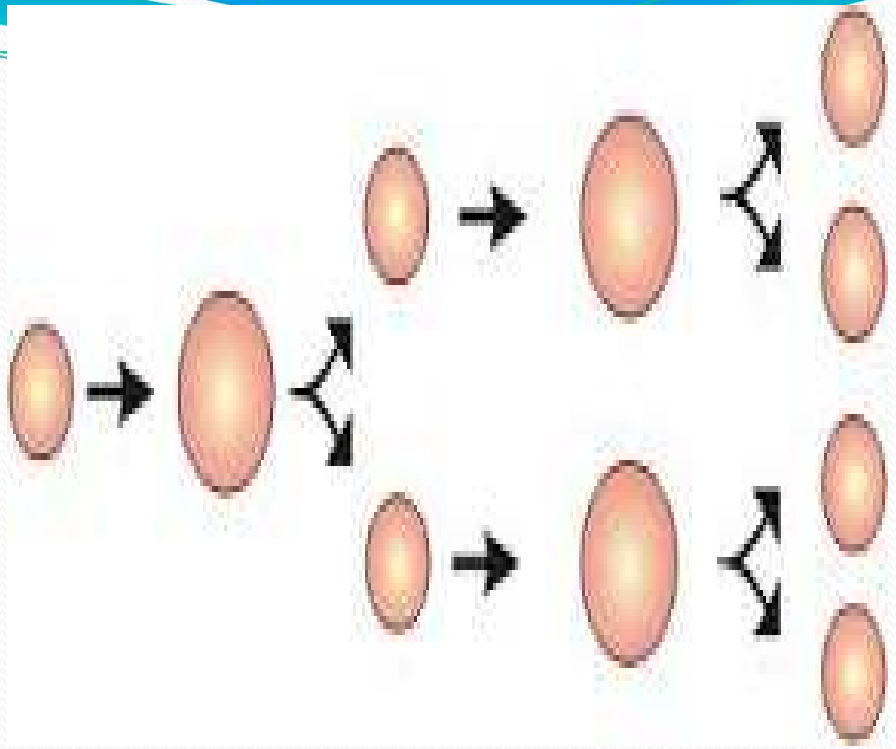
  (<□0C),
- **Thermophilic bacteria**

  (> 60C)..Common in hot spring water



Bacterial growth-

- **Bacterial growth** is the division of one bacterial cell into 2 identical daughter cells ,2,4,8, 6.. by binary fission.



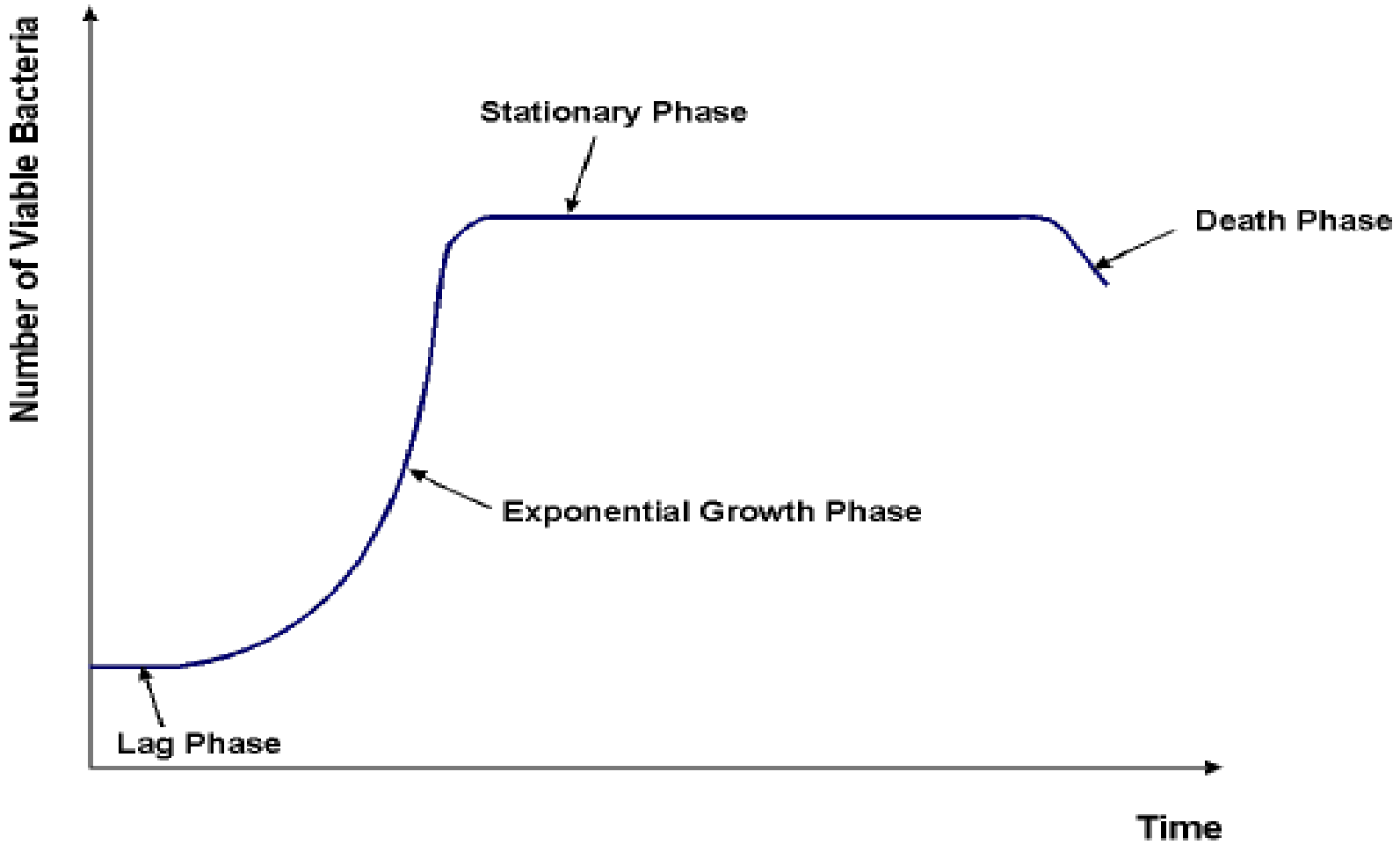
- Generation time

(5-25 min), most human commensal & pathogens.

- Each produce one colony 0^3 - 0^9 cells.

- **Baterial Growth Curve:**

- 4 phases of visible growth: Lag, Log, Stationary, death/ decline.



Measurement of bacterial growth:

A) Enumeration of cells by direct cell count

1. Microscopic
2. Using solid culture

Counting viable cells/ colony forming unit..Electronic counting using nutrient agar plates & dilution

B) Indirect counting of growth in fluid medium.

Most probable number by measuring turbidity, wet or dry weight.. G/ml..

Enumaration of bacteria is very important in study research to detect antibiotics .