Morphology of Bacteria

Dr. Atit Shah
Associate Professor

RECOMMENDED TEXTBOOK OF MICROBIOLOGY

- Ananthanarayan & Paniker – 9th edition
- C.P. Baveja – 4th edition
- P. Chakraborty – 3rd edition
- Apurava Sankar Sastry – 1st edition

- **Reference book :**
- Subhashchandra Parija- 2nd edition
- Prep manual for U.G. – B.S. Nagoba- 2nd edition
- Jawetz

Recommended Textbook of Parasitology

- **Paniker – 7th edition**
- K.D. Chatterjee – 13th edition

- **Reference Book :**
- Ajit Damle
- D.R. Arora
- S. Parija
- C.P. Baveja

Practical microbiology

- **C.P. Baveja**
- **Reference book :**
- Mackey & Mac cartneys

Learning objective

- Groups of bacteria
- Parts of bacterial cell
- Functions of each part
- Laboratory methods for demonstration of each part
- Bacterial appendages & its significance

Major groups of bacteria

- True bacteria or bacteria proper
- Actinomycetes
- Spirochetes
- Mycoplasma
- Rickettsiae
- Chlamydiae

Morphology

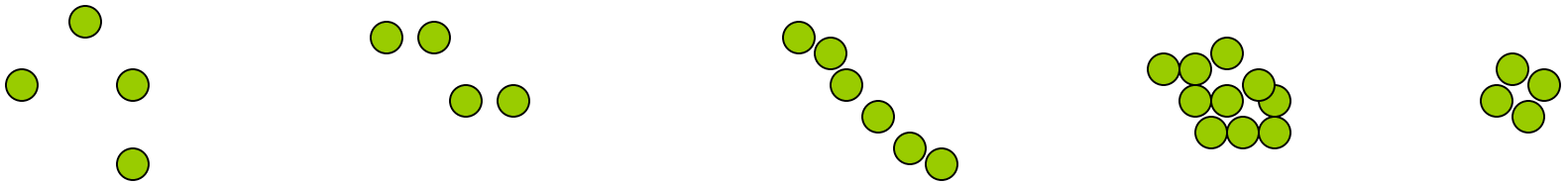
- Look – size, shape, arrangement
  - presence of flagella, fimbriae, capsule, spores

Staining characteristics
- Gram positive/negative
- Acid fast/non acid fast

Shape & Arrangement of Bacteria

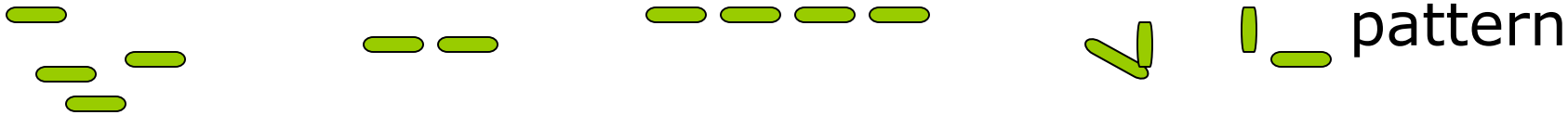
- COCCI

Singles Pair Chain Group Tetrad

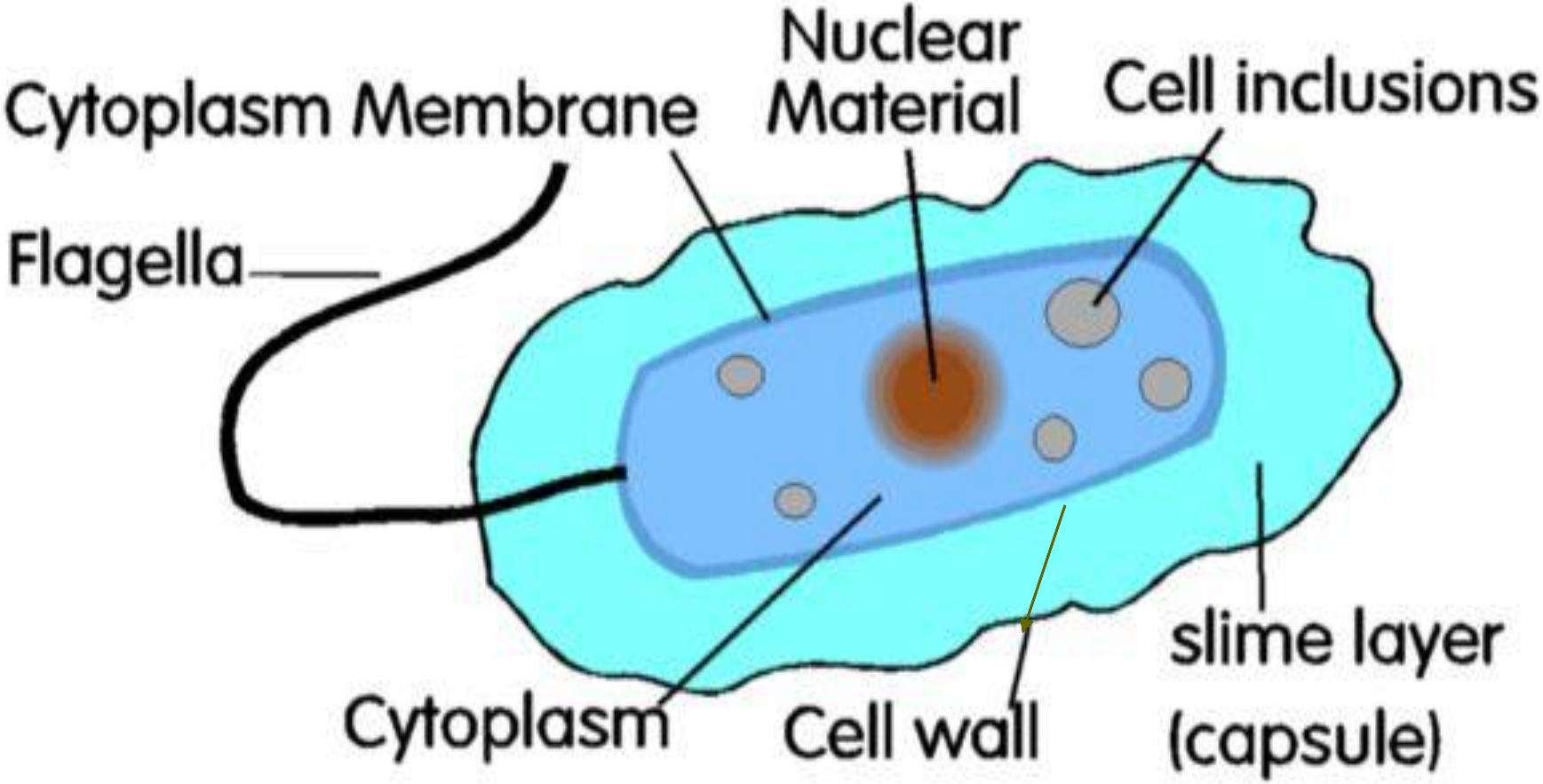


BACILLI

Chinese letter pattern

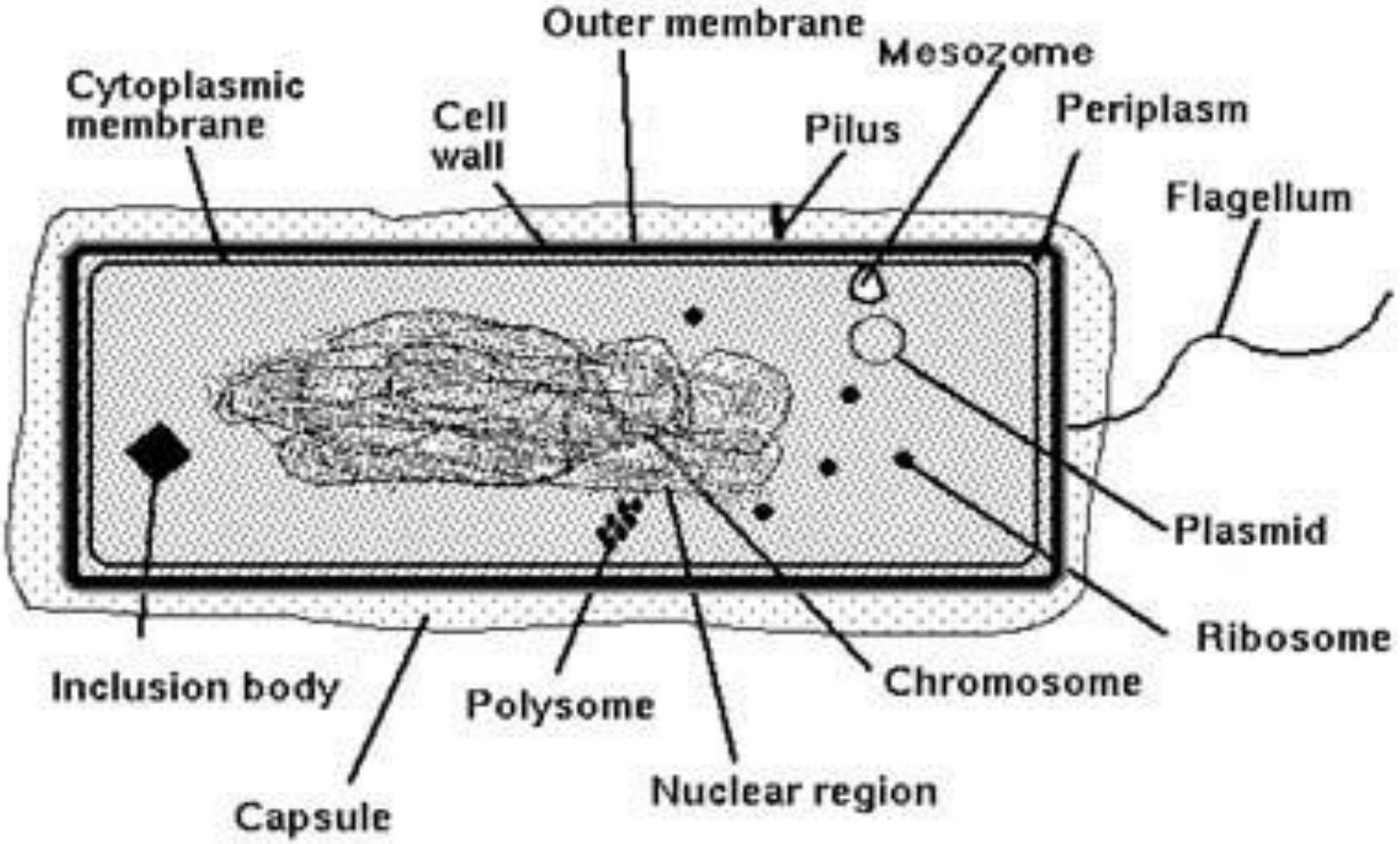


Spirochetes Coma shape



Cell structure

Structure of cell



Bacterial anatomy

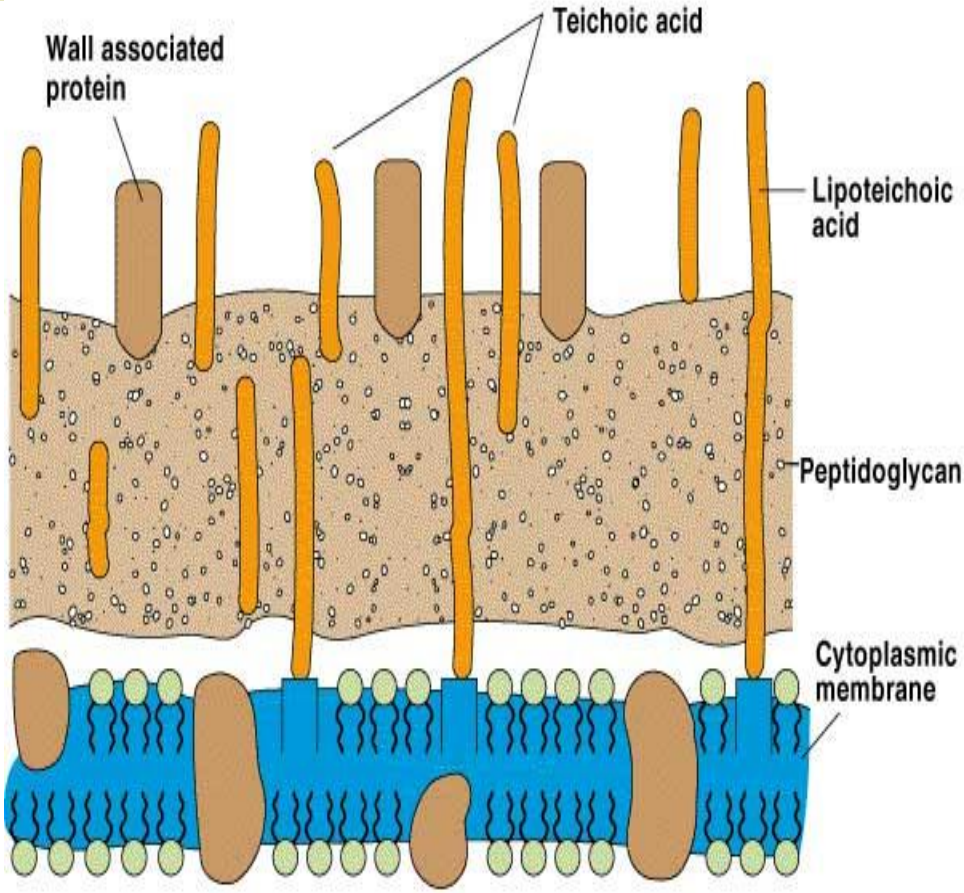
- Cell envelope
  - Cell wall
  - Underlying cytoplasmic membrane
- Cytoplasm or protoplasm
  - Ribosome, inclusion granules, mesosomes
  - Single circular chromosome of DNA
- Additional structures
  - Protective gelatinous covering -“capsule”
  - Filamentous appendage-”flagella”- organ of locomotion
  - Thin and short filamentous structures covering the whole cell-”fimbriae”- organ of adhesion

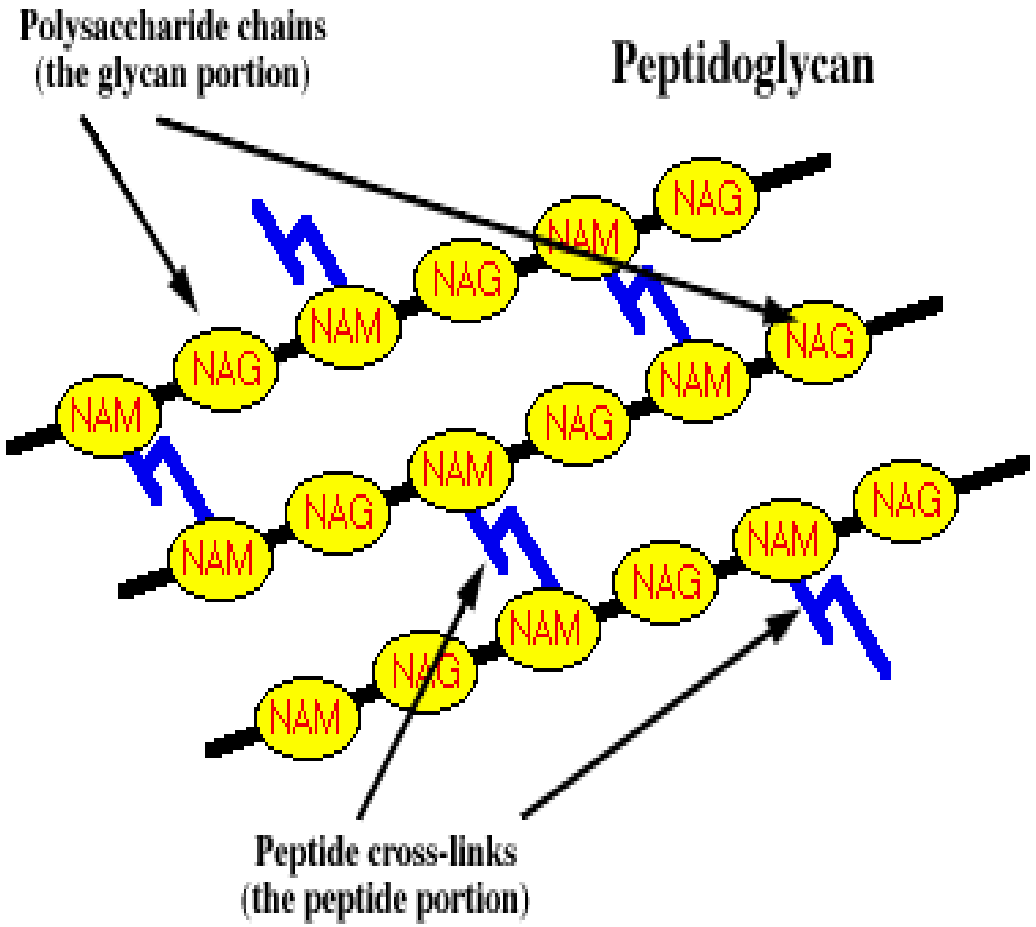
Cell wall

- Tough and rigid structure surrounding bacterial cell like shell
- 10-25 nm thick, gives rigidity and ductility
- 20-25% of dry weight of cell
- Freely permeable to solutes < 10000 mw
- Takes part in cell division by forming cross wall septa
- Back bone= Peptidoglycan- consisting of alternating units or molecules of
  - N-acetyl glucosamine
  - N-acetyl muramic acid

Gram positive cell wall:

- Inner cytoplasmic membrane
- Peptidoglycan layer
  - Thicker-16-20 nm
  - Many layers of alternating molecules of NAG-NAMA
  - Has molecules of teichoic acid
    - 50 % of dry weight of cell wall
    - Polymer containing ribitol




Polysaccharide chains
(the glycan portion)
Peptidoglycan
NAG
NAM
NAG
NAM
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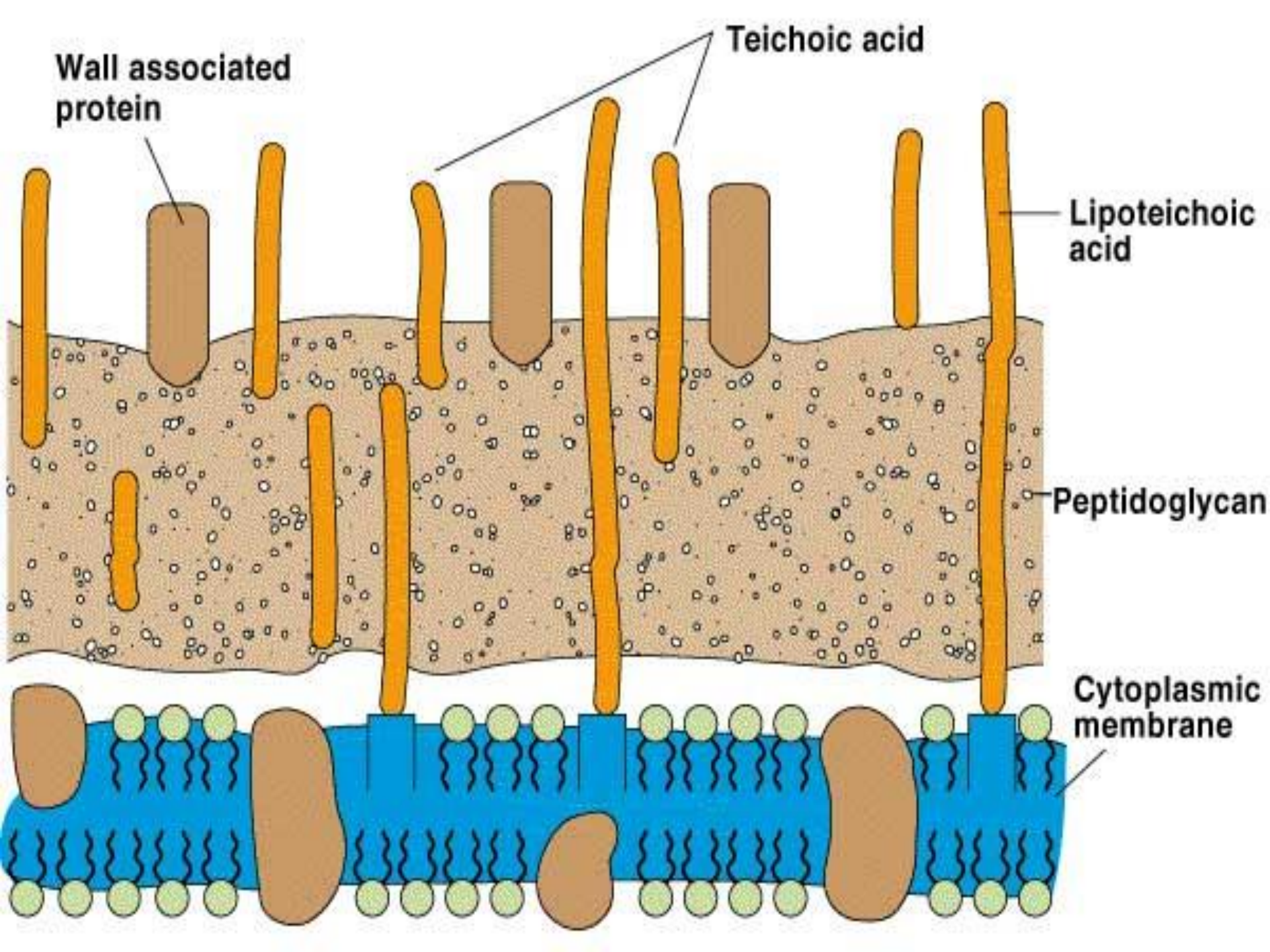
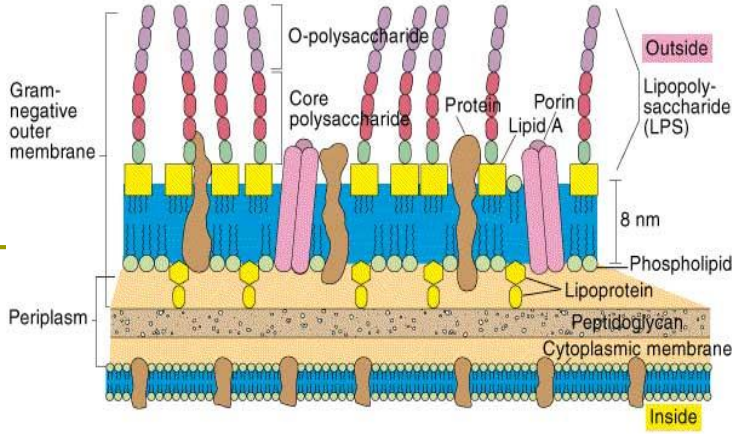
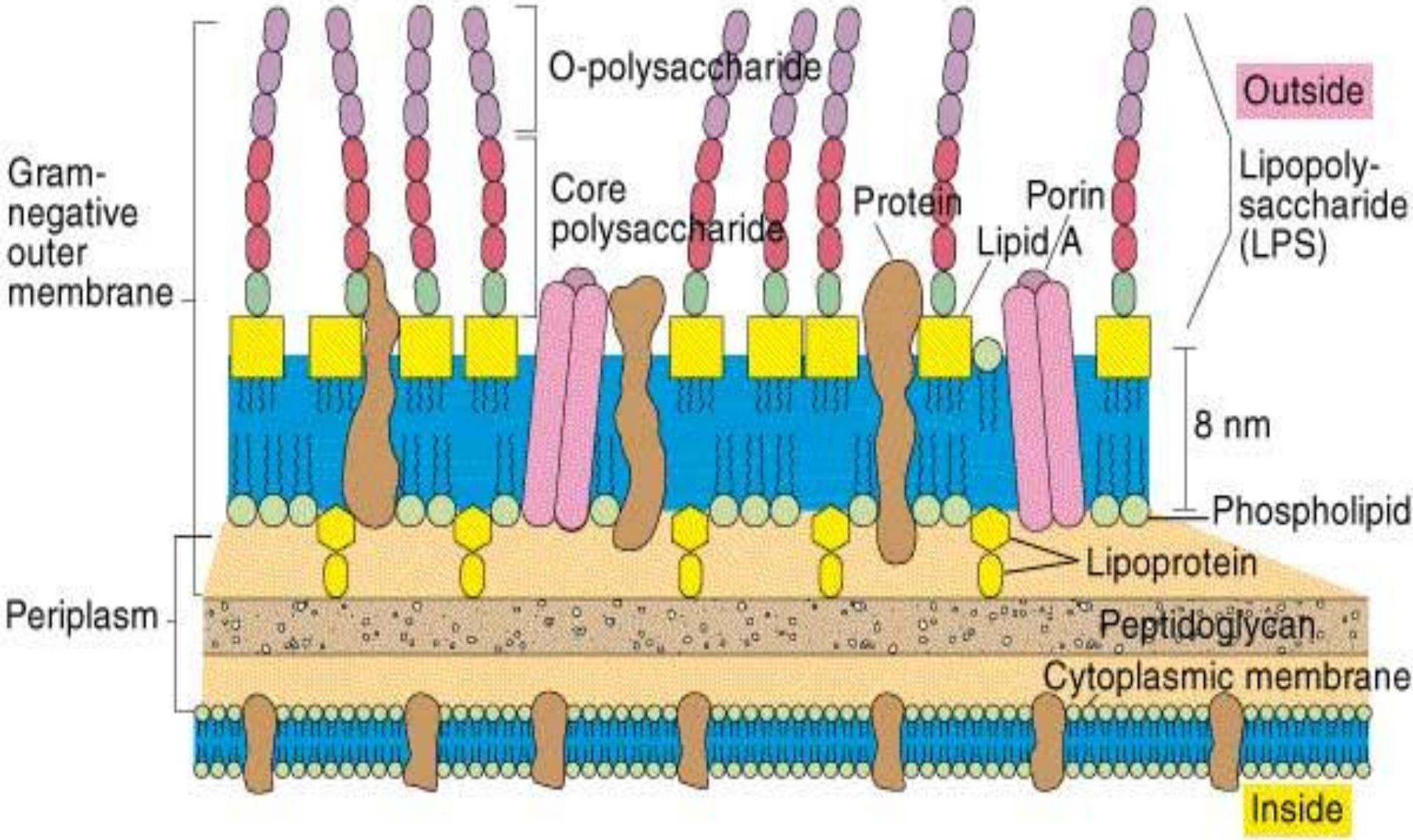
Peptide cross-links
(the peptide portion)


Wall associated protein
Teichoic acid
Lipoteichoic acid
Peptidoglycan
Cytoplasmic membrane

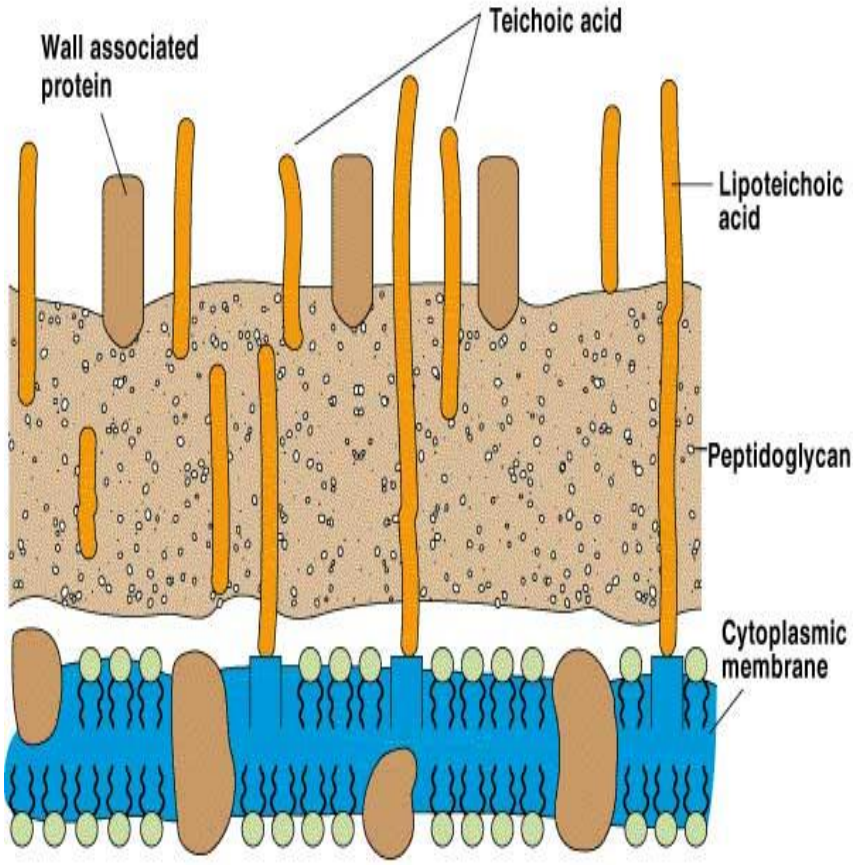
Gram negative cell wall

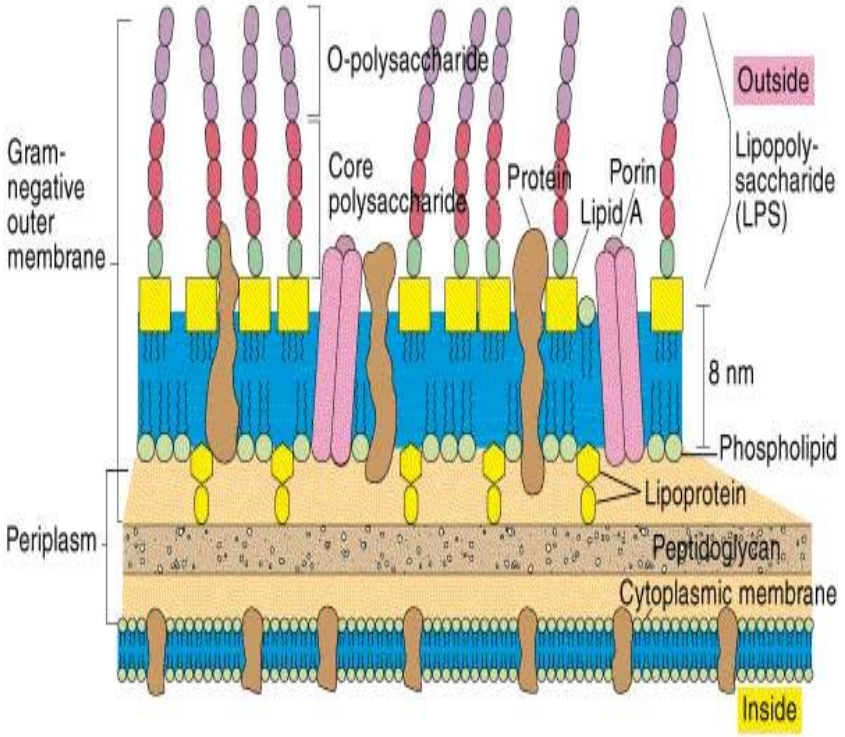


- From inside to outside
  - Inner cytoplasmic membrane
  - Thin layer of peptidoglycan
  - Periplasmic space containing lipoprotein layer which connects peptidoglycan with outer membrane
  - Outer membrane-phospholipids bilayer having lipopolysachharide(LPS)
    - Lipid A- endotoxic activity
    - Polysaccharide-Major surface antigen-O antigen
      - Core
      - Terminal series of repeat units-antigen specificity

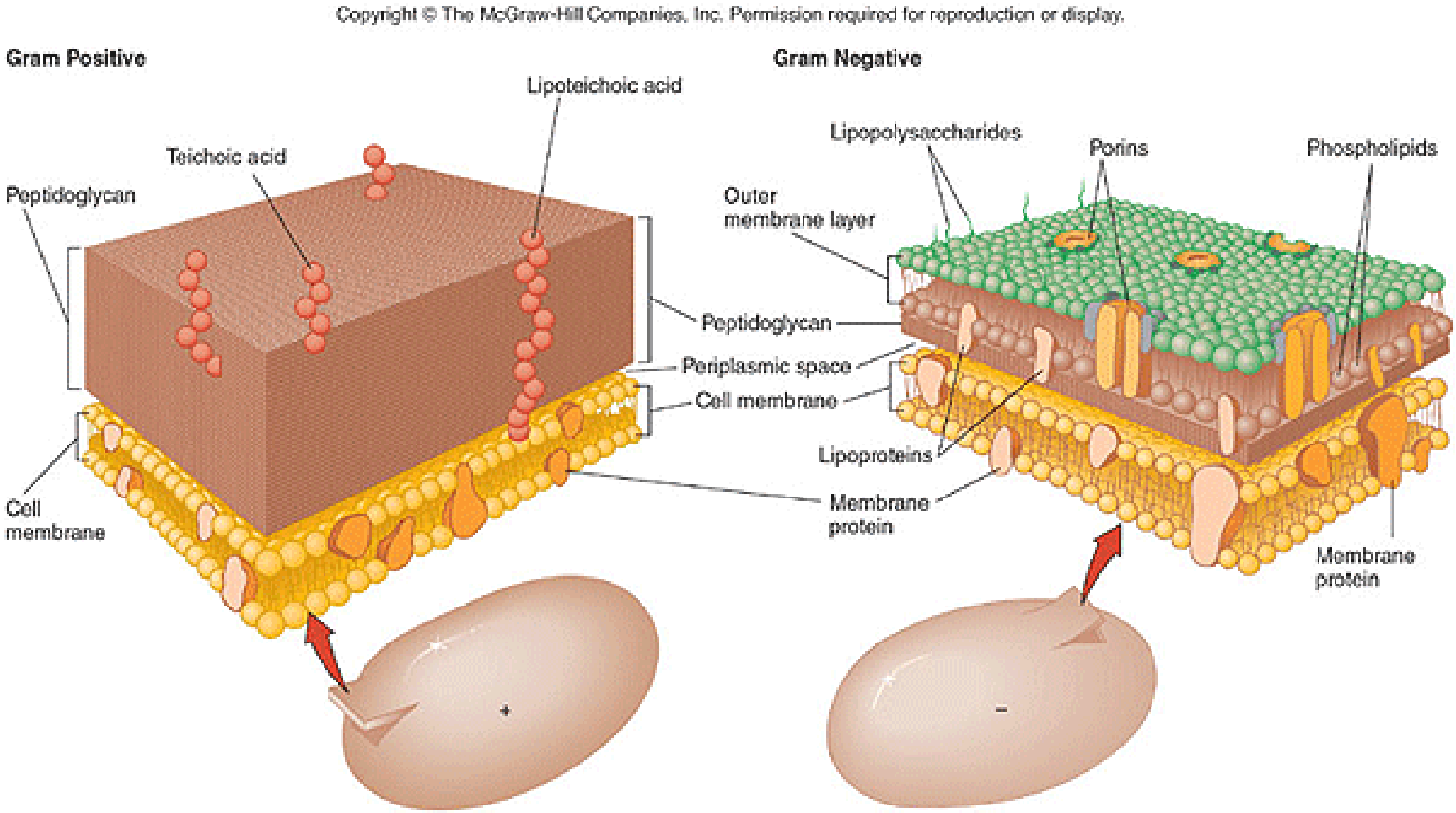

O-polysaccharide
Core polysaccharide
Protein
Porin
Lipid A
Outside
Lipopoly-saccharide (LPS)
Gram-negative outer membrane
8 nm
Phospholipid
Lipoprotein
Periplasm
Peptidoglycan
Cytoplasmic membrane
Inside

Comparison of Gram positive & Gram negative cell wall





Comparison of Gram positive & Gram negative cell wall



Gram stain

Gentian violet

Grams iodine

Acetone

Safranine

• Mechanism of Gram Staining:

- Difference between Gram(+) & Gram(-) is due to the physical nature of their cell walls.

- **Gram(+)** becomes Gram(-) when cell wall removed.

- Peptidoglycan acts as a permeability barrier preventing loss of crystal violet.

- Ethanol is thought to shrink pores of the thick peptidoglycan →dye-iodine complex retained → bacteria remain purple.

- **Gram(-)** have very thin peptidoglycan.

- Ethanol treatment extract enough lipid from wall and make more porous → purple crystal violet-iodine complex is more readily removed.

- When counterstained with safranin → Gram(-) bacteria turn pink.

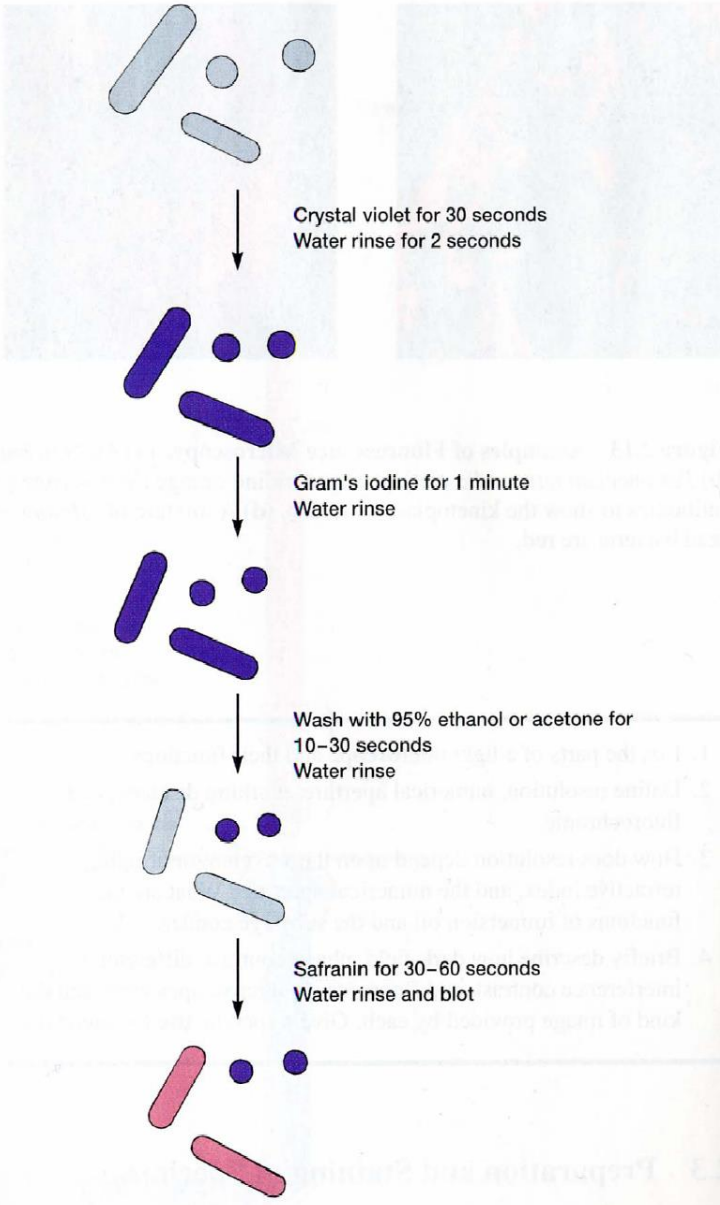


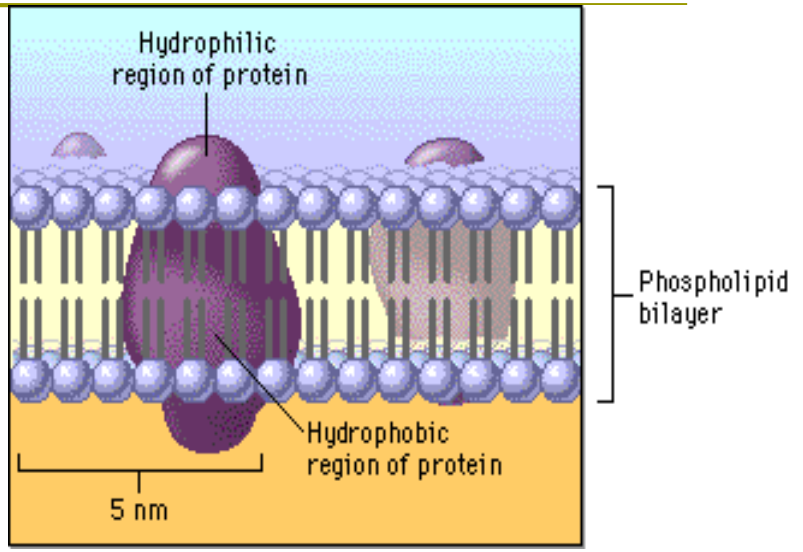
Figure 2.14 **The Gram-Staining Procedure.** Note that decolorization with ethanol or acetone removes crystal violet from gram-negative cells but not from gram-positive cells. The gram-negative cells then turn pink to red when counterstained with safranin.

Bacteria with defective cell wall

- Protoplast
  - Gram +ve bacteria in hypertonic solution
  - Contains only cytoplasmic membrane
- Spheroplast
  - Gram -ve bacteria kept in hypertonic solution
  - Cytoplasmic membrane + cell wall material
- L forms
  - Abnormal forms developed spontaneously or in presence of penicillin
  - Observed by Kleinerberger-Nobel while studying S.moniliformis at Lister institute, London

Cytoplasmic membrane

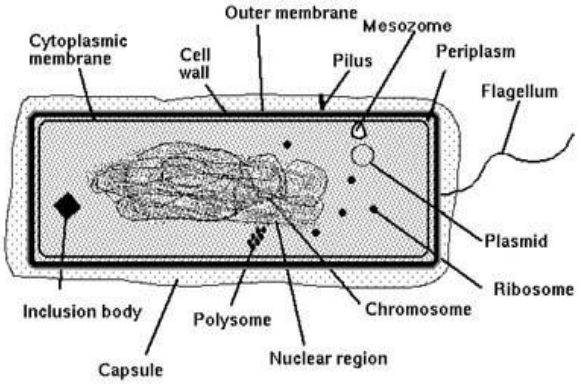
- Thin(5-10 nm), elastic semi permeable layer lies beneath the cell wall separating it from cytoplasm
- Three layers forming a single unit
- Central layer-protein and either side is lipid layer



Function :

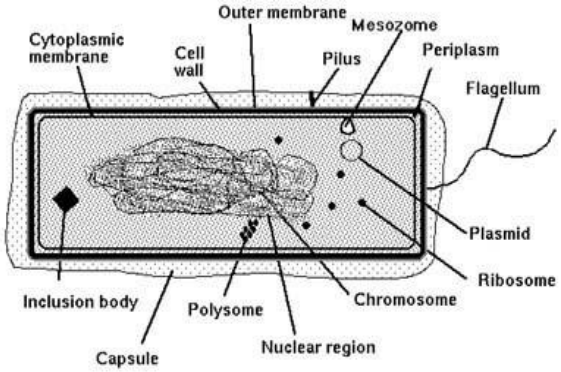
1. Active transport of molecules, acts as a osmotic barrier
2. Semi permeable membrane- passive transport of small molecules like lipid and of water occurs by diffusion
3. Contains enzymes like cytochrome oxidase, polymerizing enzymes necessary for cell wall synthesis and of tricaboxylic acid cycle

Cytoplasm



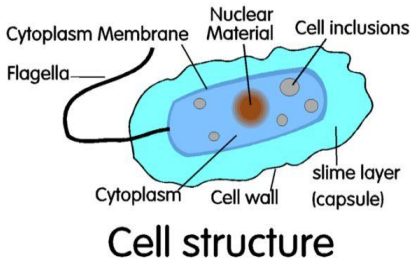
- Intracytoplasmic inclusions
  - Sources of stored energy
    - Polymetaphophate
    - Polysaccharide (starch or glycogen)
    - Poly betahydroxybutyrate (lipid)
- Ribosome
  - Complex structures of 10-20 nm size, with a sedimentation constant of 70 s
  - Sites of protein synthesis
  - Composed of RNA and proteins-organized into two subunits 30s and 50s

Contd.



- Mesosomes
  - Convoluted, multilaminated sac like structure
  - Formed due to invagination of plasma membrane into cytoplasm
  - Sites of respiratory enzymes
  - Acts like mitochondria of eukaryotic cells
- Nucleus
  - Double stranded molecule of DNA
  - No nuclear membrane, no nucleolus

Capsule

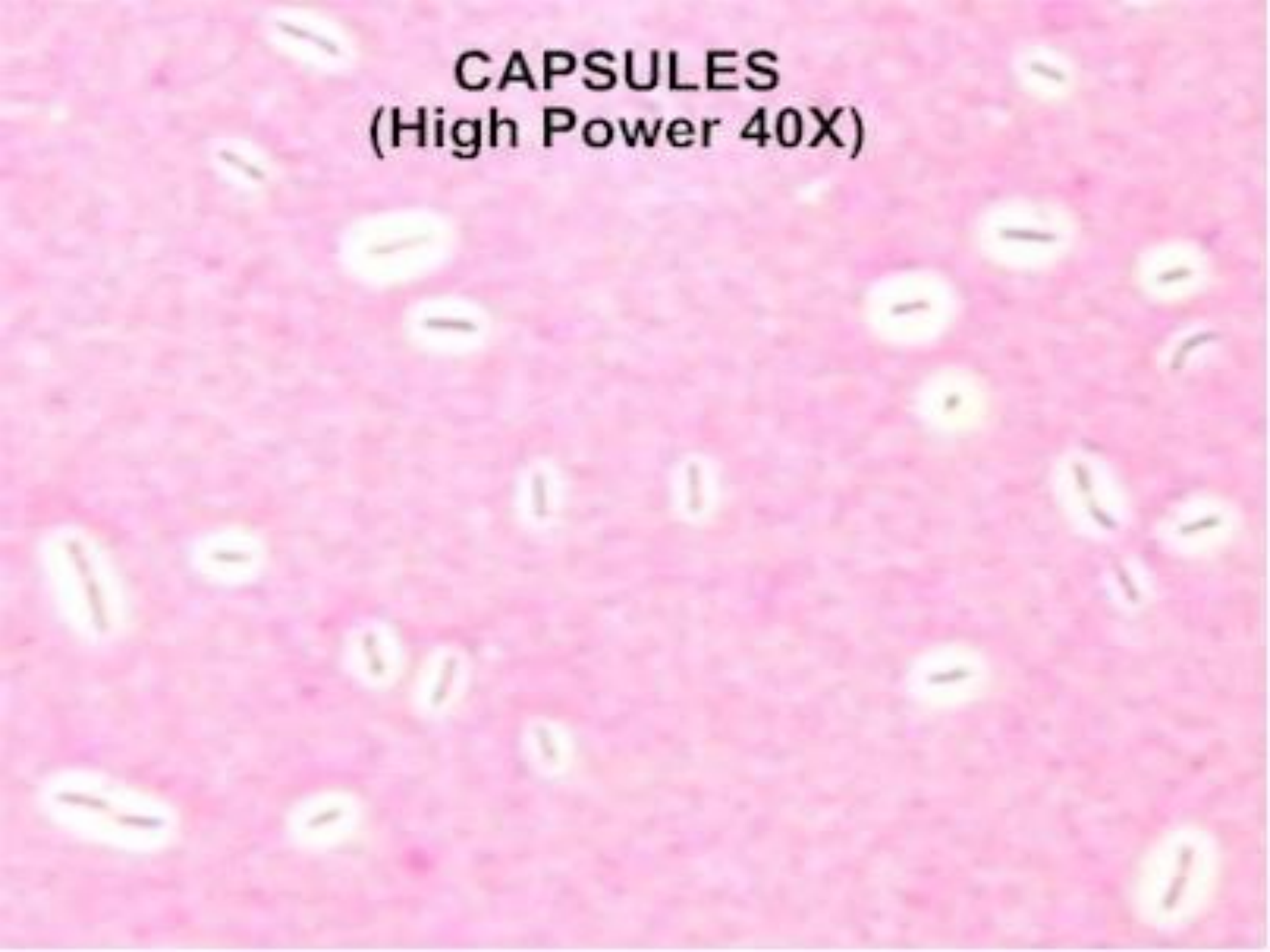


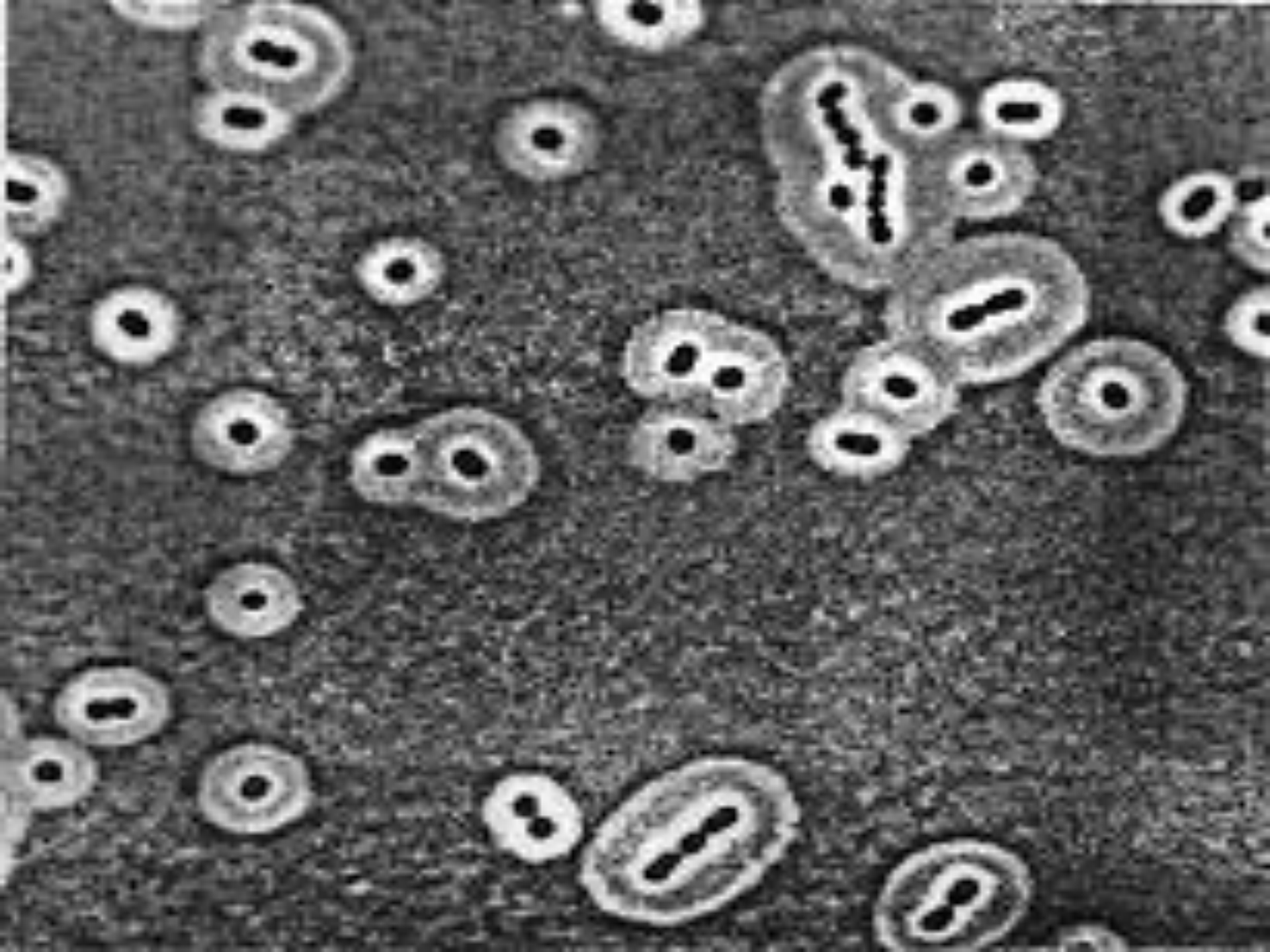
Cell structure

- Amorphous jelly like viscid material which surrounds cell, secreted by bacteria itself
  - Capsule = organized into sharply defined structure
  - Slime layer =diffuses into surrounding, remains as a loose undemarcated secretion
- Bacteria with slime production=mucoid growth on culture media
- Inhibits phagocytosis thereby enhances virulence of bacteria
- Chemically,
  - Polysaccharide- pneumococci, klebsiella
  - Polypeptide-anthrax bacilli
  - Hyaluronic acid-streptococci

Contd.

- Demonstration:
  - Best demonstrated in clinical specimens
  - Size is reduced in artificial culture medium
    - Gram stain- unstained halo around stained bacterial body
    - Negative staining - by India ink-clear halo around bacteria
    - Immunological method-when mixed with anticapsular serum, it swell becomes refractile and sharply delineated structure- ”Quellung reaction”
    - Specific capsular stain-”Hiss method” using cupper salts as mordant


CAPSULES
(High Power 40X)



Quellung reaction =capsular swelling

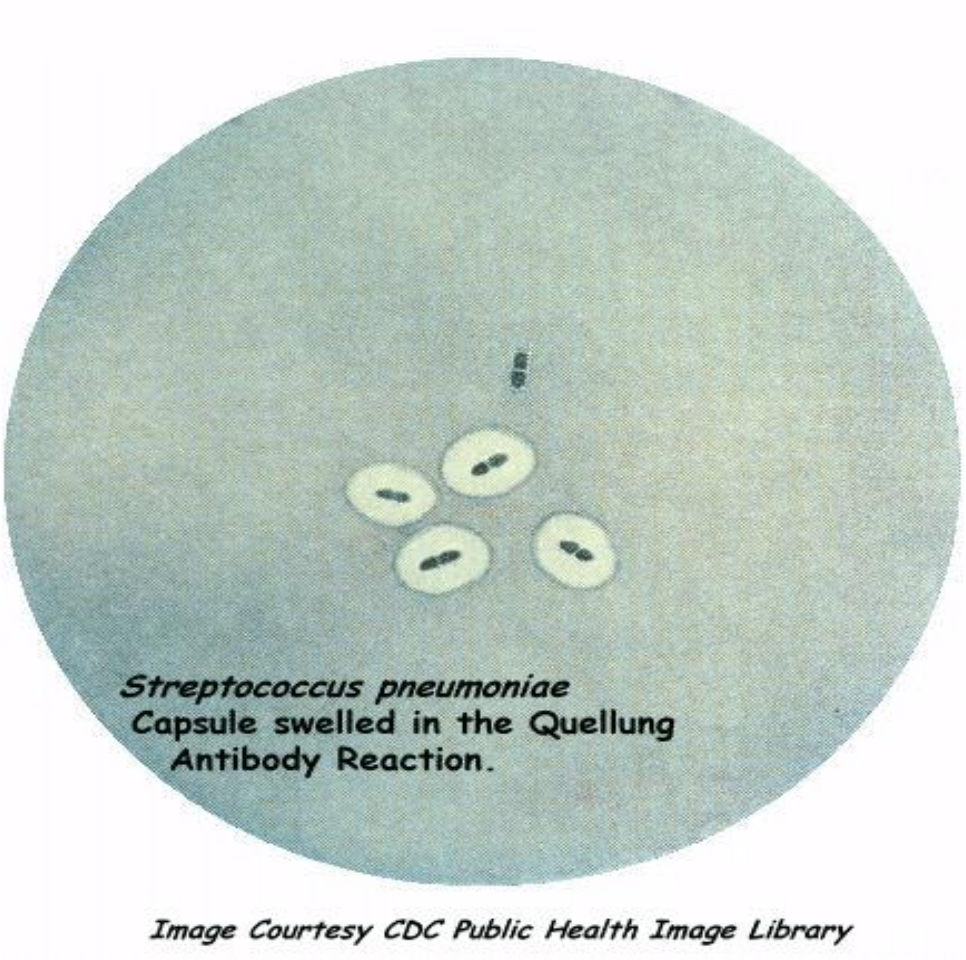
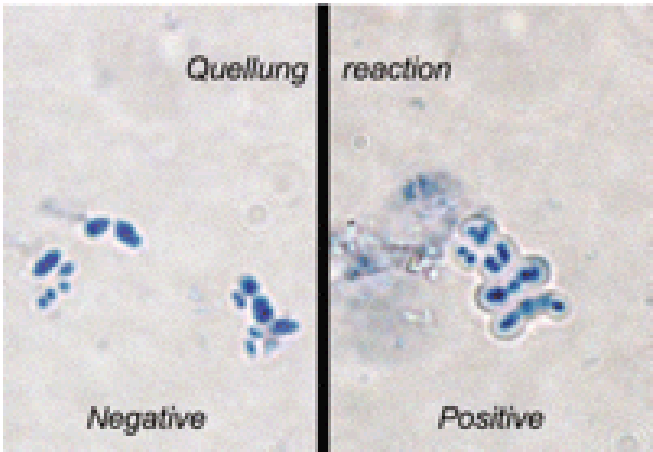
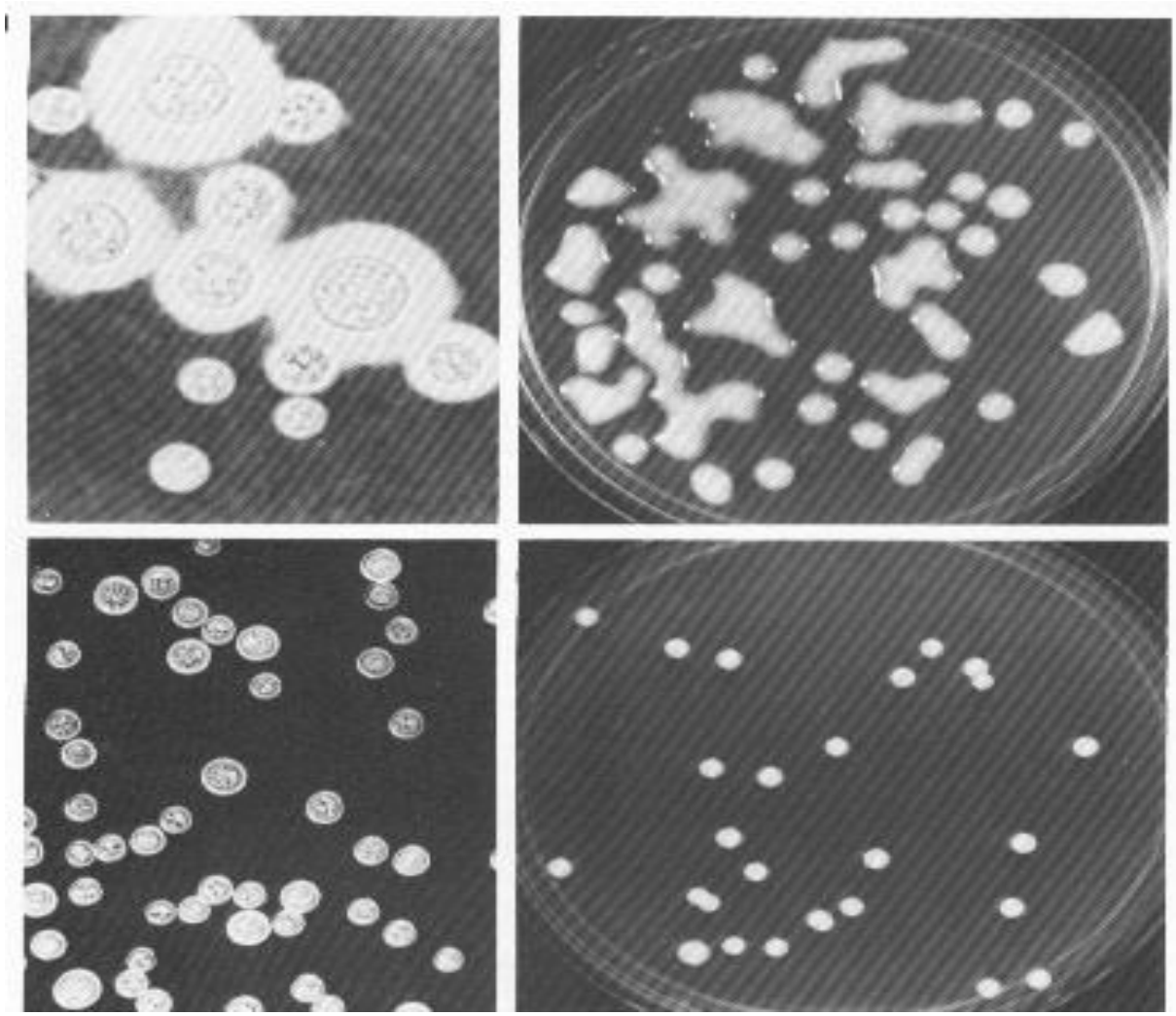


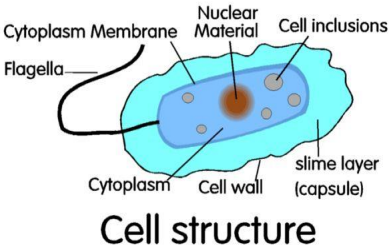
Image Courtesy CDC Public Health Image Library



Capsulated bacteria = mucoid colony



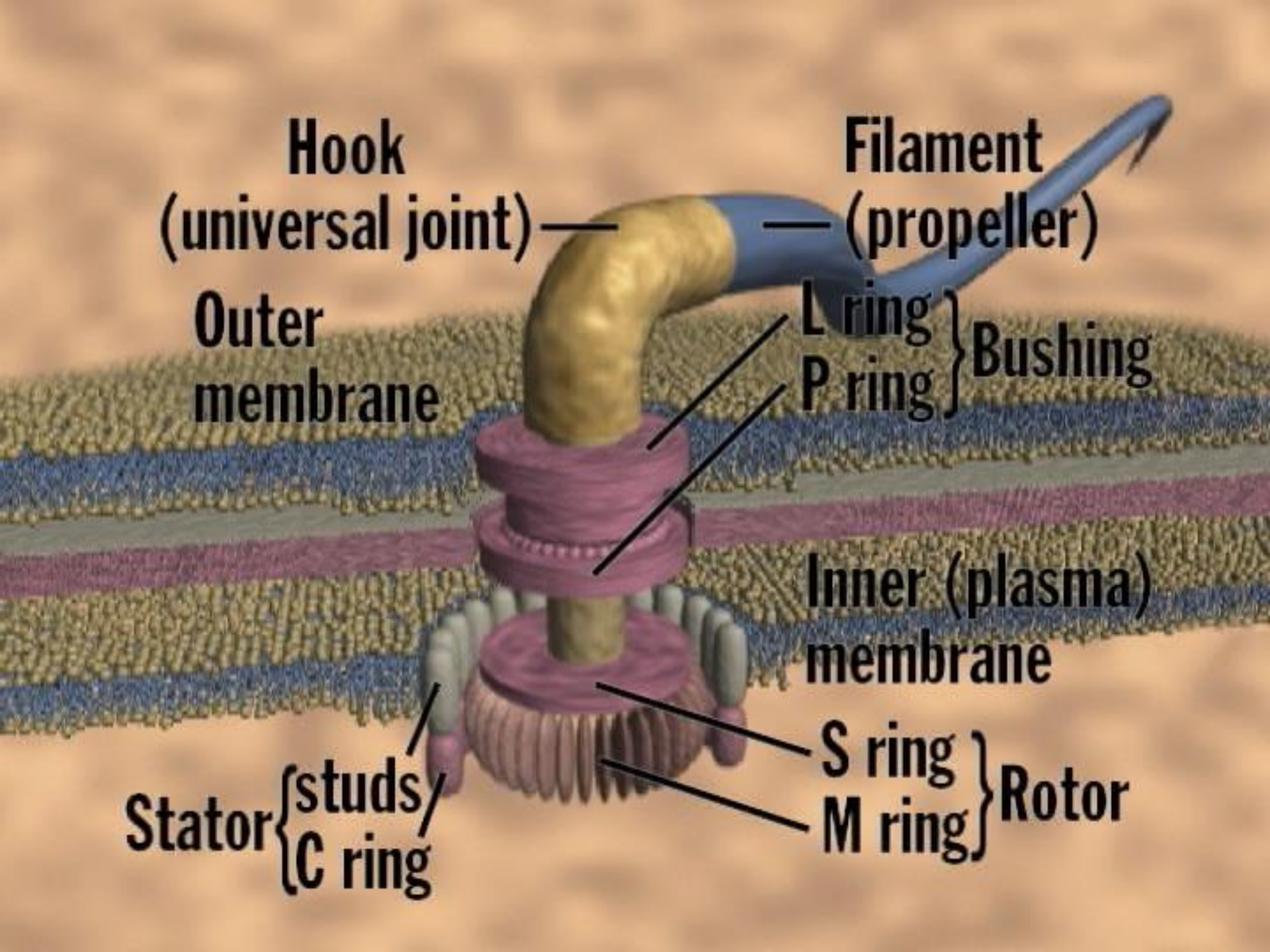
Flagella



Cell structure

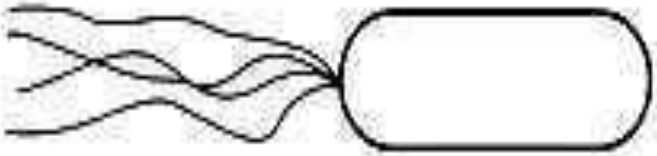
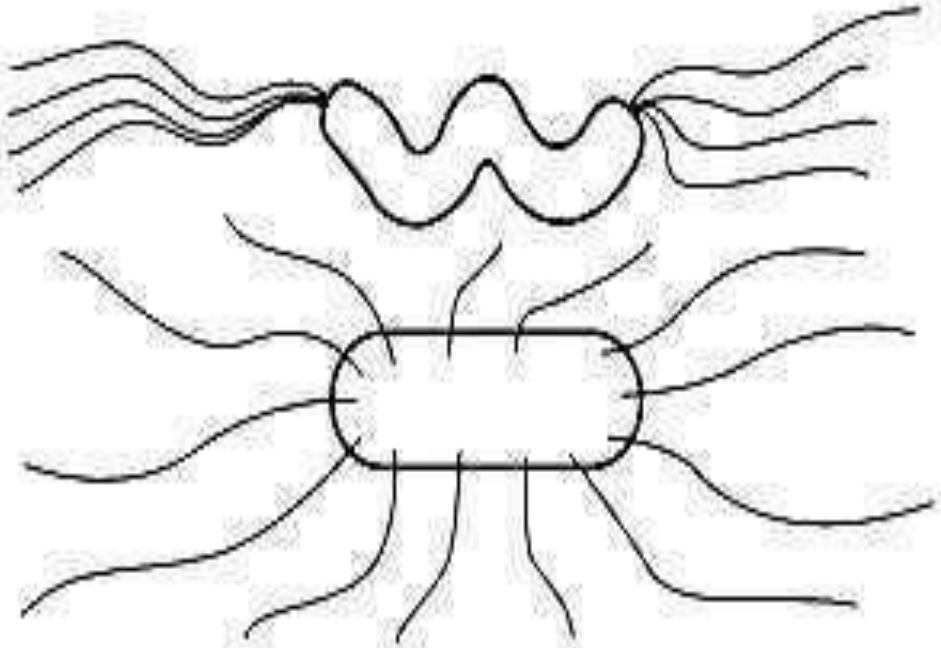
- Unbranched long thread like sinuous filaments composed of protein-flagellin
- 12-30 nm in diameter, 5-16 micron long having 3 part
  - Filament
  - Hook
  - Basal body

Hook and basal body are embedded in cell wall, filament outside cell wall


Hook
(universal joint)
Filament
(propeller)
Outer
membrane
L ring
P ring
Bushing
Inner (plasma)
membrane
S ring
M ring
Rotor
Stator
studs
C ring

Arrangement

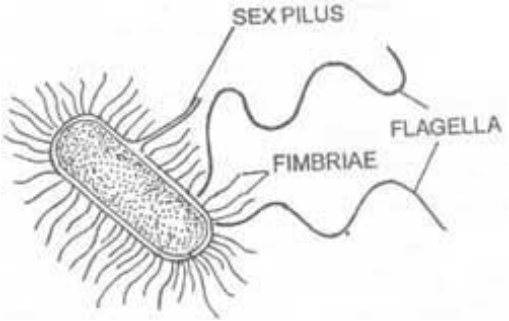
- Single or multiple, arranged in different manner
- Peritrichous- E.coli, Salmonella
- Polar-at end/s
  - Single-Monopolar — Vibrio cholerae
  - At both ends-amphitrichous — Alkaligenes sp.
  - In tufts-lophotrichous — Bartonella
  - Lophoamphitrichous — Spirilla

Structure	Flagella Type	Example
	Monotrichous	*Vibrio cholerae*
	Lophotrichous	*Bartonella bacilliformis*
	Amphitrichous	*Spirillum serpens*
	Peritrichous	*Escherichia coli*

Demonstration

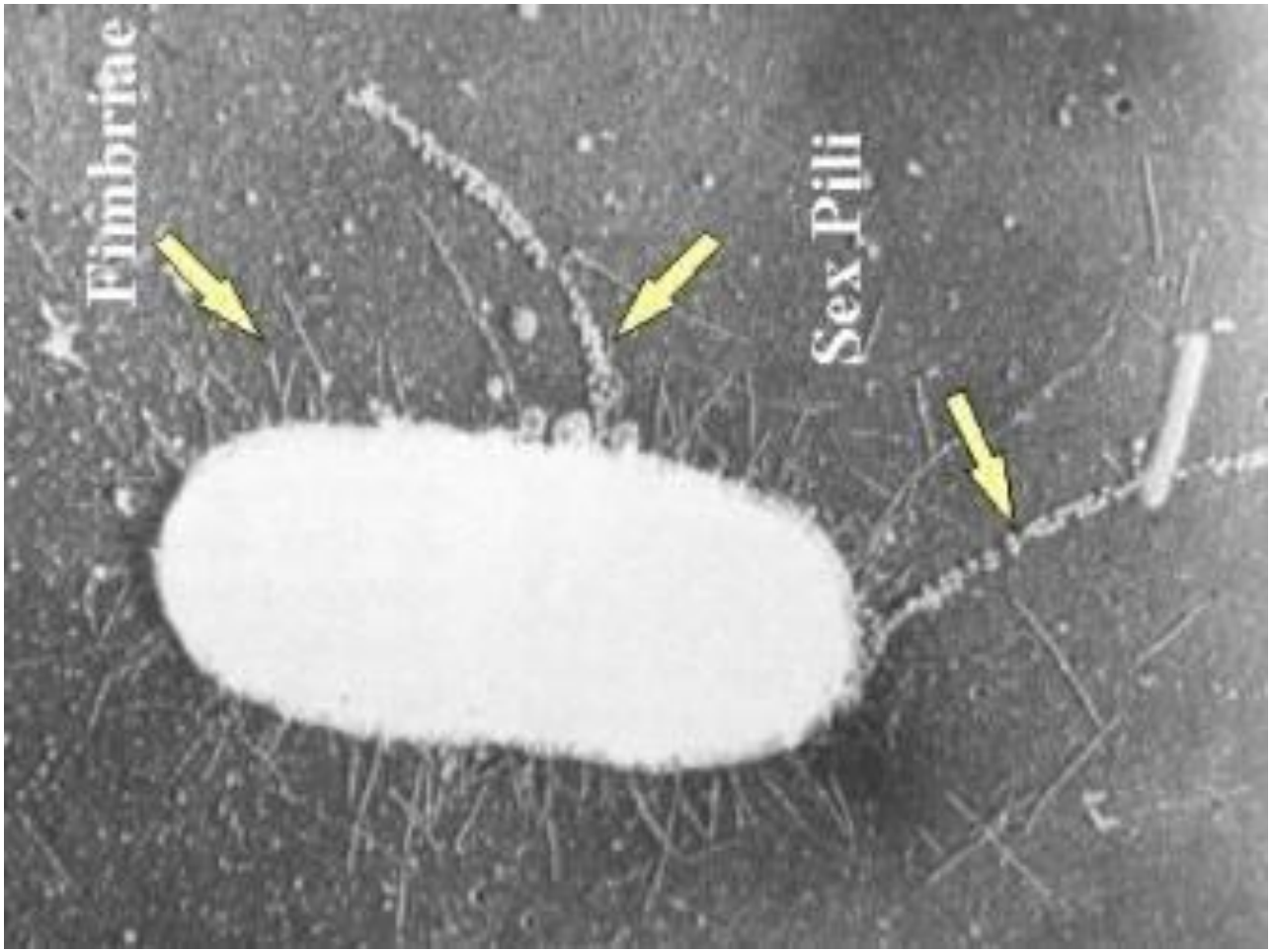
- Thin(0.02 mc) so outside limit of resolution of light microscope
- Can be demonstrated by
  - Dark ground illumination
  - Special stain- by which their thickness is increased by impregnation of silver salts like AgNO3 e.g.. Fontana's stain
  - Electron microscopy
  - By observing motility of bacteria-
    - Hanging drop preparation
    - Spreading growth over semisolid medium
    - Craigie's tube method

Fimbriae or Pili



- Gram negative bacteria carry very fine, hair-like surface appendages called- fimbriae which originate from cell membrane
- Shorter and thinner than flagella, 0.5 micron long, < 10 nm thick
- Composed of self aggregating monomer of a protein called pilin
- Can be seen only by electron microscopy
- Best seen in freshly isolated strain and in liquid culture and tend to disappear with subculture, forms surface pellicle when grown in liquid medium
- Function as organ of adhesion

Fimbriae – Electron microscope



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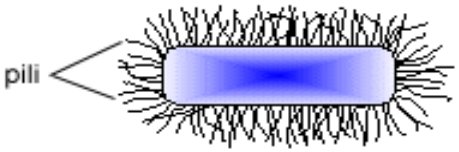
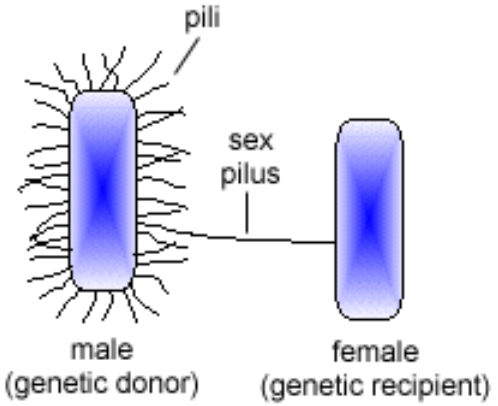
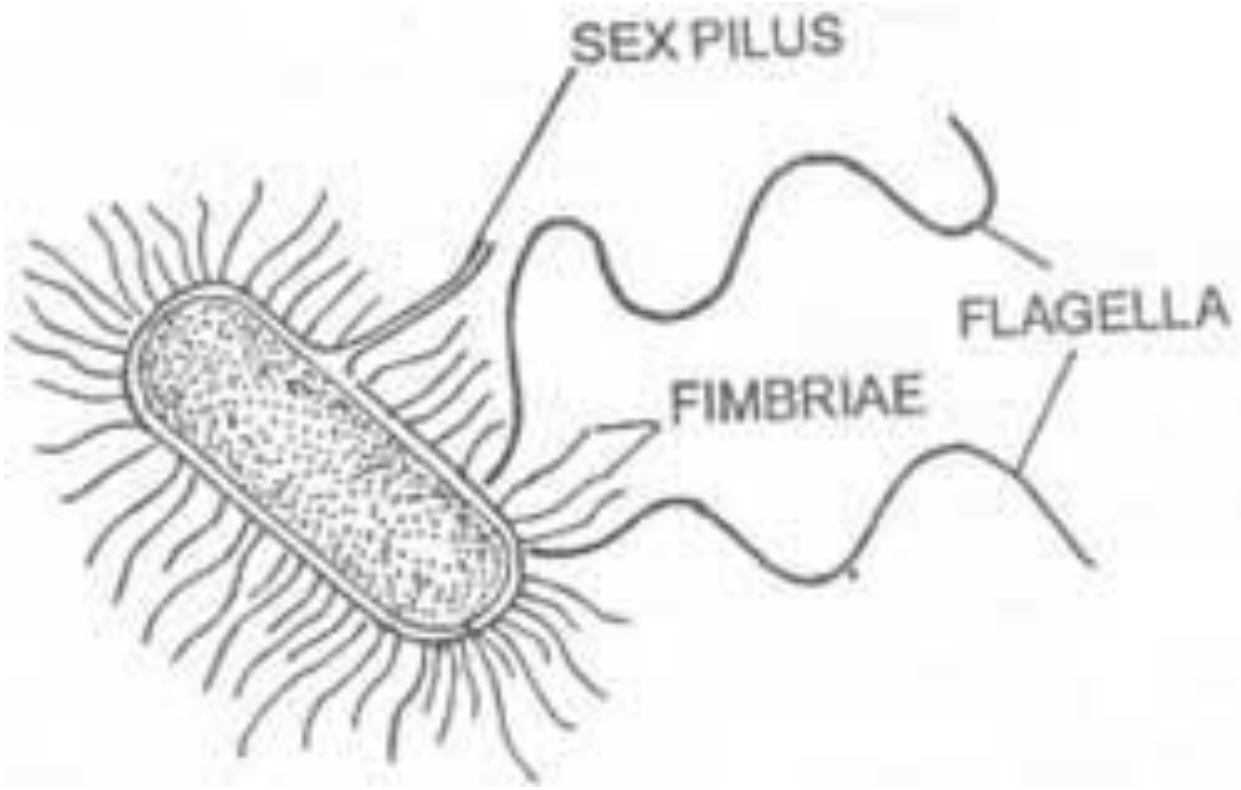
- A special type of fimbriae – Sex Pilus
  - Longer and fewer in numbers
  - Found only on some bacteria and helps in attachment to other bacteria forming hollow conjugation tube through which genetic material or plasmid is exchanged between bacteria





Flagella Vs Fimbriae



Spore

- Resistant, resting stage of bacteria
- Seen Only in few bacterial genera– e.g. Bacillus and Clostridia

- One bacteria produce single spore which on germination again produces vegetative bacteria-is *not a method of reproduction*

- Resistant to environmental stresses - heat, UV radiation, chemical disinfectants, desiccation
- Survive boiling for an hour or more.

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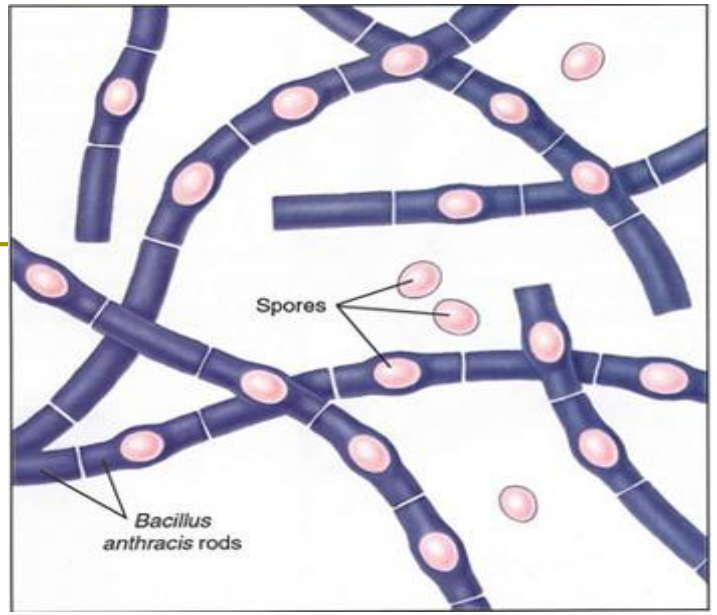
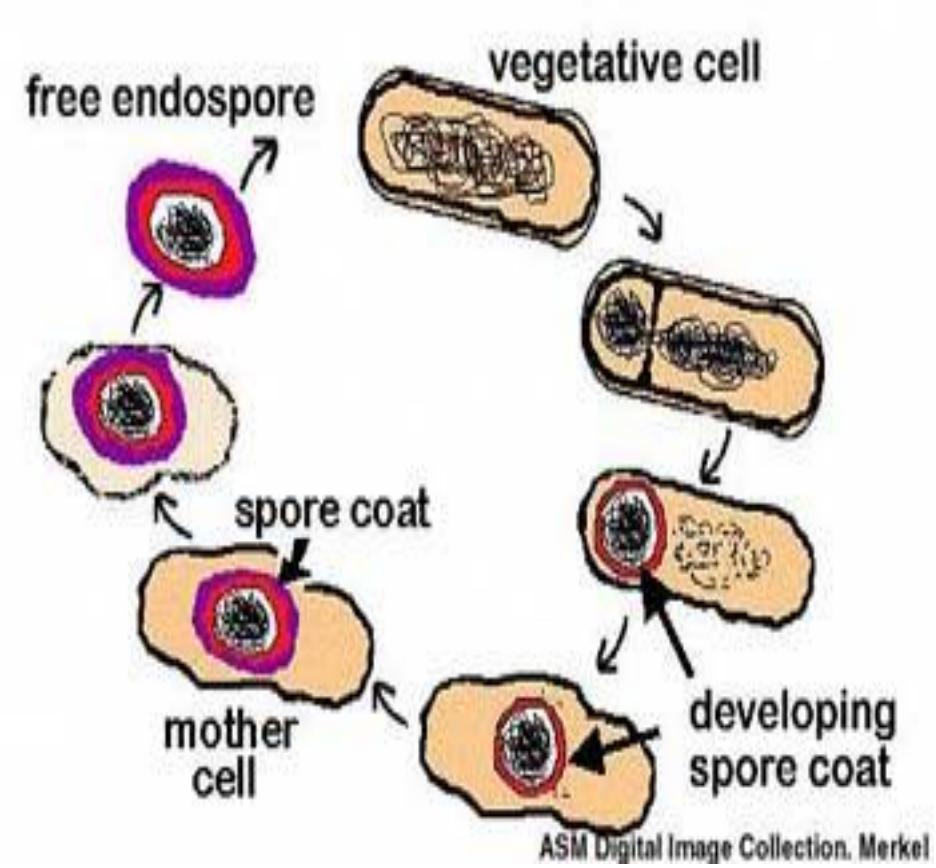


- Arranged
  - at end – terminal
  - Center- central
  - Near end- sub-terminal
- May or may not bulge bacillary body
  - bulging
  - Non-bulging
- Round or oval
  - Round at end-drum stick appearance-Cl.tetani
  - Oval at end – tennis racket shape – Cl.tertium

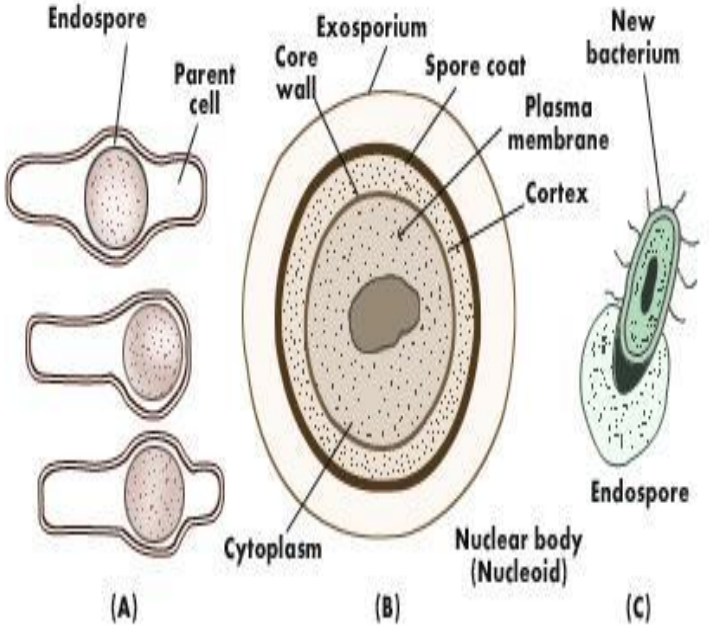
Contd.

- Sporulation is initiated by appearance of a clear area usually at one end which gradually become more opaque to form- “fore spore”



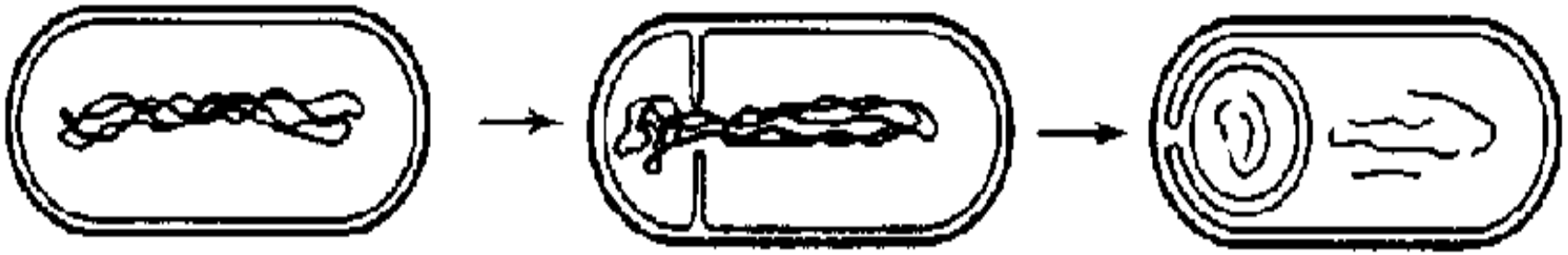
Structure of spore

- Structure from inside to outside
  - Nuclear body with cytoplasm
  - Cytoplasmic membrane
  - Spore wall
  - Thick cortex
  - Multilayered tough spore coat
  - Exosporium

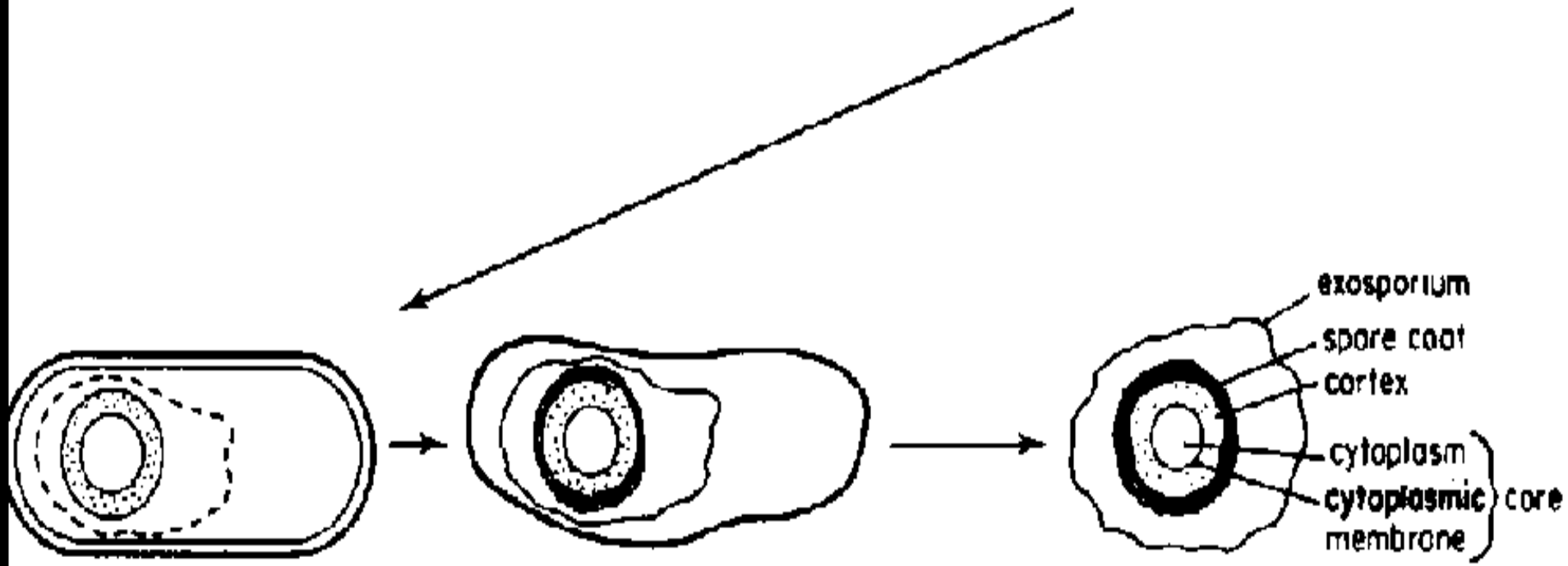


Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

Spore Formation in Gram-positive rods.



Chromosome Condensation & Septum Formation

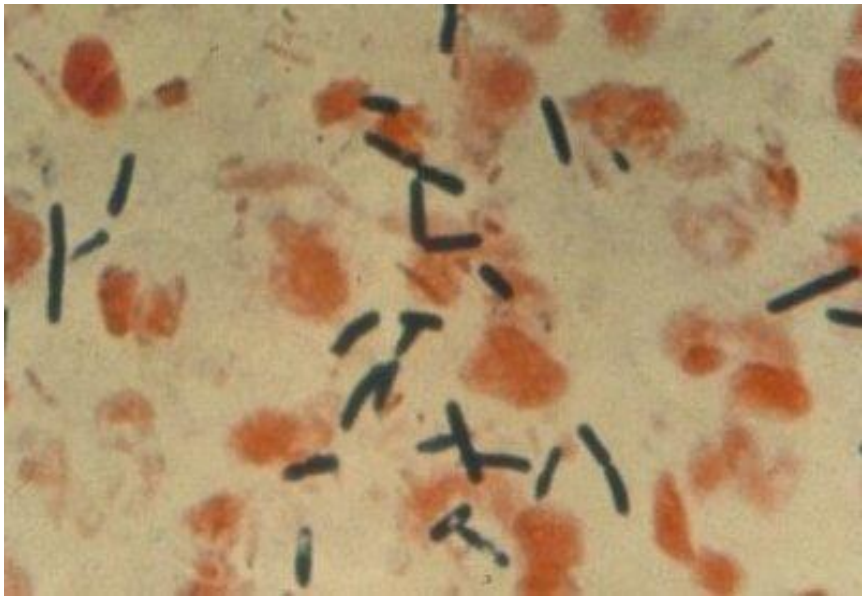


Calcium dipicolinate accumulates
in the core of the developing spore.

The Spore

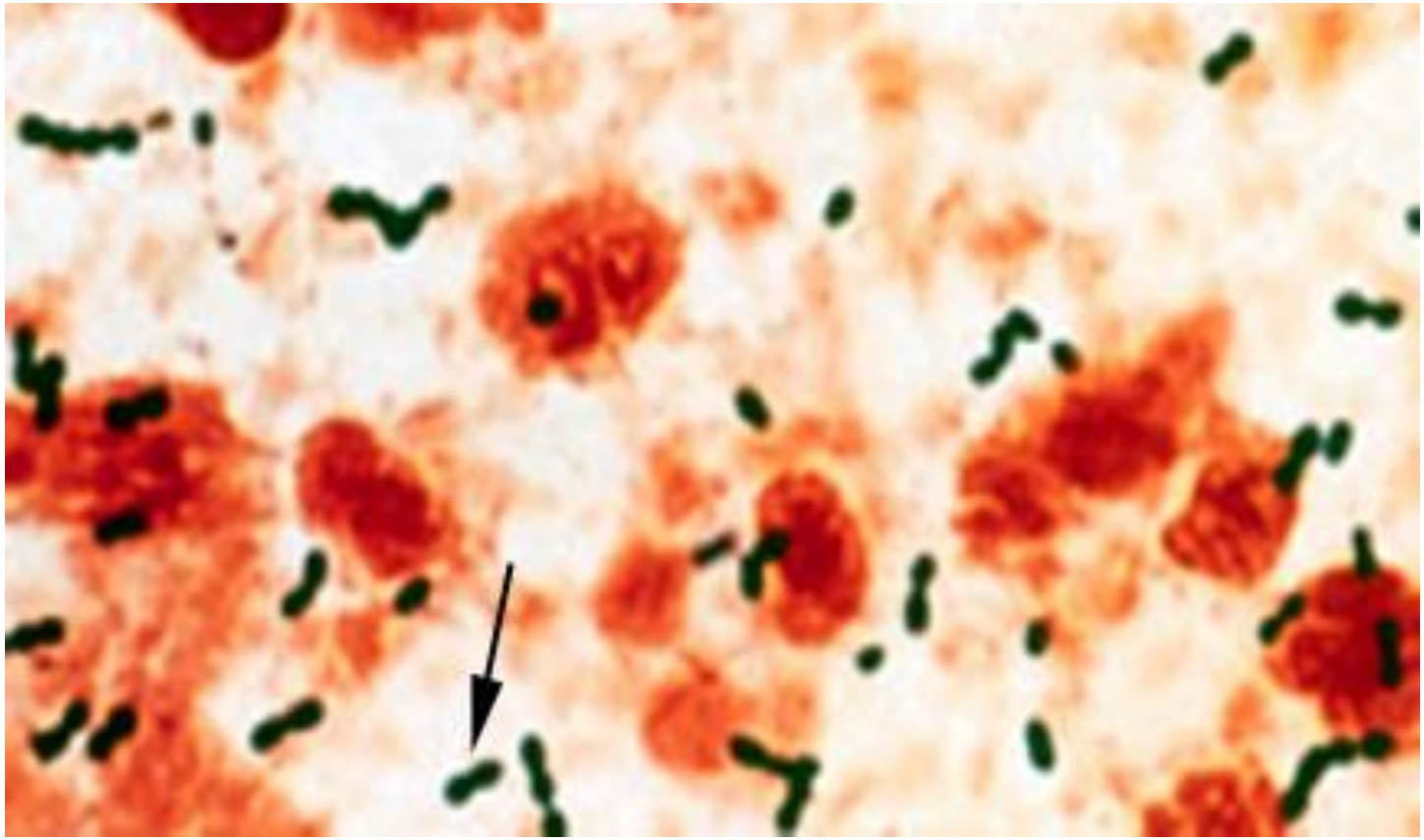
Demonstration of spores

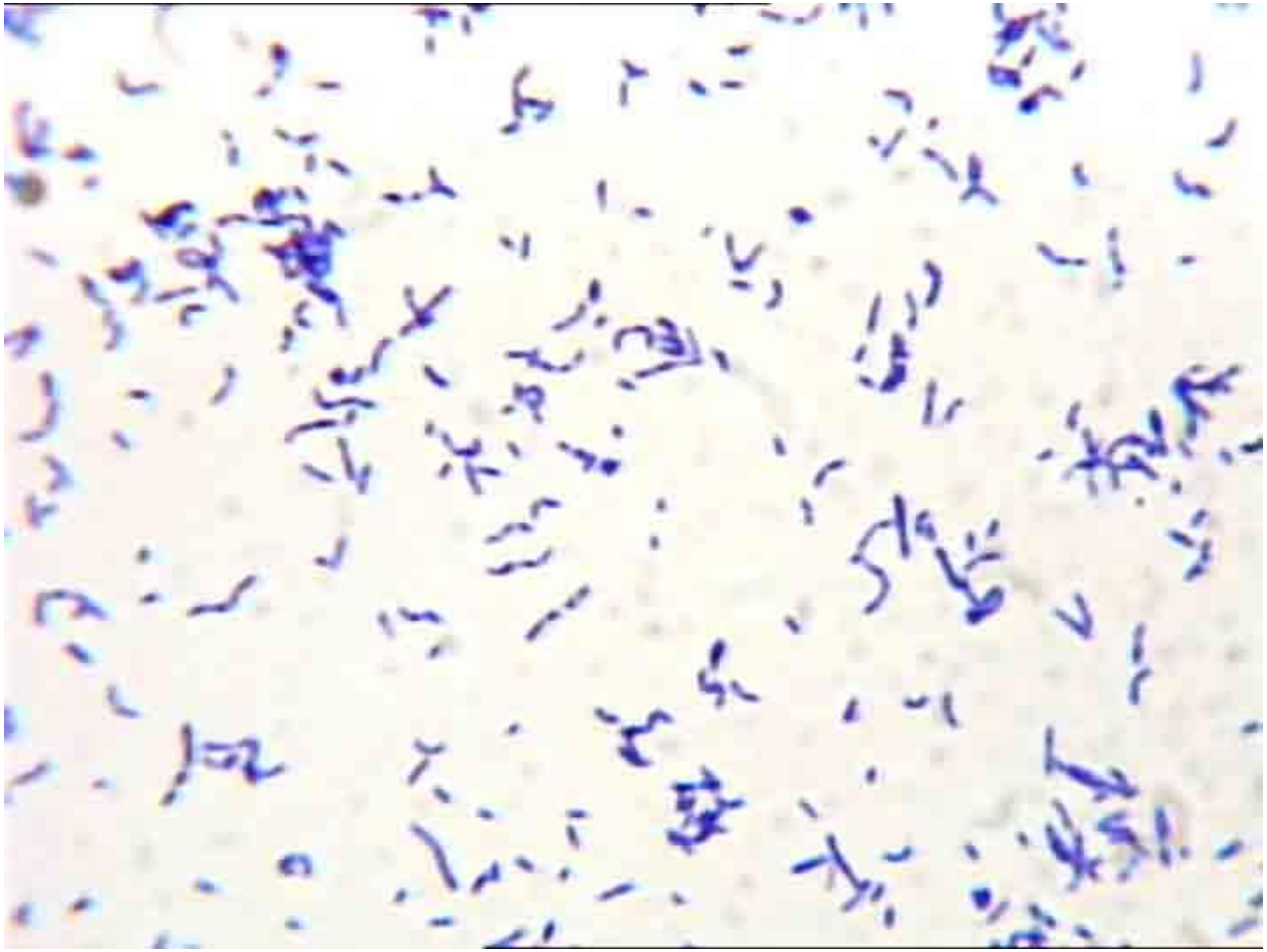
- Gram stain : appear as a clear unstained refractile body within bacterial cell
- Modified Z-N stain : using 0.25-0.5 % H_2SO_4 as a decolorizing agent, spores appear acid-fast (red).

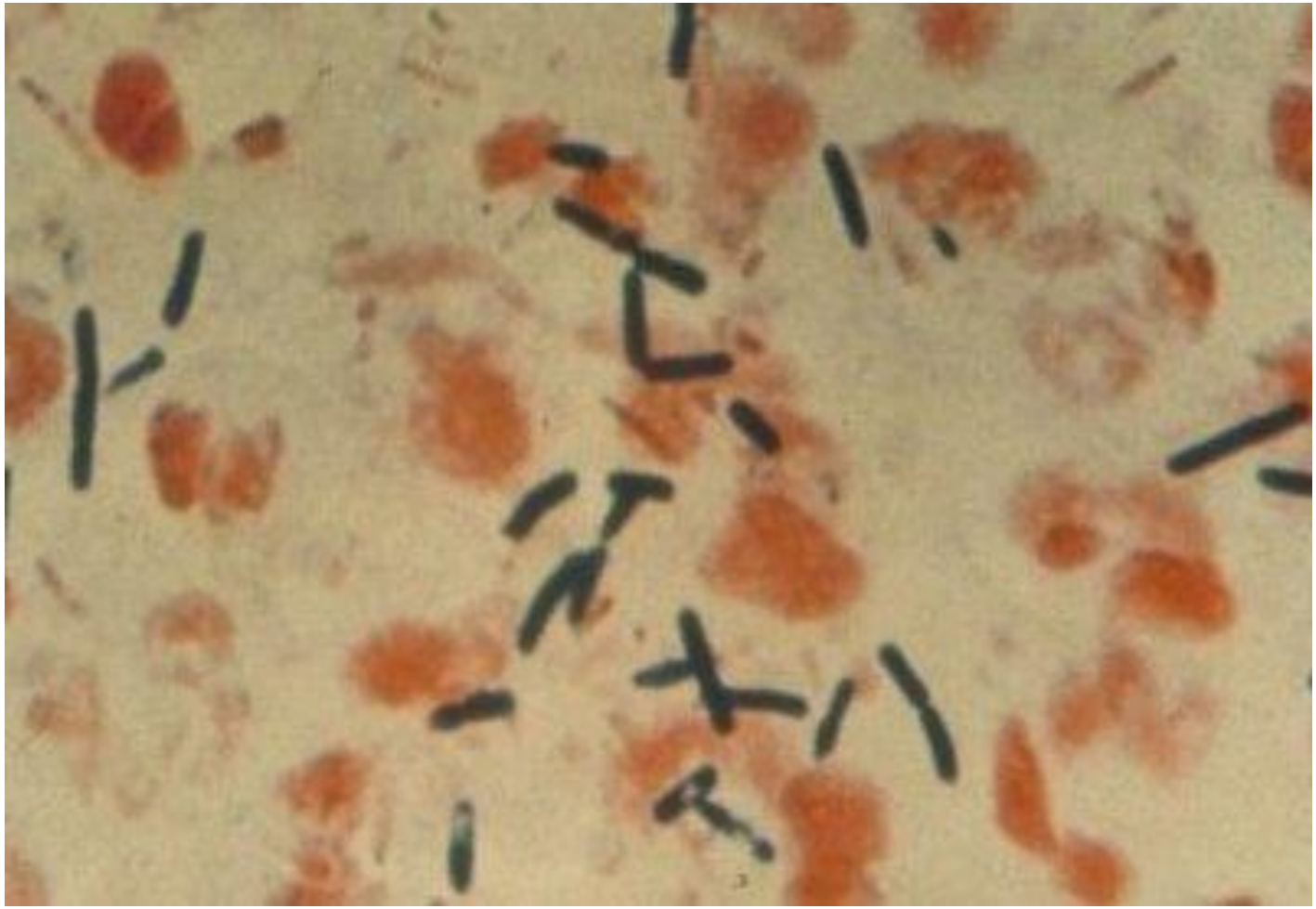


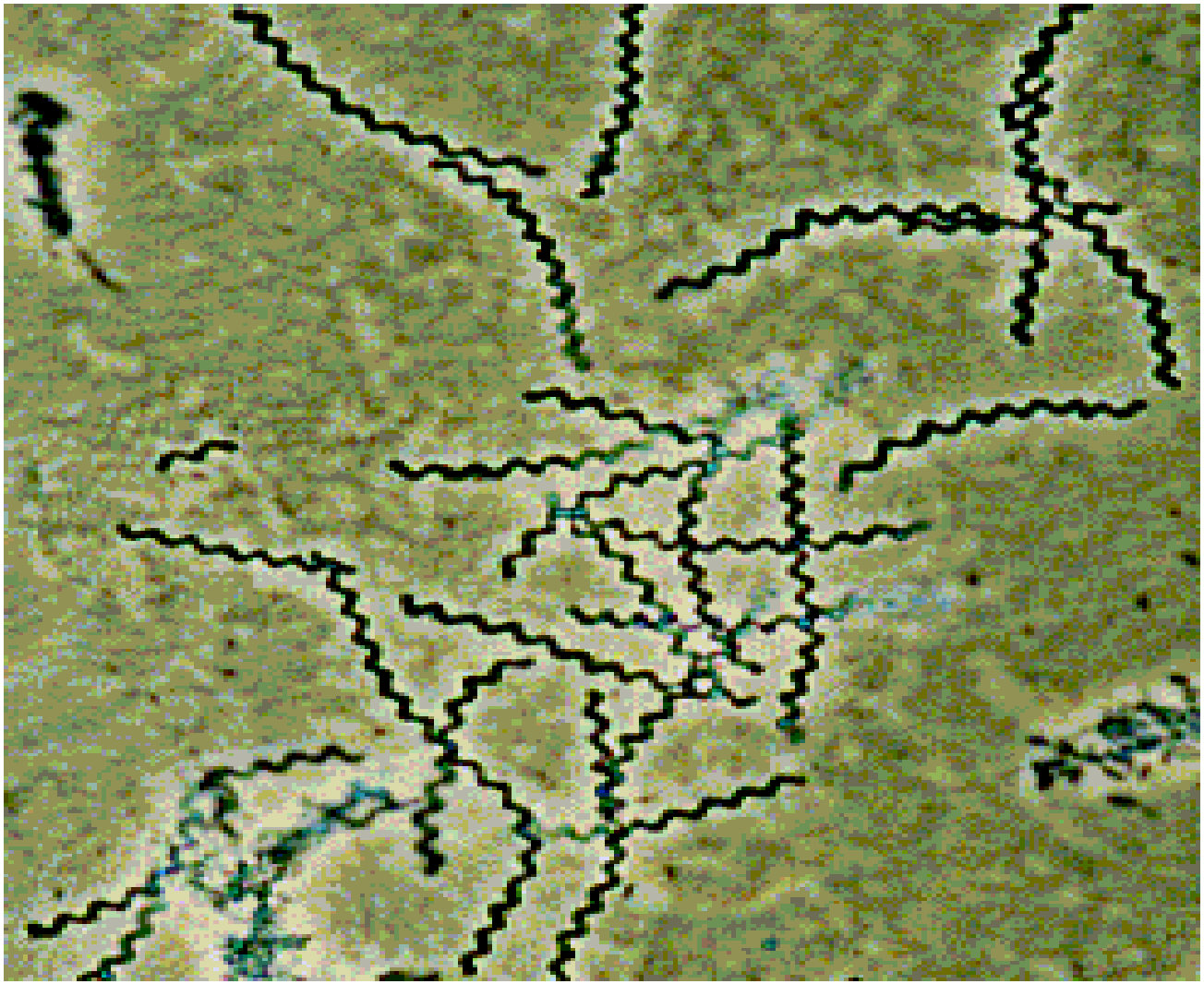
Analysis of lecture

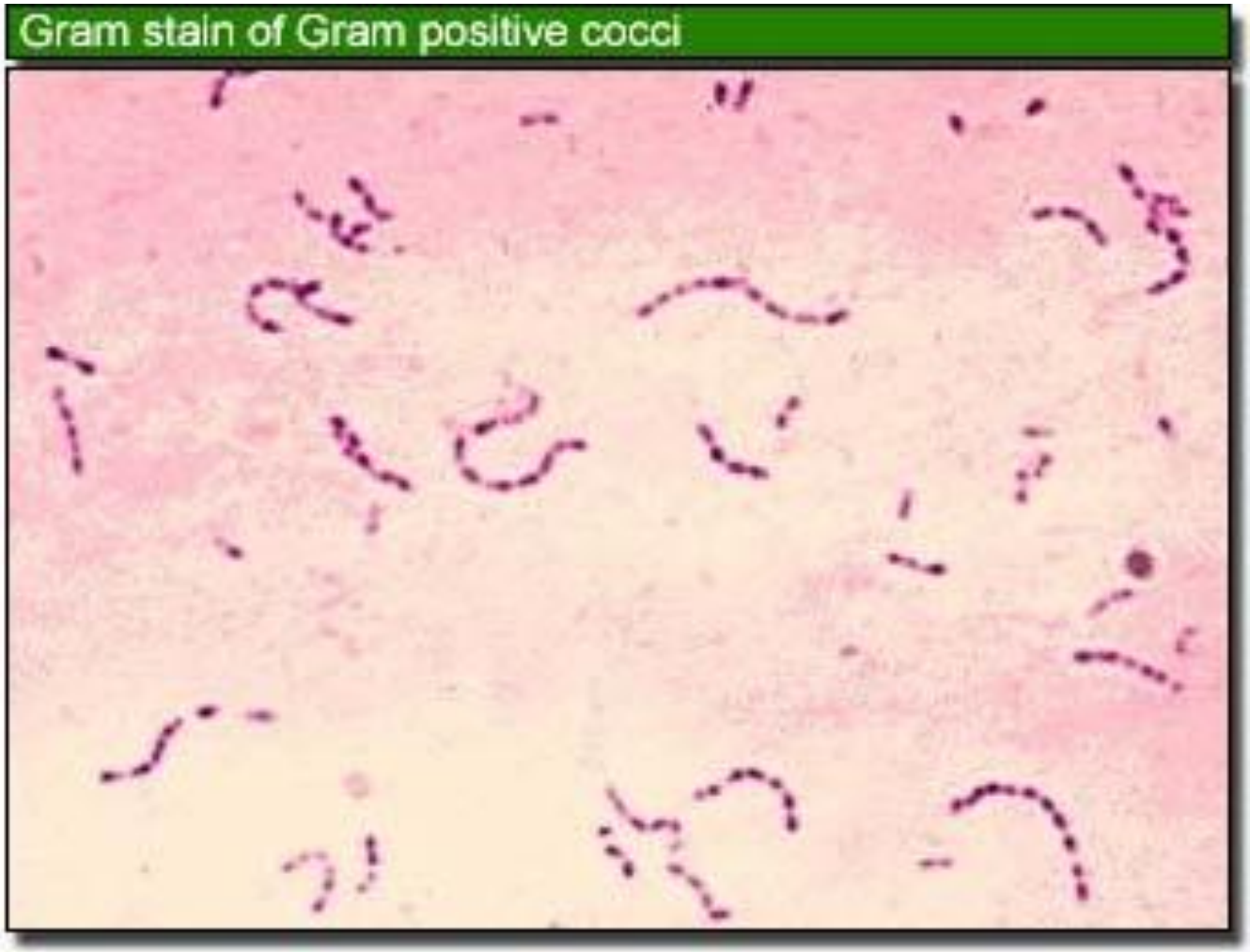

Seattle STD/HIV Prevention Training Center
Source: Negusse Ocbamichael

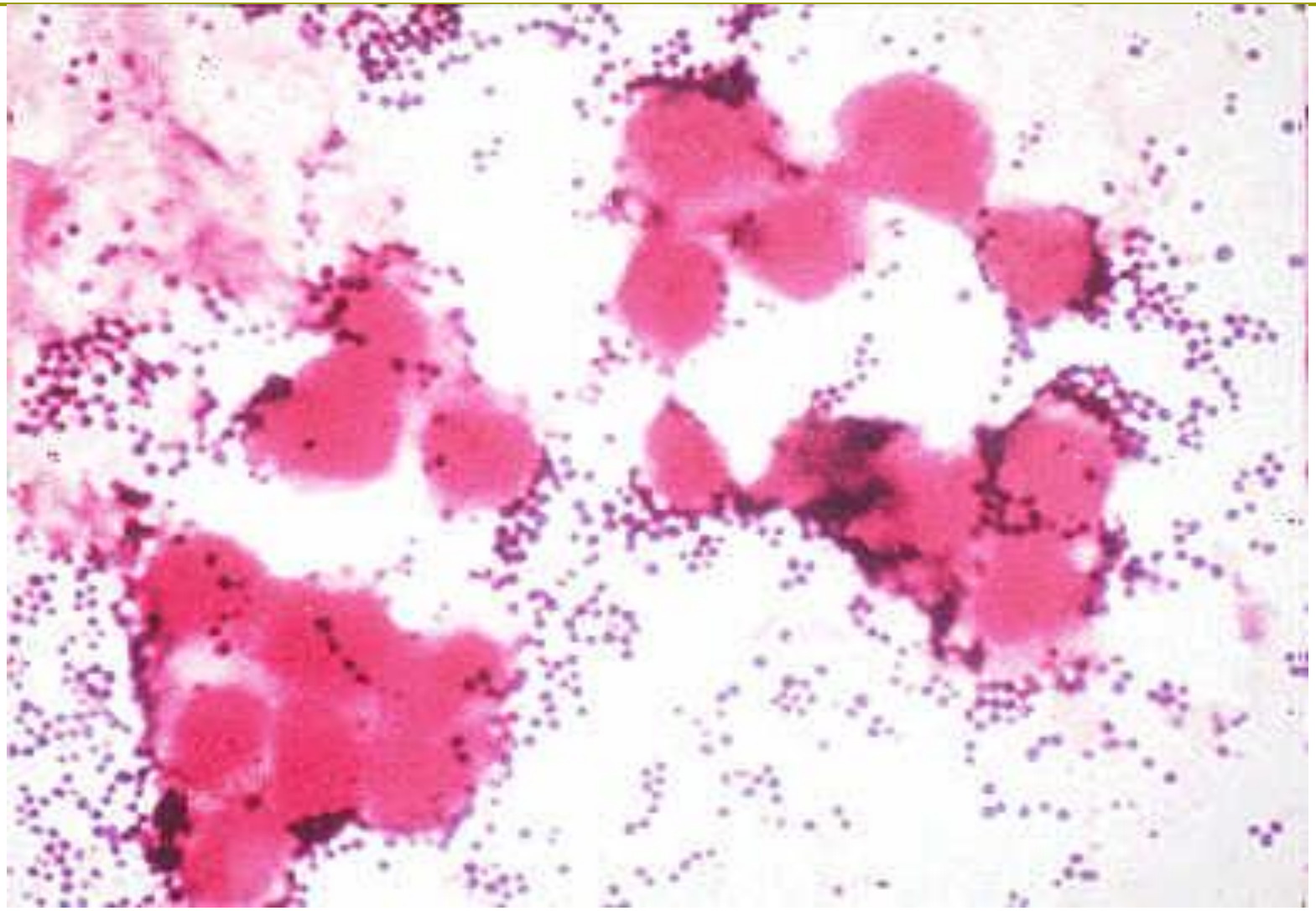









Gram stain of Gram positive cocci

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