UNIT ONE

The History and Scope of Microbiology

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INTRODUCTION

- **Microbiology-** is the science that deals with tiny organisms or microbes (micro organisms)
- **Micro organisms** are organisms that can not be seen with the naked eye
- It includes- Bacteria, Viruses, Protozoa, unicellular fungi, unicellular algae and others
- **Some organisms studies by microbiologists CAN be visualized without the aid of amplification [bread molds (fungus) and filamentous algae]**
  - **These organisms are included in the discipline of microbiology because of similarities in properties and techniques used to study them**

- The branches of Microbiology include:
  - Medical Microbiology
  - Industrial Microbiology
  - Food Microbiology
  - Soil Microbiology
  - Plant Microbiology
  - Environmental Microbiology
  - Agricultural Microbiology etc.
- **Medical Microbiology** is the study of microorganisms as causative agents of infectious diseases of human beings and their reactions to such infections

- Microorganisms exist virtually everywhere (cosmopolitan) in the biosphere
  - In soil, water, food, in the air, on the surface and inside our body
  - can also survive in most unlikely environment, like in cold air, in hot spring at temperature of 90 0c

- Microbes directly benefit or harm human beings – some of these economic importance of micro organisms are
  - microbes cause diseases both in plants and animals
  - Most of antimicrobial drugs are produced by microbes
    e.g Streptomycin – Streptomycetes, Penicilin – Peniceillium
  - Play key role in nutrient recycling- carbon and nitrogen-
    - Increase soil fertility
  - Micro organisms also involved in food production and processing
    e.g making of Yogurt, cheese, Wine, beer, bread
  - Some microorganisms (oceanic algae) contribute to the atmosphere by producing oxygen – photosynthesis
  - Biotechnology e.g. insulin
  - Biological weapons

Evolution and development of Microbiology

- **Anton van Leeuwenhoek (1632-1723)-** he was the first to make a microscope and observe bacteria, yeasts and protozoa
- **Aristotle** – believed that life comes from decaying matter
- **Johan Needham-** supporter of spontaneous generation (abiogenists)
- **Spontaneous Generation**
  - **The belief that life could originate from non-living or decomposing matter**
- **Francesco radi(1626-1697**)- opponents of spontaneous generation theory
- **Louis paster** (1822- 1895)- the father of microobiology

  - disapproved the abiogenesis theory – germ theory
  - proved that fermentation and putreification caused by microbes
  - introduced the pasteurization of milk
  - discovered vaccination against **rabies, chicken cholera, and anthrax**

Cont…

- Robert Koch (1843- 1910)- the father of Bacteriology
  - developed diagnostic techniques
  - introduced agar as a setting agent for bacteriological media
  - Koch did an experiment and formulate postulate
    - A specific disease is caused by a specific organism
    - the organism should be isolated and grown in the lab into a pure culture
    - the cultured organism must be able to produce some lesion when introduce into animal
    - the organism should be re-isolated from artificially infected animal and grown into pure culture in the lab

What are the drawbacks of Kotch's postulate ?

SCOPE OF MICROBIOLOGY

- Diagnostic use, e.g. isolation and identification of causative organism from the pathological lesions like diagnosing typhoid fever by culture methods
- Prognosis of disease, e.g. in the Widal test rising titer signifies active disease and ineffective treatment
- Guidance in treatment, e.g. by culturing the organism in pure form and then performing drug sensitivity test
- To know Source of infection, e.g. in sudden outbreak of infectious disease we can find out the source of infection

CLASSIFICATION OF BACTERIA

Taxonomy – the science that deals with **identification, nomenclature and classification** of organisms

Classification – is the arrangements of organisms into taxonomic groups (taxa) on the basis and similarities

Nomenclature- is the assignment of names to the taxonomic groups according to international rules

Identification – the process of determining that a new isolates belongs to one of the established, named taxa

Classical methods of taxonomy

1. **Numerical (phenetic taxonomy)**

- ✓ Developed with the invention of computer
- ✓ used to determine the degree of similarity between organisms

2. Morphological characters

- ✓ Concern cell **shape** and **size, staining reactions, presence or absence of spores, type of motility** etc

e. g. **Cocci:** These are spherical bacteria

Diplococcus - Binary fission occurs in one plane,

e.g. Pneumococcus

Streptococcus - Cocci arranged in chains,

e.g. *Streptococcus pyogenes*.

Staphylococcus - Cocci arranged in clusters,

e.g. *Staphylococcus aureus*.

Bacilli

Rod-shaped: eg. Enterobacteria, Bacillus species

Comma shape - e.g. *Vibrio*

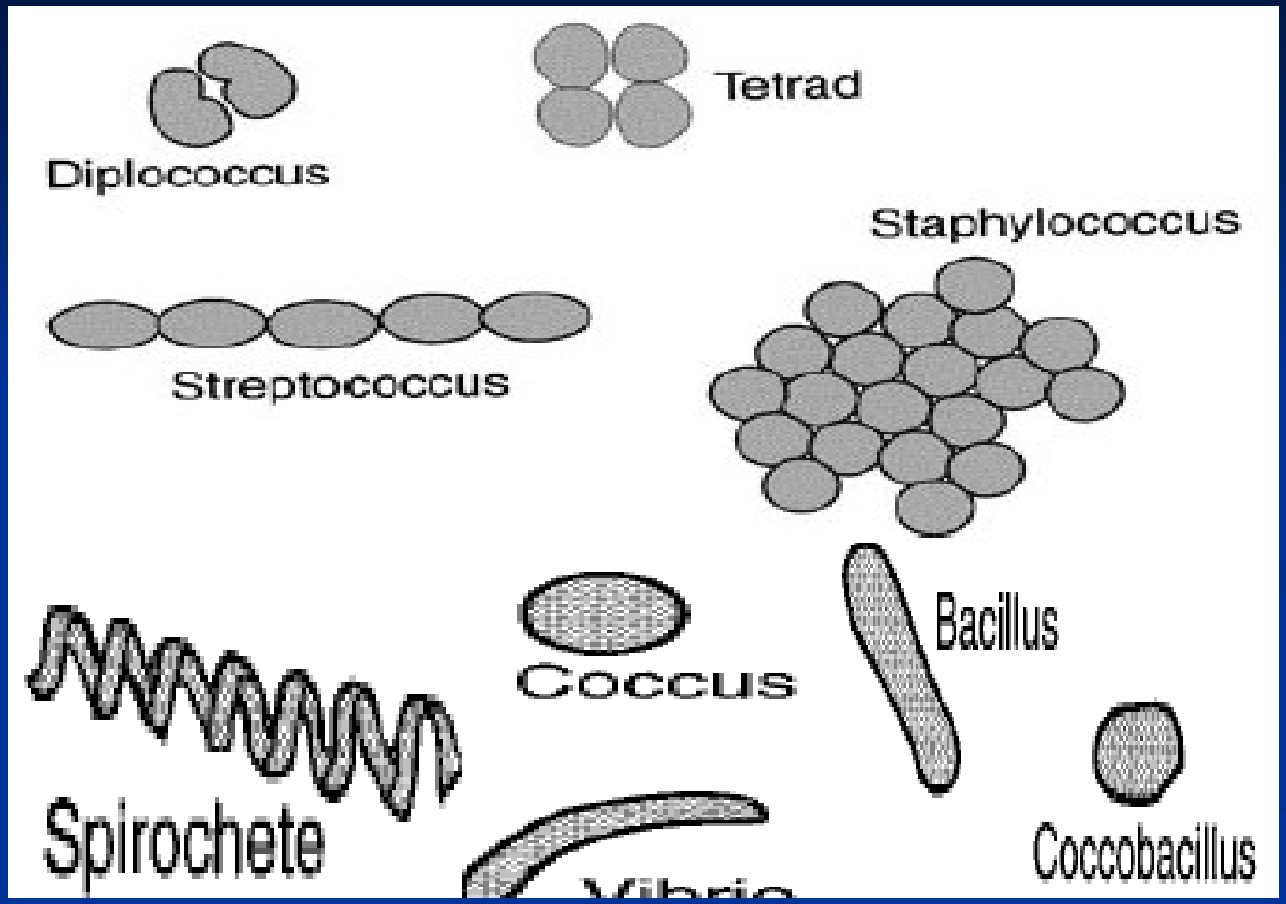
Coccobacilli- e.g. *Brucella*

Spirochaetes: these are slender, refractile and spiral filaments

e.g. *Treponema, Borrelia* and *Leptospira (fig.)*

- **based on staining properties**
- **Gram-positive bacteria**: Eg. Gram-positive cocci (Staphylococcus); Gram-positive bacilli (Bacillus)
- **Gram-negative bacteria**: Eg. Gram-negative cocci (Neisseria) Gram-negative bacilli (Salmonella)

fig. shapes of some different bacteria



3. **Cultural characters**

- Include the cultural requirements for multiplication e. g. nutrients, oxygen , temperature, liquid media , solid media

4. **Biochemical characters**

- Include the metabolic end products and the presence or absence of particular enzyme

4. **Molecular characters**

- Include the sequences of bases in the DNA

BACTERIAL CELL STRUCTURE

- are prokaryotic organisms reproduce by binary fission(Table 1)
- **Size**- Most bacteria are in the range of 1–10 µm
- Being small and simplicity is very important for bacteria Why?
  - Small size facilitates rapid exchange of nutrients and metabolic by-products with the environment,
  - Simplicity of design facilitates macromolecular synthesis, assembly of cell structures
    - rapid growth faster than eukaryotes

Table 1 Difference between Eukaryotes and Prokaryotes

Eukaryotes

- have membrane bounded nucleus
- have more than 2 chromosome
- have mitochondria, chloroplast, membrane vesicles and golgibodies
- have mitotic division
- Ribosomes size 80s
- No Peptidoglycan cell wall

Prokaryotes

- have no membrane bounded nucleus
- have 1 chromosome
- have no mitochondria, Chloroplast, membrane vesicles and golgibodies
- have no mitotic division
- ribosomes size – 70s
- have peptidoglycan cell wall

- The basic components of bacterial cell includes

1. **Cell envelope- Capsule , Cell wall** and under lying **Cytoplasmic membrane**
2. Cytoplasmic components
3. Extra cellular and surface components (external structure)

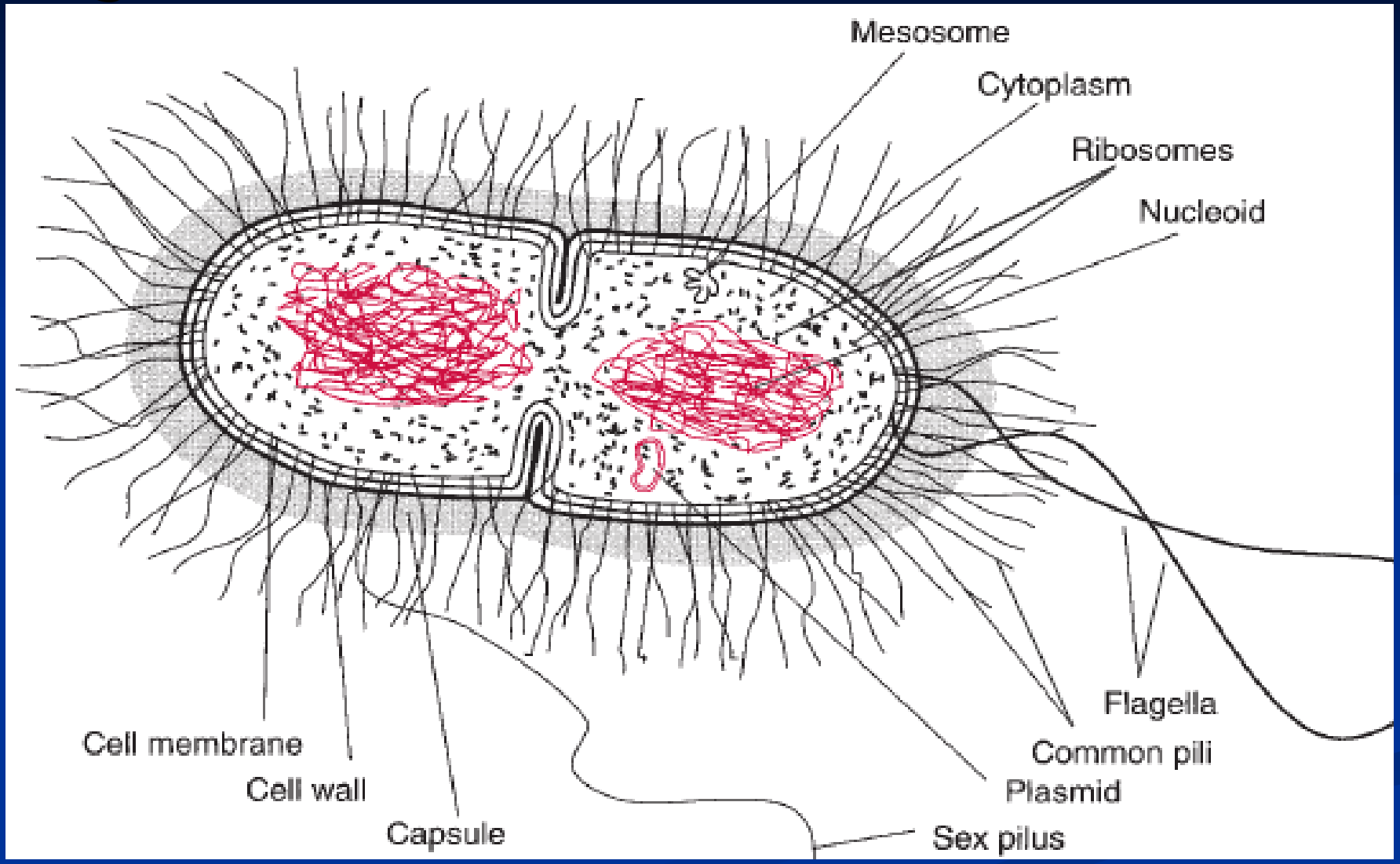
Capsule (slime layer)

- Gelatinous layer covering the entire bacteria (hydrophilic gel)
- Mainly made up of polysaccharide – (fig 1)
- May be found in both gram positive and gram negative bacteria
- Not all bacterial species produce capsules
- Resist phagocytosis (virulent factor) e.g. *Streptococcus pneumoniae*
- Some capsules promote adherence and colonization
- Used as vaccine antigens
- used for serologic identification of the species
- Capsules do not contribute to growth and multiplication

examples of bacteria producing capsule

Streptococcus pneumoniae , Klebsilla pneumoniae

fig.1


Mesosome
Cytoplasm
Ribosomes
Nucleoid
Flagella
Common pili
Plasmid
Sex pilus
Cell membrane
Cell wall
Capsule

Cell wall

- layers of the cell envelope between cytoplasmic membrane and capsule
- the cell wall is entirely absent in few unusual bacteria

  e.g. *mycoplasms , Chlamydia*
- The principal structural components of the cell wall is

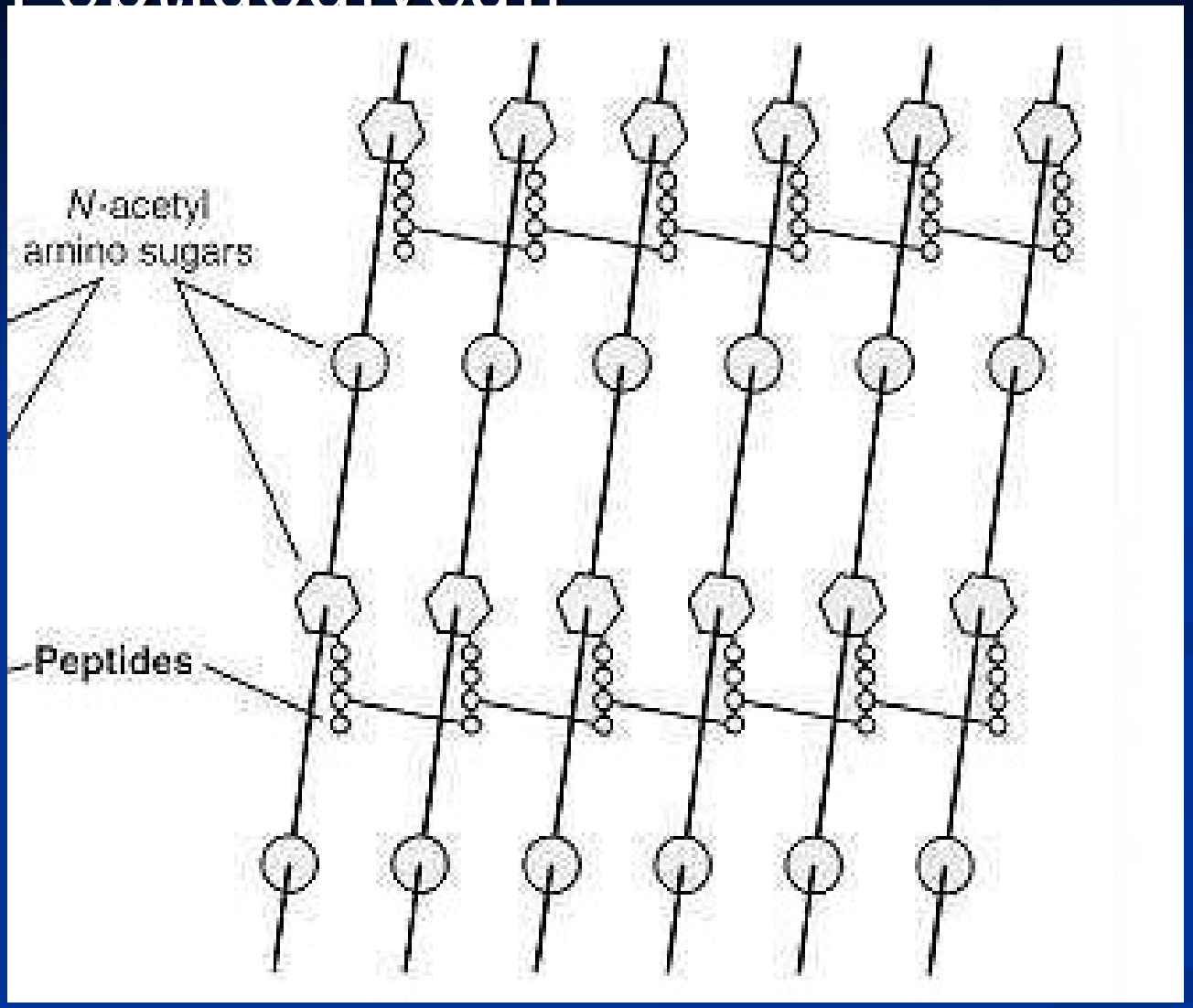
  peptidoglycan (PGn)

- Function – provide structural support
  - giving osmotic protection
  - has a role in cell division
  - various layer of the cell wall are the sites of antigenic determinants

Peptidoglycan layer- is a complex polymer consisting of :-

- **Backbone**- composed of alternating N-acetyl glucoseamine and N- acetyl- muramic acid – the same in all bacteria spp.
- A set of identical tetra peptide side chains attached to N-acetyl muramic acid – vary from species to species (fig. 2)
- A set of identical peptide cross bridges- varies species to species
- the structure of cell wall differs in gram +ve and gram – ve bacteria

fig.2 Peptidoglycan

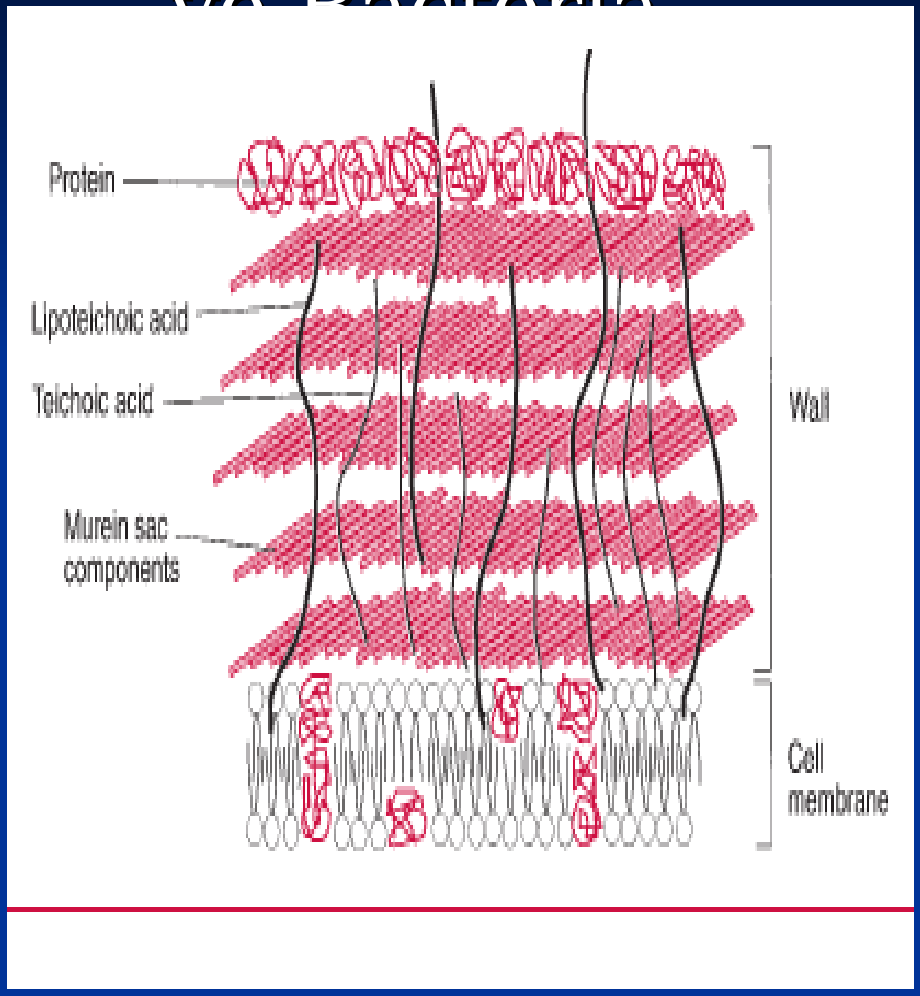


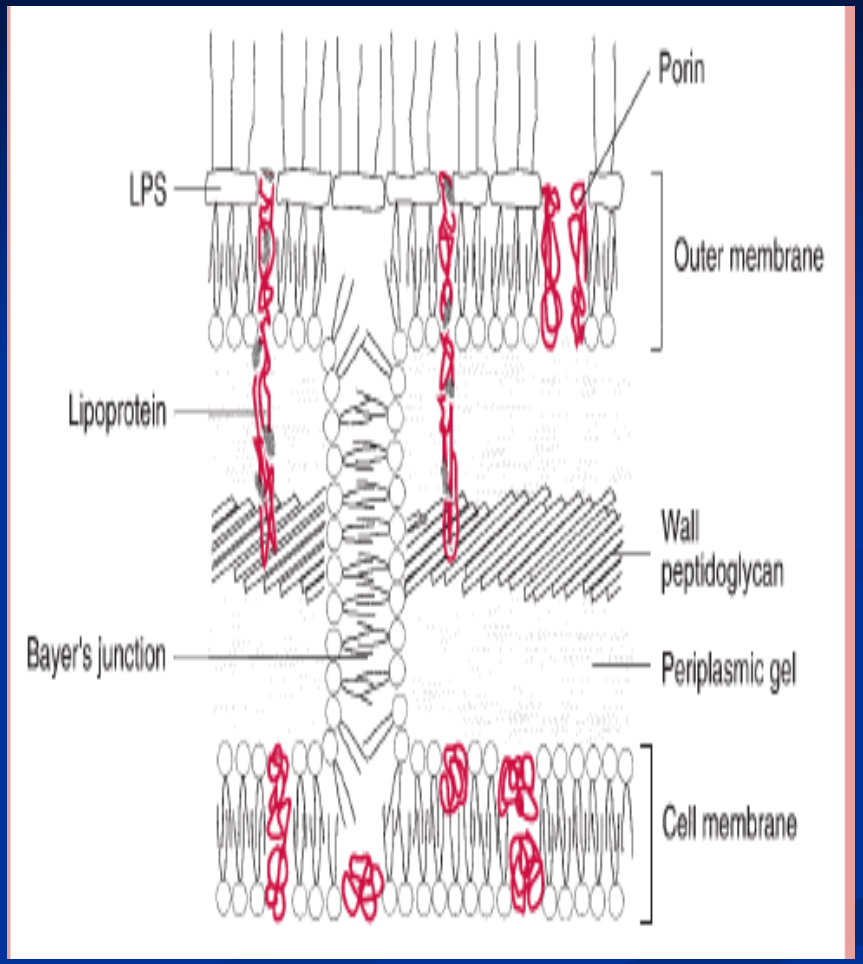
Special components of Gram positive bacteria

- many sheets of peptidoglycan
- Contain considerable amount of **teichoic acid** and **teichuronic acids**
- May contain polysaccharide molecules

- The large amount of peptidoglycan susceptible to the enzyme lysozyme and penicillin
- Penicillin specifically inhibits peptidoglycan synthesis (fig.3)

fig. 3 Gram +ve Bacteria Gram -ve Bacteria





Teichoic acid and Teichuronic acids

- Found only in gram +ve e.g. staphylococcus , lactobacilli
- Contains **glycerophosphate** or **ribitol-phosphate**
- Tow types –
  - Wall teichoic acid (WTA) - covalently linked to peptidoglycan
  - Membrane teichoic acid (LTA)- covalently linked to membrane glycolipid
- Negatively charged and serve as a cation (+ve ion) movement in to and out of the cell
- Have a role in regulating cell growth by preventing extensive wall break down

Special components of Gram negative bacteria

- less peptidoglycan
- no teichoic acid
- Outer membrane covers the cell wall thicker than peptidoglycan
  - Outer membrane is composed of Lipoprotein and Lipopolysaccharide attached to the peptidoglycan layer
- Wider area of periplasmic space between cell membrane and peptidoglycan (fig. 3)

Lipopolysaccharide (LPS)

- The LPS of the outer layer of the cell wall of gram negative bacteria is **endotoxin**
- **Endotoxin** play an important role in the pathogenesis of many Gram-negative bacterial infections e.g fever, shock

- LPS consists of
  - **a toxic lipid A** (a phospholipid containing glucosamine rather than glycerol),
  - **core polysaccharide -**containing some unusual carbohydrate residues and linked through ketodeoxyoctulonate (KDO) to lipid A
  - **O antigen polysaccharide side -**constitutes the major surface antigen (fig. 4)

Comparison of gram +ve and gram – ve bacterial cell wall

- Gram positive bacteria
- ✓ No outer membrane
- ✓ Have thick PGn
- ✓ No periplasmic space
- ✓ Have Teichoic acid

- Gram negative bacteria
- ✓ Have outer membrane
- ✓ Have thin PGn
- ✓ Have Periplasmic space
- ✓ Have no teichoic acid

Protoplasts

- A Gram-positive bacterium that has lost its Cell wall
- an enzyme in saliva and tears that destroys the peptidoglycan layer

Spheroplast - gram negative bacteria with a damaged cell wall

- The damaged caused by the action of toxic substance or antibiotics

  e. g. penicillin
- Parts of the cell wall are retained
- Spheroplast and protoplast are not reproducing

L- form- are mutant bacteria with out cell wall

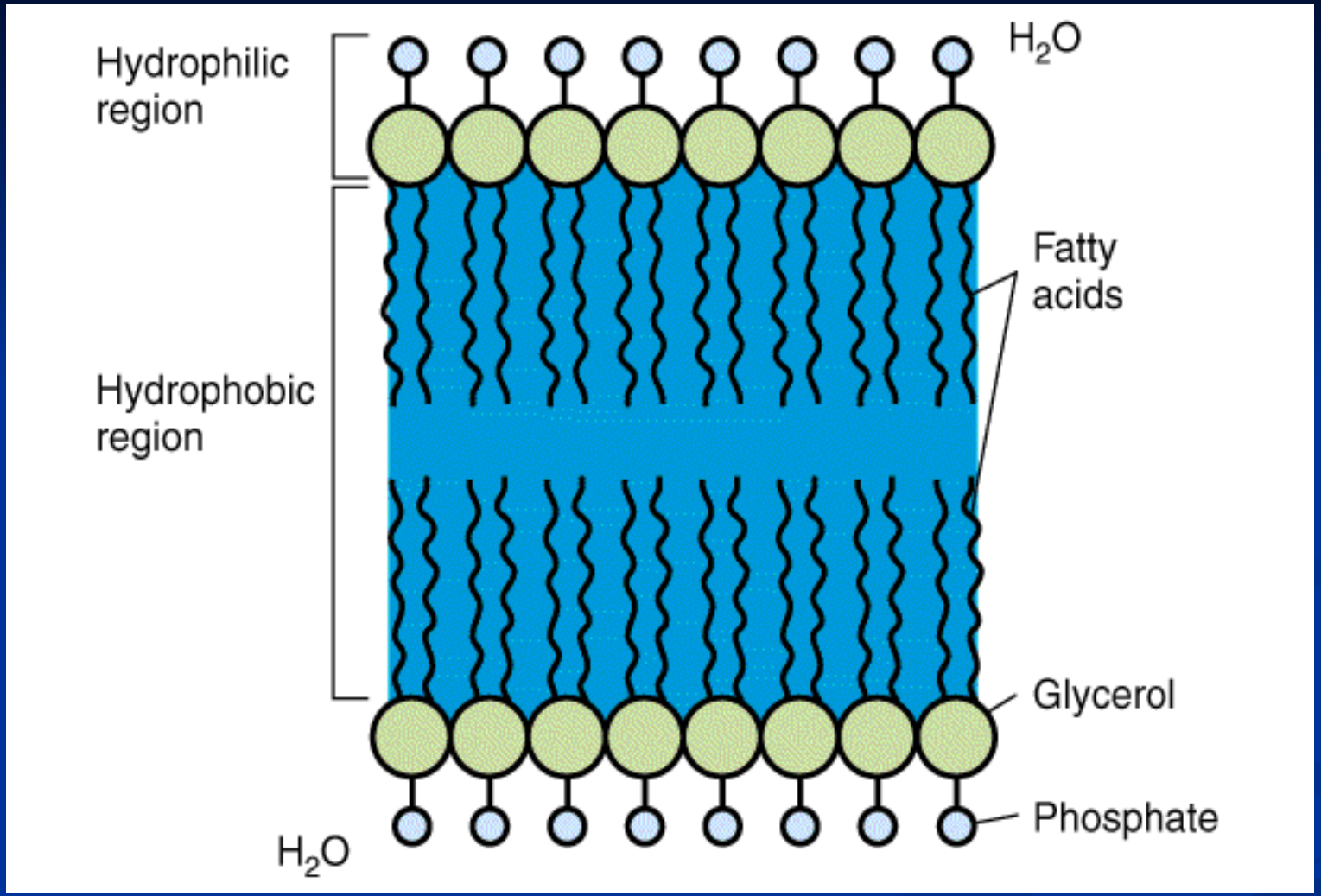
- These cell are produced when the surrounding are unfavourable
- They are able to reproduce and can be grown on special culture media with a high osmotic pressure

Mycoplasma – are highly pleomorphic bacteria with out cell wall

- Never reverse to the walled state

Cytoplasmic membrane (plasma membrane)

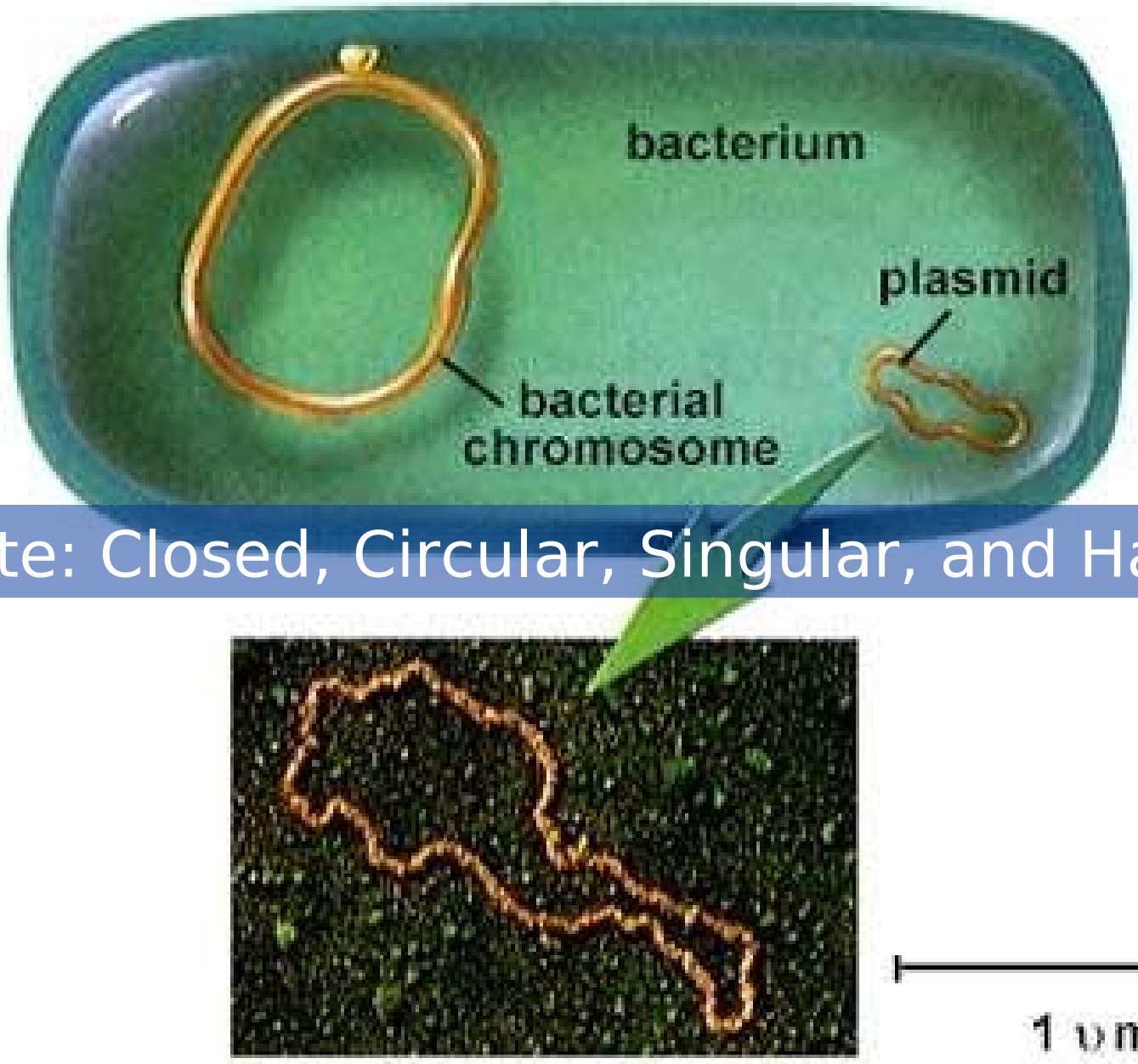
- Is the actual barrier between the interior and exterior of the bacterial cell
- Basic structure of cell membrane is phospholipid and protein bi layer, usually lacking sterols
- Membrane has roles -in synthesis of DNA and cell wall polymers
  - homeostatic,
  - excretion of enzymes,
  - electron transport processes and
  - in cell division


Hydrophilic region
Hydrophobic region
H_2O
Fatty acids
Glycerol
Phosphate
H_2O

Cytoplasmic components

- **Nucleoid**
  - consists of a large tightly packed circular chromosome of supercoiled double-stranded DNA
  - A Bacterium containing a single chromosome
  - **Functions:** codes for protein synthesis, helps in cell division and carries genetic information

Chromosomes, Bacterial



Note: Closed, Circular, Singular, and Haploid

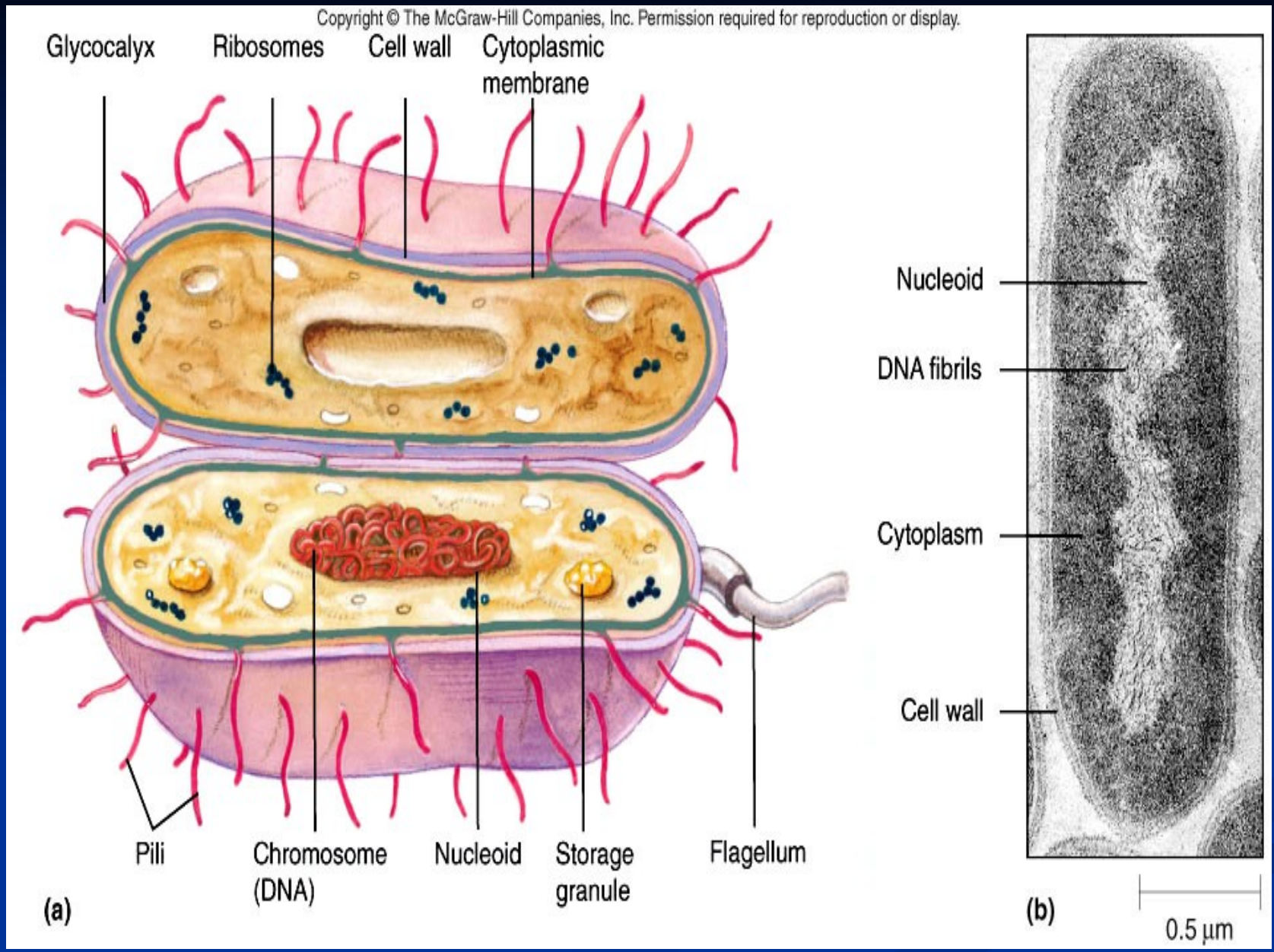
Plasmids

- are small, usually circular, double-stranded DNA molecules
- More than one type of plasmid or several copies of a single plasmid may be present in the cell
- carry genes coding for the production of enzymes that protect the cell
- attributes of virulence, such as production of some pili and of some exotoxins
- production of a sex pilus by which they promote cell conjugation
  - accomplish their own intercellular transmission

Ribosomes

- Are complex structure composed of several RNA molecules and many proteins
- A bacterium ribosome is composed of two sub units – small and large
- Active center for protein synthesis
- Streptomycin, tetracycline , Chloramphenicol inhibit protein synthesis

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Mesosomes

- Are complex invaginations of cytoplasmic membrane
- Increase in the total surface area of the membrane
- Attached to chromosomes and involved in cell division
- Others involved in to secretion of proteins and active transport

Inclusions

there are several kinds of reserve deposits in the cytoplasm

e. g. **Metachromatic granules**

- Reserves of inorganic phosphates used in the synthesis of ATP

- **Polysaccharide granules**

- Consists of glycogen and starch

-**Zinc inclusions**

- Found in *Mycobacterium, Bacillus, Azotobacter, Spirillum*

Bacterial appendages

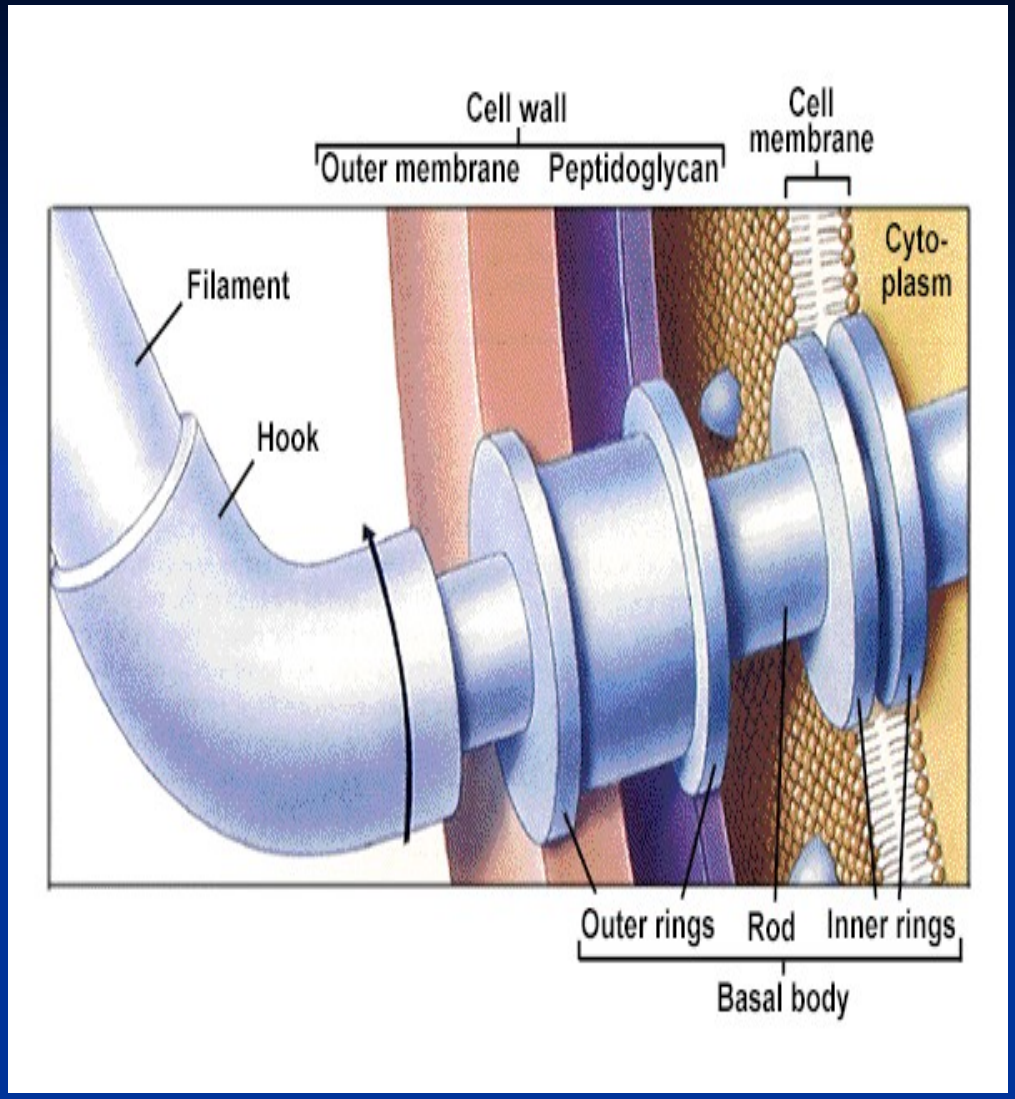
1. **Flagella**

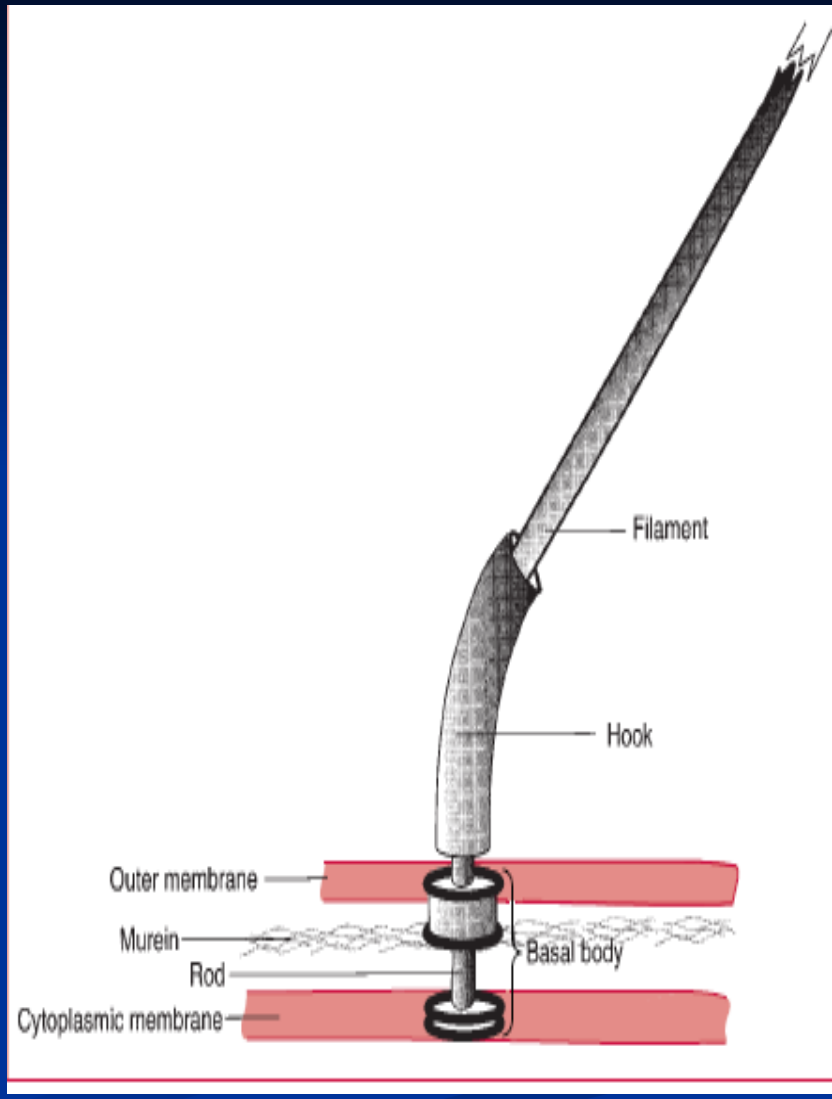
- Is an organ of locomotion
- Common in bacilli and rare in cocci
- made up of proteins called **flagellins**
- Flagellins are highly antigenic (H antigens)
- H antigens are characteristic of a given species or strain
- Number and distribution of flagella on bacterial surface are characteristics of a given species
- Can be sheared and resynthesized

Structure of flagella – consists of **basal body, a hook and filament**

- Filament -lies external to the cell surface
- Basal body –has sets of ring to which the hook is anchored and which

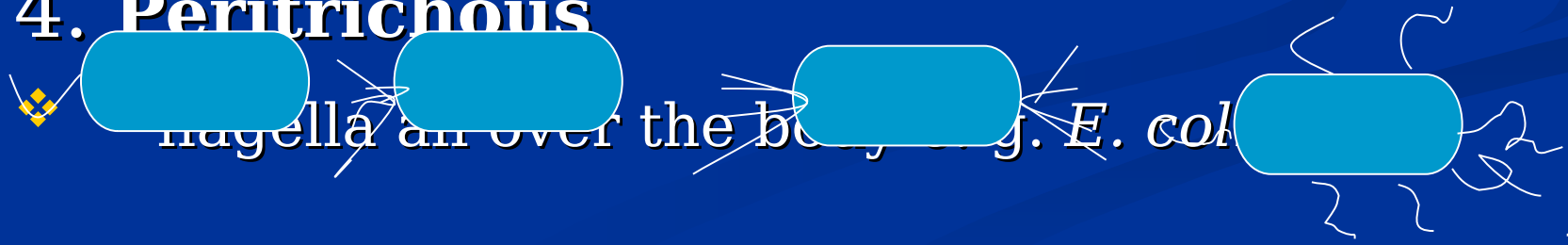
imparts motion to the flagellum


Cell wall
Outer membrane
Peptidoglycan
Cell membrane
Cyto-plasm
Filament
Hook
Outer rings
Rod
Inner rings
Basal body


Filament
Hook
Outer membrane
Murein
Rod
Cytoplasmic membrane
Basal body

Types of flagella (arrangements of flagella)

1. **Monotrichous**
   - Single flagella at a pole e. g. *V. cholerae*
2. **Lophotrichous**
   - Tuft flagella at one pole e. g. *Pseudomonas flouresence*
3. **Amphitrichous**
   - Tuft of flagella at both poles e.g. *Alcalgenes faecales*
4. **Peritrichous**
   - Flagella all over the body e. g. *E. coli*



2. **Pili (fimbriae)**

- Made up of protein like flagella
- Short, thin and filamentous
- Occur in some bacteria

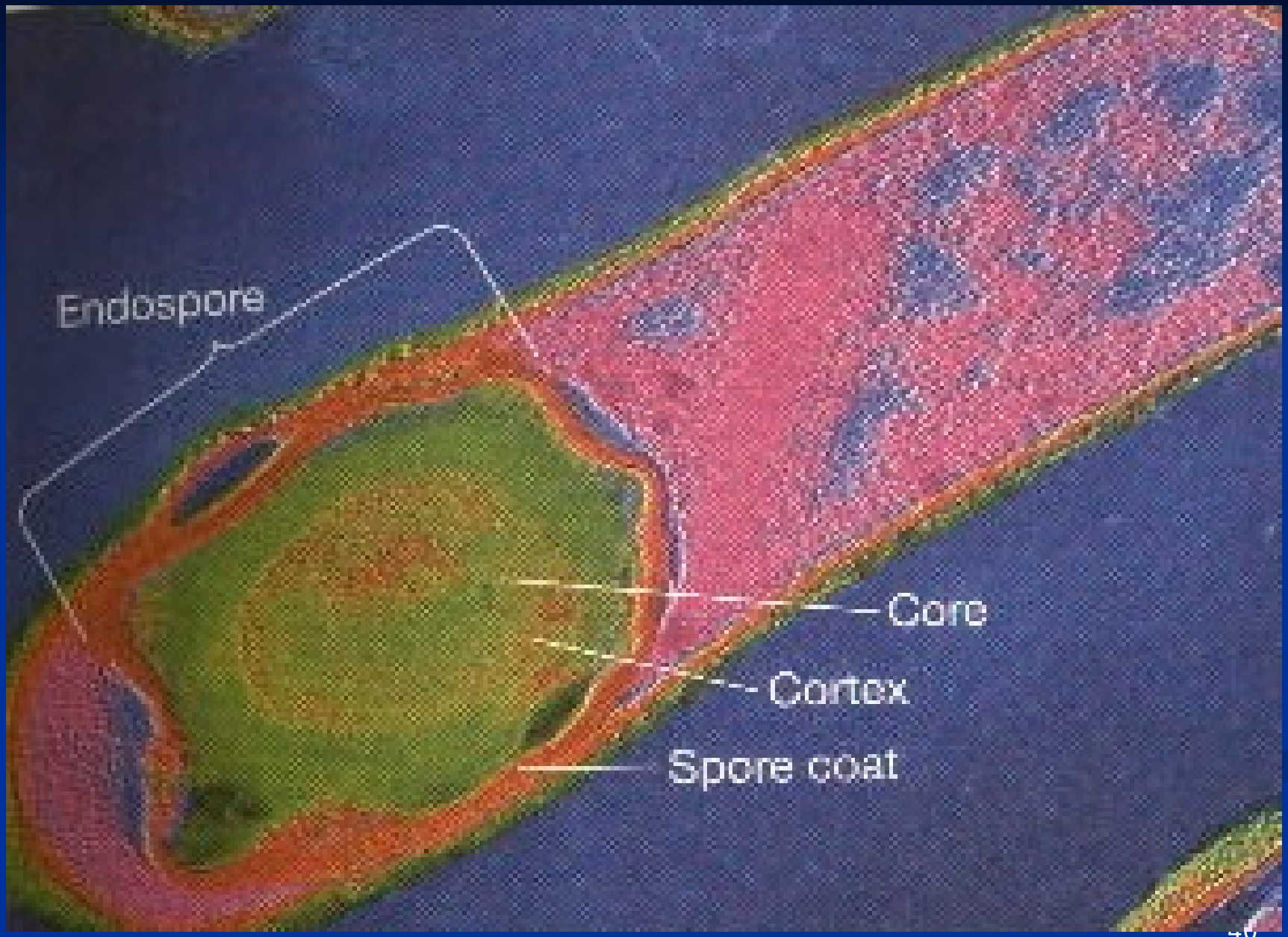
e.g. Shigella, Proteus, Salmonella, Seratia, Enterobacteriace

- There are two general classes, **common pili and sex pili**
- Common ordinary pili – used for adhesion on cells
  - are virulence factors for pathogenic MOs
- Sex pili – also called **fertility pili**
  - fewer in number
  - possessed by “males” cells
  - specialized for genetic material transfer
  - help the “male” cells attach to “non male” cells during conjugation

Endospores

- Resistant structure
  - Heat, irradiation, cold
  - Boiling >1 hr still viable
- All spore formers are Gram-positive rods

  e. g. Clostridium tetani , Bacillussubtilis
- Endospore is not a reproductive structure
- Resistance of spore is due to dehydrated state, calcium dipicolinate, and specialized coats (spore membrane, cortex , coat)
- Location is important in classification
  - Central, Subterminal, Terminal


Endospore
Core
Cortex
Spore coat