Principals of Microbial physiology

Fourth Year Biology

First semester

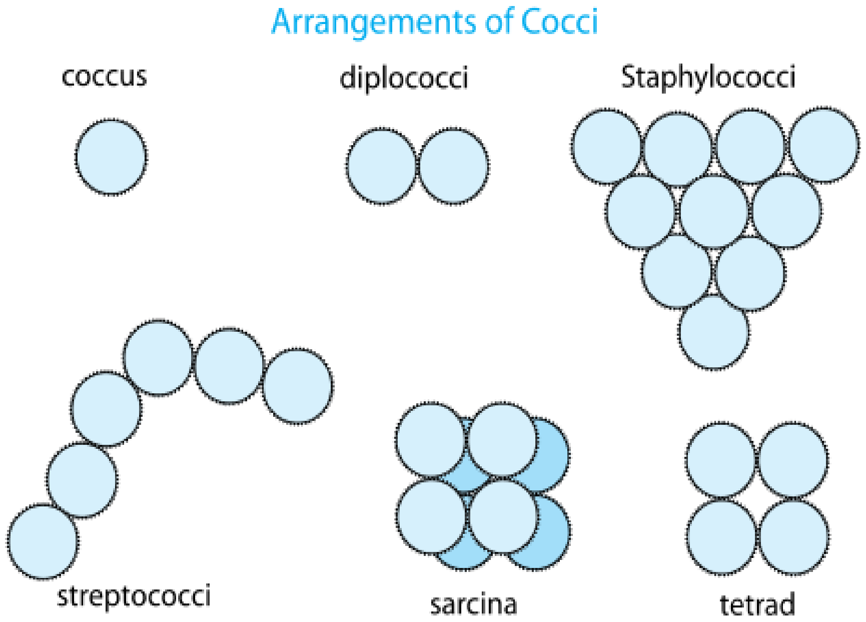
2020/2021

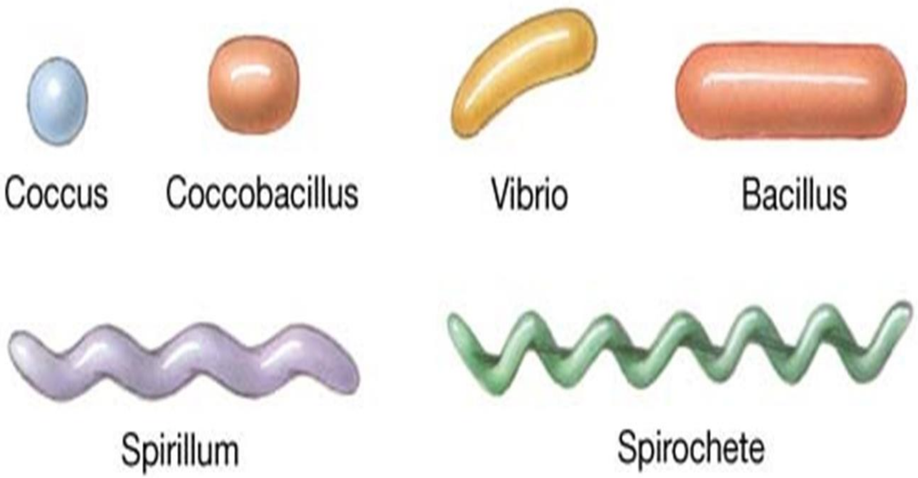
- **Microbial cell structure**
- **Bacterial cell structure**
- Bacteria, despite their simplicity, contain a well-developed cell structure which is responsible for many of their unique biological properties. Many structural features are unique to bacteria and are not found among archaea or eukaryotes. Because of the simplicity of bacteria relative to larger organisms and the ease with which they can be manipulated experimentally, the cell structure of bacteria has been well studied, revealing many biochemical principles that have been subsequently applied to other organisms.
- **Cell morphology**
- Nutrients and wastes are transported in and out the cell via the cytoplasmic membrane. The rate of transport determines the metabolic rates and therefore the growth rates of microbial cells, the smaller the size, the larger the surface area of the cytoplasmic membrane to volume and therefore the faster it's potential growth rate. Bacteria come in a wide variety of shapes, perhaps the most elemental structural property of ***bacteria*** is ***cell morphology*** (shape). Typical examples include:

Principals of Microbial physiology

- **Microbes have different shapes and is of advantage**
- Cell wall establishes the shape of a microbial cell but environmental
- conditions can change its shapes include:
  - **Spheres called cocci** (greek = berry) can divide once in one axis to produce diplococci (*Neisseria gonnorrhoeae*, *N. meningitidis*), or more than once to produce a chain (*Streptococcus pyogenes*), divides regularly in two planes at right angles to produce a regular cuboidal packet of cells or in two planes at different angles to produce a cluster of cells (*Staphyloccus aureus*)
  - **Cylinders called rods or bacilli** (Latin *bacillus* = walking stick)
  - **Spiral or spirilli** (Greek *spirillum =* little coil)
- **Shape offers an advantage to the cell:**
  - **Cocci:** more resistant to drying than rods
  - **Rods:** More surface area & easily takes in dilute, nutrients from the environment

- **Spiral:** Corkscrew motion & therefore less resistant to movement
- **Square:** Assists in dealing with extreme salinities





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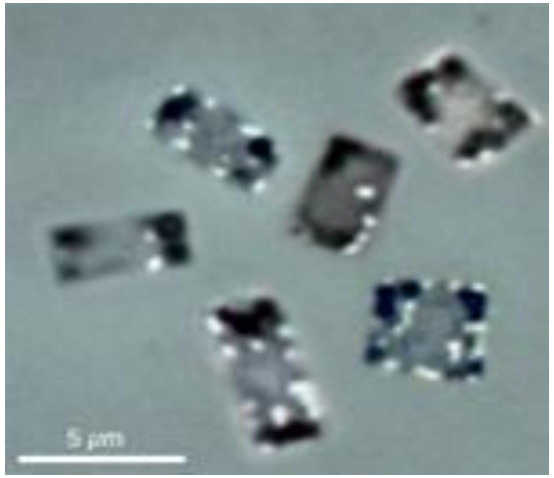
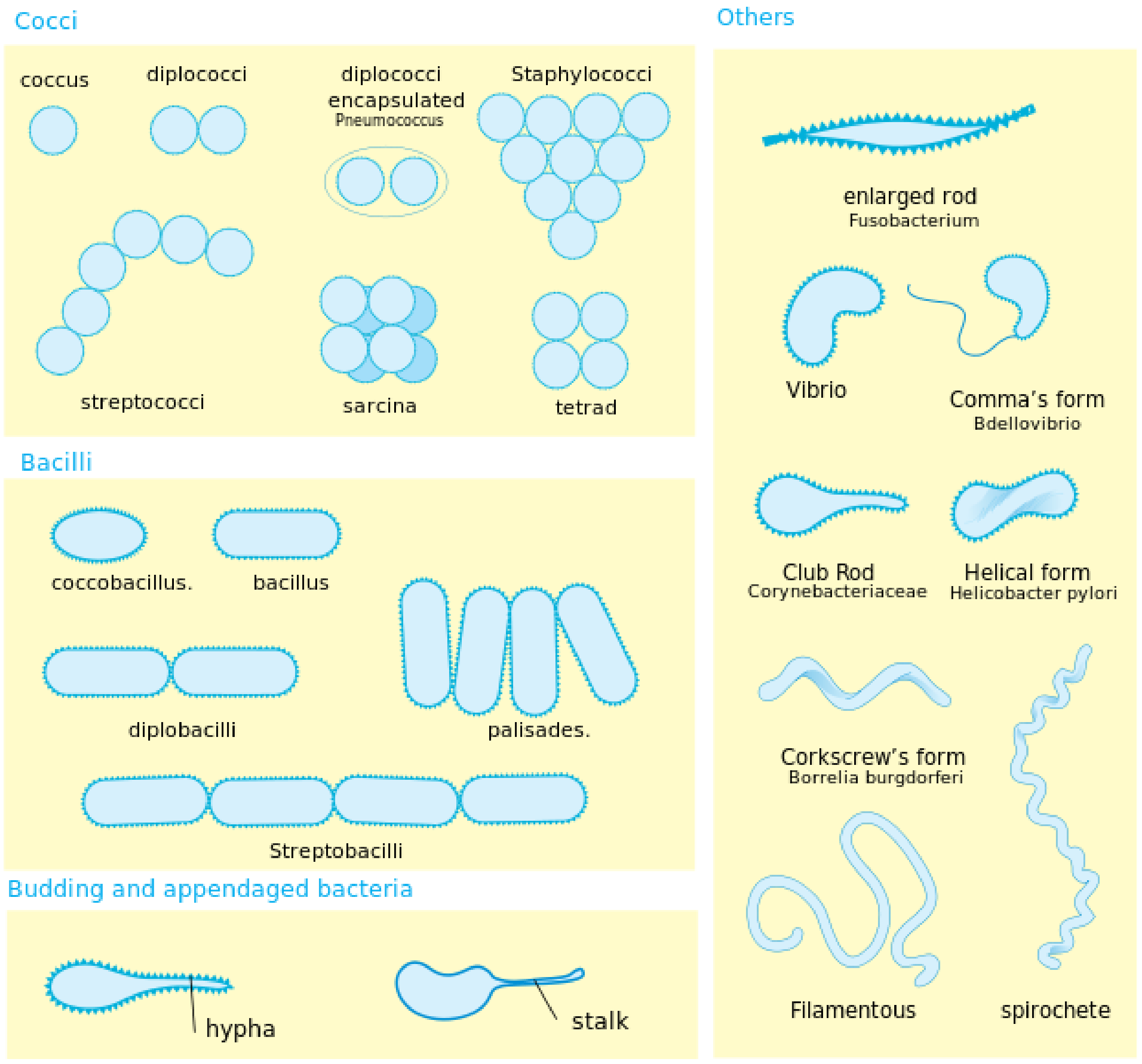


- *Haloquadratum* *wilsbyi*

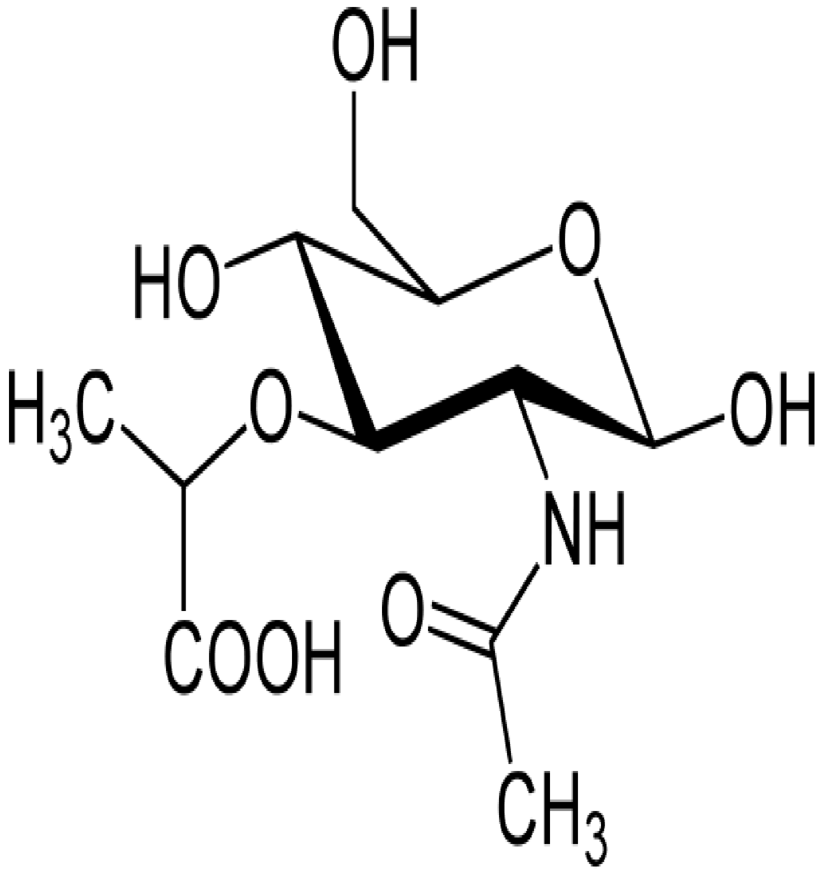

Cocci
coccus
diplococci
diplococci encapsulated
Pneumococcus
Staphylococci
streptococci
sarcina
tetrad
Bacilli
coccobacillus.
bacillus
diplobacilli
palisades.
Streptobacilli
Budding and appendaged bacteria
hypha
stalk
Others
enlarged rod
Fusobacterium
Vibrio
Comma’s form
Bdellovibrio
Club Rod
Corynebacteriaceae
Helical form
Helicobacter pylori
Corkscrew’s form
Borrelia burgdorferi
Filamentous
spirochete

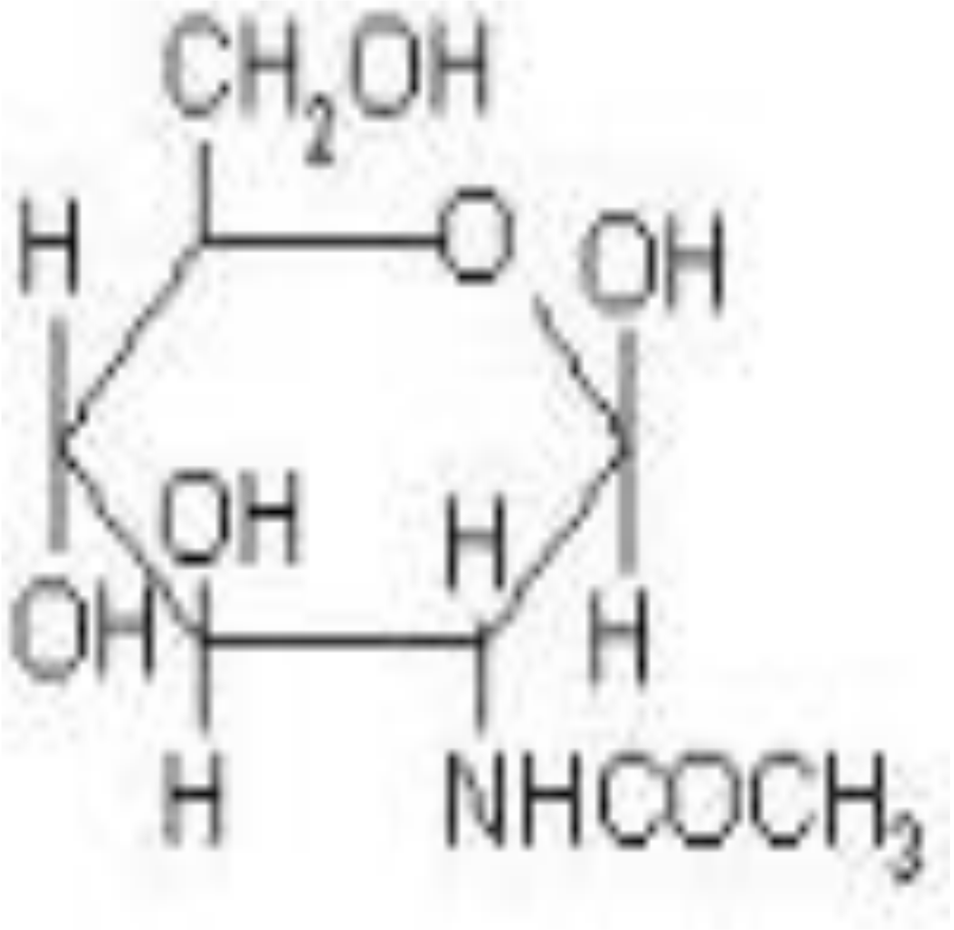
- Cell shape is generally characteristic of a given bacterial species, but can vary depending on growth conditions. Some bacteria have complex life cycles involving the production of stalks and appendages (e.g. *Caulobacter*) and some produce elaborate structures bearing reproductive spores (e.g. *Myxococcus*, *Streptomyces*). Bacteria generally form distinctive cell morphologies when examined by light microscopy and distinct colony morphologies when grown on Petri plates. These are often the first characteristics observed by a microbiologist to determine the identity of an unknown bacterial culture.

- **The importance of cell size**
- Perhaps the most obvious structural characteristic of bacteria is (with some exceptions) their small size. For example, *Escherichia coli* cells, an average sized bacterium, are about 2µm long and 0.5 µm in diameter, with a cell volume of 0.6 - 0.7 μm^3. The dry mass of a single cell can be estimated as 20 % of the wet mass. About half of the dry mass of a bacterial cell consists of carbon, and also about half of it can be attributed to proteins. Therefore, a typical fully grown 1-liter culture of *Escherichia coli* (at an optical density of 1.0, corresponding to 10^9 cells/ml) yields 1 g wet cell mass.

- **. Bacterial Cell Walls:**
- All the members of domain *Bacteria,* with the exception of the genera *Mycoplasma*, *Ureaplasma*, *Spiroplasma,* and *Anaeroplasma* contain cell walls
- Cell walls are chemically peptidoglycans ie peptides (short amino acids chains) and glycans (sugars).
  - Glycans:
    - are modified sugars N-acetyl muramic acid (NAM or M) & N-acetly glucose amine (NAG or G)
    - M and G are linked to each other by a beta 1, 4 glycosidic bond & alternate to form the wall backbone.
    - Lysozyme (an enzyme produced by organisms that consume bacteria. This enzyme digests beta 1,4 glycosidic bonds.
    - Lysozyme lyses growing or non growing cells but cell wall-less microbes are not affected
    - High osmotic pressure in high solute concentrations prevents lysis of Gram +ve & Gram -ve cells when treated with lysozyme
    - Note: sphaeroplasts = part of cell wall removed (Gram -ve)
protoplasts = complete removal of cell wall (Gram +ve)

N-acetyl muramic acid and N-acetyl glucose amine

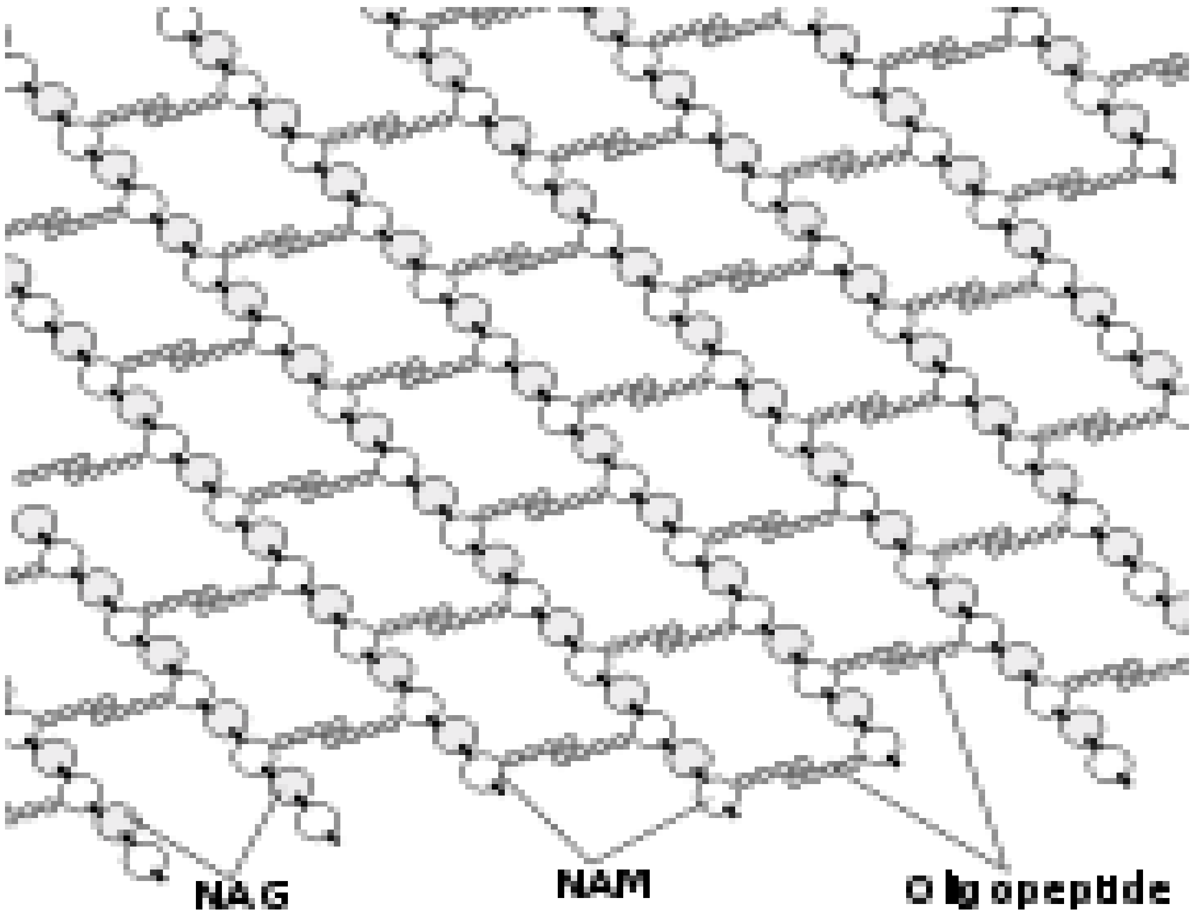




- **The structure of peptidoglygan**
- As in other organisms, the bacterial cell wall provides structural integrity to the cell. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other molecules inside the cell compared to its external environment. The bacterial cell wall differs from that of all other organisms by the presence of peptidoglycan (poly-*N*-acetylglucosamine and *N*-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. It is relatively porous and is not considered to be a permeability barrier for small substrates.

- While all bacterial cell walls (with a few exceptions e.g. extracellular parasites such as *Mycoplasma*) contain peptidoglycan, not all cell walls have the same overall structures. Since the cell wall is required for bacterial survival, but is absent in eukaryotic microorganisms, several antibiotics (penicillins and cephalosporins) stop bacterial infections by interfering with cell wall synthesis, while having no effects on human cells.
- There are two main types of bacterial cell walls, Gram positive and Gram negative, which are differentiated by their Gram staining characteristics. For both Gram-positive and Gram-negative bacteria, particles of approximately 2 nm can pass through the peptidoglycan.

Peptidoglycan structure



- **The Gram positive cell wall**
- Peptidoglycans(mucopeptides, glycopeptides, mureins) are the structural elements of almost all bacterial cell walls. They constitute almost 95% of the cell wall in some Gram positive bacteria and as little as 5-10% of the cell wall in Gram negative bacteria. Peptidoglycans are made up of a polysaccharide backbone consisting of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in equal amounts. The cell wall of some Gram positive bacteria is completely dissolved by lysozyme, as this enzyme attacks the bonds between GA and MA. In other Gram positive bacteria, e.g. *Staphylococcus aureus*, the walls are resistant to the action of lysozyme. They have acetyl groups on carbon-6 of some MA residues.

- The matrix substances in the walls of Gram positive bacteria may be polysaccharides or teichoic acids. The latter are very widespread, but have been found only in Gram positive bacteria. Teichoic acids are usually, but not always, substituted with alanine ester residues, and Teichoic acids also assist in regulation of cell growth by limiting the ability of autolysins to break the β)1-4 bond between *N*-acetyl glucosamine and the *N*-acetylmuramic acid There are two main types of teichoic acid: ribitol teichoic acids and glycerol teichoic acids. The latter one is more widespread. These acids are polymers of ribitol phosphate and glycerol phosphate, respectively, and only one type is found in the wall of any particular strain of bacteria. Teichoic acids form receptor sites for bacteriophages, and at least some of them are located on the surface of gram positive bacteria.

- **The Gram negative cell wall**
- Unlike the Gram positive cell wall, the Gram negative cell wall contains a thin peptidoglycan layer adjacent to the cytoplasmic membrane. This is responsible for the cell wall's inability to retain the crystal violet stain upon decolourisation with ethanol during Gram staining. In addition to the peptidoglycan layer, the Gram negative cell wall also contains an outer membrane composed by phospholipids and lipopolysaccharides, which face into the external environment. As the lipopolysaccharides are highly-charged, the Gram negative cell wall has an overall negative charge. The chemical structure of the outer membrane lipopolysaccharides is often unique to specific bacterial strains (i.e. sub-species) and is responsible for many of the antigenic properties of these strains.

- Differences Between Gram-positive And Gram-negative Bacterial Cell Walls

	Gram-positive	Gram negative
Peptidoglycan:	Thick layer	Thin layer
Peptidoglycan tetrapeptide: Contain:	lysine	Diaminopimelate
Peptidoglycan cross linkage:	Via pentapeptide	Direct bonding
Teichoic acid:	Present	Absent
Teichuronic acid	Present	Absent
Lipoproteins:	Absent	Present
LPS:	Absent	Present
Outer Membrane:	Absent	Present
Periplasmic Space:	Absent	Present

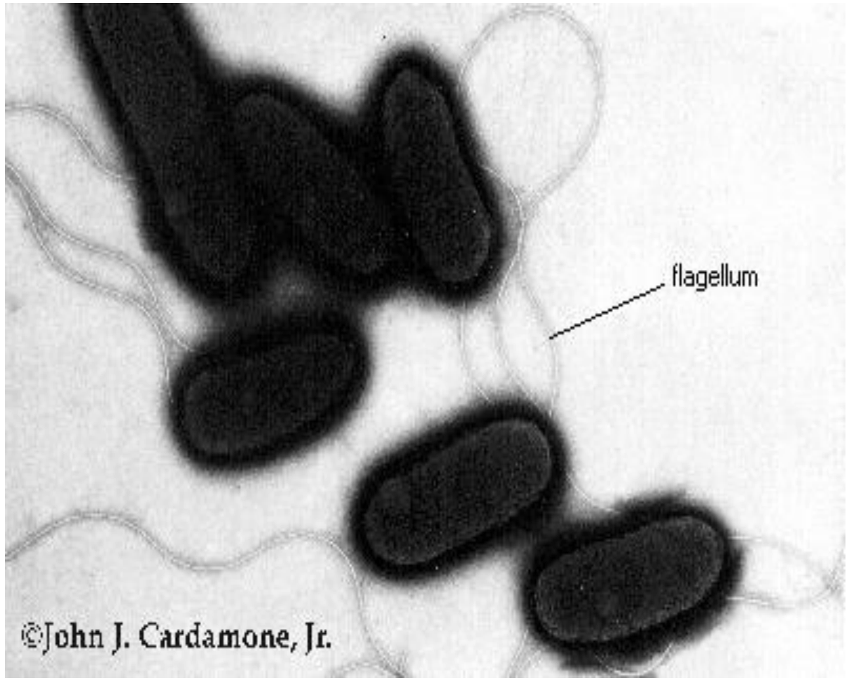
- **Other bacterial surface structures**
- ***Fimbrae and Pili***
- Fimbrae are protein tubes that extend out from the outer membrane in many members of the bacteria. They are generally short in length and present in high numbers about the entire bacterial cell surface. Fimbrae usually function to facilitate the attachment of a bacterium to a surface (e.g. to form a biofilm) or to other cells (e.g. animal cells during pathogenesis)). A few organisms (e.g. *Myxococcus*) use fimbrae for motility to facilitate the assembly of multicellular structures such as fruiting bodies. Pili are similar in structure to fimbrae but are much longer and present on the bacterial cell in low numbers. Pili are involved in the process of bacterial conjugation. Non-sex pili also aid bacteria in gripping surfaces.

- **S-layers**
- An S-layer (surface layer) is a cell surface protein layer found in many different bacteria and in some archaea, where it serves as the cell wall. All S-layers are made up of a two-dimensional array of proteins and have a crystalline appearance, the symmetry of which differs between species. The exact function of S-layers is unknown, but it has been suggested that they act as a partial permeability barrier for large substrates. For example, an S-layer could conceivably keep extracellular proteins near the cell membrane by preventing their diffusion away from the cell. In some pathogenic species, an S-layer may help to facilitate survival within the host by conferring protection against host defense mechanisms.

- **Capsules and Slime Layer (Glycocalayx)**
- Many bacteria secrete extracellular polymers outside of their cell walls. These polymers are usually composed of polysaccharides and sometimes containing protein (glycoprotein). Capsules are relatively impermeable structures that cannot be stained with dyes such as India ink. They are structures that help protect bacteria from phagocytosis and desiccation. Slime layer is involved in attachment of bacteria to other cells or inanimate surfaces to form biofilms. Slime layers can also be used as a food reserve for the cell and depot for waste products. Waste products of metabolism are excreted from the cell, and will accumulate in the capsule. This binds them up, and prevents the waste from interfering with cell metabolism.

- **Bacterial Flagella**
- **A. Structure and Composition**
- A bacterial flagellum has 3 basic parts: a filament, a hook, and a basal body.
- 1) The **filament** is the rigid, helical structure that extends from the cell surface. It is composed of the protein **flagellin** arranged in helical chains so as to form a hollow core. During synthesis of the flagellar filament, flagellin molecules of 42KD molecular weight coming off from the ribosomes, are transported through the hollow core of the filament where they attach to the growing tip of the filament causing it to lengthen .
- 2) The **hook** is a **flexible coupling between the filament and the basal body**.
- 3- The **basal body** consists of a rod and a series of rings that anchor the flagellum to the cell wall and the cytoplasmic membrane .

- Unlike eukaryotic flagella, the bacterial flagellum has no internal fibrils and does not flex. Instead, the basal body acts as a rotary molecular motor, enabling the flagellum to rotate and propel the bacterium through the surrounding fluid. In fact, the flagellar motor rotates very rapidly, (300RPS).
- The Mot proteins surround the MS and C rings of the motor and function to generate torque for rotation of the flagellum. Energy for rotation comes from the proton motive force provided by protons moving through the Mot proteins.
- Fli proteins act as the motor switch to trigger either clockwise or counterclockwise rotation of the flagellum and to possibly disengage the rod in order to stop motility


flagellum
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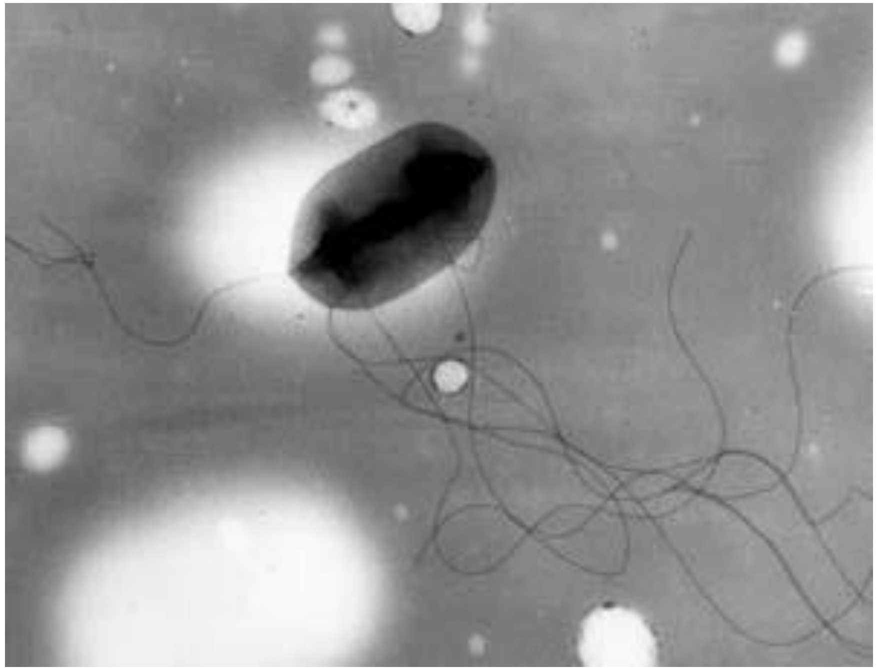
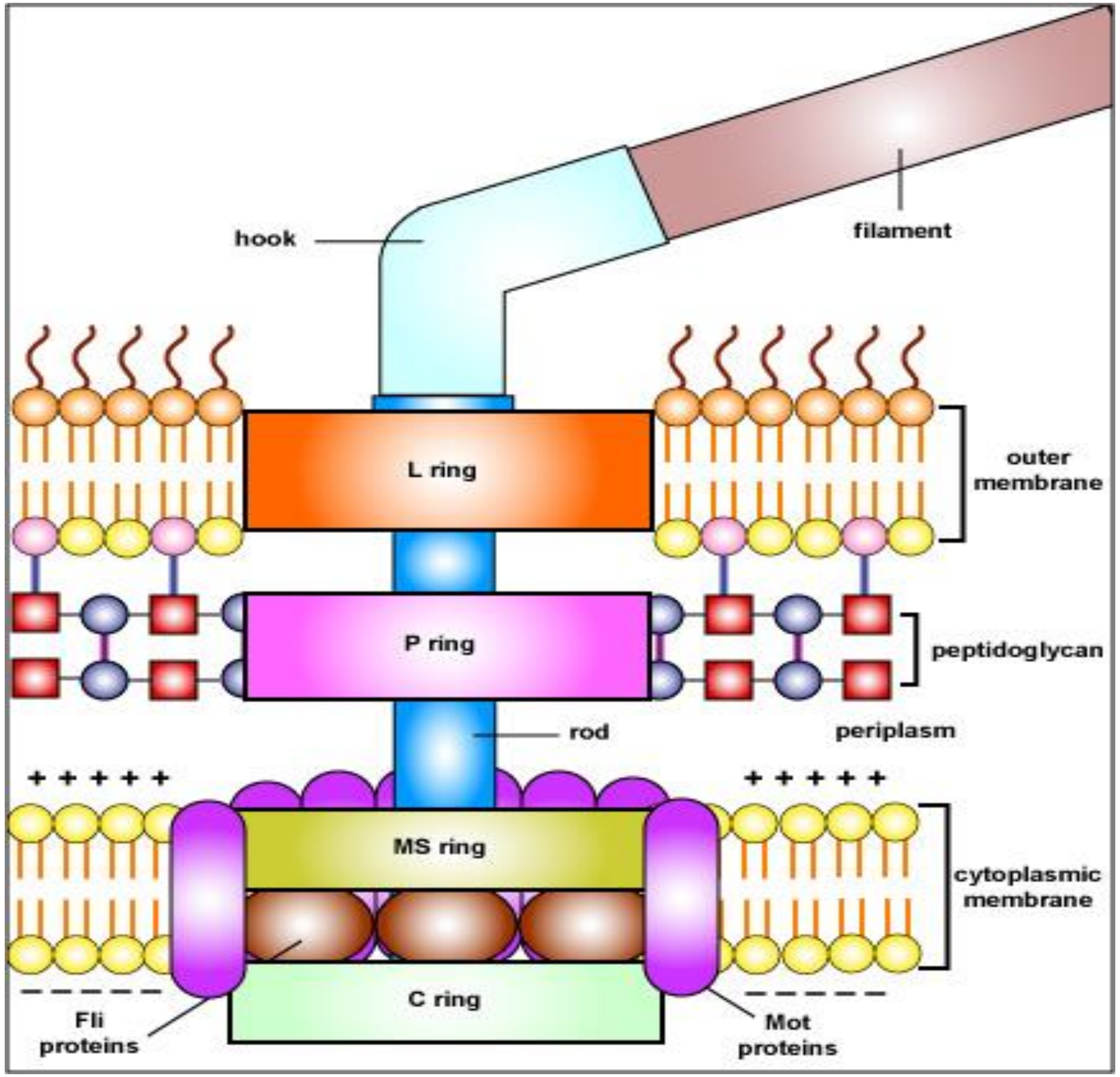
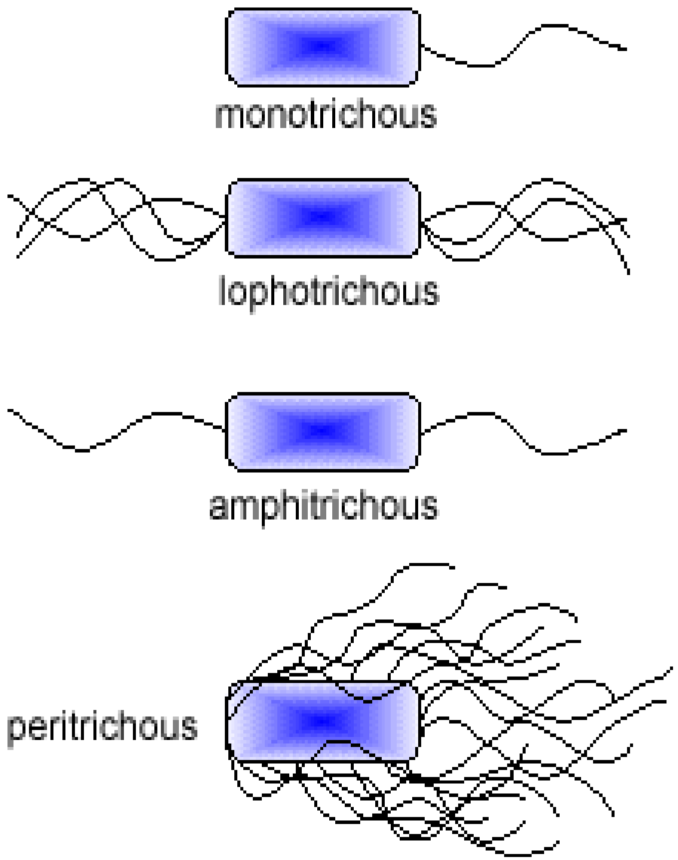


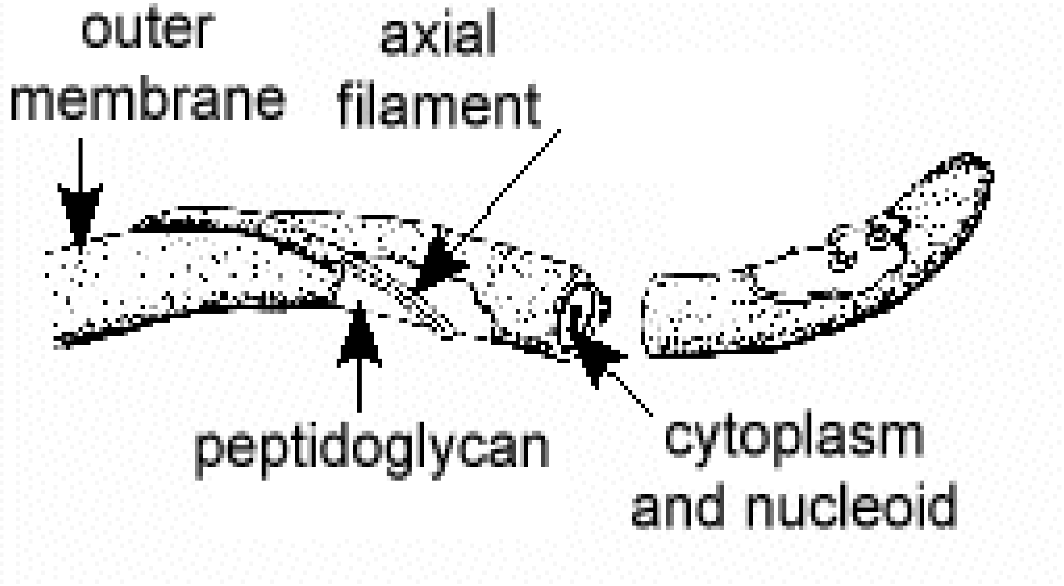
Bacterial Flagellum Ultrastructure

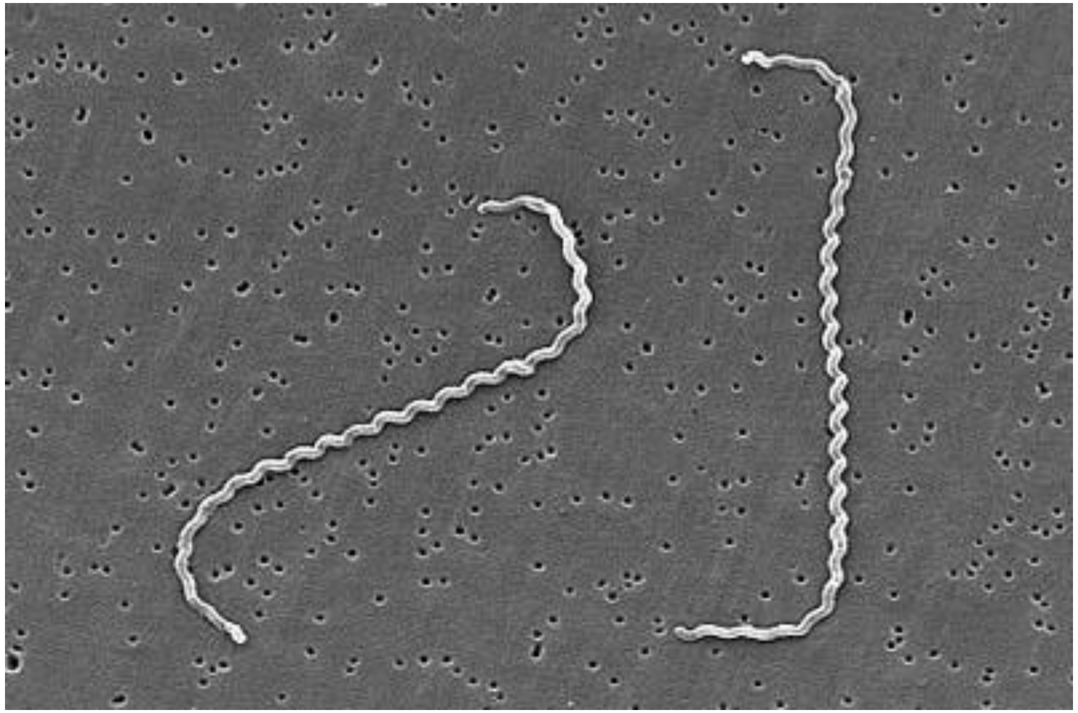


- **B. Flagellar Arrangements**
- 1. monotrichous: a single flagellum, usually
- at one pole Eg. flagellum of *vibrio*
- 2. amphitrichous: a single flagellum at both
- ends of the organism.
- Eg. *Rhodospirilum*
- 3. lophotrichous: two or more flagella at one
- or both poles. Eg. *Helicobacter*
- 4. peritrichous: flagella over the entire surface.
- *Eg. Proteus vulgaris*



- 5. **Axial filaments**: internal flagella found only in the spirochetes. Axial filaments are composed from two to over a hundred axial fibrils (or endoflagella) that extend from both ends of the bacterium between the outer membrane and the cell wall, often overlapping in the center of the cell. A popular theory as to the mechanism behind spirochete motility presumes that as the endoflagella rotate in the periplasmic space between the outer membrane and the cell wall, this could cause the corkscrew-shaped outer membrane of the spirochete to rotate and propel the bacterium through the surrounding fluid.


outer membrane
axial filament
peptidoglycan
cytoplasm and nucleoid



- **C. Functions**
- Flagella are the organelles of locomotion for most of the bacteria that are capable of motility. Two proteins in the flagellar motor, called MotA and MotB, form a proton channel through the cytoplasmic membrane and **rotation of the flagellum is driven by a proton gradient**. This driving proton motive force occurs as protons accumulating in the space between the cytoplasmic membrane and the cell wall as a result of the electron transport system travel through the channel back into the bacterium's cytoplasm. Most bacterial flagella can rotate both counterclockwise and clockwise and this rotation contributes to the bacterium's ability to change direction as it swims. A protein switch in the molecular motor of the basal body controls the direction of rotation.

- **A bacterium with peritrichous flagella:**
- If a bacterium has a peritrichous arrangement of flagella, counterclockwise rotation of the flagella causes them to form a single bundle that propels the bacterium in long, straight or curved runs without a change in direction. During a run, that lasts about one second, the bacterium moves 10 - 20 times its length before it stops. When the flagella rotate clockwise, the flagella are pushed apart from one another causing a tumbling motion. A tumble only lasts about one-tenth of a second and no real forward progress is made. After a “tumble”, the direction of the next bacterial run is random because every time the bacterium stops swimming, browinian motion and fluid currents cause the bacterium to reorient in a new direction.

- **A bacterium with polar flagella:**
- Most bacteria with polar flagella, like the peritrichous above, **can rotate their flagella both clockwise and counterclockwise**. **If the flagellum is rotating counterclockwise, it pushes the bacterium forward**. **When it rotates clockwise, it pulls the bacterium backward**. These bacteria change direction by changing the rotation of their flagella. Some bacteria with polar flagella **can only rotate their flagellum clockwise.** In this case, **clockwise rotation pushes the bacterium forward**.

- **D. Taxis**
- Around half of all known bacteria are motile. **Motility serves to keep bacteria in an optimum environment via taxis** . **Taxis is a motile response to an environmental stimulus**. Bacteria can respond to chemicals (chemotaxis), light (phototaxis), osmotic pressure (osmotaxis), oxygen (aerotaxis), and temperature (thermotaxis).