History of Microbiology. Classification of Microorganisms. Morphology and Structure of Bacteria, Fungi, Spirochetes, Chlamydia, Rickettsia.

Department of Microbiology, Immunology and Virology.

Historical Introduction

- Antony van Leeuwenhoek — First to observe live microorganisms, using a simple microscope (1685)
- John Tyndall — Developed tyndallization to destroy spores (1660)
- Louis Pasteur — Disproved the theory of spontaneous generation (1861). Contributed to the understanding of fermentation (1858). Developed technique for selective destruction of the microorganisms (pasteurization) (1866). Study of bacterial contamination of wine (1866) and diseases of silkworms (1868). Attenuated vaccines for anthrax (1881) and chicken cholera. Immunization against rabies (1885)
- Joseph Lister — Contributed to concept of aseptic technique (1865-1870)
- Robert Koch — Developed postulates for proving the cause of infectious diseases (1884) and pure culture concept. Observed anthrax bacilli (1876). Developed solid culture media (1882). Discovered organisms causing tuberculosis (1882)
- Paul Ehrlich — Formulated humoral theory of resistance. Developed new staining techniques. Developed first chemotherapeutic agent (1890s to 1900)
- Elie Metchnikoff — Formulated cellular theory of resistance (1890s)

Definition of Microbiology

Medical microbiology is the study of microbes that infect humans,
the diseases they cause, their diagnosis, prevention measures, aseptic techniques, treatment of infectious diseases, immunology, and production of vaccines to protect against infectious diseases.

Classification of Microorganisms

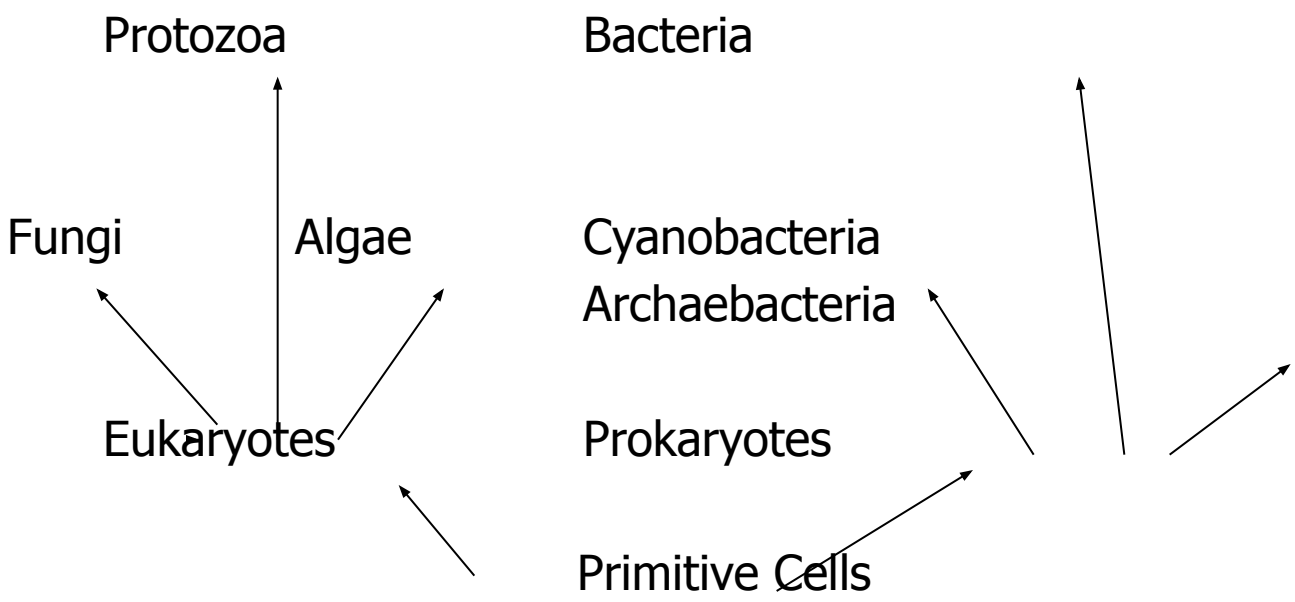
- Scientific nomenclature includes a hierarchial scheme. The lower down in the system the more specific or narrowly defined is the group.

Kingdom → Phylum → Class → Order → Family → Genus → Species

Species- is fundamental unit as outlined above the concept is that all bacteria, which share a specific set of defined properties, belong to a particular species.

- Classification of Bacteria is based on Gram staining characteristic, morphology, and metabolism type. Bergey's Manual of Systematic Bacteriology is the bible of bacterial taxonomy.
- Classification of Viruses is based on nucleic acid type, host organism, and morphology.

Family Tree of Microorganisms



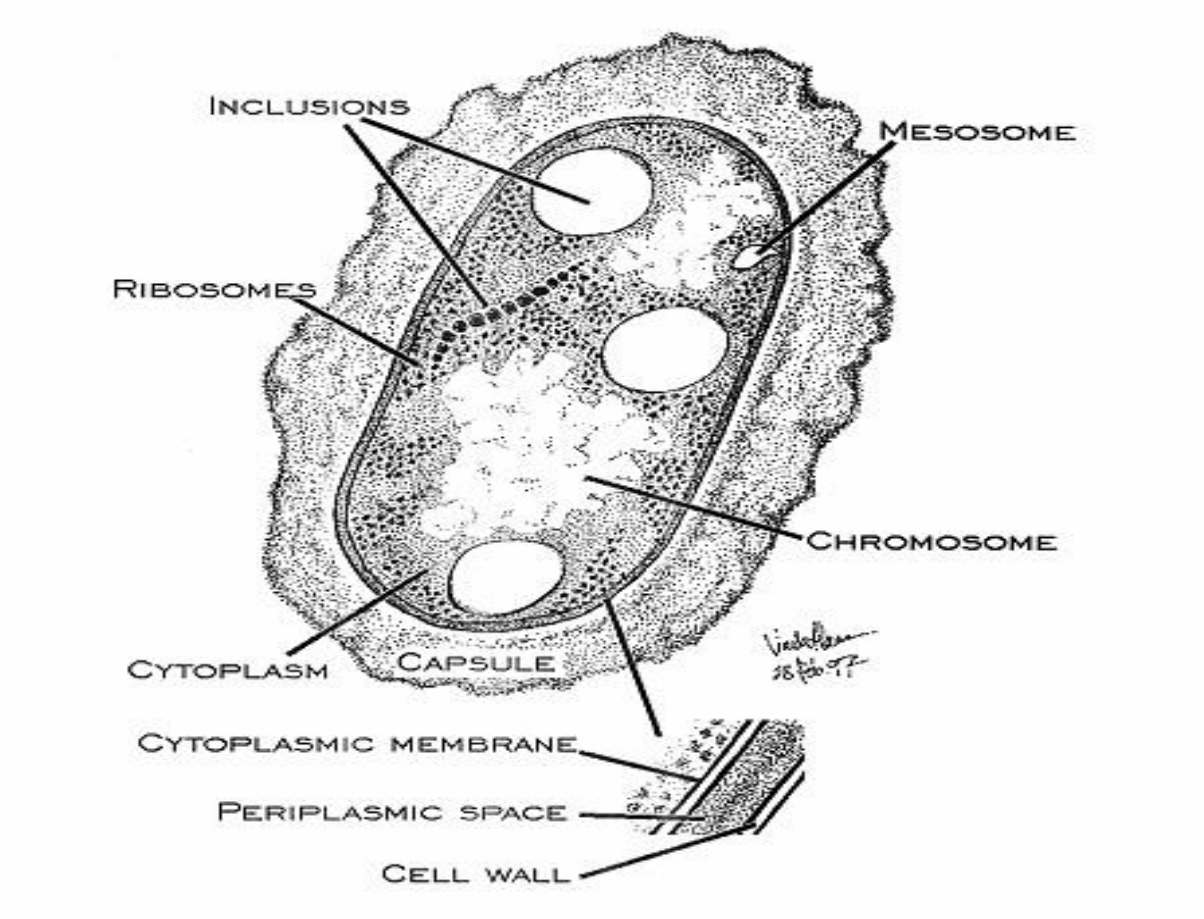
Differences between Prokaryotic and Eukaryotic Cells

Structure	Prokaryotes	Eukaryotes
Nucleus		
Nuclear membrane	Absent	Present
Nucleus	Absent	Present
Chromosome	One	More than one
Deoxyribonucleprotein	Absent	Present
Division	By binary fission	By mitosis
Cytoplasm		
Mitochondria, Golgi apparatus, Lysosomes, Pinocytosis, Endoplasmic reticulum	All are absent	All are present
Chemical composition		
Sterols	Absent	Present
Muramic acid	Present	Absent

Prokaryotic Cell Structure

- Prokaryotes are unicellular organisms of relatively simple construction.
- A prokaryotic cell has five essential structural components: a genome (DNA), ribosomes, cell membrane, cell wall, and some sort of surface layer.
- Structurally a prokaryotic cell has three architectural regions: **appendages** (attachment to the cell surface) in the form of **flagella** and **pili (or fimbriae);** a **cell envelope** consisting of a **capsule, cell wall and plasma membrane;** and a **cytoplasmic region** that contains the cell **genome (DNA)** and **ribosomes** and various sort of **inclusions.**

Cell structure



Characteristic of typical bacterial cell structures

Sturcture	Function(s)	Predominant chemical composition
Flagella	Swimming movement	Protein
Pili		
Sex pilus	Mediates DNA transfer during conjugation	Protein
Common pili or Fimbriae	Attachment to surfaces; protection against Phagotrophic engulfment	Protein
Capsules (includes "slime layers" and glycocalyx)	Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; reserve of nutrients or protection against desiccation	Usually polysaccharide; occasionally polypeptide
Cell wall		
Gram-positive bacteria	Prevents osmotic lysis of cell protoplast and confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
Gram-negative bacteria	Peptidoglycan prevents osmotic lysis and confers rigidity and shape; outer membrane is permiability barrier; associated LPS and proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-lipopolysacharide "outer membrane"

Plasma membrane	Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems	Phospholipid and protein
Ribosomes	Sites of translation (protein synthesis)	RNA and protein
Inclusions	Often reserves of nutrients; additional specialized functions	Highly variable; carbohydrate, lipid, protein or inorganic
Chromosome	Genetic material of cell	DNA
Plasmid	Extrachromosomal genetic material	DNA

Appendages

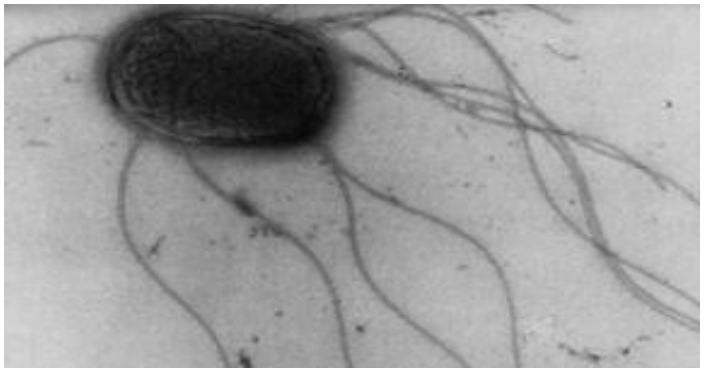
Flagella-are filamentous protein structures attached to the cell surface that provide the swimming movement for most motile prokaryotes.

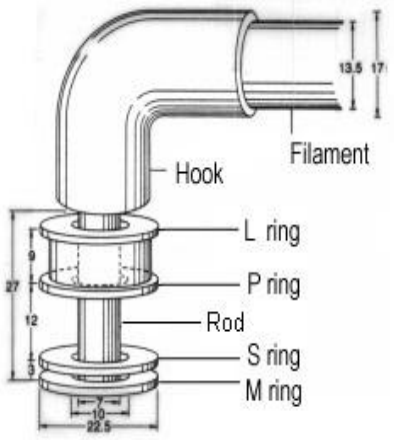
The diameter is about 20 nanometers.

The flagellar apparatus consists of several **distinct proteins**: a system of **rings** embedded in the cell envelope (the **basal body**), a **hook-like structure** near the cell surface, and the **flagellar filament**.

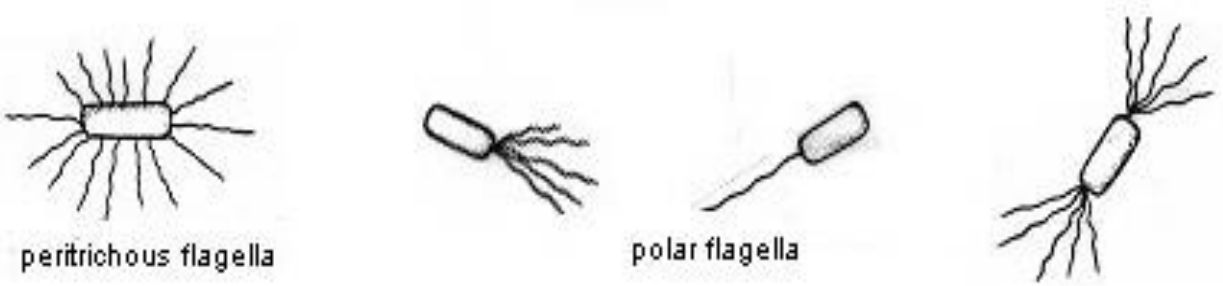
The innermost rings, the M and S rings located in the plasma membrane, comprise the motor apparatus.

The outermost rings, the P and L rings, located in the periplasm, function as bushings to support the rod where it is joined to the hook of the filament on the cell surface.



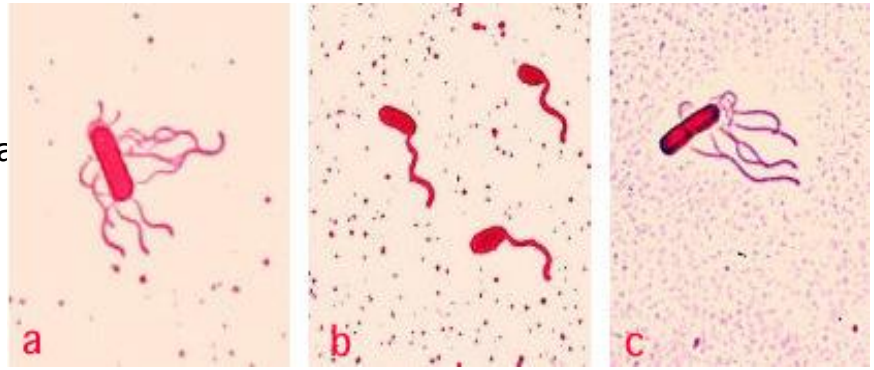


- Flagella may be variously distributed over the surface of bacterial cells.
- Arraingment of flagella: monotrichous, amphitrichous, lophotrichous, peritrichous.



Detecting Bacterial Motility

- **Flagellar stains** (show their pattern of distribution)
- **Motility test medium** demonstrates if cells ca swim in a semisolid medium (Proteus)
- **Direct microscopic observation** of living bacteria in a wet mount
- **Dark ground Illumination**
- **Electron microscopy**



Fimbriae

Fimbriae and **pili** are short, hair-like structures

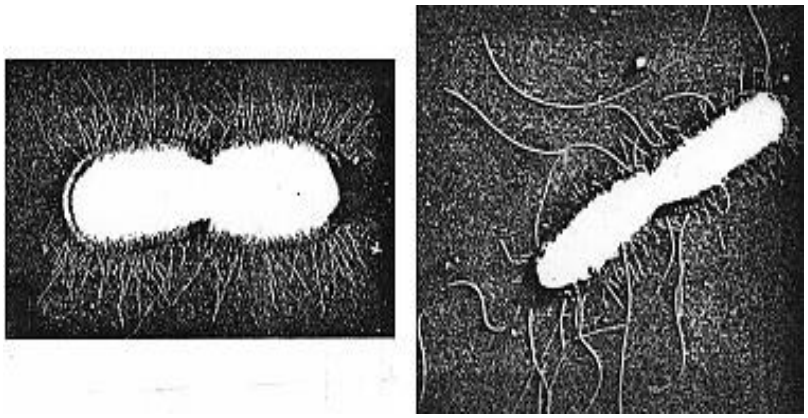
- they are composed of protein
- shorter, stiffer, smaller in diameter
- very common in Gram-negative bacteria, but occur in some archaea and Gram-positive bacterias
- involved in adherence of bacteria to surfaces, substrates and other cells or tissues in nature

F or sex pilus, specialized type of pilus in *E.coli* mediates the transfer of DNA between mating bacteria during the process of **conjugation,**

Common pili (almost always called **fimbriae**)

- usually involved in specific attachment of prokaryotes to surface in nature
- they are major determinants of bacterial virulence: they allow pathogens to attach to (colonize) tissues, resist attack by phagocytic white blood cells

Col I (colicin) pili



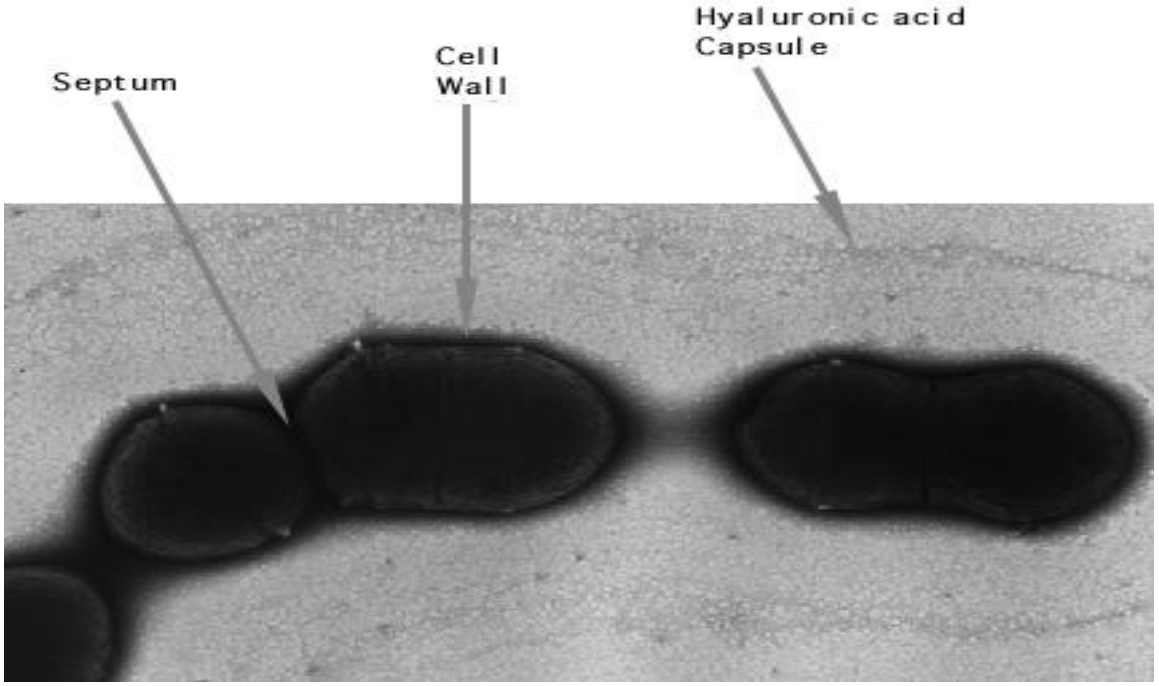
The Cell Envelope

The cell envelope consists:

plasma membrane
a cell wall
a capsule

Capsules

- Polysaccharide layer outside of the cell wall polymer



The function of capsules:

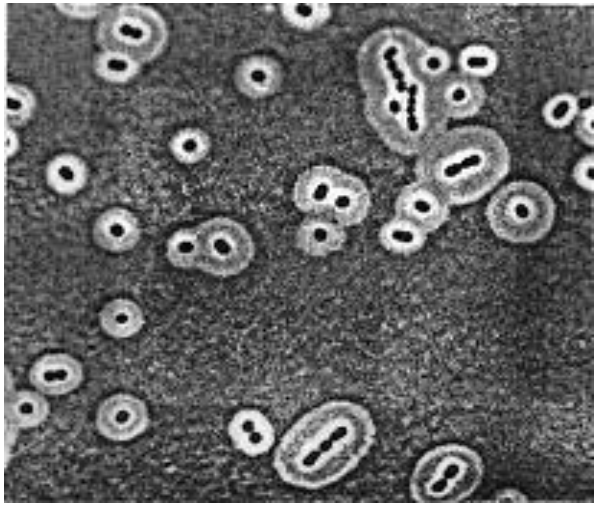
- Mediate adherence of cells to surface
- Protect bacterial cells from engulfment by predatory protozoa, white blood cells (phagocytes)
- Protect from attack by antimicrobial agents of plant or animal origin
- Protect cells from perennial effects of drying desiccation

Capsulated Organisms

Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella sp., Bacillus anthracis, Haemophilus influenzae**,** Yersinia pestis etc.

Demonstration of Capsule

- India ink staining (nagative staining)
- Serological mathods (capsule swelling reaction)
- Special capsule staining



Cell Wall

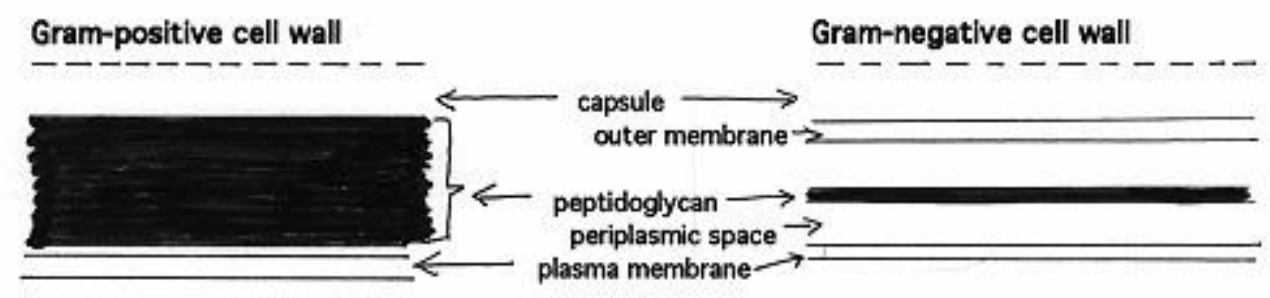
- is essential rigid structure for viability (protection cell protoplasm from mechanical damage and osmotic rupture or lysis)
- composed of unique components found nowhere else in nature
- one of the most important sites for attack by antibiotics
- provide ligands for adherence and receptor sites for drugs or viruses
- cause symptoms of disease in animals
- provide for immunological distinction and immunological variation among strains of bacteria
- It is 10-25 nm in thickness and weighs about 20-25% of the dry weight cell wall.

Cell wall structure

contains a unique type of **peptidoglycan** called **murein- (N-acetylmuramic acid)**
By cell wall structure there are two groups of bacterias

Gram-Positive Cell Envelope(15-80nm)
consists in two or three layers:
cytoplasmic membrane,
a thick **peptidoglycan layer,**
and outer layer or, **capsule** or,
glycoprotein **(S-layer)**

Gram-Negative Cell Envelope (10nm)
cytoplasmic membrane **(inner membrane),**
single planar sheet of peptidoglycan,
outer membrane contains a unique component
lipopolysaccharide (LPS or **endotoxin),**
the space between inner and outer membrane is the **periplasmic space**



Bacteria with Defective Cell Wall

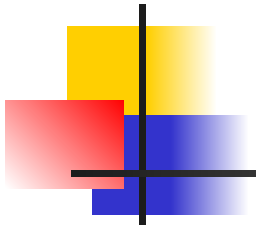
The synthesis of cell wall may be inhibited or interfered by many factors such as, antibiotics, bacteriphages, and lysozyme.

- **Mycoplasma:** This is a naturally occuring bacteria without cell walls. They don't require hypertonic environment for maintenance and are stable in culture medium
- **L-forms:** L-forms develop either spontaneously or in the presence of penicillin or other agents that interfere with synthesis of cell wall. These are difficult to cultivate and require agar containing solid medium having right osmotic strength. L-forms are more stable than protoplasts and spheroplasts
- **Protoplasts:** These are derived from Gram positive bacteria. They contain cytoplasmic membrane and cell wall is totally lacking. These are produced artificially by lysozyme in a hypertonic medium. These are unstable.
- **Spheroplasts:** These are derived from Gram positive bacteria. They are produced in presence of penicillin. They are osmotically fragile and must be maintained in hypertonic culture medium. They differ from protoplast in that some cell wall material retained.

The Plasma Membrane

Functions of the prokaryotic plasma membrane.

- 1. Osmotic or permeability barrier
- 2. Location of transport systems for specific solutes (nutrients and ions)
- 3. Energy generating functions, involving respiratory and photosynthetic electron transport systems, establishment of proton motive force, and transmembranous, ATP-synthesizing ATPase
- 4. Synthesis of membrane lipids (including lipopolysaccharide in Gram-negative cells)
- 5. Synthesis of murein (cell wall peptidoglycan)
- 6. Assembly and secretion of extracytoplasmic proteins
- 7. Coordination of DNA replication and segregation with septum formation and cell division
- 8. Chemotaxis (both motility per se and sensing functions)
- 9. Location of specialized enzyme system



- It is 5-10 nm thick elastic semipermiable layer which lies beneath the cell wall separating it from the cell cytoplasm.
- The plasma membrane of procaryotes may invaginate into the cytoplasm or form stacks or vesicles attached to the inner membrane surface. These structures are sometimes referred to as **mesosomes** Such internal membrane systems may be analogous to the cristae of mitochondria or the thylakoids of chloroplasts which increase the surface area of membranes to which enzymes are bound for specific enzymatic functions
- They are the principal centers of respiratory enzyme
- Mesosomes may also represent specialized membrane regions involved in DNA replication and segregation, cell wall synthesis, or increased enzymatic activity.
- There are two types of mesosomes- septal and lateral. The septal mesosome attached to bacterial chromosome and is involved in DNA segregation and in the formation of cross-walls during binary fission.

The Cytoplasm

- The bacterial cytoplasm is a colloidal system containing a variety of organic and inorganic solutes in a viscous watery solution.
- The cytoplasmic constituents of procaryotic cells invariably include the **procaryotic chromosome** and **ribosomes**.
- The chromosome is typically one large circular molecule of **DNA**, more or less free in the cytoplasm.
- Procaryotes sometimes possess smaller extrachromosomal pieces of DNA called **plasmids**.
- The total DNA content of a procaryote is referred to as the cell **genome**.
- The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of **ribosomes,** procaryotic ribosomes are 70S in size

Nucleus

- Bacterial nucleus has no nuclear membrane or nucleolus
- The genomic DNA is double stranded in the form of a circle.
- It measures about 1mm (1000µm) when straightened
- The bacterial DNA is haploid, replicates by simple fission and maintains bacterial genetic characteristic

Plasmids

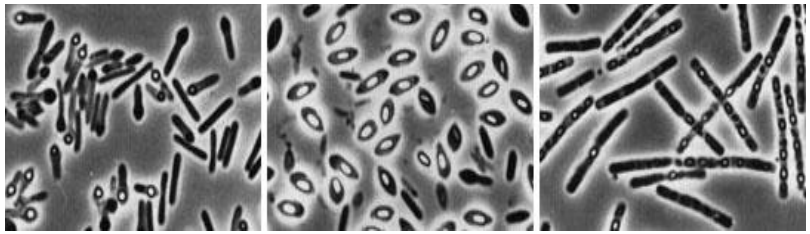
- Some bacteria may possess extranuclear genetic material in the cytoplasm consisting of DNA named as plasmids or episomes
- The plasmid replicates autonomously.
- They are not essential for the life of the cell, but may confer on the bacteria certain properties, such as drug resistance and toxigenecity which constitute a survival advantage to the bacteria.
- These plasmids can be transmitted from one bacterium to another. either by conjugation or by the agency of bacteriophage.
- Plasmids also may be transferred to daughter cells during cell division.

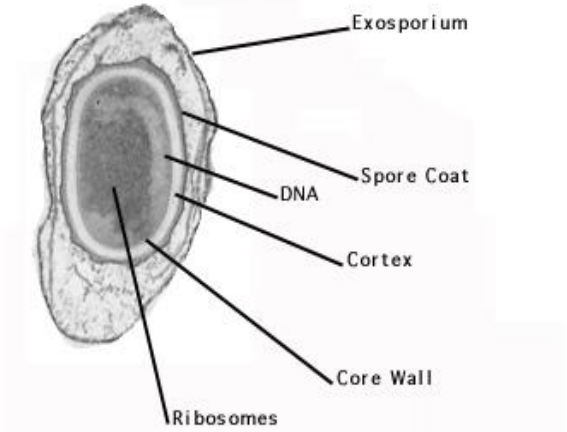
Inclusions

- Often contained in the cytoplasm of prokaryotic cells is one or another of some type of inclusion granule. **Inclusions** are distinct granules that may occupy a substantial part of the cytoplasm
- Inclusion granules are usually reserve materials of some sort
- Many bacteria accumulate granules of **polyphosphate** which can be used in the synthesis of ATP
- These granules are termed **volutin garnules** or **metachromatic granules**
- They are characteristic features of the corynebacteria
- They can be stained

Endospores

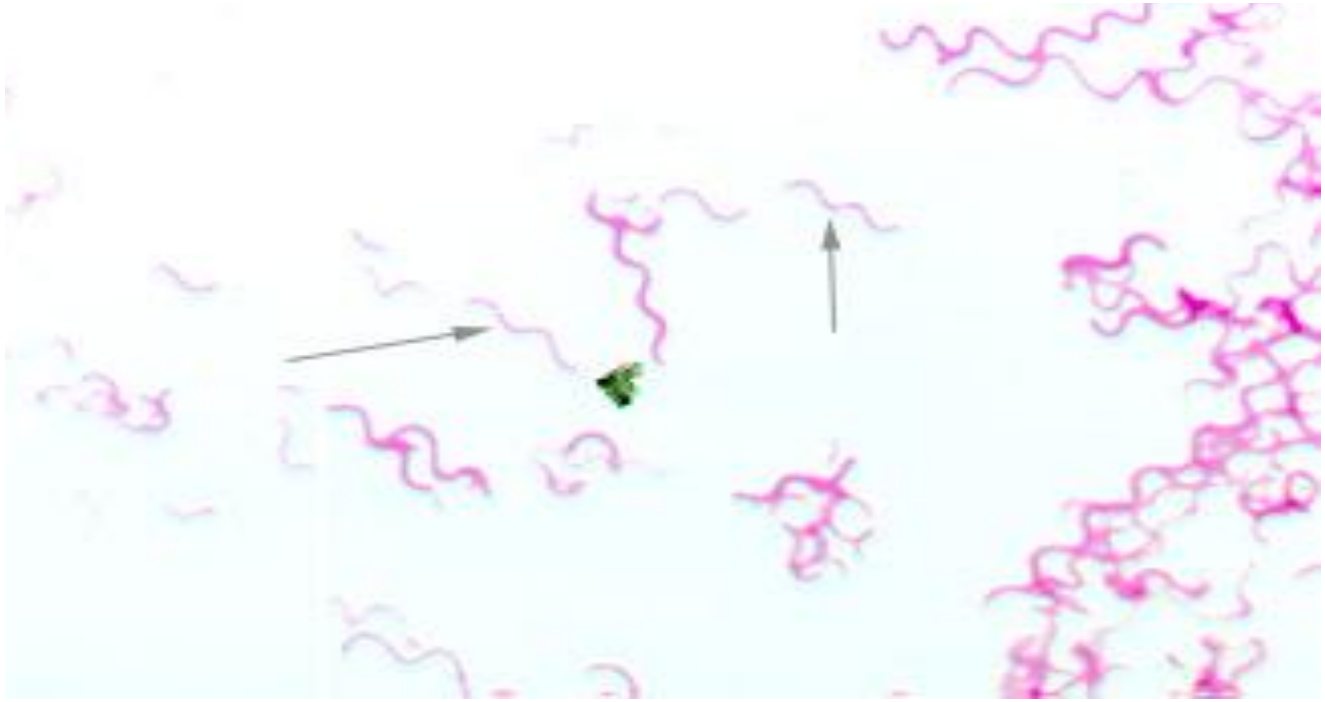
- A bacterial structure sometimes observed as an inclusion is actually a type of dormant cell called an **endospore**.
- Endospores are formed by a few groups of **Bacteria** as intracellular structures
- Highly resistant to environmental stresses
- Endospores are formed by vegetative cells in response to environmental signals that indicate a limiting factor for vegetative growth
- Under appropriate environmental conditions, they germinate back into vegetative cells.
- There are eight stages, O,I-VII, in the sporulation cycle of a *Bacillus* species, and the process takes about eight hours**.**

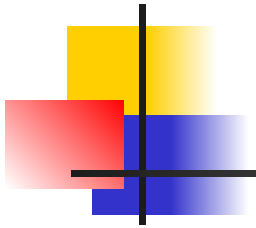




Morphology of the Spirochetes

- The spirochetes
- Long
- Thin
- Corkscrewlike
- Gram-negative
- Anaerobic bacteria
- There are three families are pathogen for human: Leptospira,Treponema, and Borrelia

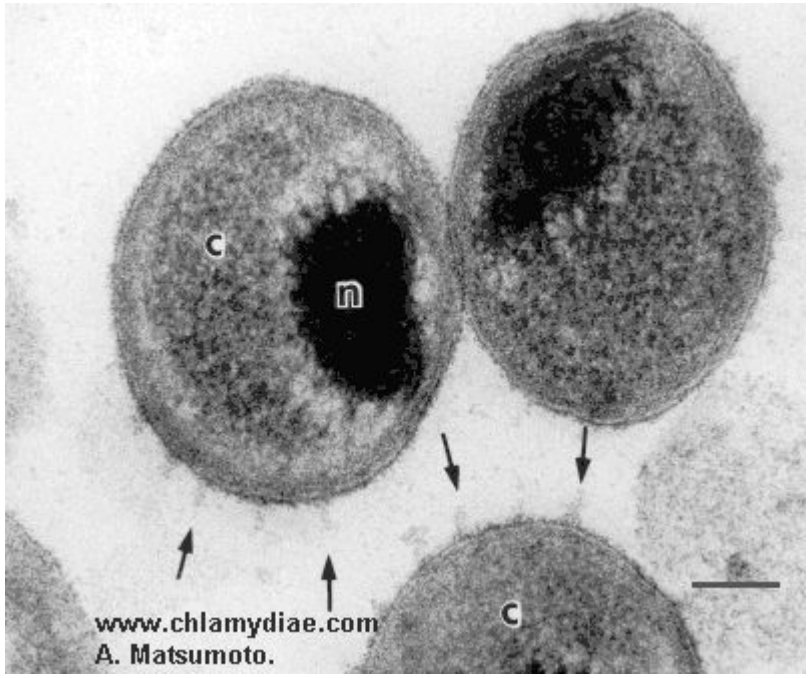




- The spirochetes - very difficult to culture
- This is due to their extreme anaerobic requirements their unique nutritional requirements (require α1-globulin)
- Over the last decade or so, some **have** been cultured and their characteristics determined
- But many remain uncultured
- Because they were so hard to grow in culture, their differentiation was based primarily on size and other morphological characteristics Three sizes were seen, giving rise to the categories: small, intermediate, and large

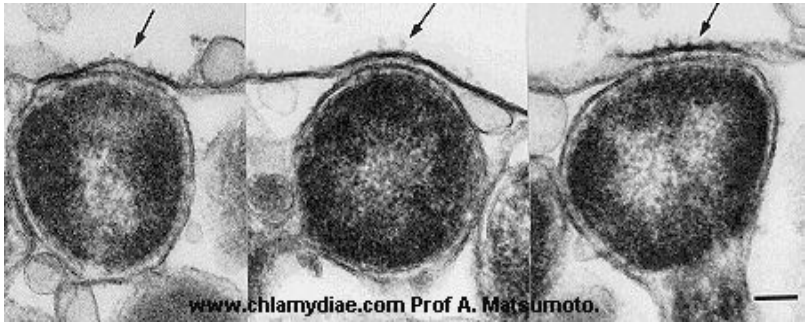
Chlamydia

- *Chlamydia* are obligate intracellular bacteria that multiply in host cells
- There are three species associated with human disease: *C. psittaci, C. trachomatis,* and *C. pneumoniae.*
- *Chlamydia* are small rounded organisms that vary in morphology during their replicative cycle.
- Chlamydiae are not culturable on synthetic media.
- The replicative cycle of *Chlamydia* involves two forms, the **elementary body** and the **reticulate body**.
- The elementary body represents the infectious form, and is resistant to environmental stresses. The elementary body is taken up by the host cells by endocytosis to form a phagosome.
- Within 8-12 hours, the elementary body reorganizes to the larger reticulate body, with division by binary fission until the entire cell is filled with the organisms.



- A transmission electron microscope picture of a thin section through an elementary body of *C. psittaci*

- *Reticulate bodies of C. Psittaci*



Mycoplasma

- Mycoplasma are bacteria that lacks cell walls.
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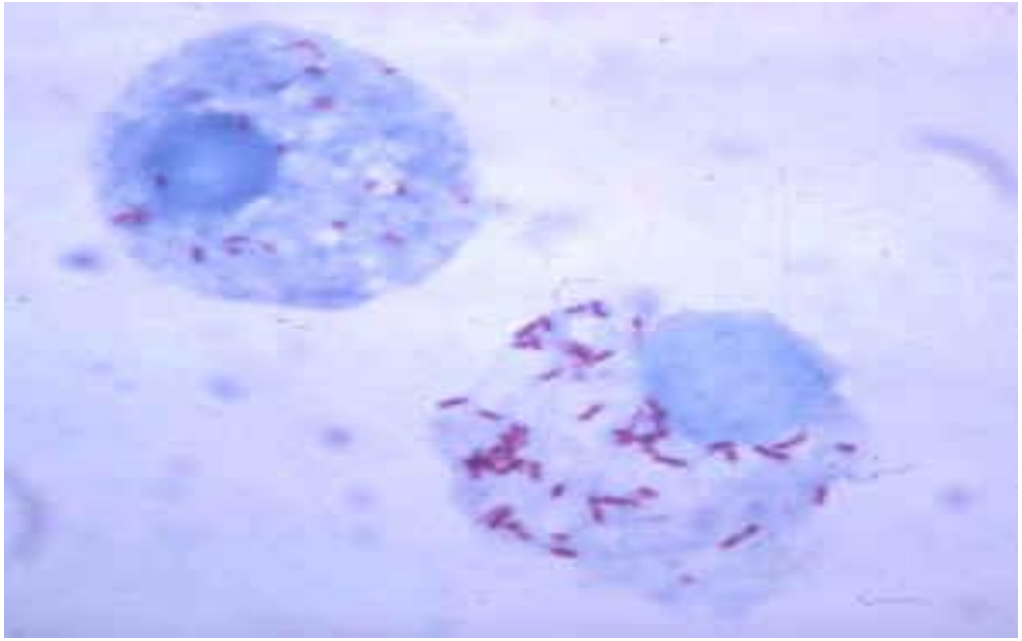
- Two human species are associated with disease: *M. pneumoniae* (pneumonia) and *M. hominus* associated with genital tract infections.
- The bacteria are very small (0.2 □M) but pleomorphic.
- They are bounded by a single triple layered membrane that contains sterols. They do not stain well with usual stains.
- Organisms can grow on enriched liquid culture medium and *Mycoplasma* agar to give tiny colonies after several days, with a denser center appearance like an inverted fried egg.

Rickettsia

- The rickettsia are bacteria which are obligate intracellular parasites.
- They are considered a separate group of bacteria because they have the common feature of being spread by arthropod vectors (lice, fleas, mites and ticks).
- The cells are extremely small (0.25 u in diameter) rod-shaped, coccoid and often pleomorphic microorganisms
- They have typical bacterial cell walls, no flagella (except for *Rickettsia prowazekii*), are gram-negative and multiply via binary fission only inside host cells.
- They occur singly, in pairs, or in strands.
- Most species are found only in the cytoplasm of host cells, but those which cause spotted fevers multiply in nuclei as well as in cytoplasm.
- In the laboratory, they may be cultivated in living tissues such as embryonated chicken eggs or vertebrate cell cultures.

- Gimenez stain of tick hemolymph cells infected with *R. rickettsii*



Fungi

- Fungi are eukaryotic organisms
- Include mushrooms, molds and yeasts
- They have no chlorophyll or other photosynthetic pigments
- Their cell walls contain a substance called chitin
- Fungal infections are **mycoses**
- Growth in two basic forms, as **yeasts** and **molds**
- They growth in synthetic mediums