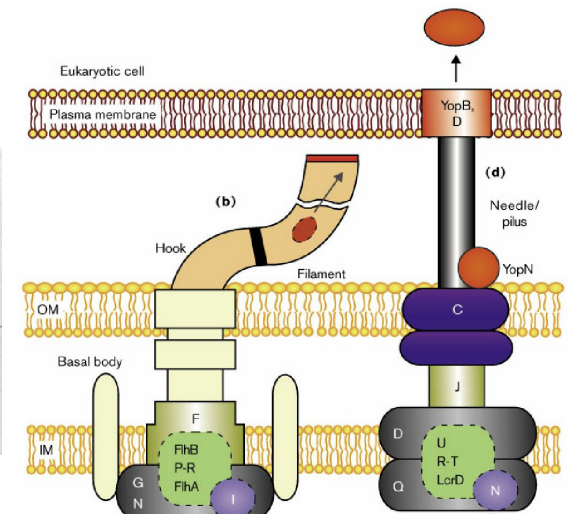
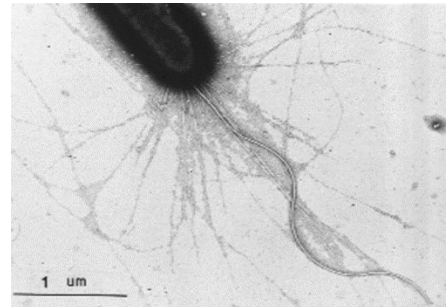
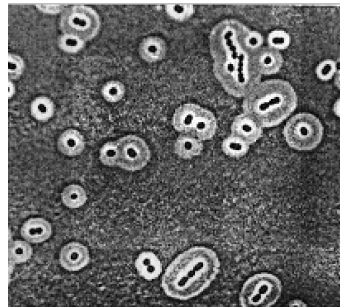
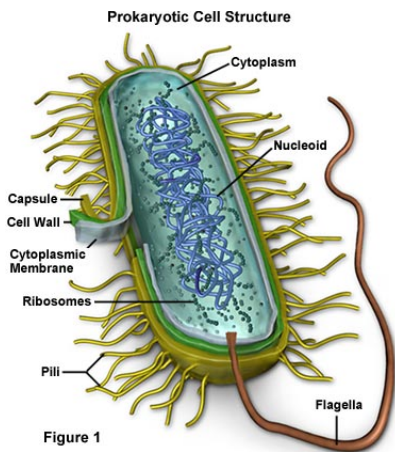
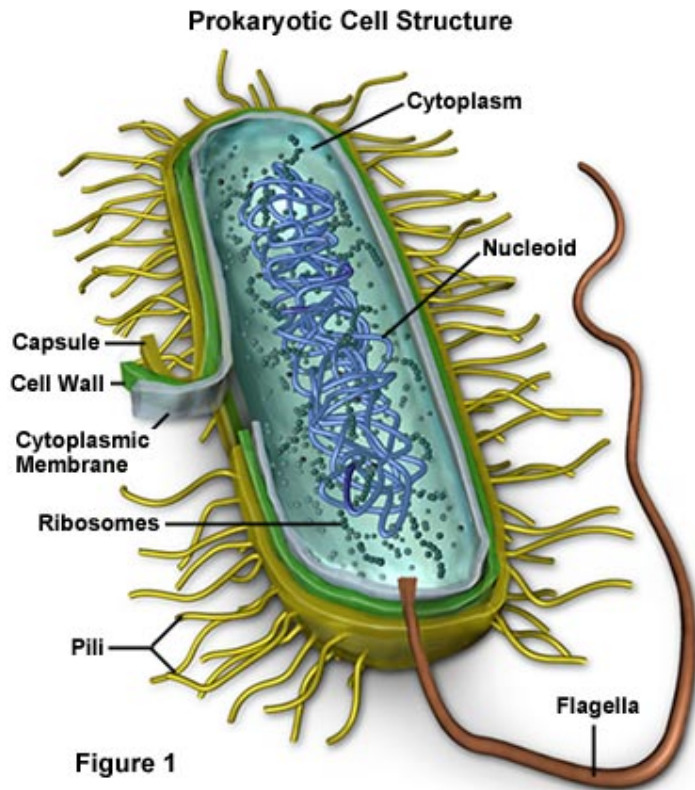


# Bacterial physiology: envelopes & beyond

- Bacterial envelopes **GP** & **GN**
- Specialized surface structures: flagella, pili, capsule
- Protein export systems



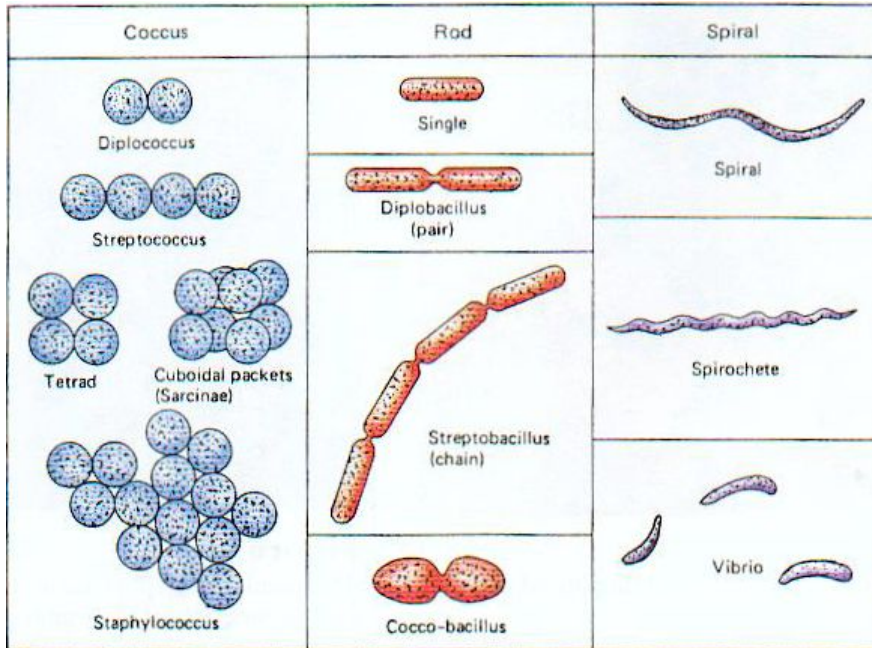
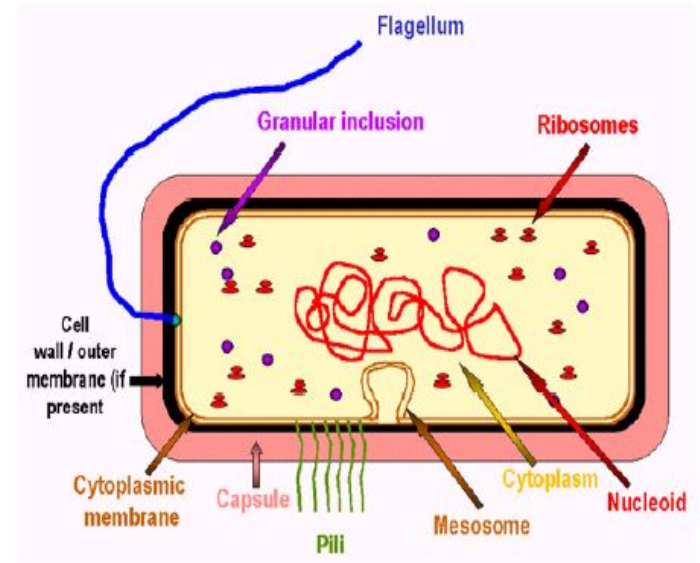
# Bacteria have complex envelopes and surface structures



- Essential for viability
- Composed of unique components
- Target for antibiotics
- Protects against environmental stresses
  - Bile salts, low pH, low osmotic pressure
- Ligands for adherence
- Resist phagocytosis
- Trigger innate immune response, sepsis
- Antigenic variation between bacteria

# Essential structures

cell wall  
cell membrane  
Cytoplasm  
nuclear material



## Particular structures

capsule

flagella

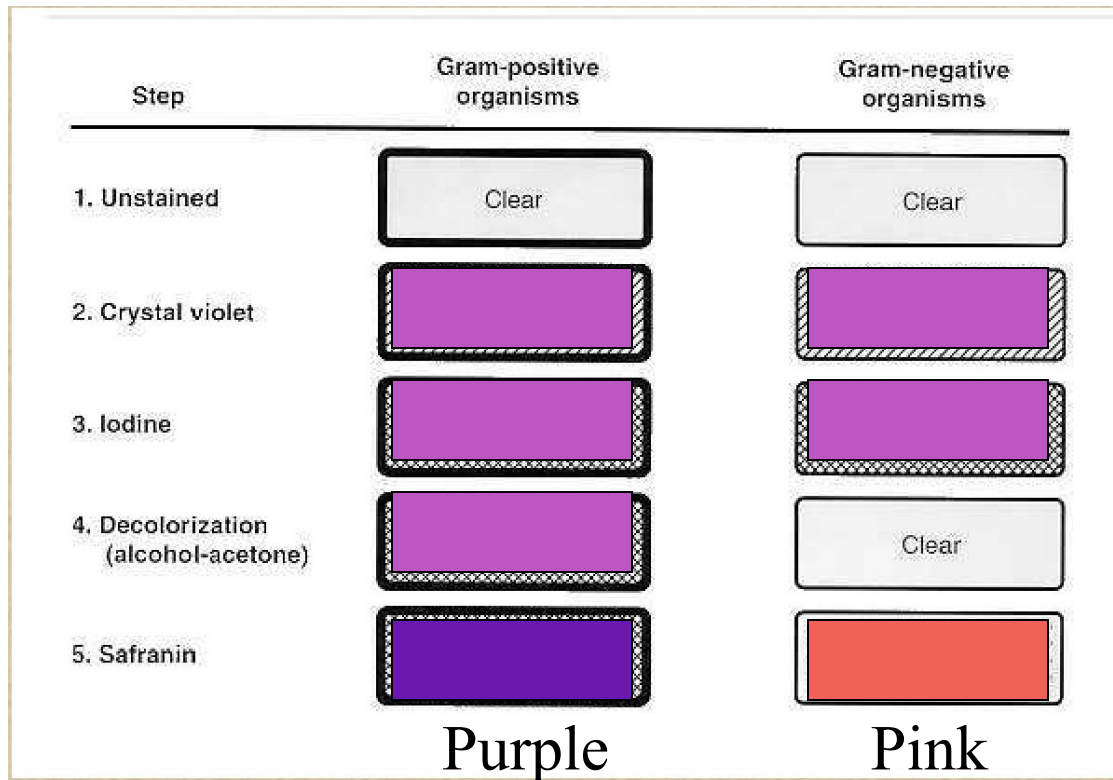
pili

spore

# Gram stain

*Hans Christian Gram 1844*

- **Divides bacteria into 2 (3) classes**
  - **Gram positive, gram negative, poorly staining (includes TB ((Acid fast))**



# Examples

**GNC**



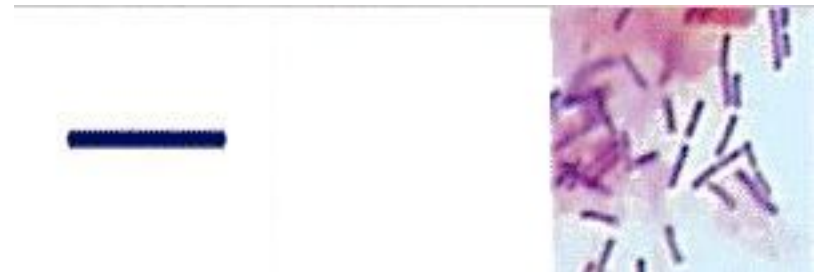
**GPC**



**GNR**

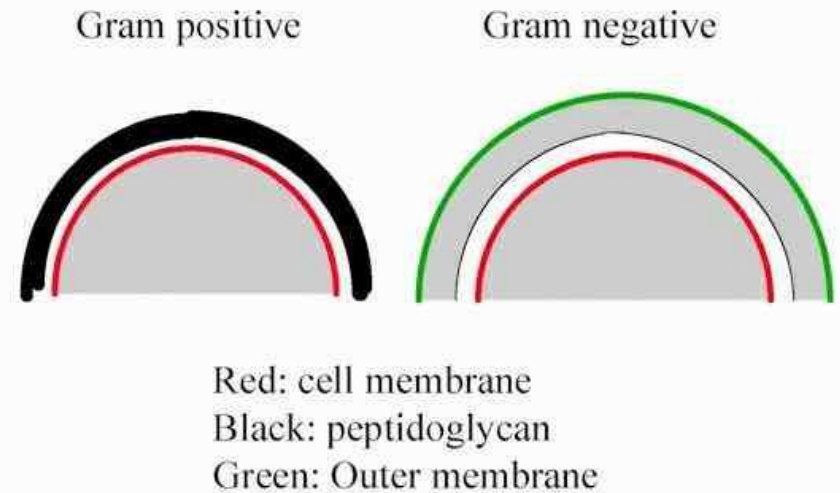


**GPR**

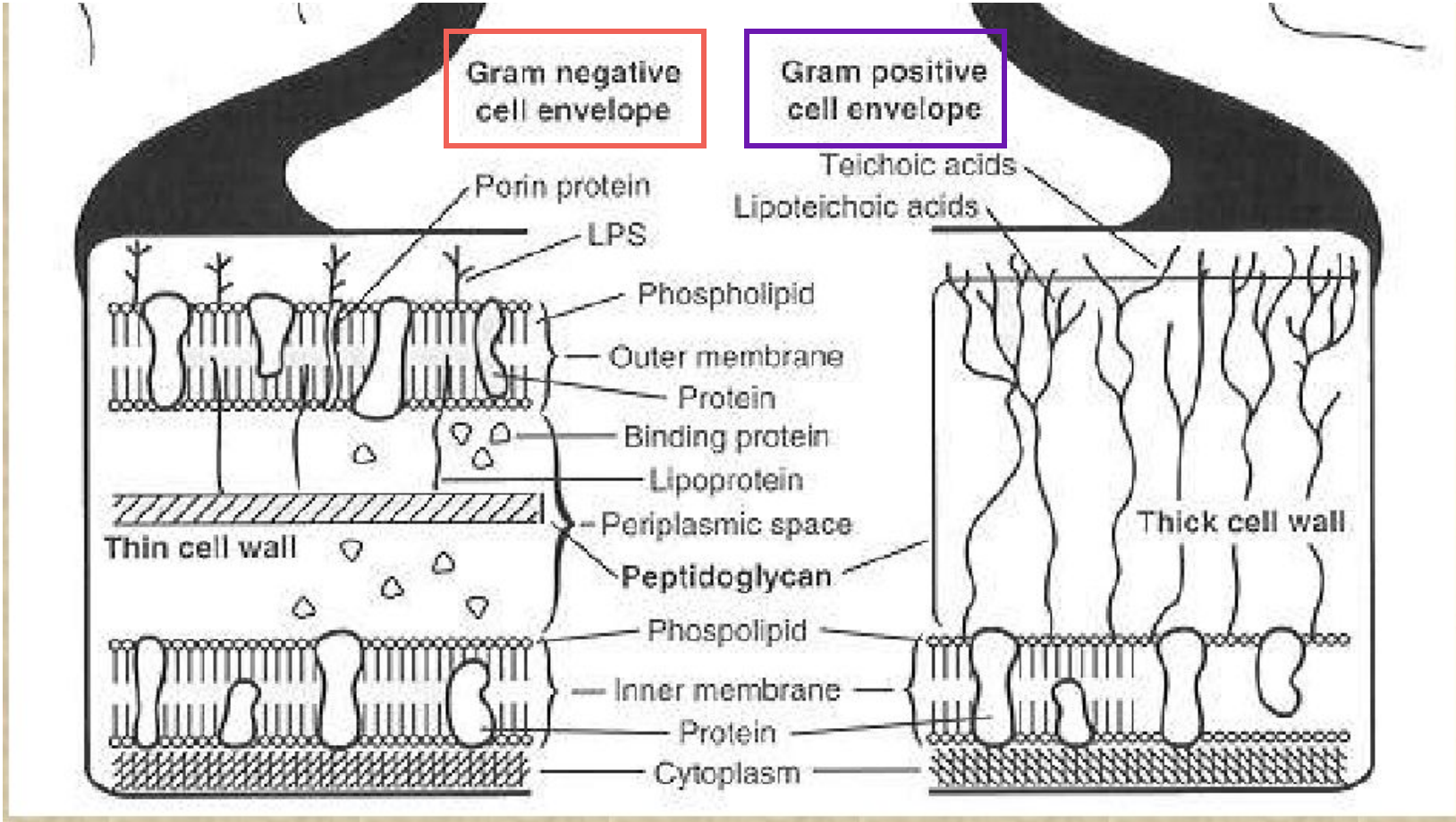


# Cell Envelope

- The cell envelope is all the layers from the cell membrane outward, including the cell wall, the periplasmic space, the outer membrane, and the capsule.
  - All free-living bacteria have a cell wall
  - periplasmic space and outer membrane are found in Gram-negatives
  - the capsule is only found in some strains



# Gram negative vs gram positive envelope

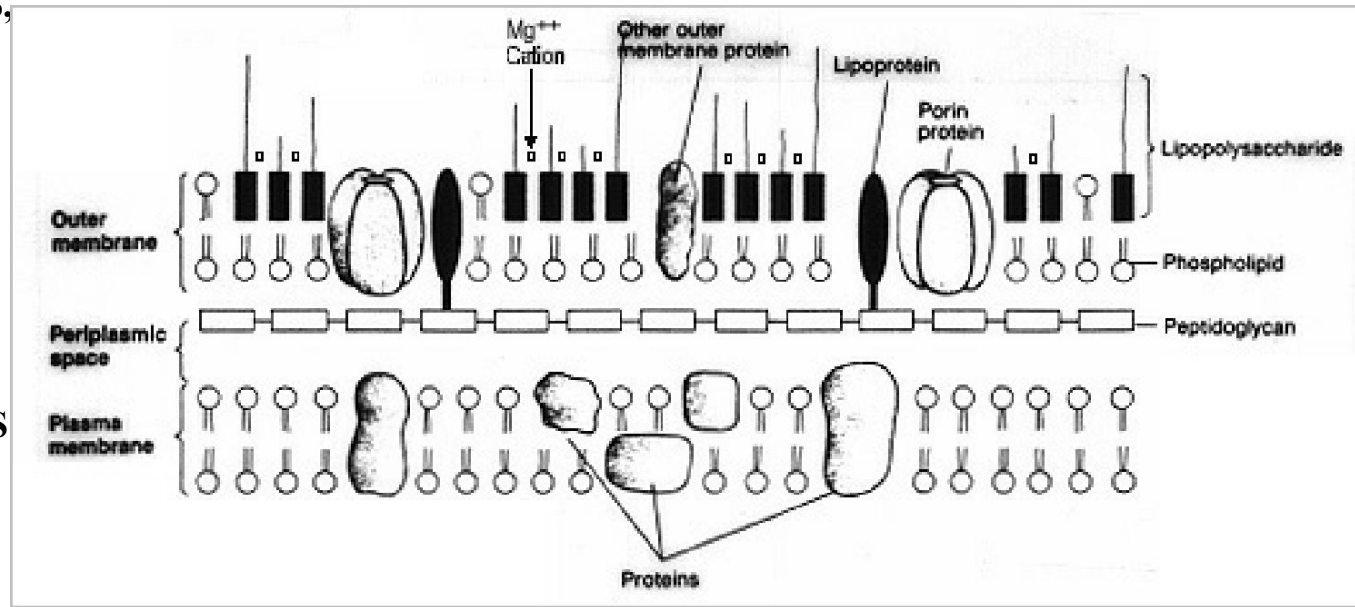


# Gram Negative Membranes

- **Inner (cytoplasmic) membrane**
  - Active transport
  - Respiratory chain components
  - energy transducing systems
  - H<sup>+</sup>-ATPase proton pump
  - Biosynthetic enzymes for membrane phospholipids, PG, LPS, capsule

- **Periplasm**
  - Peptidoglycan
  - Degradative enzymes, B-lactams
  - Binding proteins, signaling molecules

- **Outer membrane**
  - Porins, transporters, LPS
  - Specific uptake of maltose, B12, nucleosides, iron
  - Murein lipoprotein “tethers OM to PG”
  - Asymmetric:
    - inner phospholipid leaflet
    - Outer leaflet: LPS, Mg

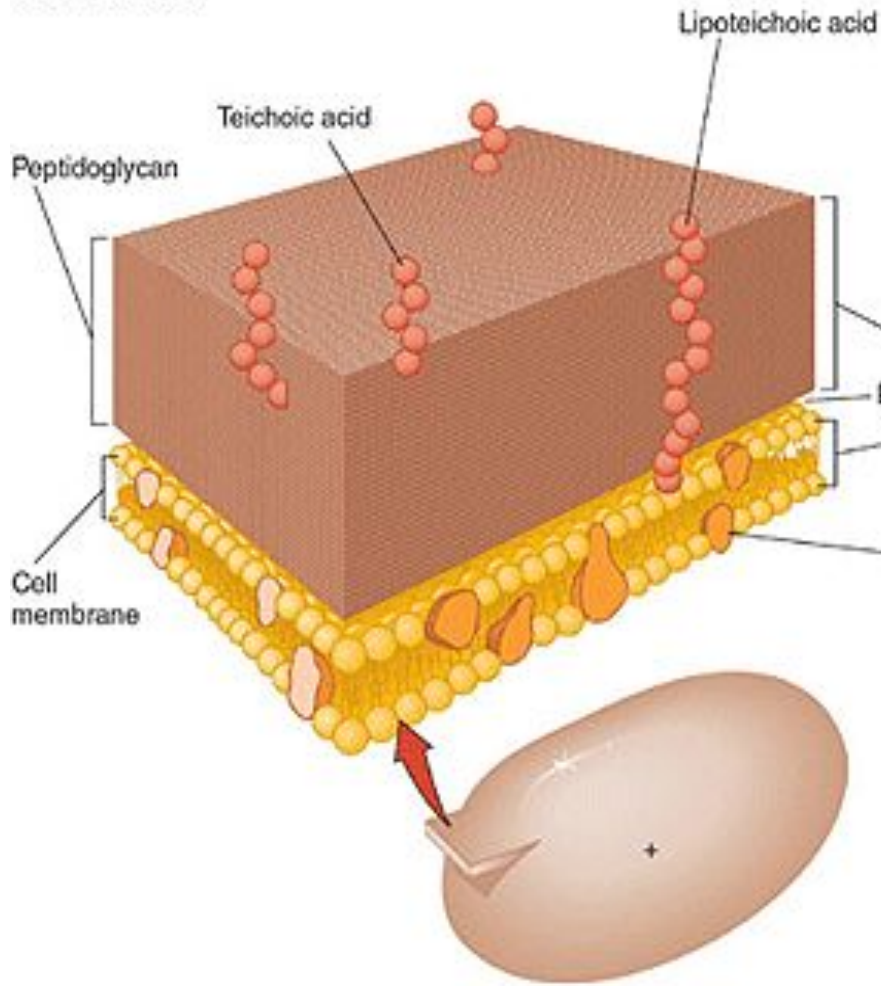




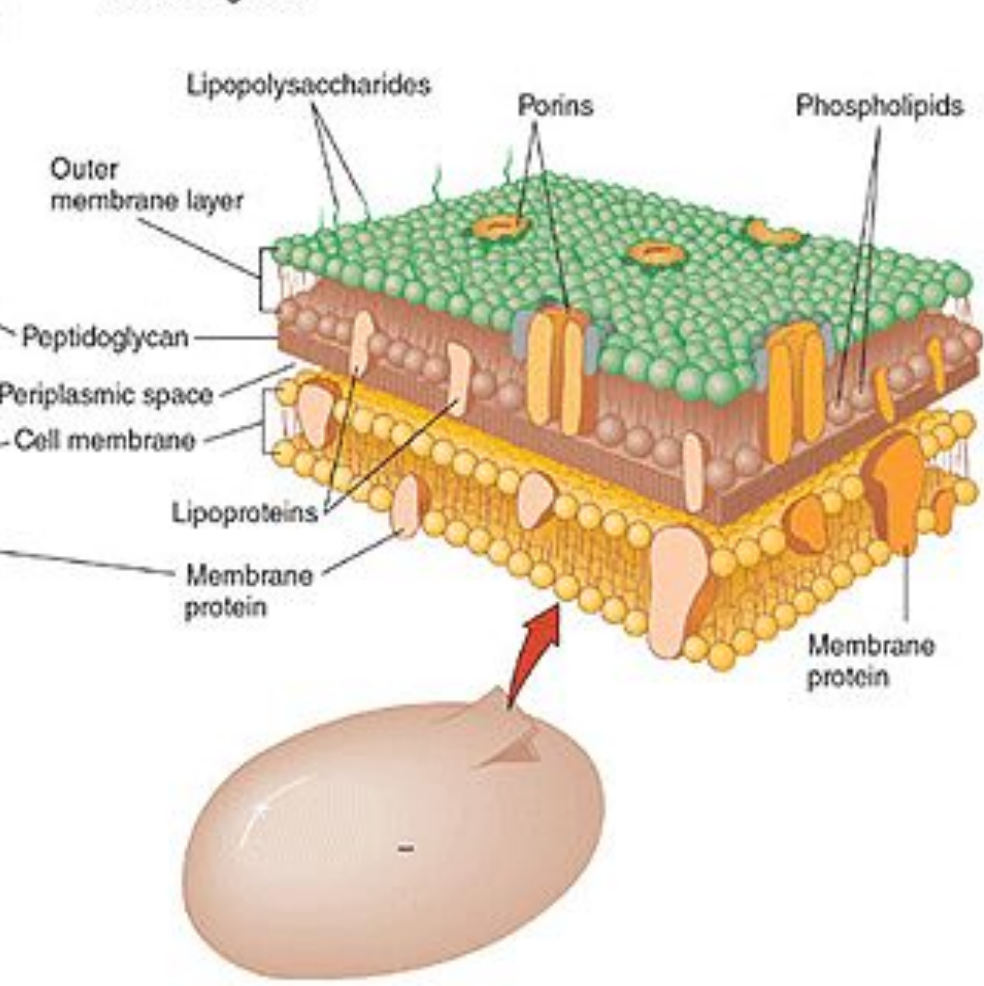
# Differences between GP & GN PG

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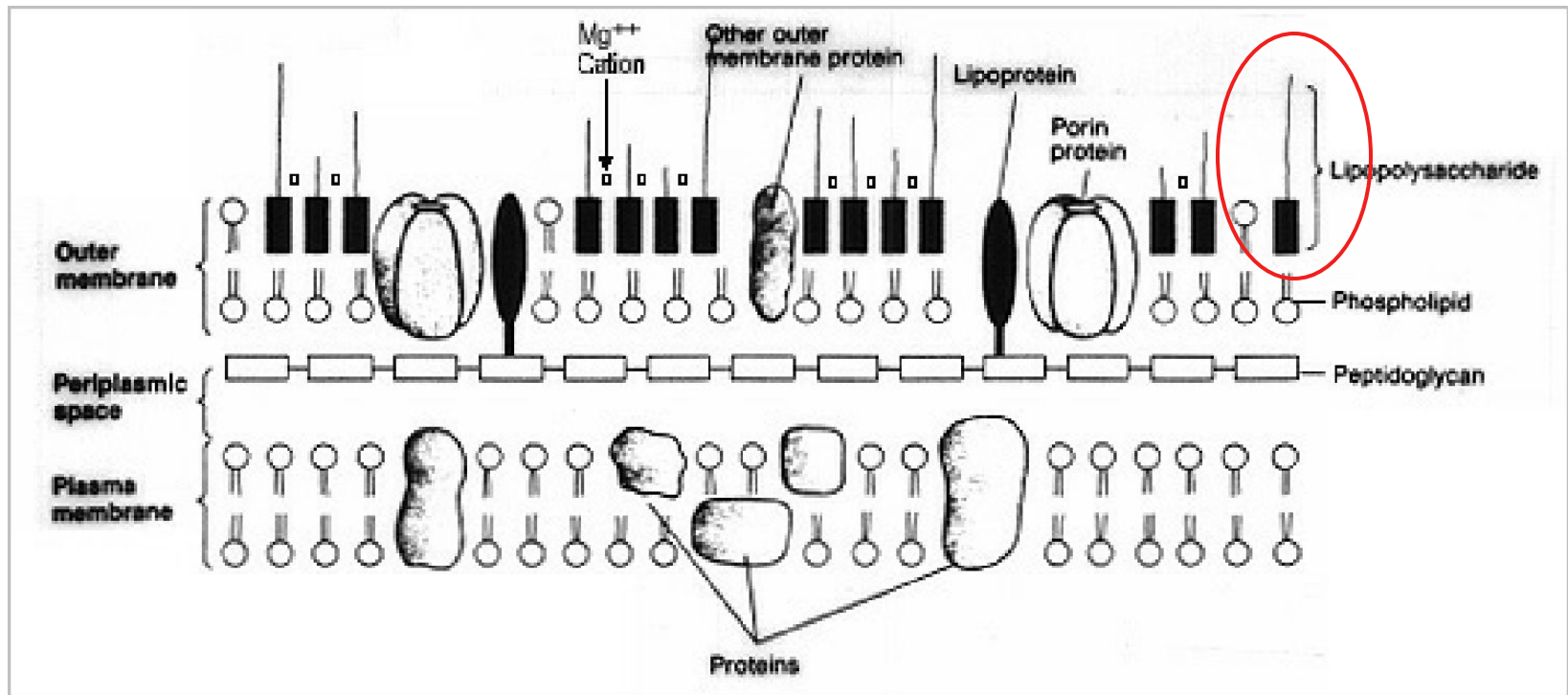
Gram Positive



Gram Negative



# GN Outer membrane



# LPS has 3 components

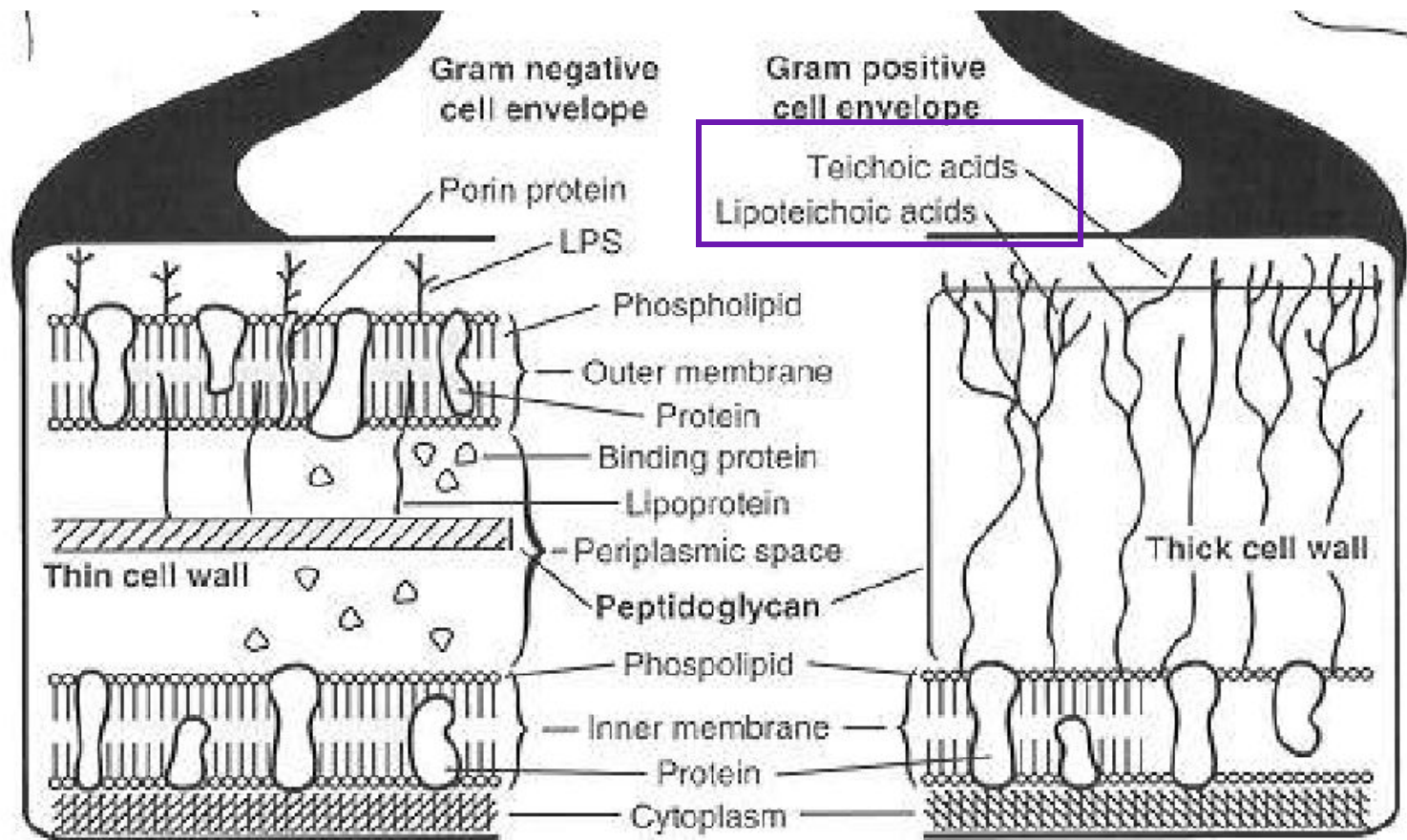
Lipid A	Core	O Antigen
Glucosamine $\beta$ -hydroxymyristate Fatty acids	Ketodeoxyoctonate Phosphoethanolamine Heptose Glucose, galactose, <i>N</i> -acetylglucosamine	Polysaccharide chains: repeating units of species-specific mono- saccharides, e.g., gal- actose, rhamnose, mannose and abequose in <i>S typhimurium</i> LPS

**Toxic,  
Anchors in OM,  
TRL4 ligand**

**Genus-specific ag**

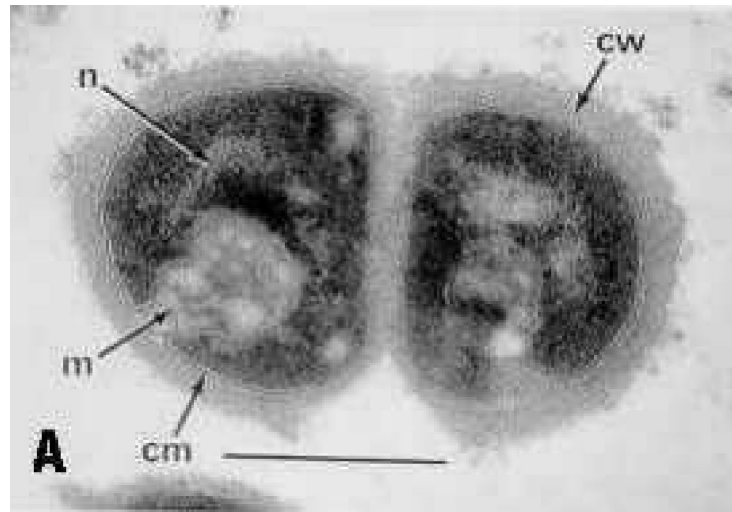
**Species or serotype-  
specific**

# Gram positive envelope



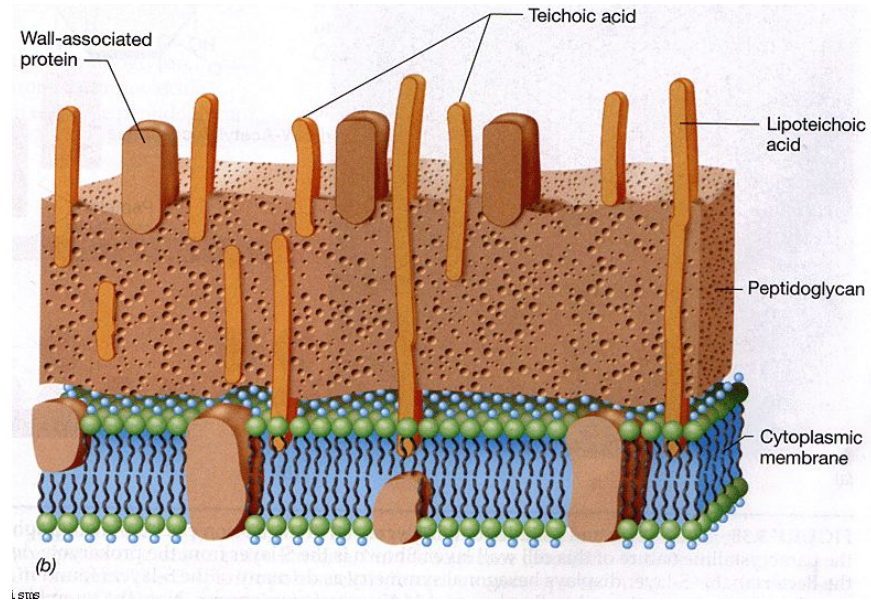
# Gram positive cell wall

- **Peptidoglycan is major constituent**
  - Sugar chains (glycan backbone) cross-linked via peptides
  - target of PCN, cephalosporins, vancomycin
  - Ligand for TLR2
  - Polar 2° to sugars & charged amino acids



# Teichoic & Lipoteichoic acid

- **Teichoic acid: Negatively charged polyglycerol or polyribitol phosphate polymers, covalently linked to PG in GP's**
- **Lipoteichoic acid: Teichoic acid w/lipophilic glycolipid anchored in cytoplasmic membrane**
- **TLR2 ligand**
- **Adhesin for Streptococcus spp. and cause **septic shock****

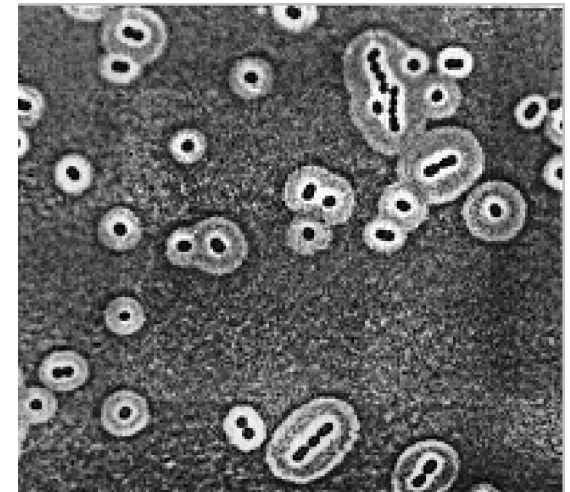
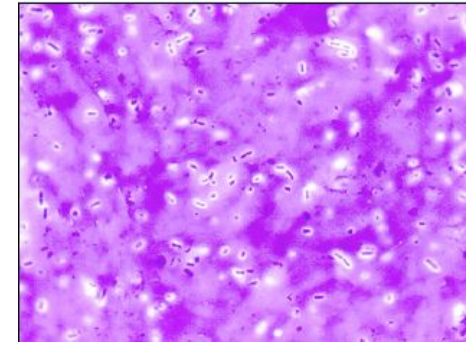


# Glycocalyx

- Coating of molecules external to the cell wall, made of sugars and/or proteins
- Two types:
  1. Slime layer - loosely organized and attached
  2. Capsule - highly organized, tightly attached
- Functions:
  - Protect cells from dehydration and nutrient loss
  - Inhibit killing by white blood cells by phagocytosis, contributing to pathogenicity
  - Attachment - formation of **biofilms**

# Bacterial capsules

- **Outermost layer (up to 10 u) of some GP & GN bacteria**
- **Usually viscous polysaccharide**
- **Not essential for viability**
- **Sometimes is less discrete:**
  - Slime
- **Help microbes resist phagocytosis**
  - Shield from complement



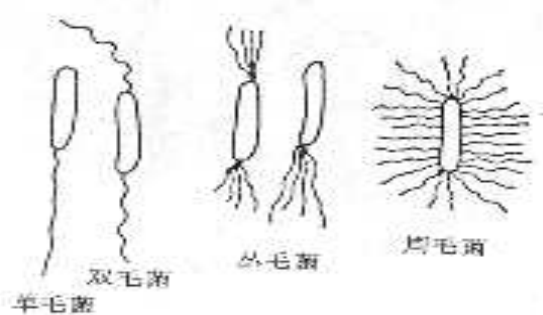
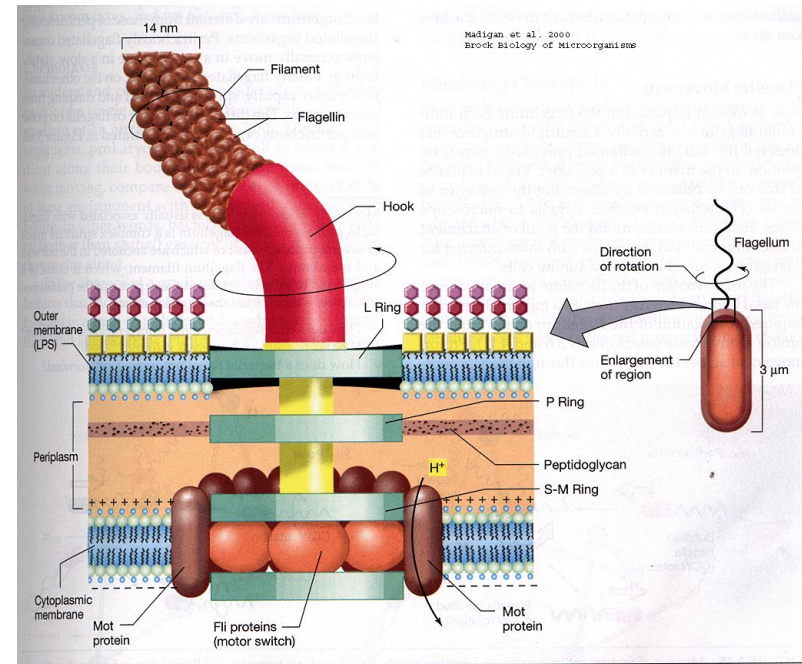


# *Functions of Cell Wall*

- Maintaining the cell's characteristic shape- the rigid wall compensates for the flexibility of the **phospholipid membrane** and keeps the cell from assuming a spherical shape
- Countering the effects of **osmotic pressure**
- Providing **attachment sites** for bacteriophages
- Providing a rigid platform for surface appendages- **flagella, fimbriae, and pili** all emanate from the wall and extend beyond it
- Play an essential role in **cell division**
- Be the sites of major **antigenic determinants** of the cell surface.
- **Resistance of Antibiotics**

# Flagella

- Some bacterial species are mobile and possess locomotory organelles - flagella. Flagella consist of a number of proteins including flagellin
- The diameter of a flagellum is thin, 20 nm, and long with some having a length 10 times the diameter of cell. Due to their small diameter, flagella cannot be seen in the light microscope unless a special stain is applied. Bacteria can have one or more flagella arranged in clumps or spread all over the cell.



- Identification of Bacteria
- Pathogenesis
- Motility of bacteria

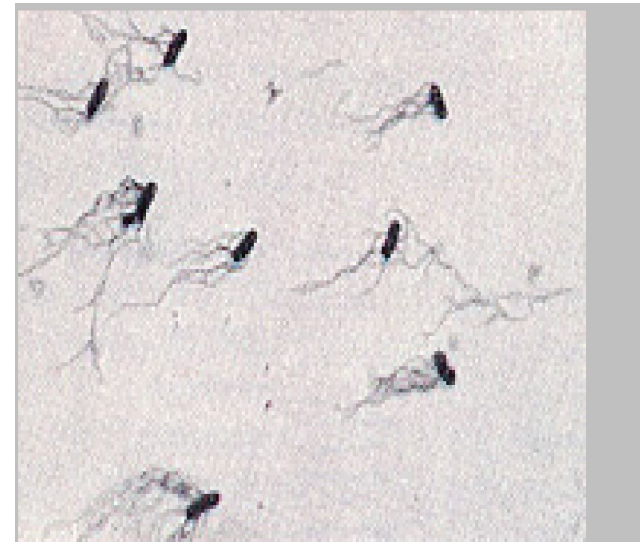
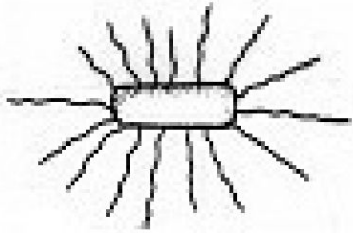


Figure 3. *Salmonella typhi* flagellar stain.

# Flagellar localization

- Polar or peritrichous location



peritrichous flagella

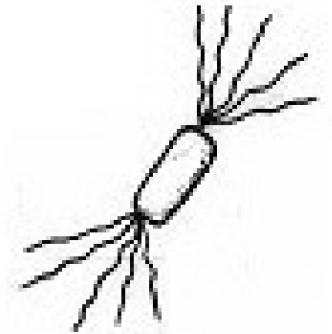
**E. coli**



**Bartonella**

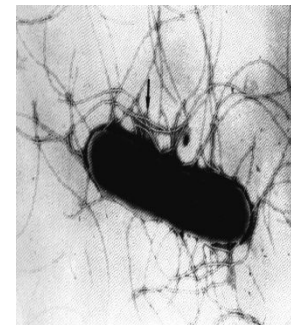
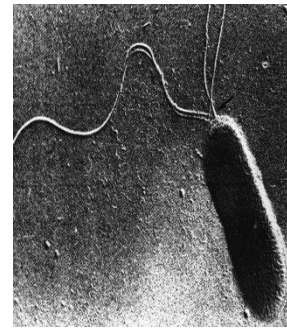
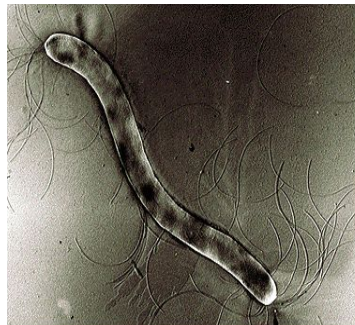
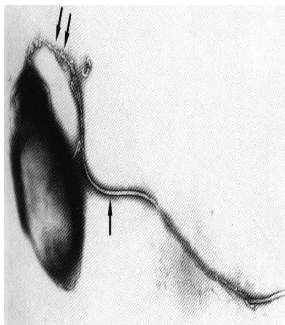


**Vibrio**

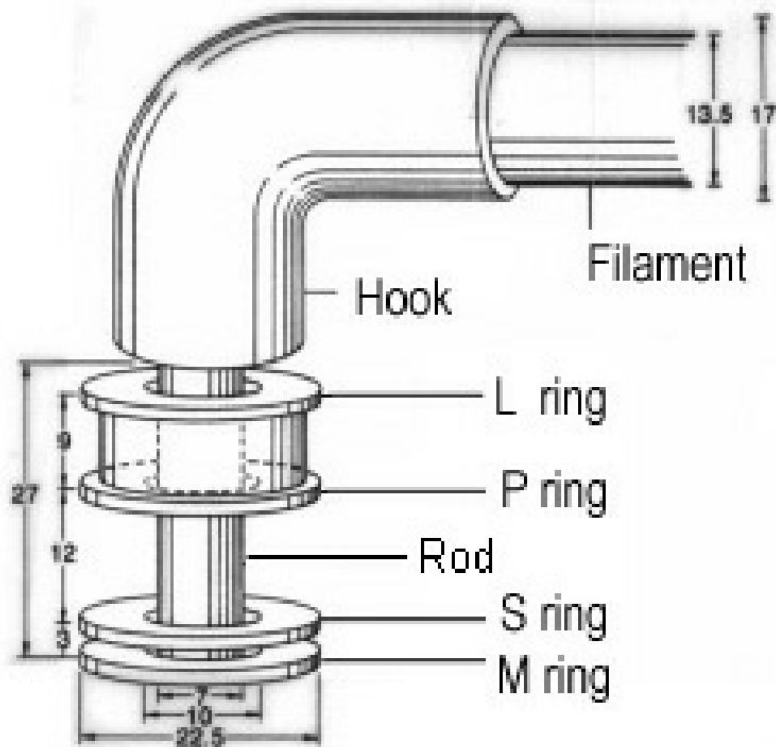


**Spirillum**

Monotrichate/Amphitrichate/Lophotrichate/Peritrichate



# Flagellar structure



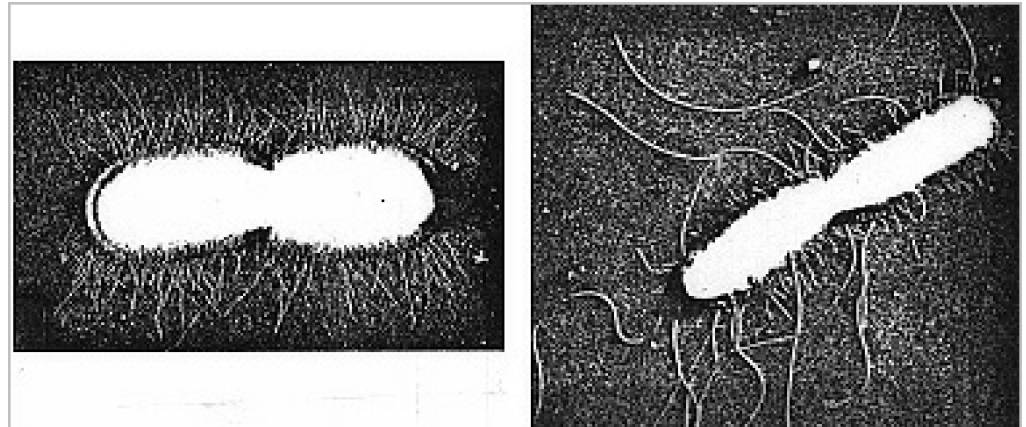
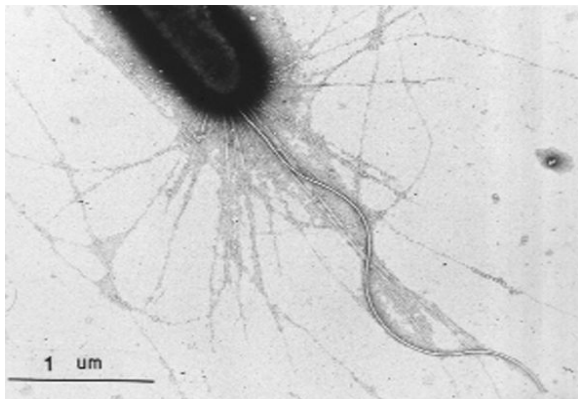
- **Filament (flagellin protein)**
- **Hook: connects filament to basal body**
- **Basal body: anchors hook, imparts motion**
  - **Motor:**
  - **M & S rings Cytoplasmic membrane**
  - **P ring: periplasm**
  - **L ring: outer membrane**
- **Highly regulated protein machine**

# Flagella and pathogenesis

- **Swimming/chemotaxis important for motility and virulence**
  - **Co-regulated with other virulence factors (TCP in *V. cholera*) toxin – coregulated pili**
  - **Adhesion (Salmonella, Pseudomonas)**
- **Protein export apparatus (Salmonella)**
- **Early stages in biofilm formation**
- **Flagellin protein is immunogenic**
- **Salmonella flagellin is a presumptive ligand for TLR5**

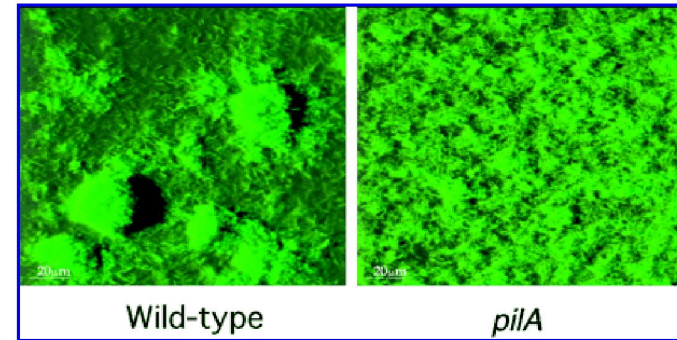
# Pili/fimbriae

- **Mostly found on GNR, rarely GPR**
- **Short, hairlike structures**
- **Not involved in swimming or swarming motility**



# Functions of Pili

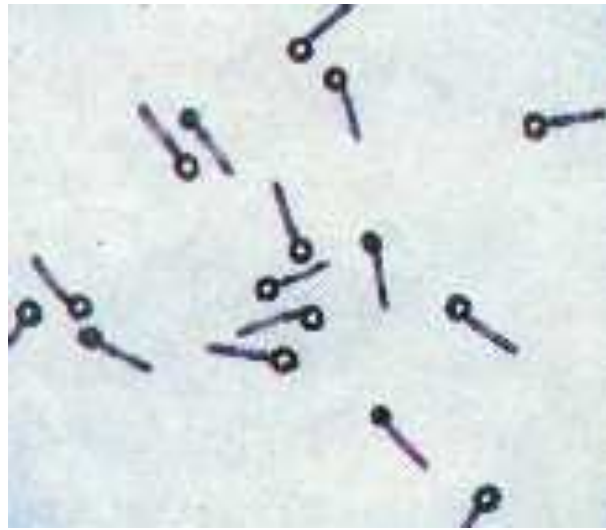
- Conjugation (*E. coli* F pilus)
- DNA uptake (*Neisseria*)
- Phage receptors
- Adhesion
- Early steps in biofilm formation
- Twitching motility (*Pseud aeruginosa*)
- Resist phagocytosis
- Antigenic variation (*Neisseria gonorrhoea*)



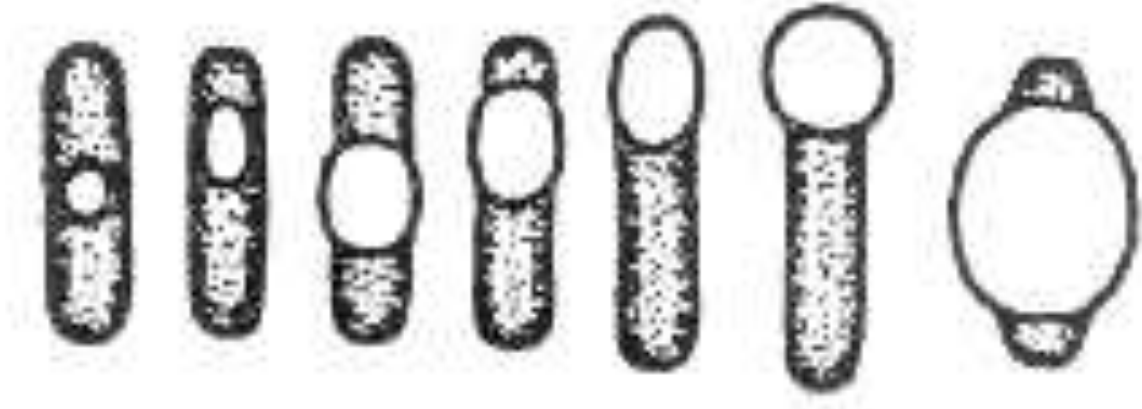
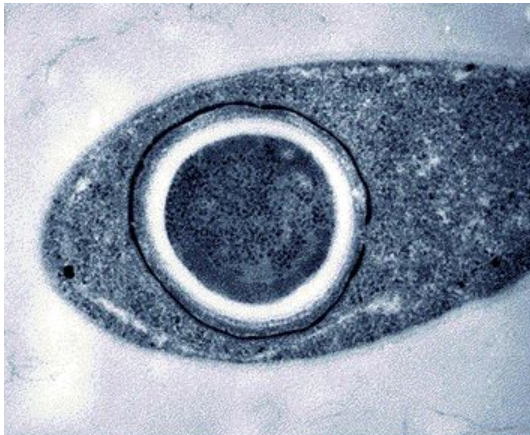
Confocal microscopy of twenty four-hour biofilms formed in continuous flow chambers by *P. aeruginosa* wild-type and (B) type IV pili-defective mutant. This image shows a top-down view of the biofilm formed by the wild-type strain. The characteristic mound-shaped architecture of the *P. aeruginosa* biofilm can be seen. The type IV pili mutant forms a biofilm lacking this normal architecture. Images courtesy of Matt Parnell, University of Manchester.

QuickTime™ and a  
Cinepak decompressor  
are needed to see this picture.

# Endospores (spores)



- Identification of Bacteria
- Pathogenesis
- Resistance



- Dormant cell
- Resistant to adverse conditions
  - high temperatures
  - organic solvents
- Produced when starved
- Contain calcium dipicolinate DPA, Dipicolinic acid
- *Bacillus* and *Clostridium*



# Spores

- Some bacteria can form very tough spores, which are metabolically inactive and can survive a long time under very harsh conditions.
- Spores can also survive very high or low temperatures and high UV radiation for extended periods. This makes them difficult to kill during sterilization.
  - Anthrax
- Spores are produced only by a few genera in the Firmicutes:
  - *Bacillus* species including *anthracis* (anthrax) and *cereus* (endotoxin causes ~5% of food poisoning)
  - *Clostridium* species including *tetani* (tetanus), *perfringens* (gangrene), and *botulinum* (botulism: food poisoning from improperly canned food)

