Cell Wall

- The cell wall is a ***rigid structure*** that ***surrounds*** the bacterial cell just outside of the plasma membrane

Functions to:

- gives the bacterium its ***shape***

- gives ***mechanical strength*** to the cell and ***protects*** it from exploding due to osmotic lysis

(*shape and strength due to the peptidoglycan*)

(Most bacteria have a strong walls except for mycoplasma and Archaea)

- *Many bacteria have cell walls that **contain compounds** that contribute to the **virulence of that organism***

- *The bacterial cell wall is also the **site of action of several antibiotics**, most notably the beta-lactamase family which includes penicillin.*
  - *Penicillin prevent cell wall synthesis*

- The substrate for a number of degradive enzymes such as lysozyme, [which is contained in human saliva and lacrimal secretions (tears)]
  - These ***compounds attack the cell wall and cause the bacteria to lyse.***

Cell Wall Composition

The cell wall of a ***eubacteria*** is composed of a unique polymer called ***peptidoglycan,*** that is not found in the ***archaeobacteria*** nor ***the eukaryotes.***

Peptidoglycan or Murein

Petidoglycan is a linear polymer of alternating subunits of *N-acetyl glucosamine (NAG)* and *N-acetyl muramic acid (NAM*) - (Backbone)

- a side chain of four amino acids (tetrapeptide) is attached to each NAM subunit.

- The side chains of linked peptidoglycan units are joined together through a peptide interbridge that forms covalent bonds between the tetrapeptide side chains

- ***Provides much of the strength and rigidity possessed by bacterial cell walls.***

- Most gram negative organisms do not utilize a peptide interbridge, they will simply form covalent bonds between specific residues of adjacent tetrapeptide side chains.

- The crosslinked peptidoglycan units form an enormous polymer that is analogous to chain mail armour, and completely enclosed the cell

- lysozyme degrades the cell wall by breaking the beta 1-4 bonds that connect the NAM and NAG subunits (G-M-X-G-M)
  penicillin breaks the tetrapeptide bridge extending from the NAM residues.

Gram Positive Cell Wall

- A gram positive cell wall is a single layer that is 20-80 nm thick and makes up 50-90% of the dry weight of the cell

- Primarily composed of peptidoglycan containing a peptide interbridge

- It does not possess an outer membrane

- It usually contains large amounts of negatively charged teichoic acids, which helps to give the bacteria its negative charge, and may be important in maintaining the strength of the wall.

- ***Teichoic acids*** are polymers of glycerol and ribitol joined by phosphate groups.
  - Teichoic acids are not found in gram negative bacteria.
  - Teichoic acids are connected to either the peptiodglycan itself or to plasma membrane lipids (lipoteichoic acids)
  - Teichoic acids are not present in gram negative bacteria.

Gram Negative Cell Wall

More Complex than the gram positive cell wall.

- The Gram Negative cell wall has a peptidoglycan layer that is about 2-3 nm thick (thin compared to the gram positive) and makes up about 5-20% of the dry weight of the cell.

- There is an outer membrane than lies outside of the thin peptidoglycan layer.

- ***Braun's Lipoprotein:***
  - Most abundant membrane protein
  - Covalently joined to the peptidoglycan and embedded in the the outer membrane.

- The peptidogylcan and the outer membrane are firmly linked by this protein.

- ***Adhesion Site:***
  - Strengthens the wall and holds the outer membrane in place
  - ******Regions of direct contact or possibly true membrane fusions.
  - ******Proposed that substances can move into the cell through these adhesion sites rather than through the periplasm.
  -

Peptidoglycan Layer:

The peptidoglycan layer is covered by an outer membrane that contains various proteins as well as lipopolysaccharides (LPS)

Lipopolysaccharides (LPS) is a large complex molecule containing both lipids and carbohydrates.

LPS consists of three parts:
(1) Lipid A
(2) Core polysaccharide
(3) O-side chain

Lipid A

- consists of two glucosamine sugar derivitives each attached to three fatty acids and phosphate

- The lipid A residue is buried within the outer membrane and the remaining components are projected from the cell surface

Core Polysaccharide

- The core polysaccharide consists of 10 sugar residues, most of which have an unusual structure, and are attached to the lipid A moiety.

O-Side Chain

- The LPS structure ends with a terminal O side chain. The O antigen is a short polysaccharide chain that varies in composition and generally contains a number of unusual sugar residues

LPS Importance

- The LPS is negatively charged an contributes to the overall negative charge of the cell (like the teichoic acids of Gm+ cells)

- More importantly, the Lipid A molecule is toxic and serves as a super-antigen to induce an inflammitory immune response (endotoxin).

- (Teichoic acids are the major antigens of Gm+ cells, LPS is the major antigen of GM- cells)

Outer Membrane Function:

- The outer membrane functions as a permeability barrier that protects the cell from a number of toxic agents, including some dyes and several antibiotics

- Outer membrane is more permeable than the plasma membrane and permits the passage of small molecules like glucose, due to porins.

- The outer membrane contains a number of protein channels called ***porins*** that make the lipid bilayer relatively permeable to small molecules. Three porins molecules band together, span the membrane to form narrow channels through which smaller molecules can pass.

Periplasmic Space

The periplasmic space refers to a gap that exists between the cell membrane and the outer membrane that is clearly visible in electron micrographs of ***gram negative cells****. It is rarely seen in GM positive cells and the gap is smaller.*

Recent evidence suggest that the PS may be filled with a loose network of peptidogylcan.

The ***periplasm*** is a gel like substance that fills the *periplasmic space* in GM -- bacteria, and is contained in the cell wall layer of GM+ bacteria.

- GM + cells may have periplasm, even it lack an obvious PS

The periplasm contains numerous digestive enzymes, transport proteins, osmoprotectants, etc.

ASM NEWS 61;125-130 (1995)

- ***In Gram Negative Bacteria***
  - contain many proteins that participate in nutrition acquisition.
  - enzymes involved in peptidoglycan synthesis

- the modification of toxic compounds harmful to the cell

- ***Gram Positive Bacteria***
  - appear not to have as many proteins, rather secrete ***exoenzymes***: enzymes that ordinarily would be periplasmic in gram negative bacteria.

- ***Archae***
  - may be gram + or gram –
  - cell walls are distinctive in structure & chemical composition
  - walls lack peptidoglycan and are composed of proteins, glyocoproteins, or polysaccharide.

Capsule

A *capsule* is a layer of mucoid material that surrounds the bacterial cell, lying outside the cell call.

- When this ***mucoid layer is thick, well organized, and difficult to remove***, it is called a ***capsule***

- If it is ***thin, diffuse, and easy to wash off***, it is called a ***slime layer***

Capsules can easily be seen with a light microscope using a capsule stain.

- Most capsules are composed of a network of polysaccharide fibers that have been secreted from the cell, called the ***glycocalyx.***

The amount of capsule produced by a cell depends on the culture conditions. Growth in high carbon, low nitrogen medium promote capsule formation.

The general ***Function of a Capsule or slime layer*** is to:

- resist desiccation, they contain a great deal of water
- to help the bacterium adhere to surfaces
- prevent or inhibit phagocytosis by a host phagocyte
  - Streptococcus pneumoniae:
    When it lacks a capsule, it is destroyed easily and does not cause disease.
    When capsulated, quickly kills mice.
- Gliding bacteria often produce slime which aids in their motility.

S-Layers

- Common in Archae, where they may be the only wall structure outside the plasma membrane.

- Most bacteria possess a surface layer of a crystalline protein arrayed in a two dimensional matrix.
    - The composition of this paracrystalline surface layer (or S-layer) is widely varied.
    - Composed of protein and glycoprotein

- In gram negative bacteria: adheres directly to the outer membrane
- In gram positive bacteria: associated with the peptidoglycan surface.

Functions:

- May protect cell against ion and pH fluctuations, osmotic stress, enzymes, or predacious bacterium (Bdellovibrio)
- It is speculated to serve as a ***permeability barrier*** to large molecular weight compounds
- May also ***confer some type of protection against host defense mechanisms to many pathogenic bacteria.***
- *Helps to maintain the shape and envelope rigidity of some bacterial cells.*

Can promote cell adhesion to surface.

Fimbrae

Fimbrae, are short, fine hairlike structures that are thinner than flagella and not involved in motility.

- Used by bacteria to attach to surfaces.

- Smaller and more numerous than flagella and can only be seen with the electron microscope.

- Only found in the ***Gram Negative bacteria***.

A ***fimbra*** is a:

- straight cylinder with an outer diameter of about 7 nm and is composed of protein subunits called pilin.
- Each fimbrae contains about 1000 pilin subunits.

- At the tip of each fimbrae is a protein called an ***adhesion,*** which recognizes and binds to specific receptors on a "host cell surface".

- This is one of the factors that contributes to the ability of a bacterium to cause an infection.

The primary function of a fimbrae ***is to facilitate attachment***.

- Can attach to receptors on a host cell, which is required for invasion.

- They can also attach to liquid-solid or liquid-gas surfaces and take advantage of nutrient pools.

- They can also attach to other bacterial cells in order to promote gene transfer by a process referred to as conjugation.

Sex pili

- The structures that mediate conjugation are called ***sex pili*** and differ from the standard fimbrae in that they are ***genetically encoded by sex factors or conjugative plasmids.***

Pili are also longer than fimbrae and much less abundant, only one or two pili are found on the cell surface.

Flagellum (Flagella)

- A slender, rigid rod.

- Flagella are the organelles of motility

Not all bacteria are motile; ***Nonmotile bacteria, especially cocci, do not possess flagella.***

Consequently, ***motility is a stable property of a species that can aid in its identification***

We can also use the number and patterns of flagellar attachment as an identifying characteristic:

- Cells with ***one single***, polar flagellum are referred to as ***Monotrichous***
  (mono means one and trichous means hair)
  Polar flagellum – located at the end

- If a cell has single flagellum at both ends it is called ***Amphitrichous***
  (amphi means on both sides)

- Cells with a tuft of flagella at one or both ends are referred to as ***Lophotrichous***
  (lopho means tuft-cluster)

- Cells with flagella all over the surface of the cell are called ***Peritrichous***
  (peri means around)

Flagella cannot be seen with the light microscope unless a specialize staining procedure is used.

- These protocols use a mordant to increase the diameter of the flagellum, and then the mordant/flagellum complex is stained.

- Flagella can be easily seen with the electron microscope.

A ***flagellum*** is composed of ***three parts***:
(1) Filament
(2) Hook
(3) Basal body

Filament

- The filament is a rigid, helical rod, about 14 nm in diameter, that extends from the surface of the cell to the tip of the flagellum and acts like a propeller.

- The filament is composed of multiple protein subunits called flagellin

Hook

- The hook structure is essentially a flexible universal joint that connects the filament to the basal body

Basal Body

- The basal body is essentially a rod that spans the cell wall and passes through a series of rings. The basal body anchors the flagellum within the cell membrane and serves as a motor to rotate the filament.

- A series of proteins (mot) attached to the inner ring effect the rotation, and the fli proteins serve as a molecular switch, changing the direction of rotation in response to environmental signals.

- The energy used to drive the flagellar rotation comes from the proton motive force. As a proton enters the cell through the mot complex, its energy is coupled to movement.

- In order to achieve a single rotation, 1000 protons must be translocated.

- The speed of rotation is directly proportional to the proton motive force.

Flagellar rotation can be extraordinarily fast. E. coli can rotate its flagellum as fast as 270 rotations per second and the marine bacterium *Vibrio alginolyticus* can achieve rotations up to 1700 revolutions per second.

Chemotaxis

- ***Movement toward chemical attractants and away from repellents, an advantage to the bacteria.***

- ***Bacteria do not always swim aimlessly but are attracted by sugars and amino acids and are repelled by harmful substances and baterial waste products.***

- ***They also respond to other environmental cues such as temperature, light and gravity.***

Chemoreceptors

- *Attractants and repellants are detected by* ***chemorecptors: special proteins tha bind chemicals and transmit signals to the other components of the chemosensing system.***

- *20 attractants chemoreceptors and 10 chemoreceptors for repellents*

- *These chemorecptorm proteins may be located in the perisplamic space or the plasma membrane.*

- *Some receptors are involved with the initial stages of sugar transport into the cell.*

Basic Travel of bacteria:

Bacteria travel in a straight or slightly curved line called ***a run****, for a few seconds*
*Then, it will stop and t****umble*** *or* ***twiddle*** *about*
The tumble is followed by a run in a different direction.
Read pages 67 and 69
Figure 3.39

Endospore

- A special resistant, dormant structure formed by gram positive bacteria.

- Develop within vegetative bacterial cells of several general
  Examples: Bacillus, Clostridium (both rods) and Sporosarcina (cocci)

- Extraordinarily resistant to environmental stresses:
  - Heat
  - Ultraviolet radiation
  - Gamma radiation

  - Chemical disinfectants
  - Desiccation
- Some have remained viable for 100,000 years
- Actinomycetes: recovered after 7,500 years.

The autoclave is important in microbiology because endospores often survive boiling for an hour or more.

Can be examined with light and electron microscopes.

The position of the spore in the mother cells ***Sporangium*** is often used for identification purposes, because of the differences in different species.

(1) Central Center
(2) Subterminal close to one end
(3) Terminal At the end
(4) Terminal with swollen sporangium

Endospore Structure:

- ***Exosporium:*** *thin delicate covering surrounding the spore*

- ***Spore Coat:*** - *lies beneath the exosporium*
  - *composed of several protein layers*
  - *may be fairly thick*
  - *impermeable and responsible for the spore's resistance to chemicals.*
- ***Cortex:*** - *may occupy half of the spore volume, rests beneath the spoore Coat.*
  - *made of peptiodgycan that is less cross-linked than the vegetative cells.*
- ***Spore Cell Wall (Core Wall)***
  - *inside the cortex and surrounds the protoplast* ***or core***
  - *the core has the normal cell structures such as the nucleiod, but is metabolically inactive.*

Read page 68,69, 70