Environmental Microbiology

Course 2: Basic Bacteriology

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Two Kingdoms of Bacteria

Archaebacteria

- These are ancient bacteria
- They exist in extreme environments
- They are anaerobic

Eubacteria

- Live everywhere that archaebacteria don't

  (like on and in you!)
- Some are autotrophic and some are heterotrophic

San Francisco Bay Salt Ponds

A variety of eubacteria

Comparing Archae- and Eu-bacteria

Archaebacteria

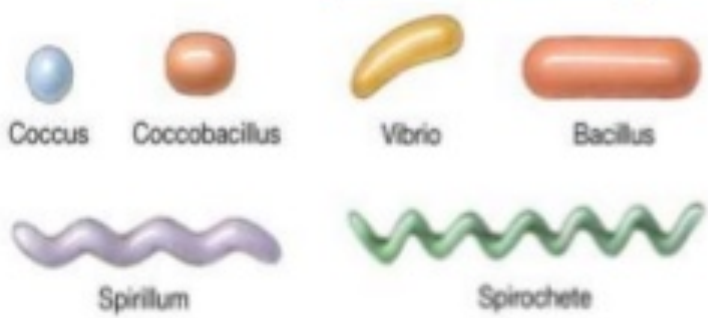
- Live in harsh environments
  - Hot sulfur springs, the Great Salt Lake, Volcanic deep-sea vents
  - Love extreme pH level (acidic or basic)
  - Love heat
- Have similar RNA gene sequences that are different from Eubacteria
- Most are Autotrophs
  - Anaerobic (without oxygen)
  - Chemosynthesis

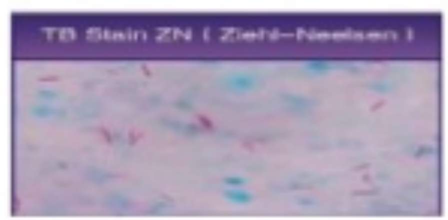
Eubacteria

- Most familiar bacteria
- Some are disease-causing
- Classified by
  - Shape (coccus, bacillus, spirillum
  - Gram stain (positive or negative)
- Can obtain nutrients as
  - Heterotrophs
  - Parasites
  - Autotrophs
  - Saprotrophs
- Most have flagella for movement

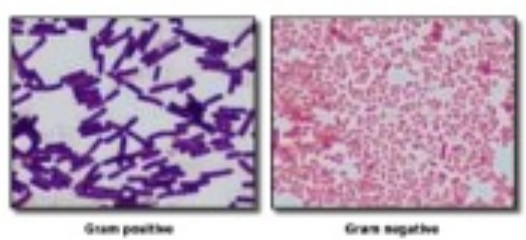
Classification of Bacteria

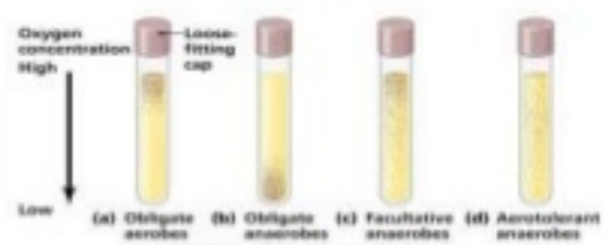
- **Based on Shape**
  - Cocci (Spherical)
  - Bacilli (Straight rod)
  - Spiral (Curved Rod)
- **Based on Stain**
  - Gram Stain (Positive or negative)
  - Ziehl- Nielson Stain (ex. M. tuberculosis)
- **Based on Oxygen requirement**
  - Strict/ Obligate Aerobes
  - Anaerobes
  - Facultative Anaerobes

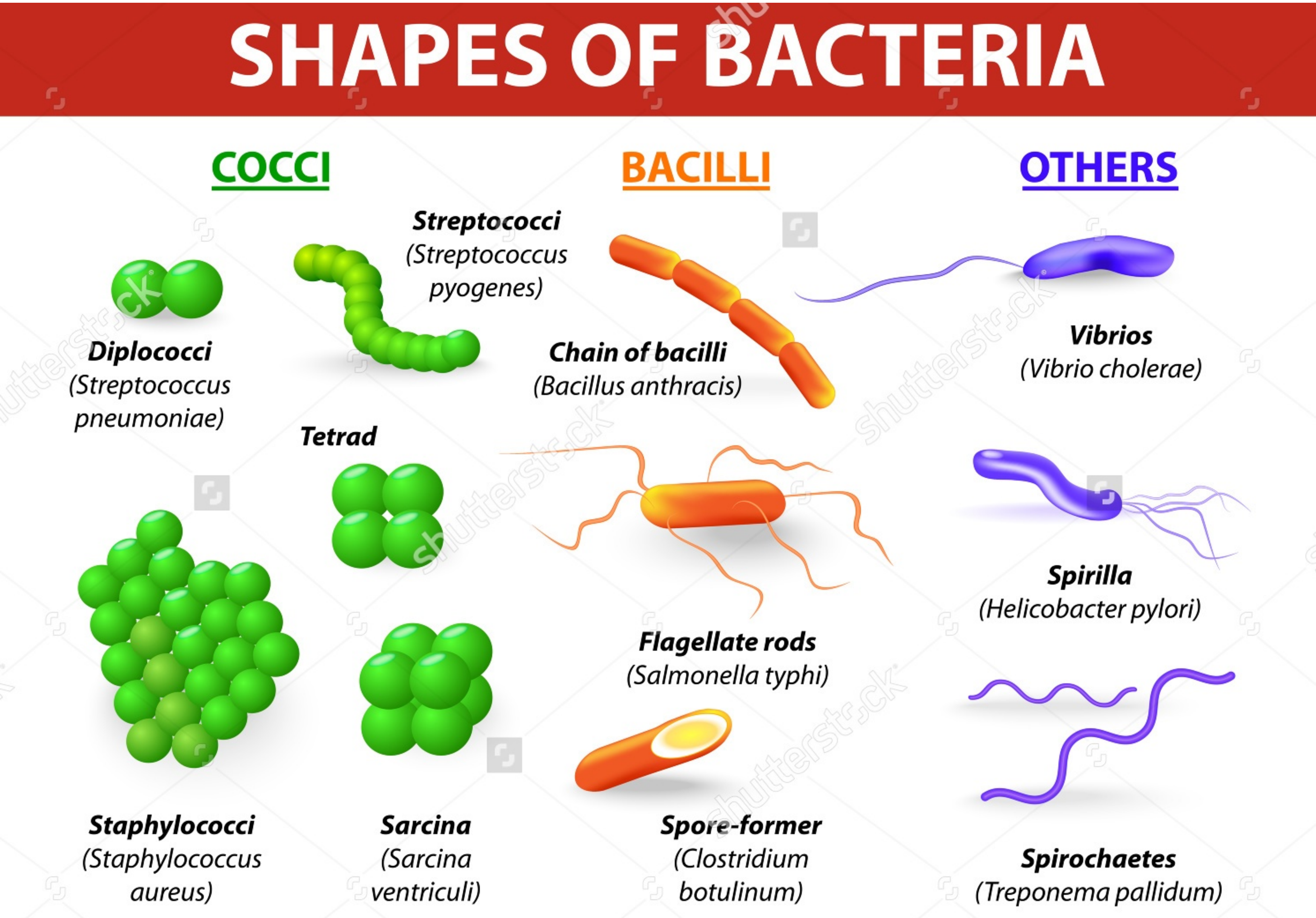




Differential Staining - Gram Staining

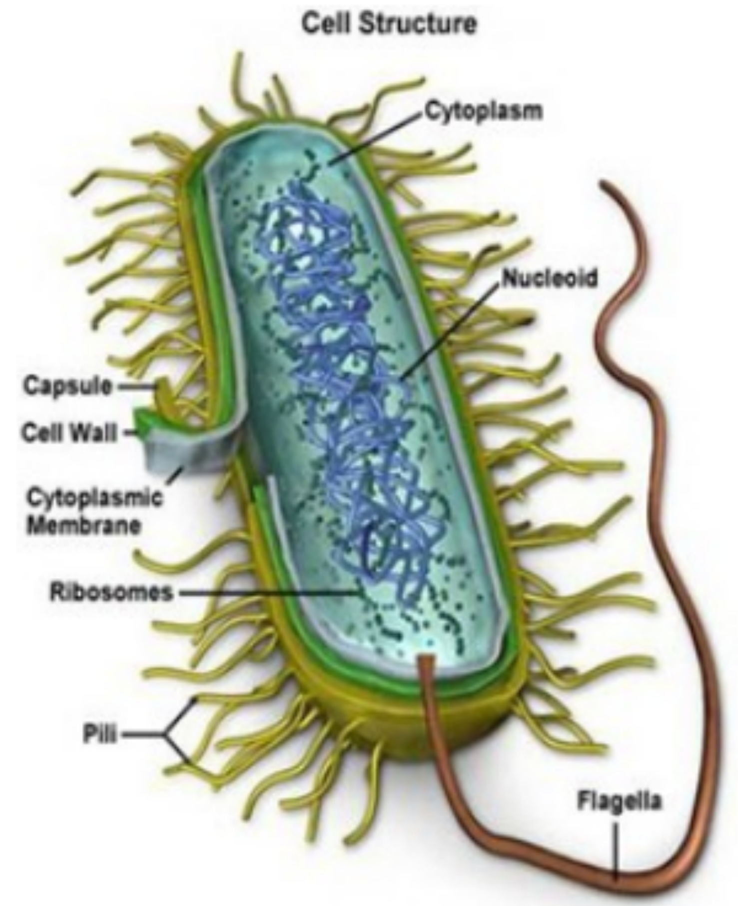





SHAPES OF BACTERIA
COCCI
BACILLI
OTHERS
Streptococci
(Streptococcus pyogenes)
Diplococci
(Streptococcus pneumoniae)
Tetrad
Staphylococci
(Staphylococcus aureus)
Sarcina
(Sarcina ventriculi)
Chain of bacilli
(Bacillus anthracis)
Flagellate rods
(Salmonella typhi)
Spore-former
(Clostridium botulinum)
Vibrios
(Vibrio cholerae)
Spirilla
(Helicobacter pylori)
Spirochaetes
(Treponema pallidum)

Bacterial cell structure

- Cytoplasmic structures
  - Nucleoid
  - Ribosome
  - Cytoplasmic membrane
- Cell wall
- External structures



Basic Bacterial Cell Structure

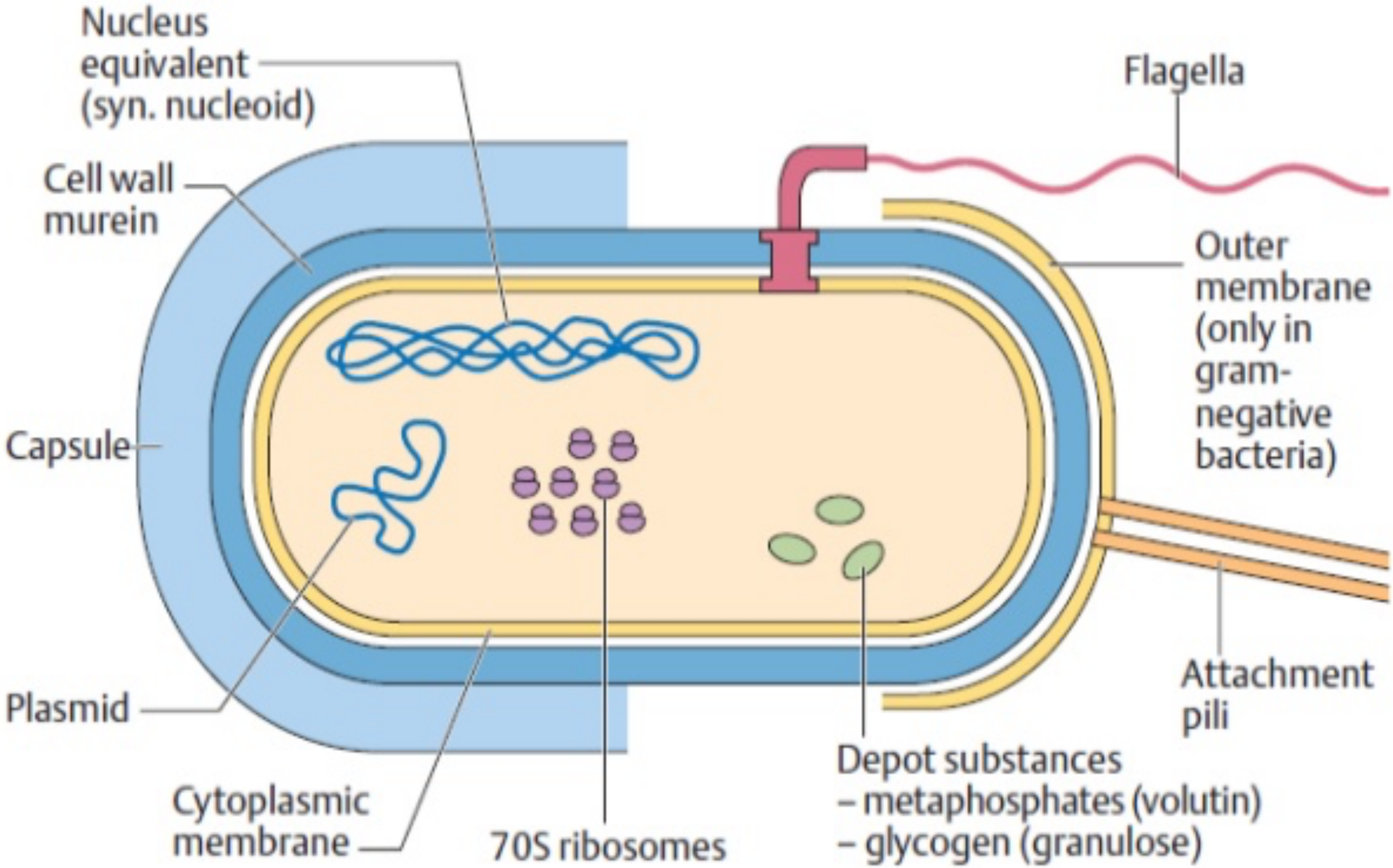
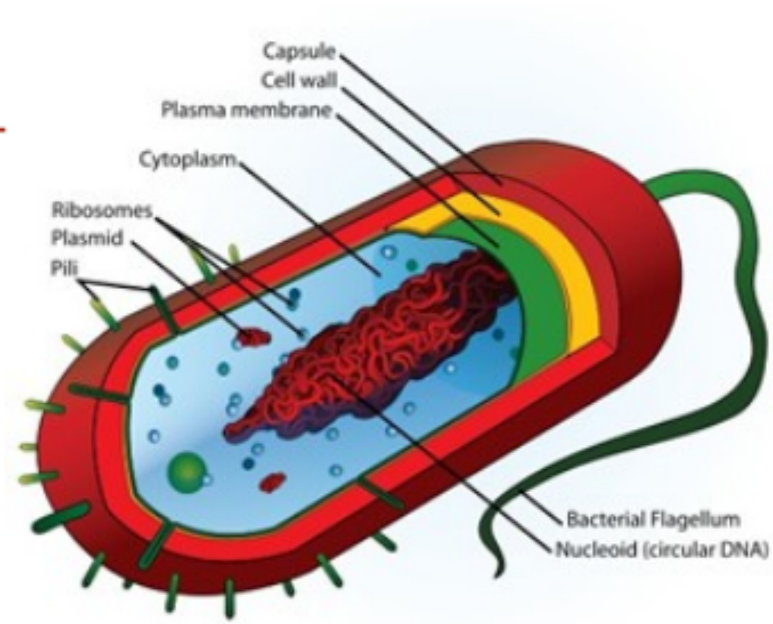
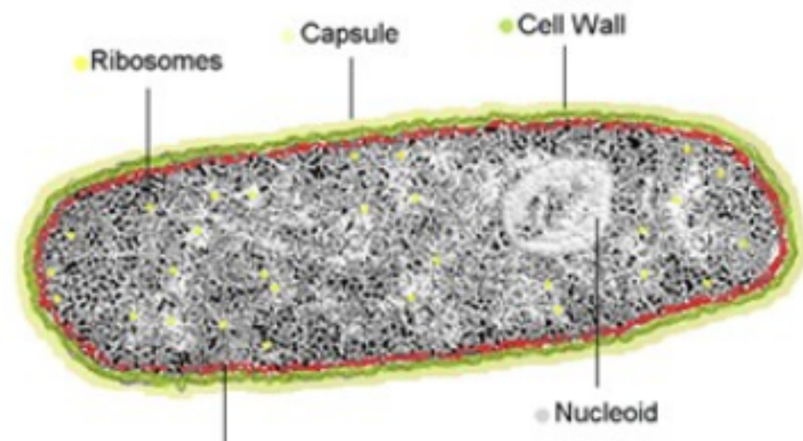


Fig. 3.7 All bacteria have the same basic structure (not to scale).

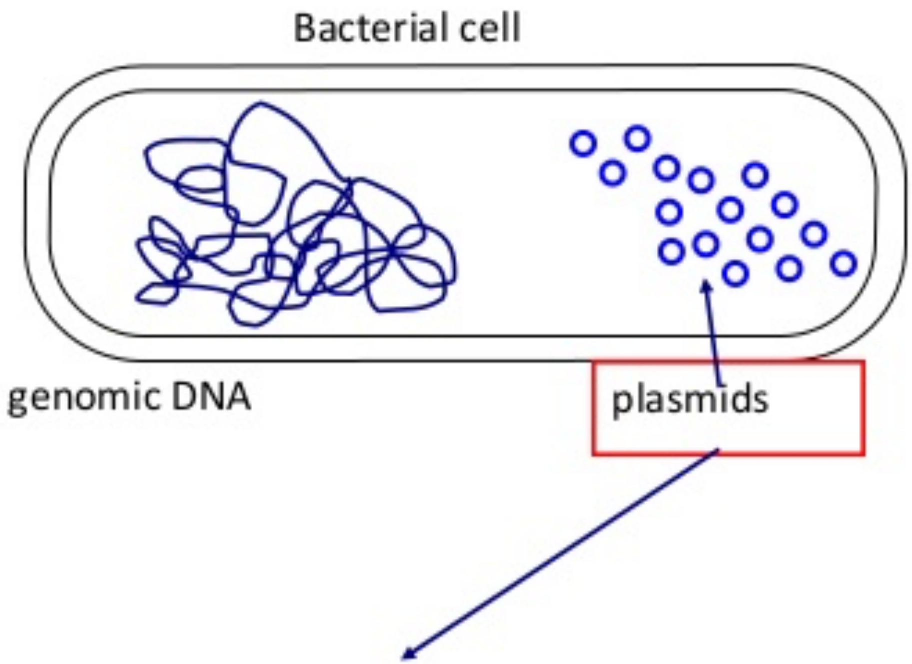
Bacterial cell structure
Nucleoid

- Prokaryotes:
  - No true nucleus; no nuclear membrane, no nucleolus
- Bacterial chromosome:
  - Single, double-stranded circle found in the **nucleoid**





Plasmids: vehicles of recombinant DNA

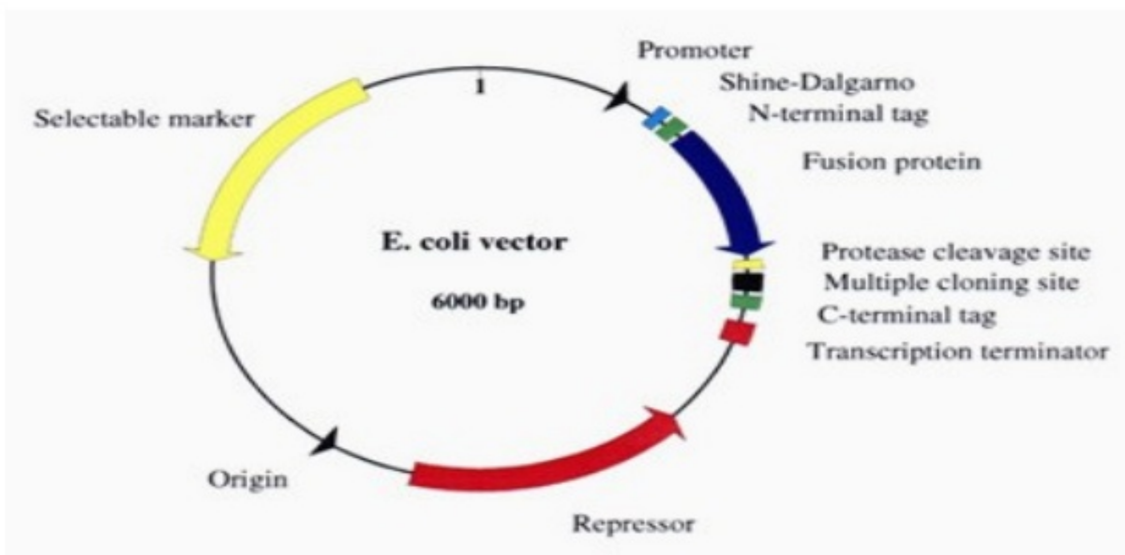


Non-chromosomal DNA
Replication: independent of the chromosome
Many copies per cell
Easy to isolate
Easy to manipulate

Applications of plasmids

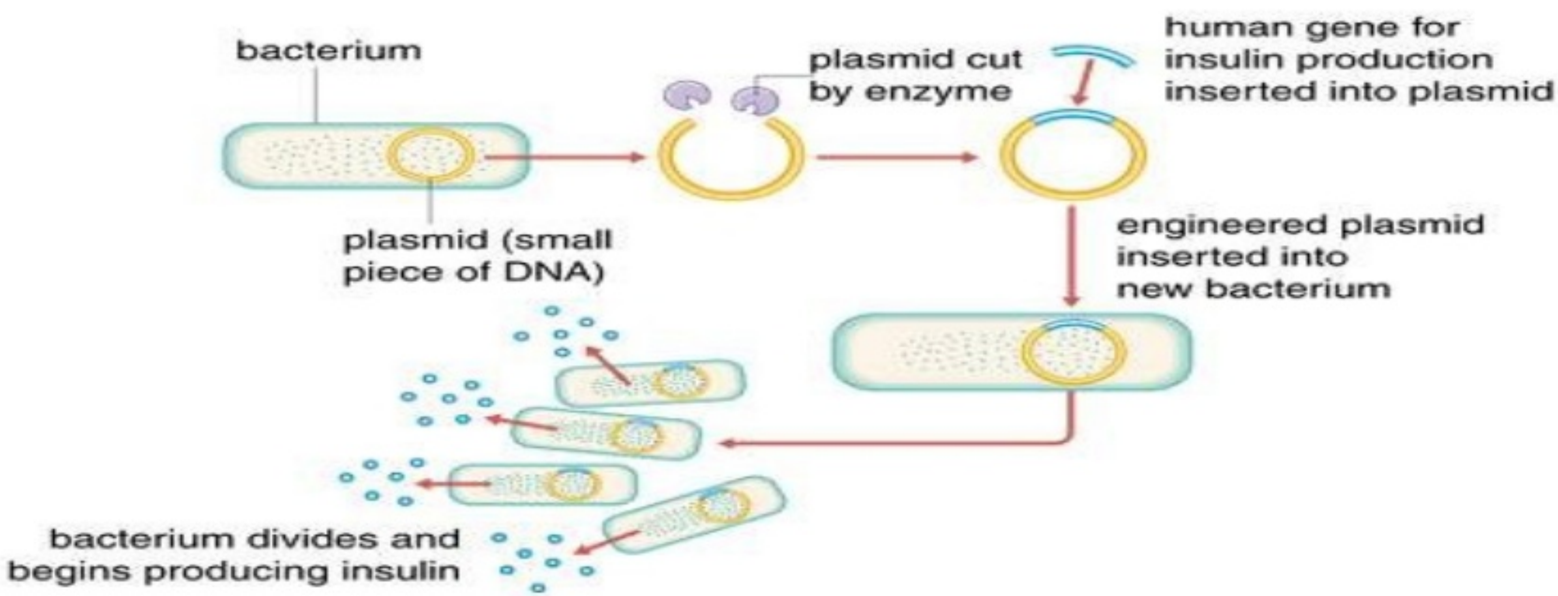
- Plasmids are extremely valuable tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering where they are commonly used to multiply (make many copies of) particular genes .

- Plasmids in this conditions are called vectors .



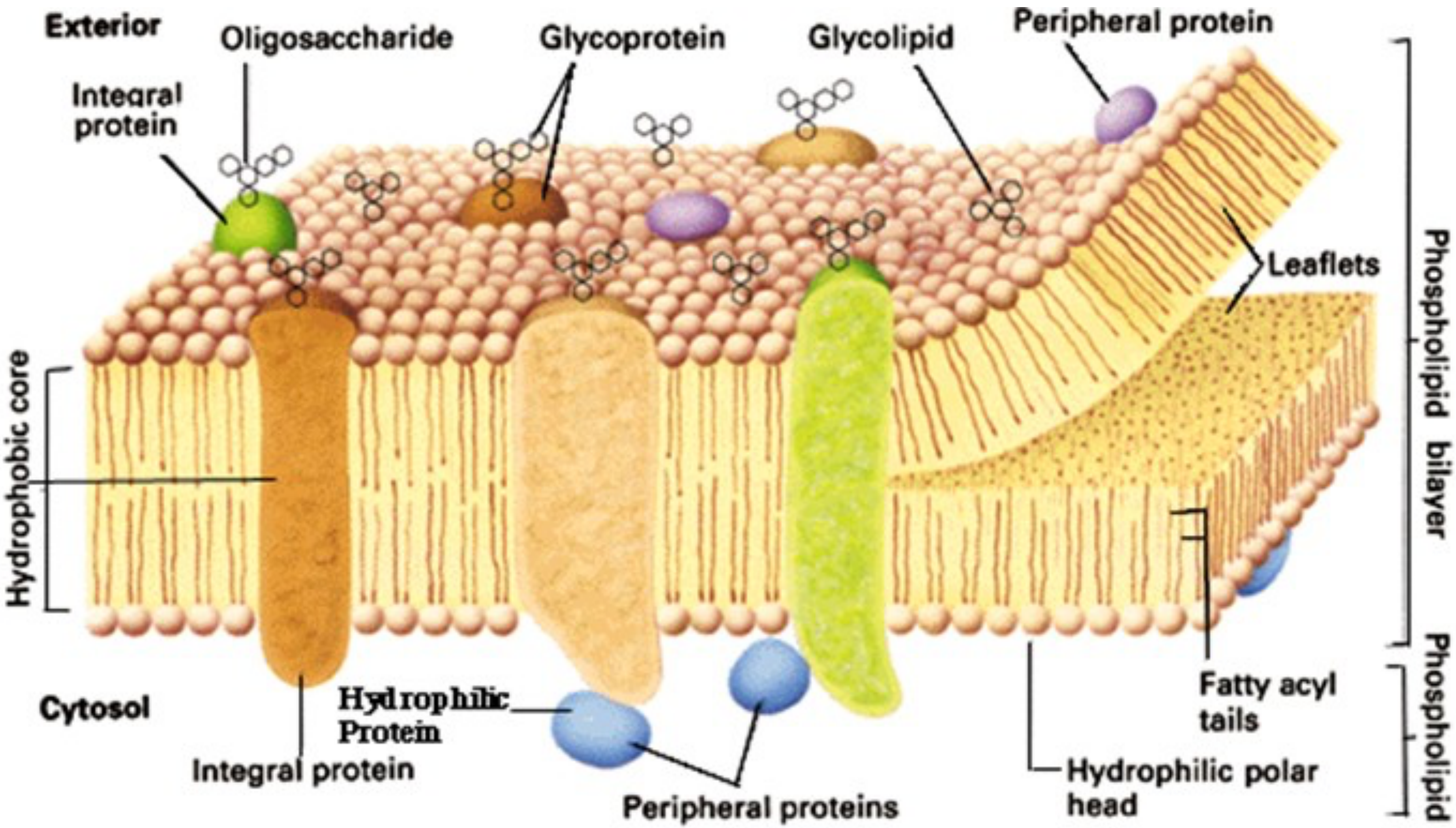
They play a critical role in :

- gene cloning, recombinant protein production (e.g., of human insulin).



1. The Cell Membrane

- Phospholipid bilayer: 2 surface layers of hydrophilic of polar head and inner layer of hydrophobic nonpolar tail.
- Peripheral proteins: Function as enzyme scaffold for support and mediator for cell movement
- Integral proteins: disrupting lipid bilayer. Other types known as transmembrane protein
- Glycoprotein: protein attached to the carbohydrate
- Glycolipid: Lipid attached to carbohydrate


Exterior
Oligosaccharide
Glycoprotein
Glycolipid
Peripheral protein
Integral protein
Leaflets
Phospholipid bilayer
Hydrophobic core
Fatty acyl tails
Cytosol
Hydrophilic Protein
Integral protein
Peripheral proteins
Hydrophilic polar head
Phospholipid

Bacterial cell structure
Cell membrane – Functions

- Selective permeability and transport of solutes
- Electron transport and oxidative phosphorylation
- Excretion of hydrolytic exoenzymes
- Functioning in DNA and cell wall synthesis
- Bearing the receptors of the chemotactic and other sensory transduction systems

Bacterial Cell Wall

- provides overall strength to the cell.
- maintains the cell shape, which is important for how the cell will grow, reproduce, obtain nutrients, and move.
- protects the cell from **osmotic lysis**, as the cell moves from one environment to another or transports in nutrients from its surroundings.
- The cell wall can keep out certain molecules, such as toxins, particularly for gram negative bacteria.
- the bacterial cell wall can contribute to the pathogenicity or disease –causing ability of the cell for certain bacterial pathogens.

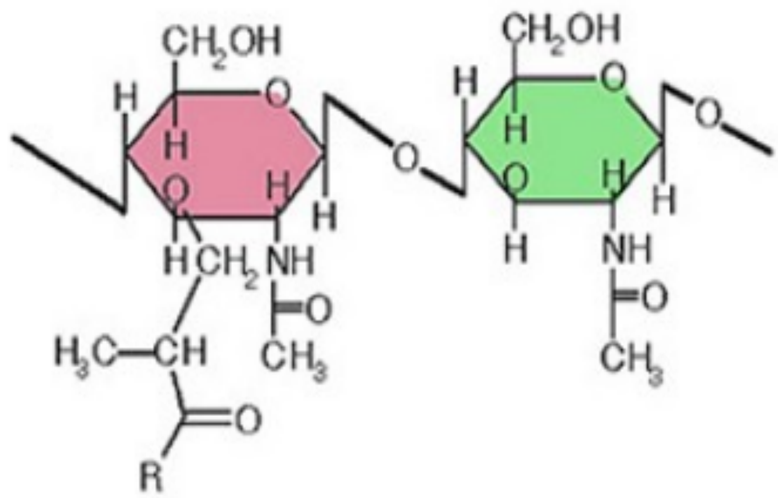
Bacterial cell structure
Cell wall

- **Gram-positive bacteria**
  - Peptidoglycan
  - Teichoic acid
  - Lipoteichoic acid

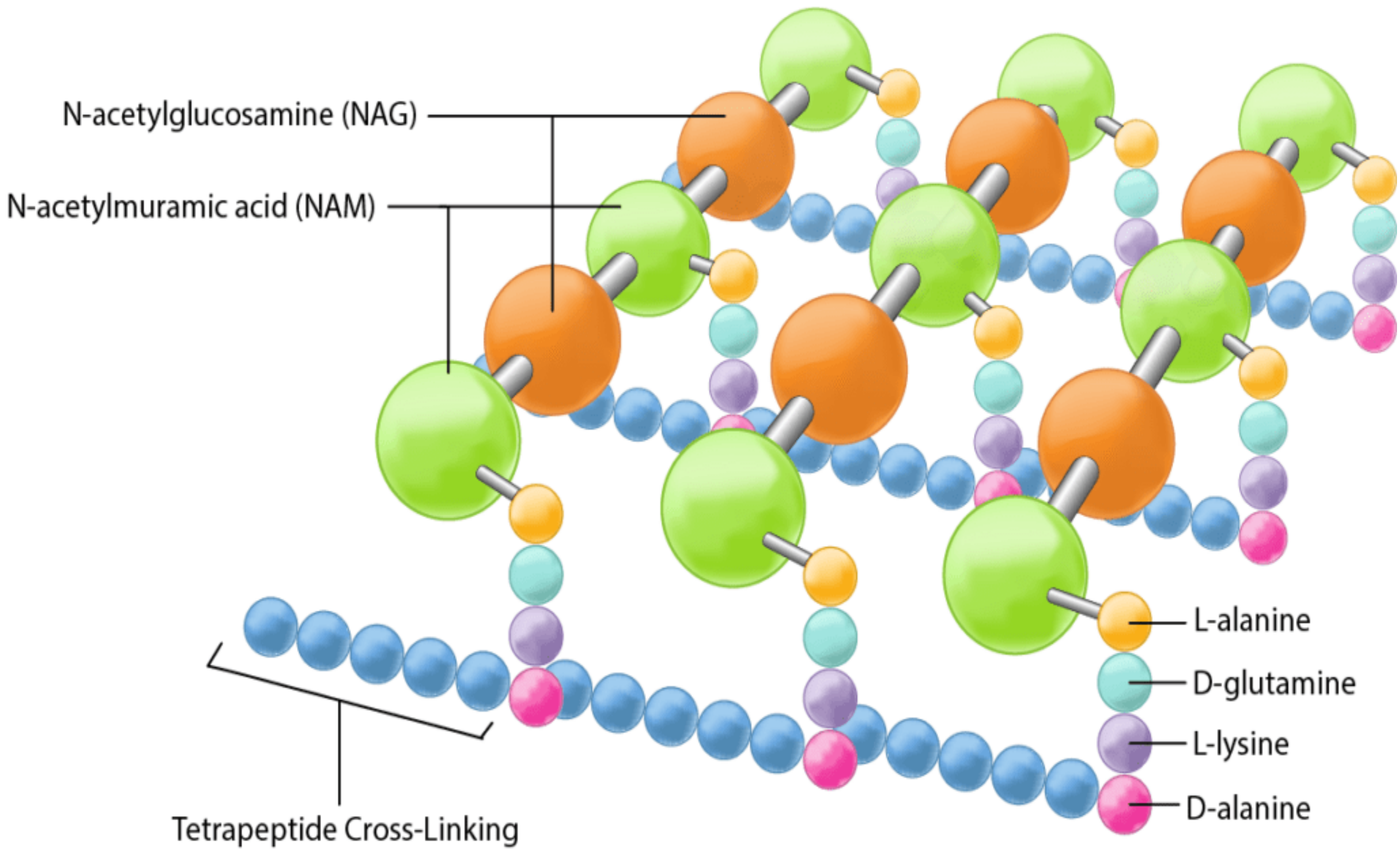
- **Gram-negative bacteria**
  - Peptidoglycan
  - Periplasmic space
  - Outer membrane
  - Proteins
  - Lipopolysaccharide

Peptidoglycan

- Polymer of disaccharide
- Also known as murein,
- is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of bacteria (but not Archaea), forming the cell wall.
- The sugar component consists of alternating residues of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM).
- Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids.

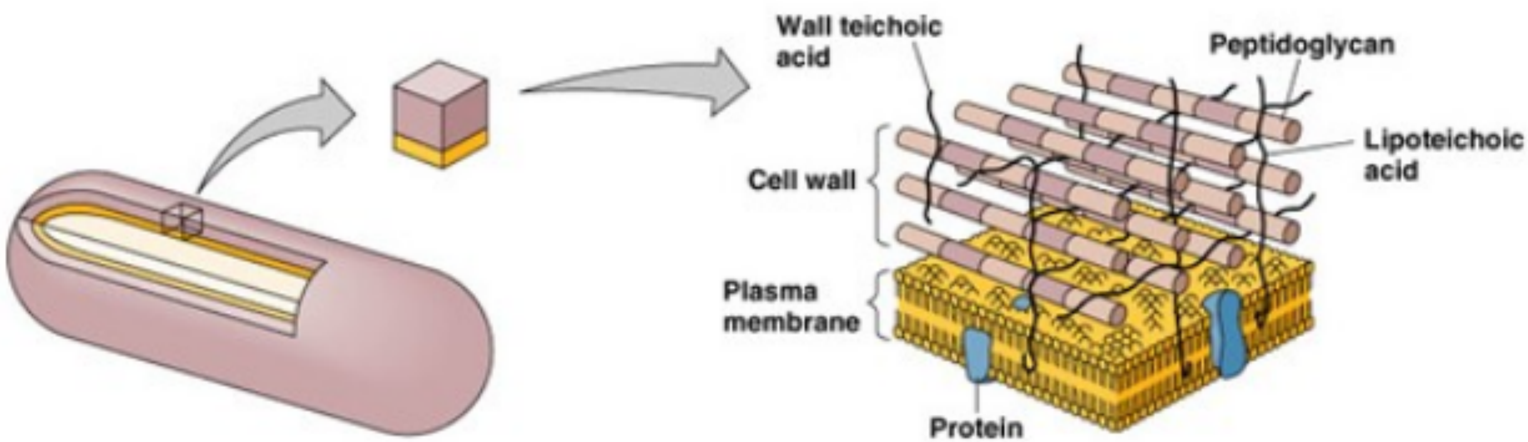


N-acetylmuramic acid-N-acetylglucosamine


N-acetylglucosamine (NAG)
N-acetylmuramic acid (NAM)
L-alanine
D-glutamine
L-lysine
D-alanine
Tetrapeptide Cross-Linking

Gram positive bacterial cell wall

- Teichoic acid
  - Lipoteichoic acid links to plasma membrane
  - Wall teichoic acid links to peptidoglycan
- May regulate movement of cations
- Polysaccharides provide antigenic variation



(b) Gram-positive cell wall

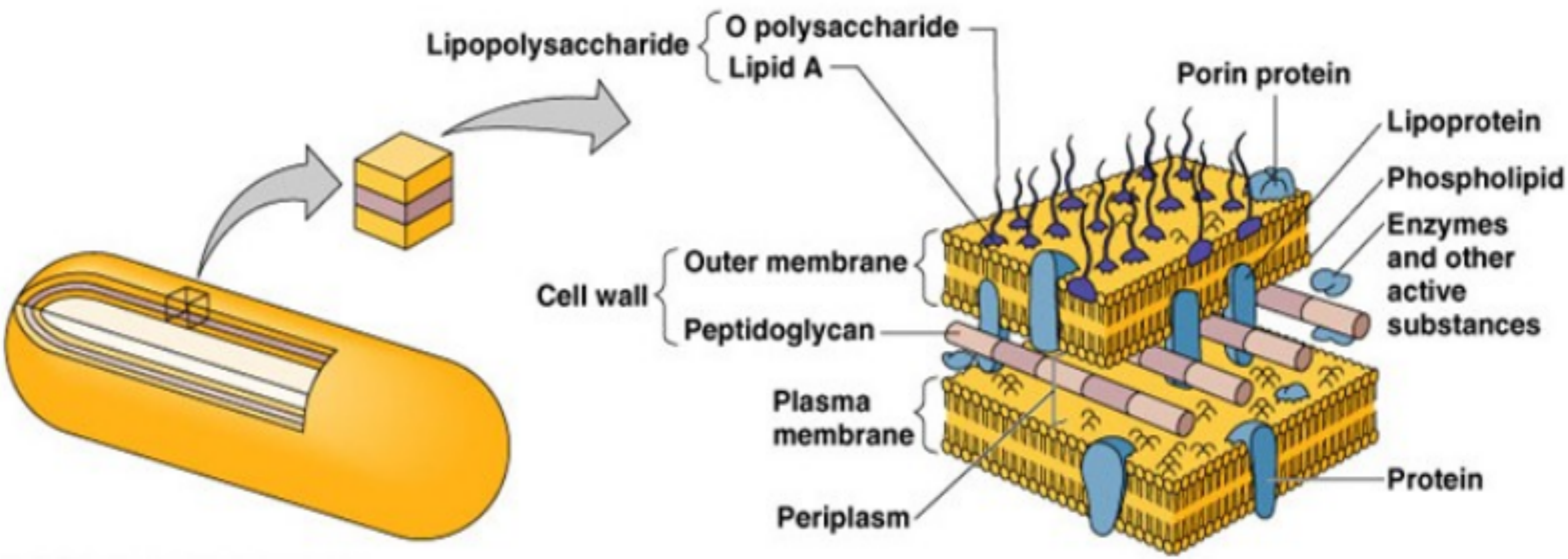
Teichoic Acids

- Teichoic acid is believed to play several important roles for the cell, such as generation of the net negative charge of the cell, which is essential for development of a proton motive force.
- Teichoic acid contributes to the overall rigidity of the cell wall, which is important for the maintenance of the cell shape, particularly in rod-shaped organisms.

Teichoic Acids

- There is also evidence that teichoic acids participate in cell division, by interacting with the peptidoglycan biosynthesis machinery.
- Lastly, teichoic acids appear to play a role in resistance to adverse conditions such as high temperatures and high salt concentrations, as well as to β-lactam antibiotics.
- Teichoic acids can either be covalently linked to peptidoglycan (**wall teichoic acids or WTA**) or connected to the cell membrane via a lipid anchor, in which case it is referred to as **lipoteichoic acid**.

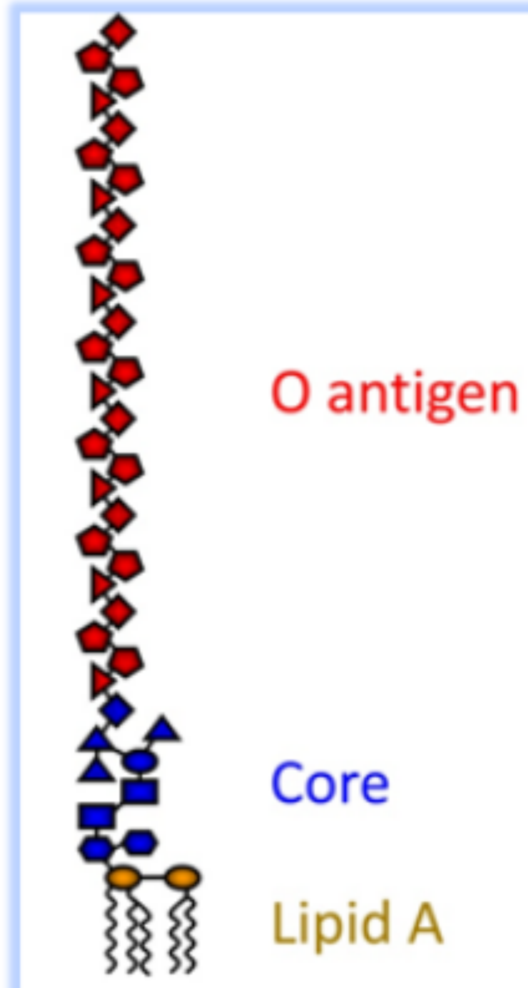
Gram negative bacterial cell walls



(c) Gram-negative cell wall

Bacterial cell structure
Cell wall – Lipopolysaccharide

- **O-specific polysaccharide:**
  - Induces specific immunity **(O-antigen)**
- **Common core polysaccharide:**
  - Same in all Gram-negative bacteria
- **Lipid A:**
  - Responsible for **primary toxicity**

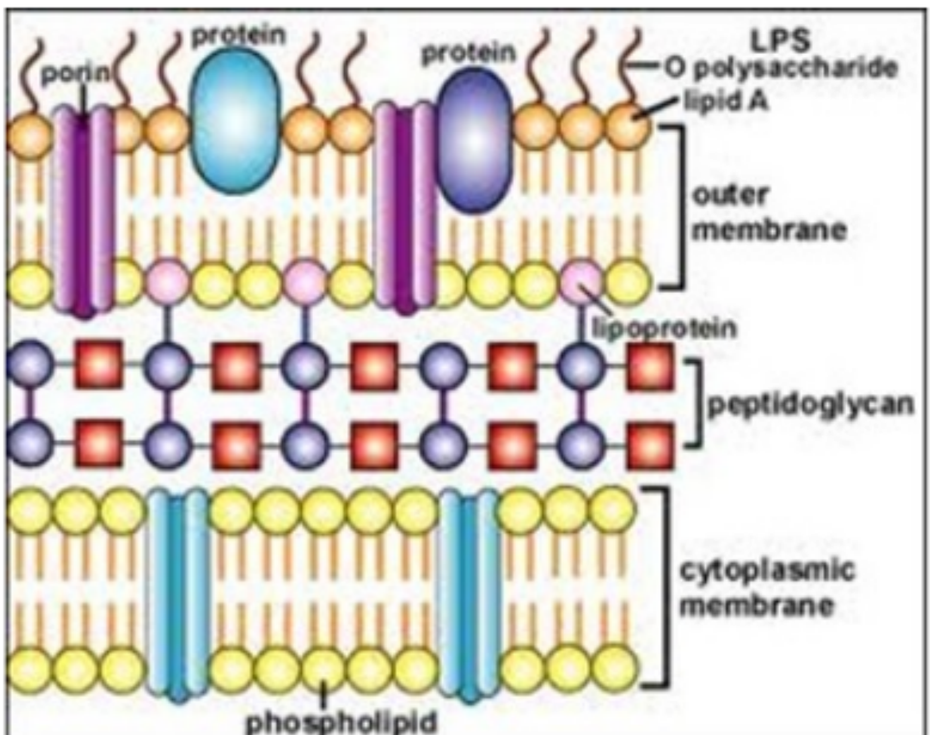


Lipopolysacharide (LPS)

LPS is known to serve many different functions for the cell, such as contributing to the net negative charge for the cell, helping to stabilize the outer membrane, and providing protection from certain chemical substances by physically blocking access to other parts of the cell wall. In addition, LPS plays a role in the host response to pathogenic gram negative bacteria.

Bacterial cell structure
Cell wall – Lipopolysaccharide

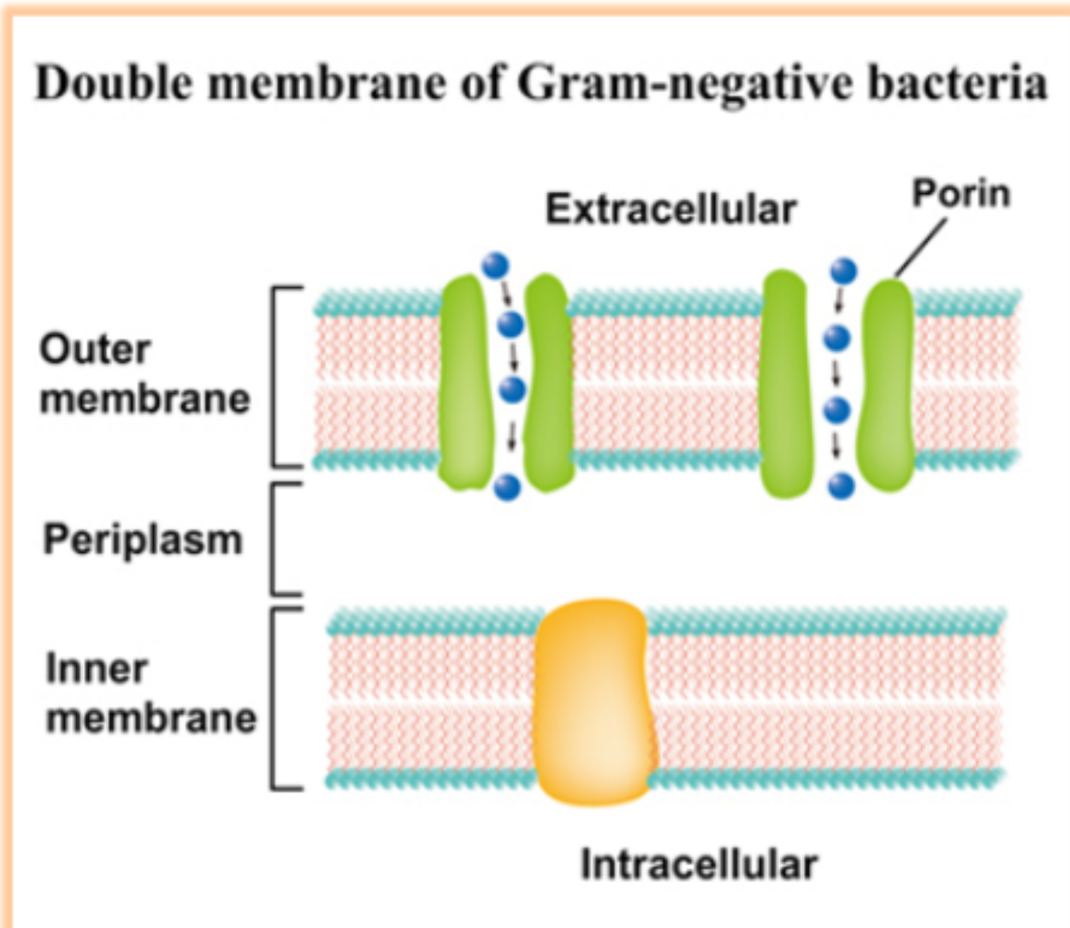
- Lipopolysaccharide (LPS) → Endotoxin of Gram-negative bacteria



Bacterial cell structure
Cell wall – Outer membrane

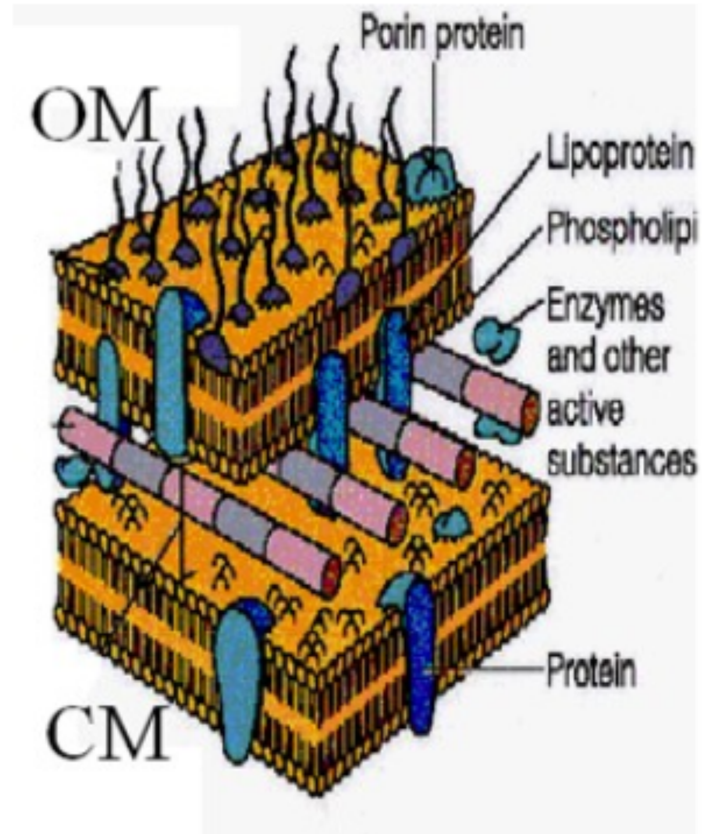
- Possess special channels **(porins)** : passive diffusion of low-molecular-weight hydrophilic compounds (sugars, amino acids and ions)
- Large antibiotic molecules penetrate slowly: antibiotic resistance!

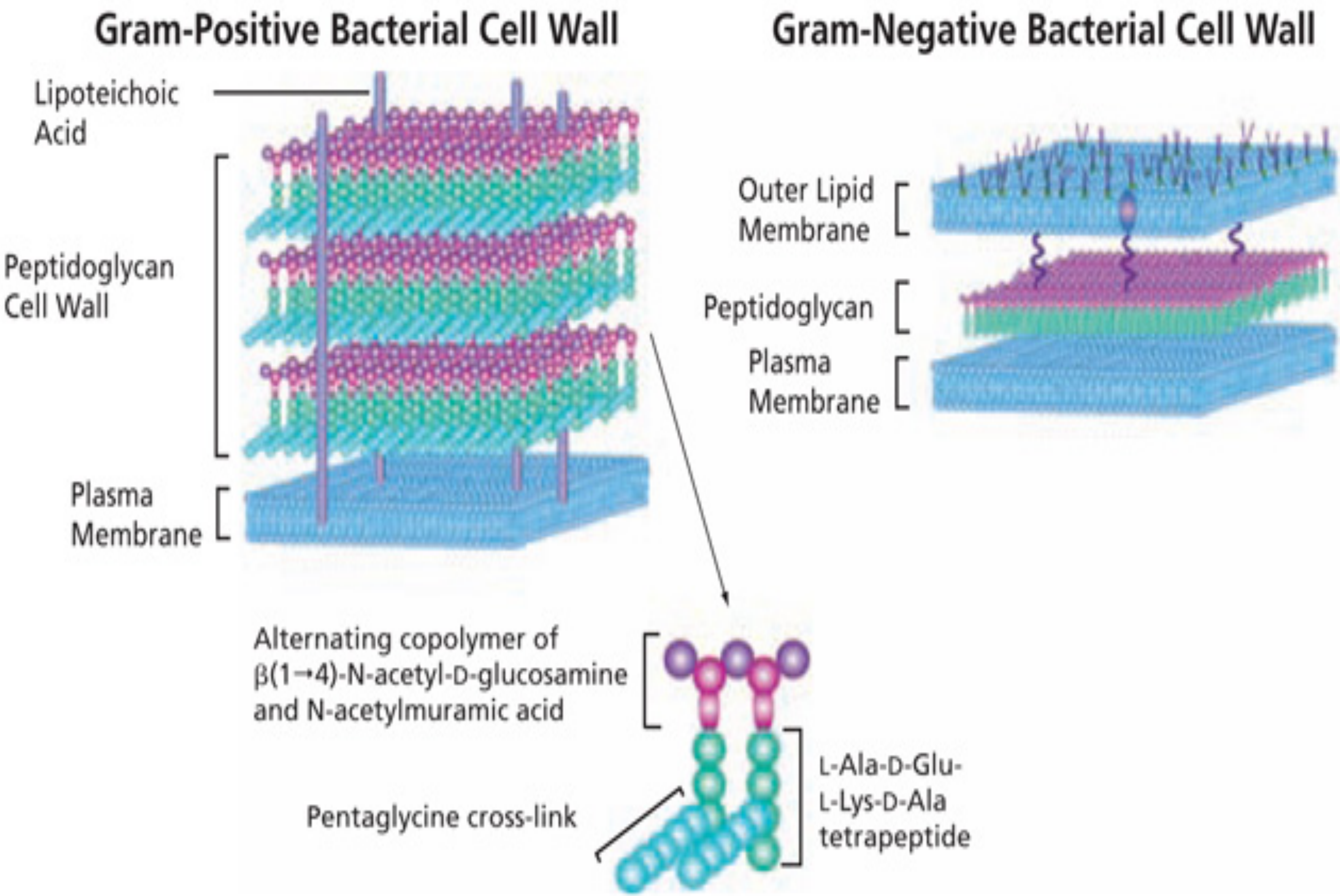
Bacterial cell structure
Cell wall – Outer membrane



Periplasm

- The periplasm is the "stuff" in that space,
  - A hydrated gel including the PG
  - Binding proteins that aid in transport
  - Hydrolytic enzymes for breaking down large molecules
  - Chemoreceptor proteins that help direct swimming
  - Enzymes for synthesizing PG, OM

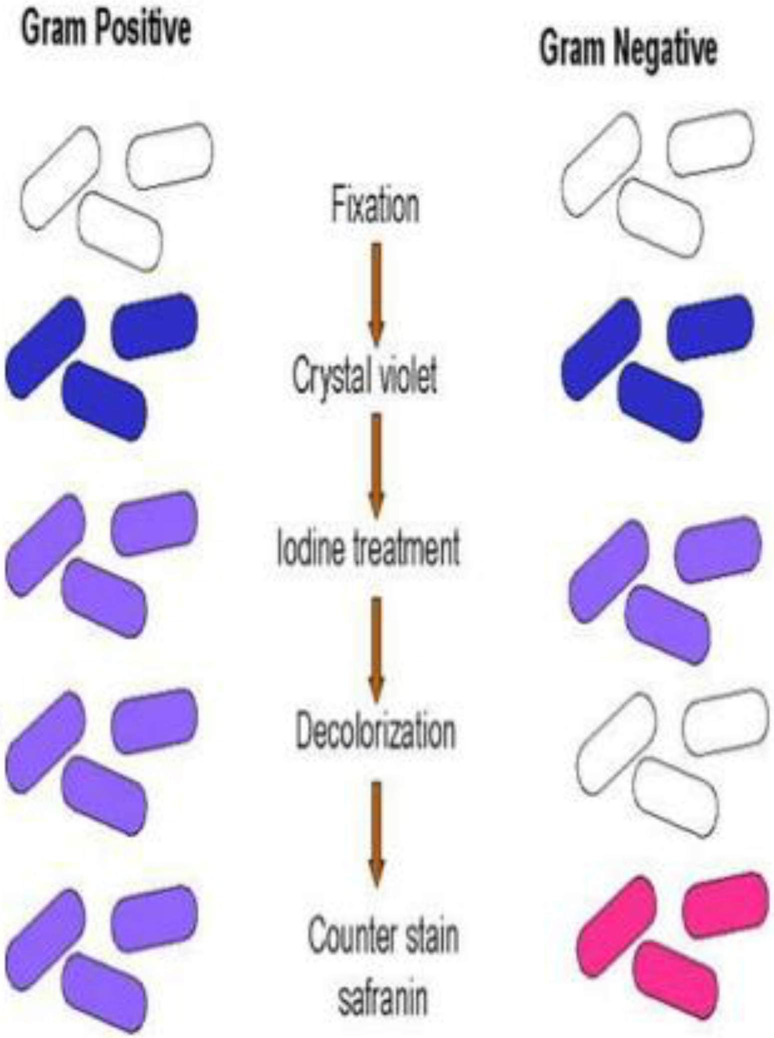


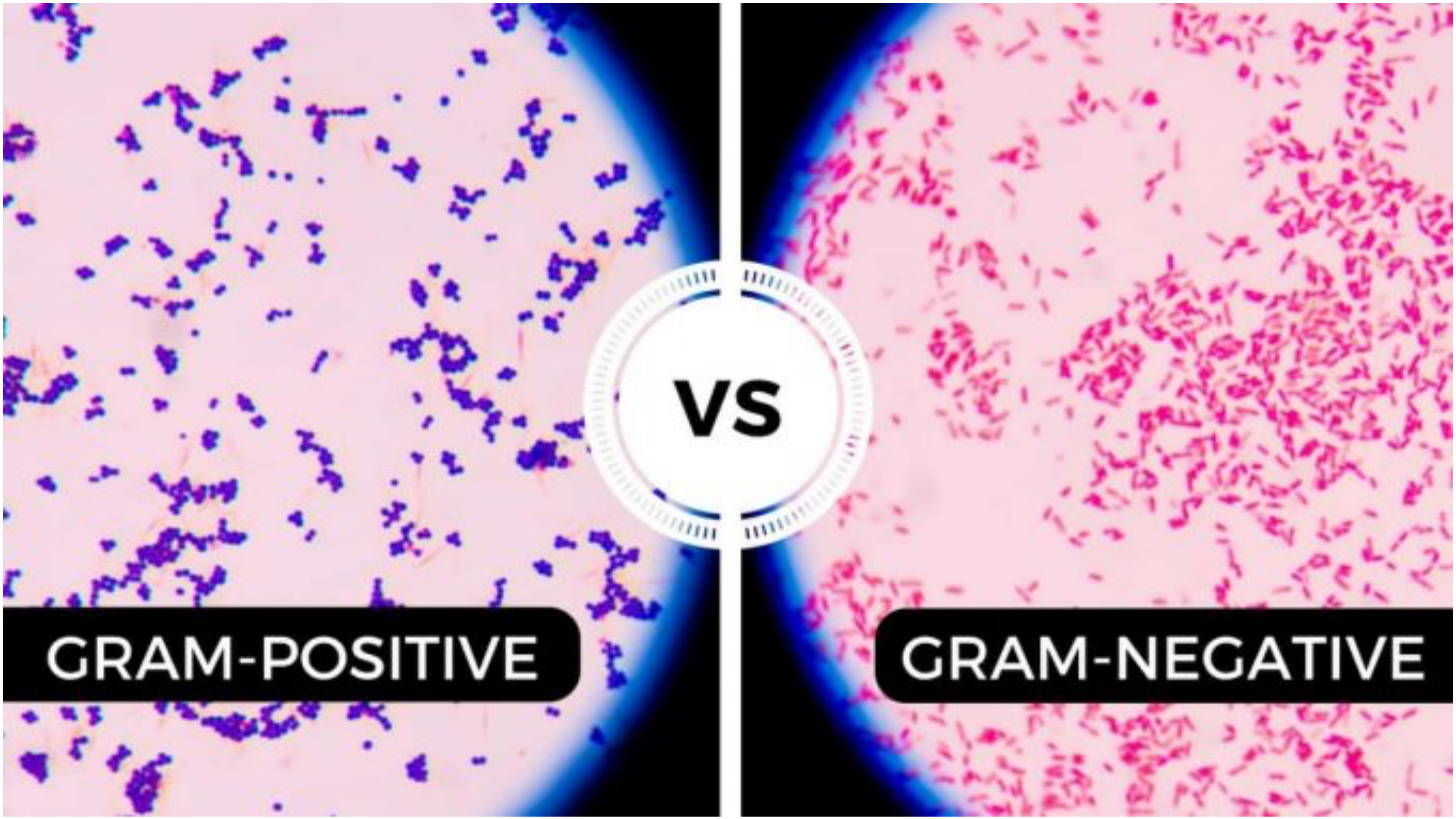

Gram-Positive Bacterial Cell Wall
Gram-Negative Bacterial Cell Wall
Lipoteichoic
Acid
Peptidoglycan
Cell Wall
Plasma
Membrane
Outer Lipid
Membrane
Peptidoglycan
Plasma
Membrane
Alternating copolymer of
β(1→4)-N-acetyl-D-glucosamine
and N-acetylmuramic acid
Pentaglycine cross-link
L-Ala-D-Glu-
L-Lys-D-Ala
tetrapeptide

Gram Stain

Principle of staining technique:

1. Primary stain:- Crystal Violet
2. Mordant(fixes the dye):- Iodine
3. Decolorizing agent:-Alcohol/Acetone
4. Counter stain;- Safranin



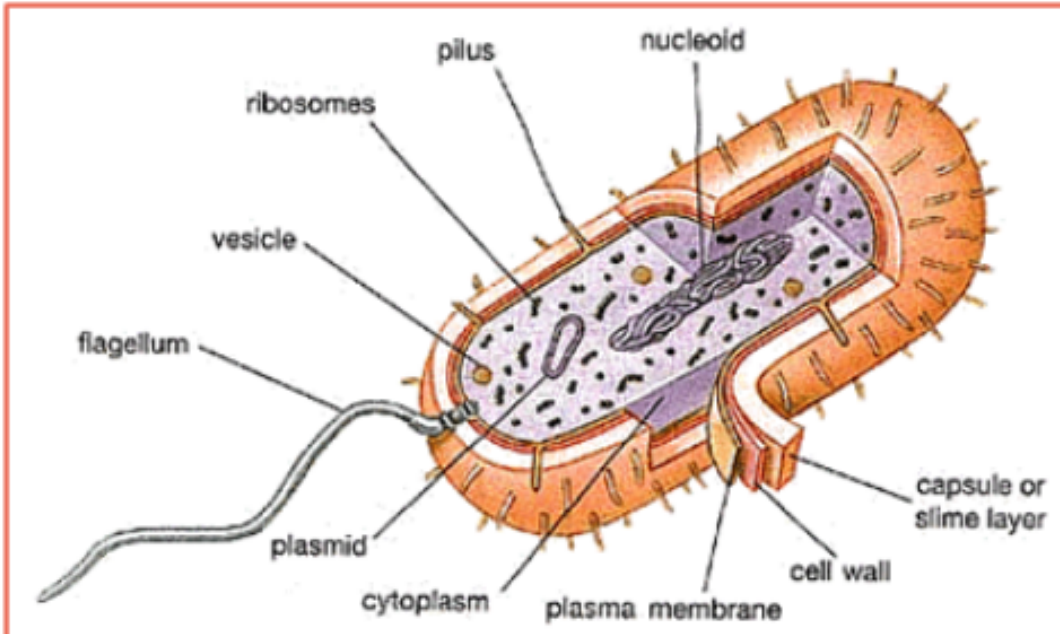

GRAM-POSITIVE
VS
GRAM-NEGATIVE

Inclusion Bodies

Metachromatic granules (volutin)	Phosphate reserves Corynebacterium diptheria
Polysaccharide granules Example: Glycogen	Energy reserves
Lipid inclusions (PHBs)	Energy reserves Mycobacterium, Bacillus, Azotobacter
Sulfur granules	Energy reserves Corynebacterium diptheria
Carboxysomes	Ribulose 1,5-diphosphate carboxylase for CO_2 fixation Nitrifying Bacteria
Gas vacuoles/Gas vesicles	Protein covered cylinders
Magnetosomes	Iron oxide (destroys H_2O_2) Aquaspirillum magnetotacticum

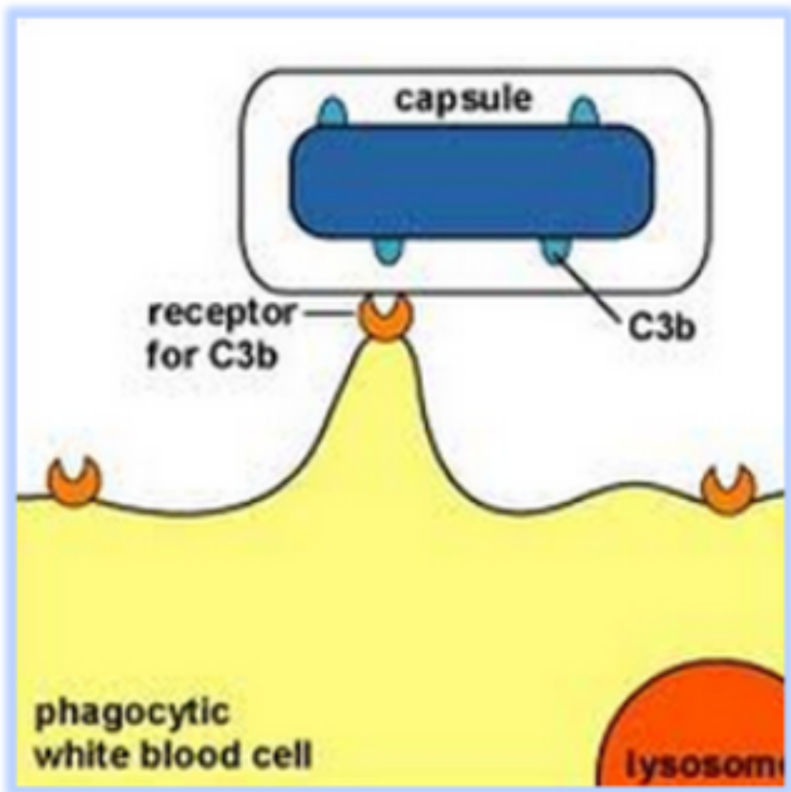
Bacterial cell structure
External structures

- Capsule/slime layer
- Flagella
- Fimbriae (pili)



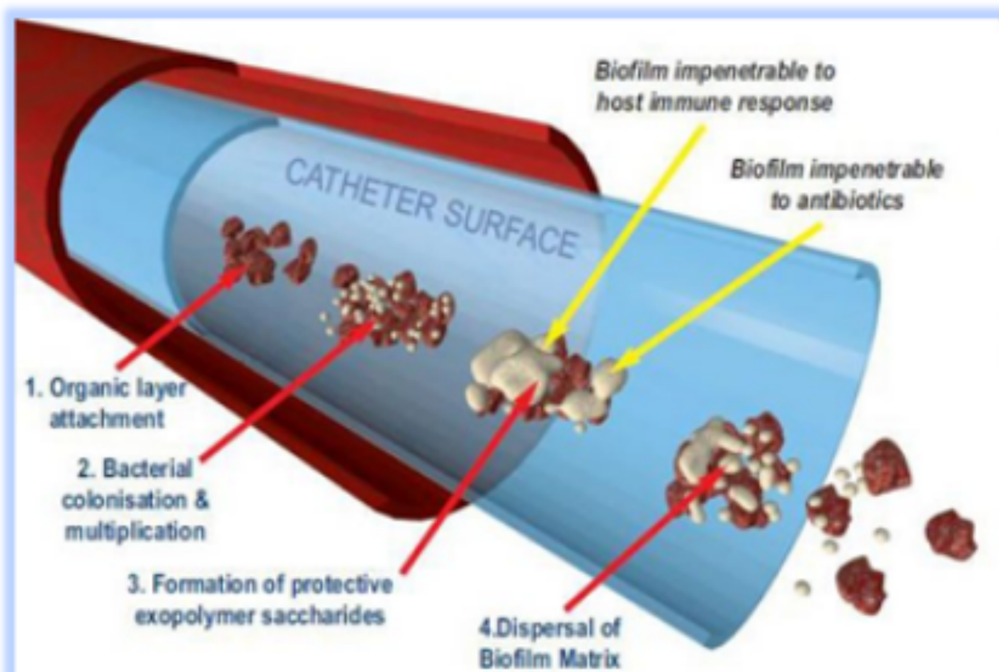
Bacterial cell structure
External structures – Capsule/slime layer

- Protects bacteria against **phagocytosis**

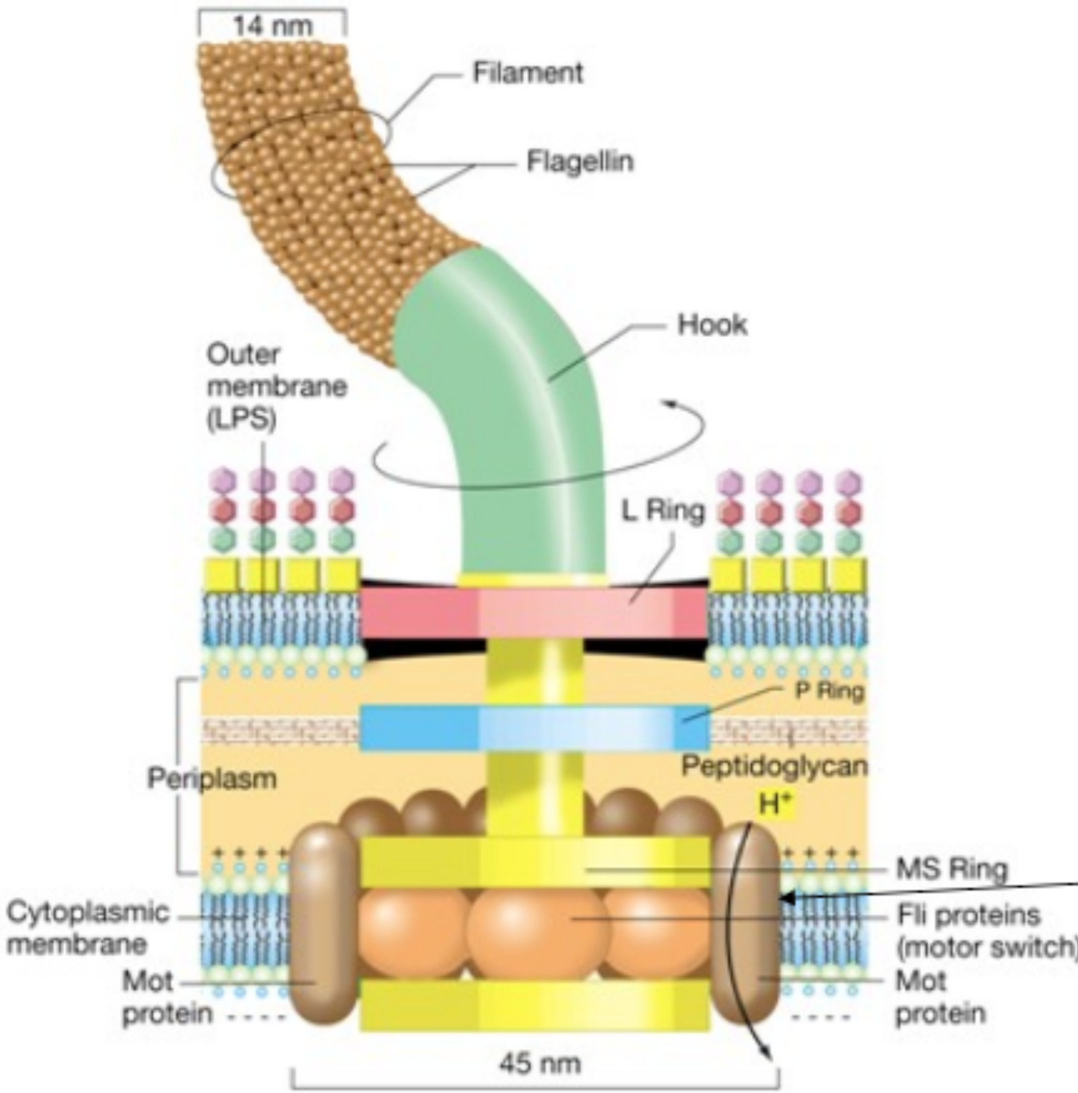


Bacterial cell structure
External structures – Capsule/slime layer

- Plays a role in **adherence** (biofilm formation)
- Artificial valves, catheters,...

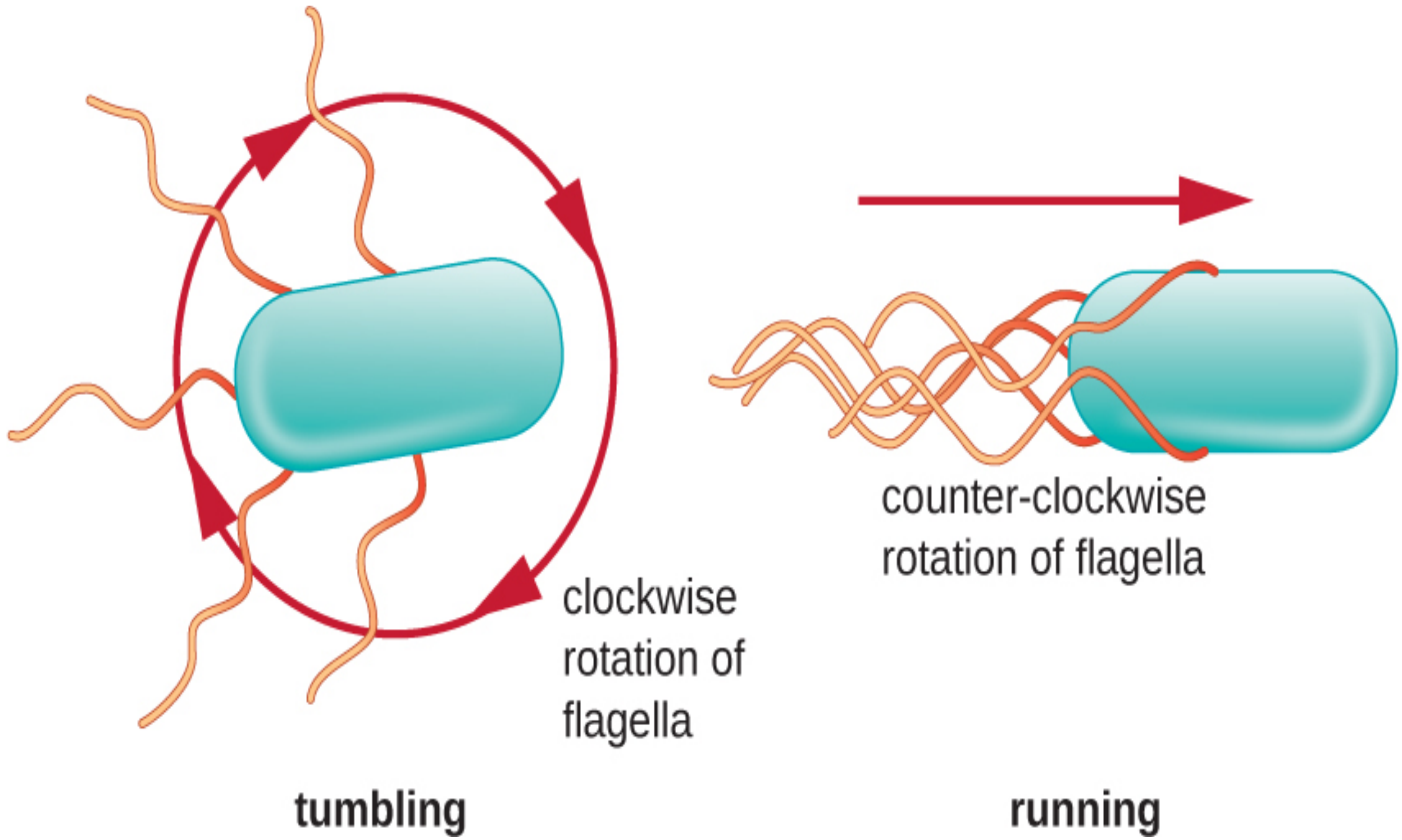


The Flagellum



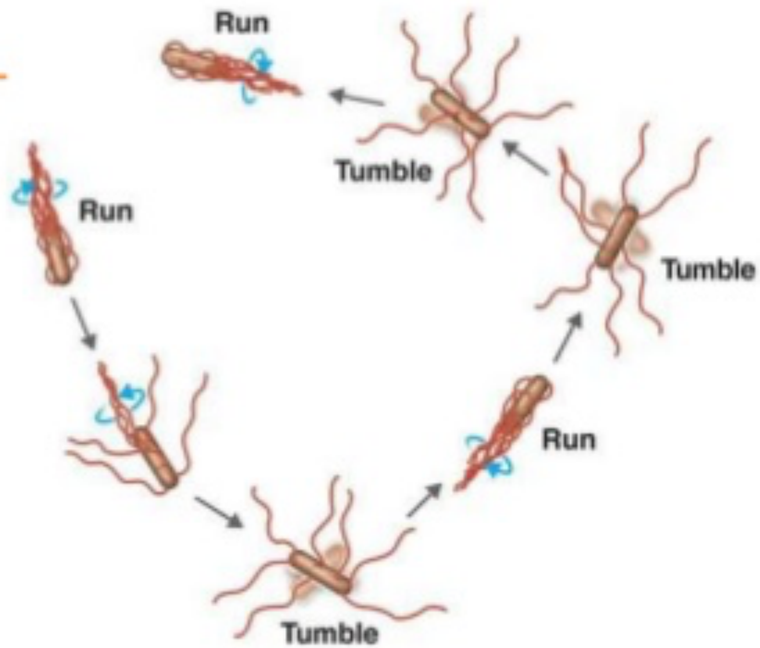
1000 H^+ / rotation

> 40 genes involved

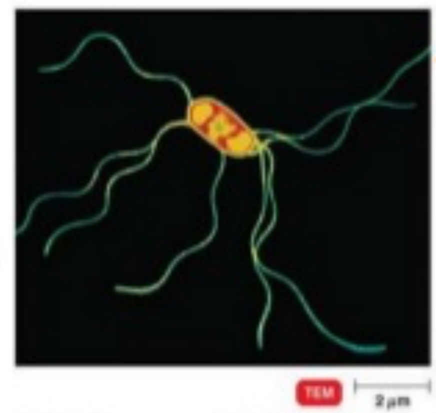

clockwise
rotation of
flagella
tumbling
counter-clockwise
rotation of flagella
running

Motility

- Rotate flagella to run or tumble
- Move toward or away from stimuli (taxis)
- The stimuli include chemicals like oxygen, ribose, galactose – Chemotaxis.
- Stimuli can be light – Phototaxis.



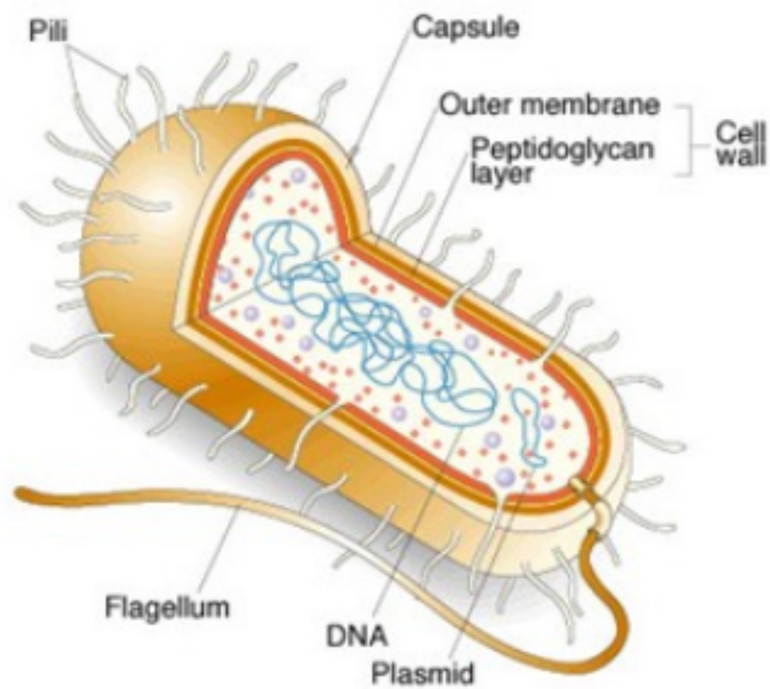
(a) A bacterium running and tumbling. Notice that the direction of flagellar rotation (blue arrows) determines which of these movements occurs. Gray arrows indicate direction of movement of the microbe.



(b) A *Proteus* cell in the swarming stage may have more than 1000 peritrichous flagella.

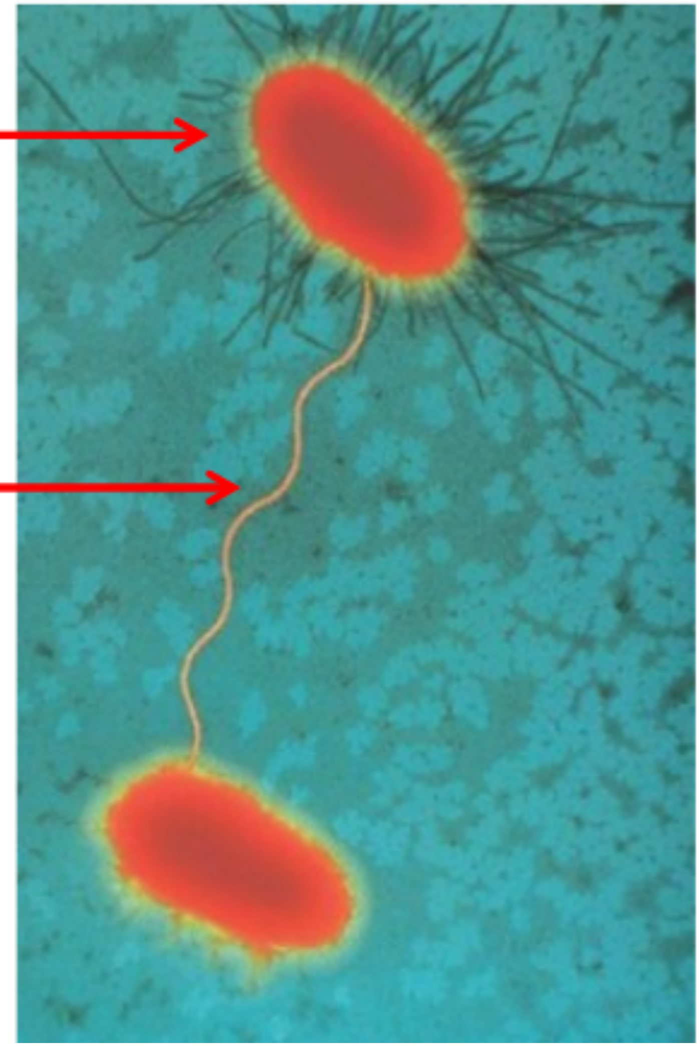
Bacterial cell structure
External structures – Fimbriae (pili)

- **Gram-negative bacteria**
- Shorter and finer than flagella
- Composed of protein subunits called **pilins**
- **Adhesins:** minor proteins at the tips of pili; responsible for attachment



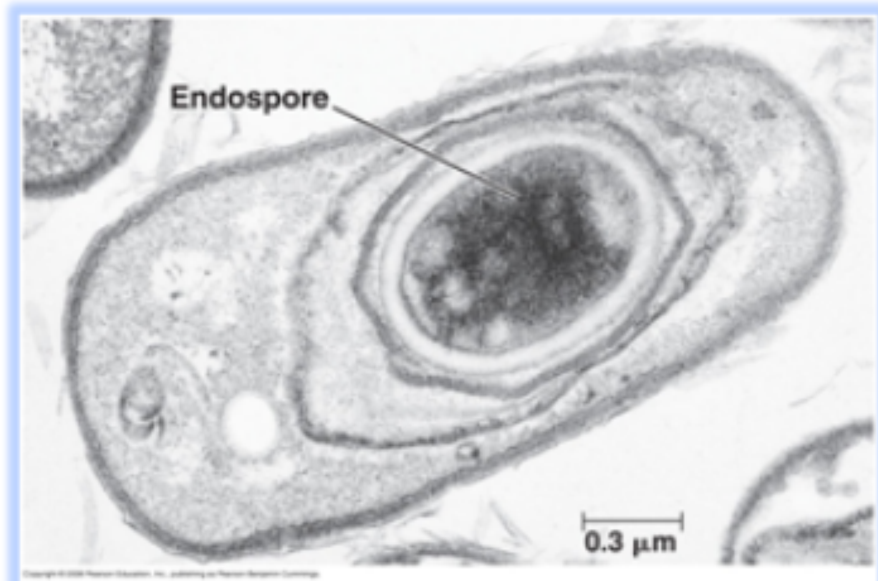
Bacterial cell structure
External structures – Fimbriae (pili)

- **Ordinary pili:**
  - **Adherence** of bacteria to host cells
- **Sex pili:**
  - Attachment of donor and recipient cells in bacterial **conjugation**



Bacterial cell structure
Endospores (spores)

- Distinct bacterial genera; the most commons:
    - *Bacillus* (Gram-positive aerobic rod)
    - *Clostridium* (Gram-positive anaerobic rod)
- Response to environmental conditions (depletion of nutrients)



Endospores form within the Cell

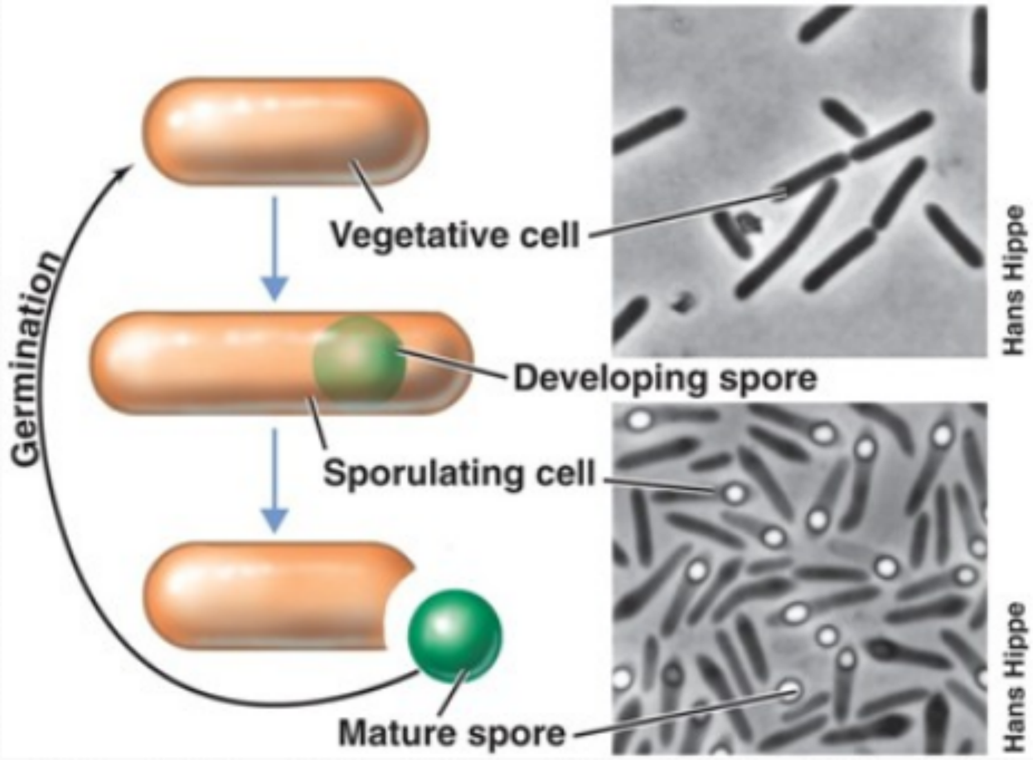
- Endospore is dormant stage of some bacterium that allows it to survive unfavorable conditions that would normally be lethal such as extreme drought or heat
- Endospores are resistant against;
  - Drought
  - Low nutrient conditions
  - Radiation
  - High temperatures
  - Various chemical disinfectants

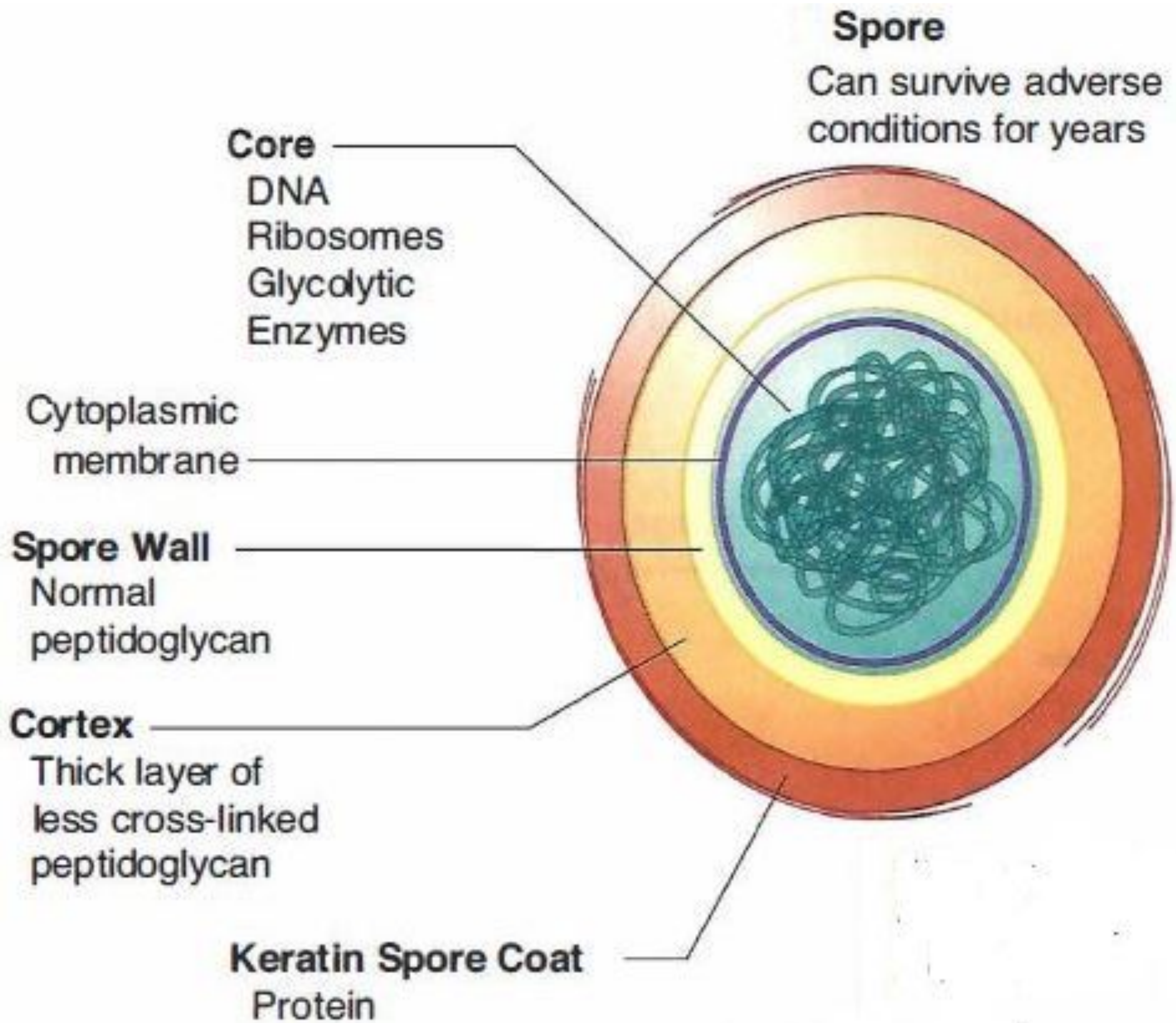
Endospore Function

- Endospores are ultimately <u>protection for the bacterial genome</u>
- Spores form within the cell and contain a full copy of the bacterial genome
- Endospores are not a form of reproduction, because only one new cell germinates from each spore
- Spores can be variable in size and location within the cell

Sporulation or Sporogenesis

- Process of endospore formation within a vegetative (parent) cell
- Germination = return of an endospore to its vegetative state




Spore
Can survive adverse conditions for years
Core
DNA
Ribosomes
Glycolytic
Enzymes
Cytoplasmic membrane
Spore Wall
Normal peptidoglycan
Cortex
Thick layer of less cross-linked peptidoglycan
Keratin Spore Coat
Protein

Structure	Chemical Composition	Function
Essential components		
Cell wall		
Peptidoglycan	Glycan (sugar) backbone with peptide side chains that are cross-linked	Gives rigid support, protects against osmotic pressure, is the site of action of penicillins and cephalosporins, and is degraded by lysozyme
Outer membrane of gram-negative bacteria	Lipid A	Toxic component of endotoxin
	Polysaccharide	Major surface antigen used frequently in laboratory diagnosis
Surface fibers of gram-positive bacteria	Teichoic acid	Major surface antigen but rarely used in laboratory diagnosis
Plasma membrane	Lipoprotein bilayer without sterols	Site of oxidative and transport enzymes
Ribosome	RNA and protein in 50S and 30S subunits	Protein synthesis; site of action of aminoglycosides, erythromycin, tetracyclines, and chloramphenicol
Nucleoid	DNA	Genetic material
Mesosome	Invagination of plasma membrane	Participates in cell division and secretion
Periplasm	Space between plasma membrane and outer membrane	Contains many hydrolytic enzymes, including β-lactamases
Nonessential components		
Capsule	Polysaccharide[1]	Protects against phagocytosis
Pilus or fimbria	Glycoprotein	Two types: (1) mediates attachment to cell surfaces; (2) sex pilus mediates attachment of two bacteria during conjugation
Flagellum	Protein	Motility
Spore	Keratinlike coat, dipicolinic acid	Provides resistance to dehydration, heat, and chemicals
Plasmid	DNA	Contains a variety of genes for antibiotic resistance and toxins
Granule	Glycogen, lipids, polyphosphates	Site of nutrients in cytoplasm
Glycocalyx	Polysaccharide	Mediates adherence to surfaces

[1]Except in *Bacillus anthracis*, in which it is a polypeptide of D-glutamic acid.

Bacterial Pathogenesis

1. Infection: growth and multiplication of a microbe in or on the body with or without the production of disease.
2. The capacity of a bacterium to cause disease reflects its relative "Pathogenicity."
3. Virulence is the measure of the pathogenicity of a microorganism.
4. Pathogenesis refers both to the mechanism of infection and to the mechanism by which disease develops.

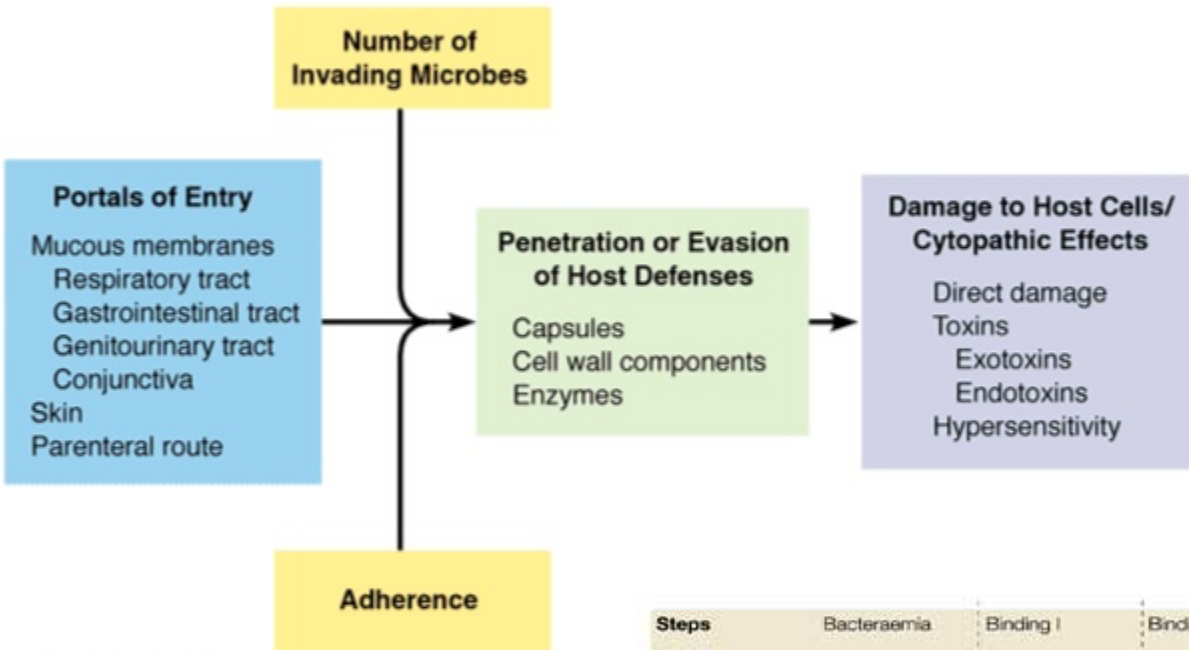
Characteristics of Pathogenic Bacteria

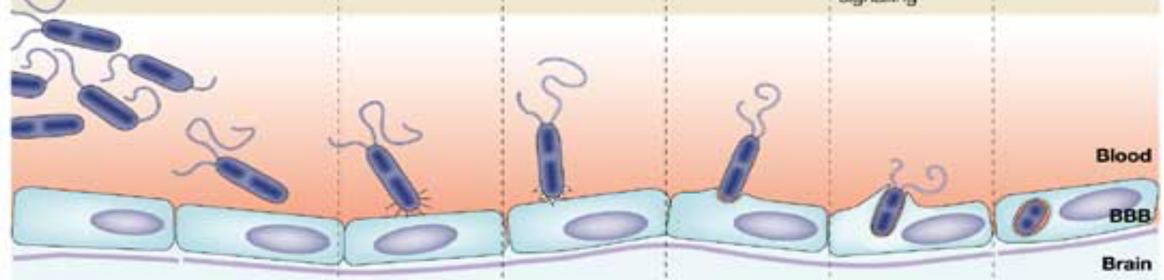
1. Transmissibility
2. Adherence to host cells
3. Invasion of host cells and tissue
4. Evasion of the host immune system
5. Toxigenicity

A bacterium may cause diseases by

1. Destroying tissue (invasiveness)
2. Producing toxins (toxigenicity)
3. Stimulating overwhelming host immune responses

Bacterial Mechanisms of Pathogenicity: How Microorganisms Cause Disease





Steps	Bacteraemia	Binding I	Binding II	Invasion	Cytoskeletal rearrangement/ signalling	Intracellular survival
Bacterial determinants	K1 capsule O-LPS	Type 1 fimbriae (FimH) S fimbriae (?)	OmpA	CNF1 Ibe proteins AslA TraJ	CNF1 OmpA Ibe proteins (?)	K1 capsule
Host factors	Serum Neutrophils	CD48 sialoglycoprotein/ glycolipid (?)	gp96	37LRP / 67LR 45-kDa receptor (IbeA)	FAK PI3K Rho cPLA2	Rab7
References	12,26	28,30–32	27,29,38	33–37,39	37,42–44	24,47,48

Virulence factors of bacteria

I. adherence / colonization:

- pili
- motility / chemotaxis (flagella)
- outer membrane proteins

II. infectious process:

- exotoxins / endotoxins
- type III / type IV secretion processes
- intracellular growth (invasion)

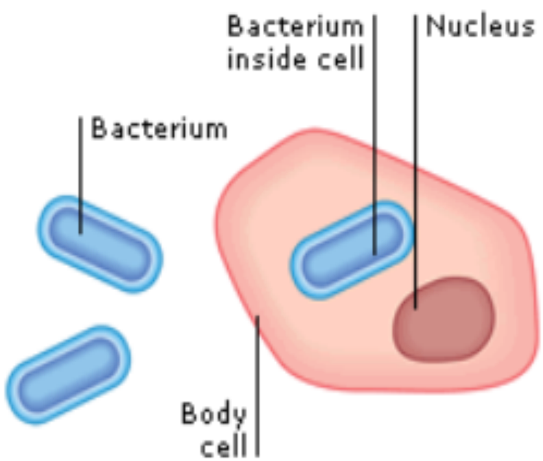
III. protection against host defense:

- capsule / cell wall / outer membrane
- antigenic variation
- biofilm formation

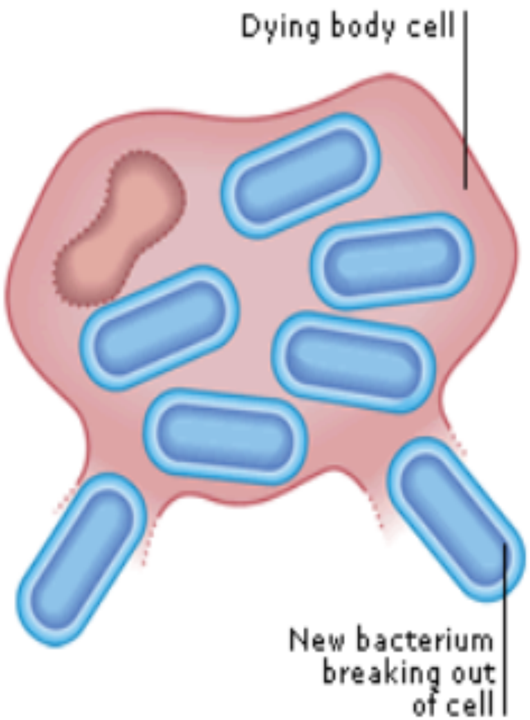
Bacterial invasion of a cell

A few bacteria damage tissues in the human body not by secreting toxins but by directly invading the cells. Once inside body cells, the bacteria reproduce and eventually burst out, rupturing the cell membrane.

Different bacteria are specifically attracted to certain body cells. Bacteria enter the cell through the membrane and use the cell nutrients.



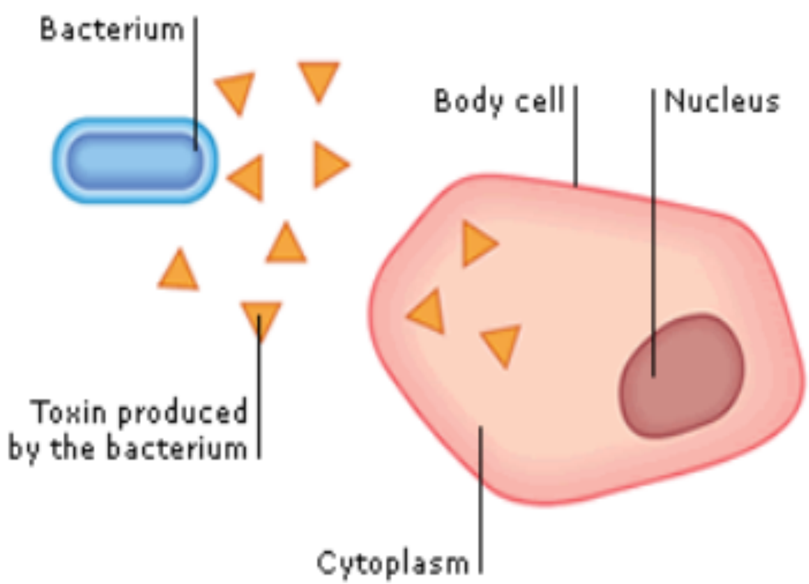
The bacteria multiply rapidly in the cell. They kill the cell by breaking its membrane then spread to other areas of the body.



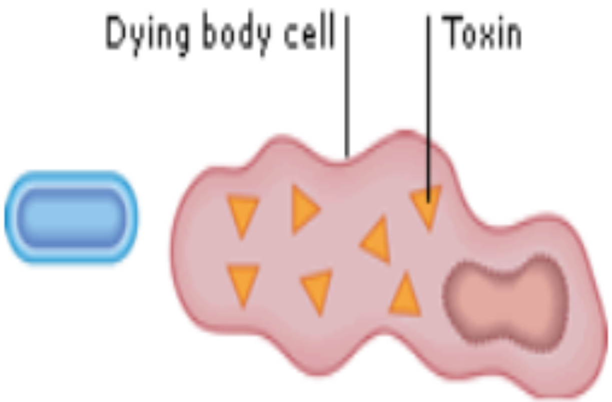
Effects of toxins

Some bacteria cause disease by producing poisonous chemicals known as toxins. These chemicals may destroy specific body cells or enter cells and alter their chemical processes. Some toxins are released from bacteria when they die and may cause shock and fever.

The toxin is released into the body by the bacterium. The toxin attaches to a body cell and is absorbed into the fluid cytoplasm.

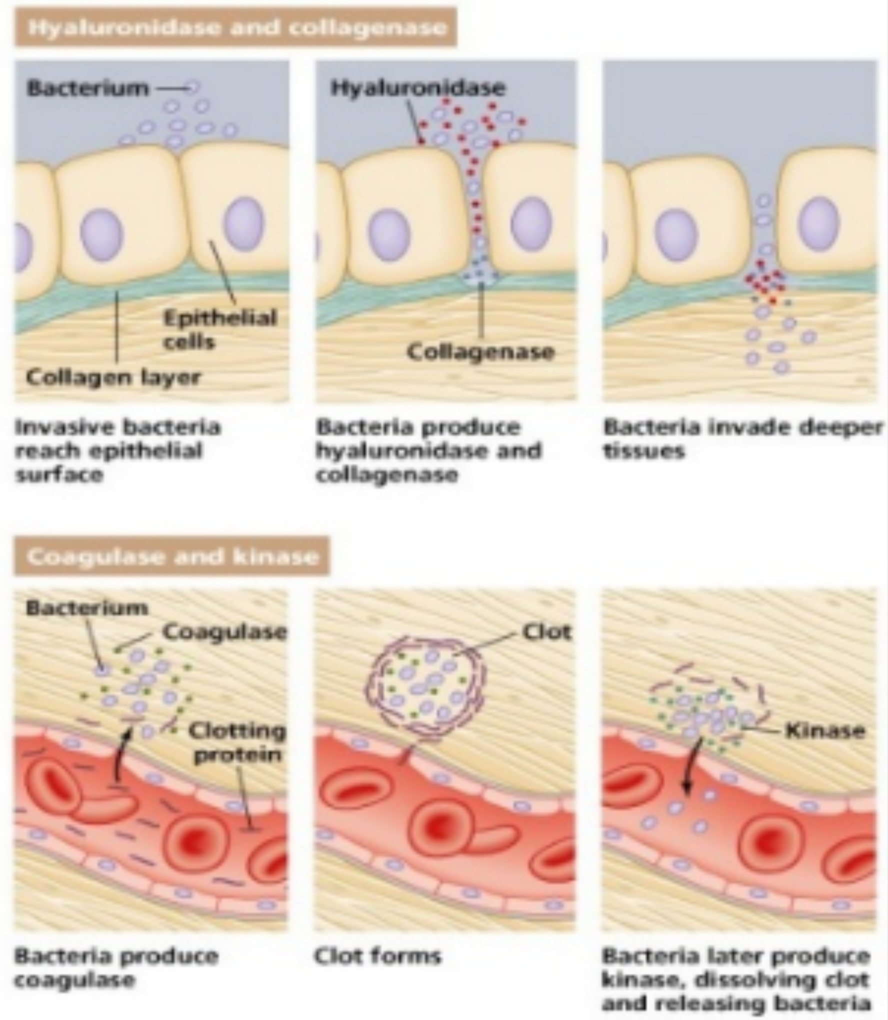


The toxin disrupts normal chemical reactions inside the cell, so that the cell is unable to function and dies.



Invasiveness

- Hyaluronidase
- Coagulase
- Streptokinase (dissolves Clots)



(a) Extracellular enzymes

Table 19.5 Basic properties of exotoxins and endotoxins

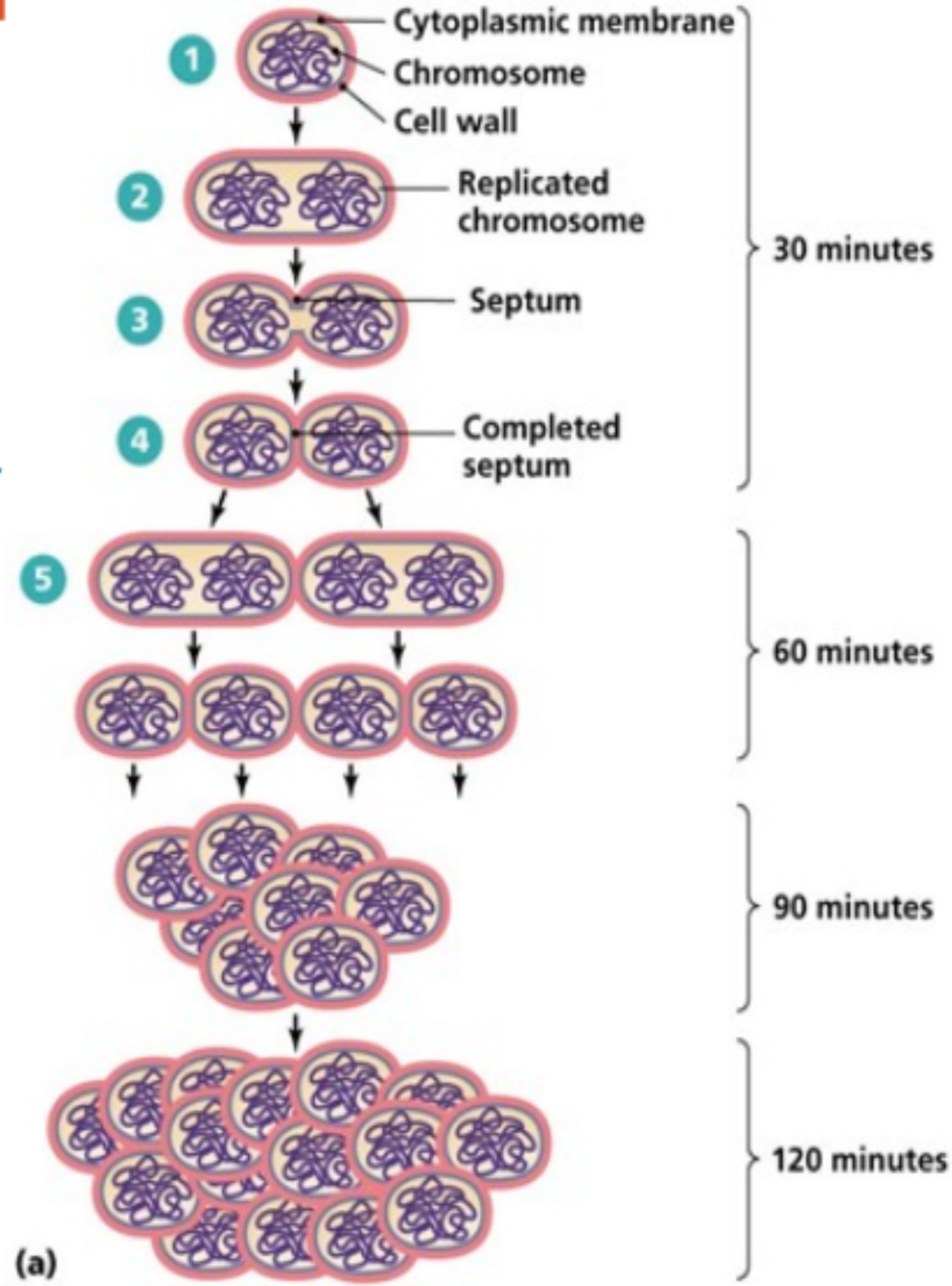
Property	Exotoxin	Endotoxin
Chemical properties	Proteins, excreted by certain gram-positive or gram-negative Bacteria; generally heat-labile	Lipopolysaccharide-lipoprotein complexes (see Figures 3.33 and 3.34); released on cell lysis as part of the outer membrane of gram-negative Bacteria; extremely heat-stable
Mode of action; symptoms	Specific; either cytotoxin, enterotoxin, or neurotoxin with defined specific action on cells or tissues	General; fever, diarrhea, vomiting
Toxicity	Highly toxic, often fatal	Weakly toxic, rarely fatal
Immunogenicity	Highly immunogenic; stimulate the production of neutralizing antibody (antitoxin)	Relatively poor immunogen; immune response not sufficient to neutralize toxin
Toxoid potential	Treatment of toxin with formaldehyde will destroy toxicity, but treated toxin (toxoid) remains immunogenic	None
Fever potential	Do not produce fever in host	Pyrogenic, often produce fever in host

Reproduction of Bacteria

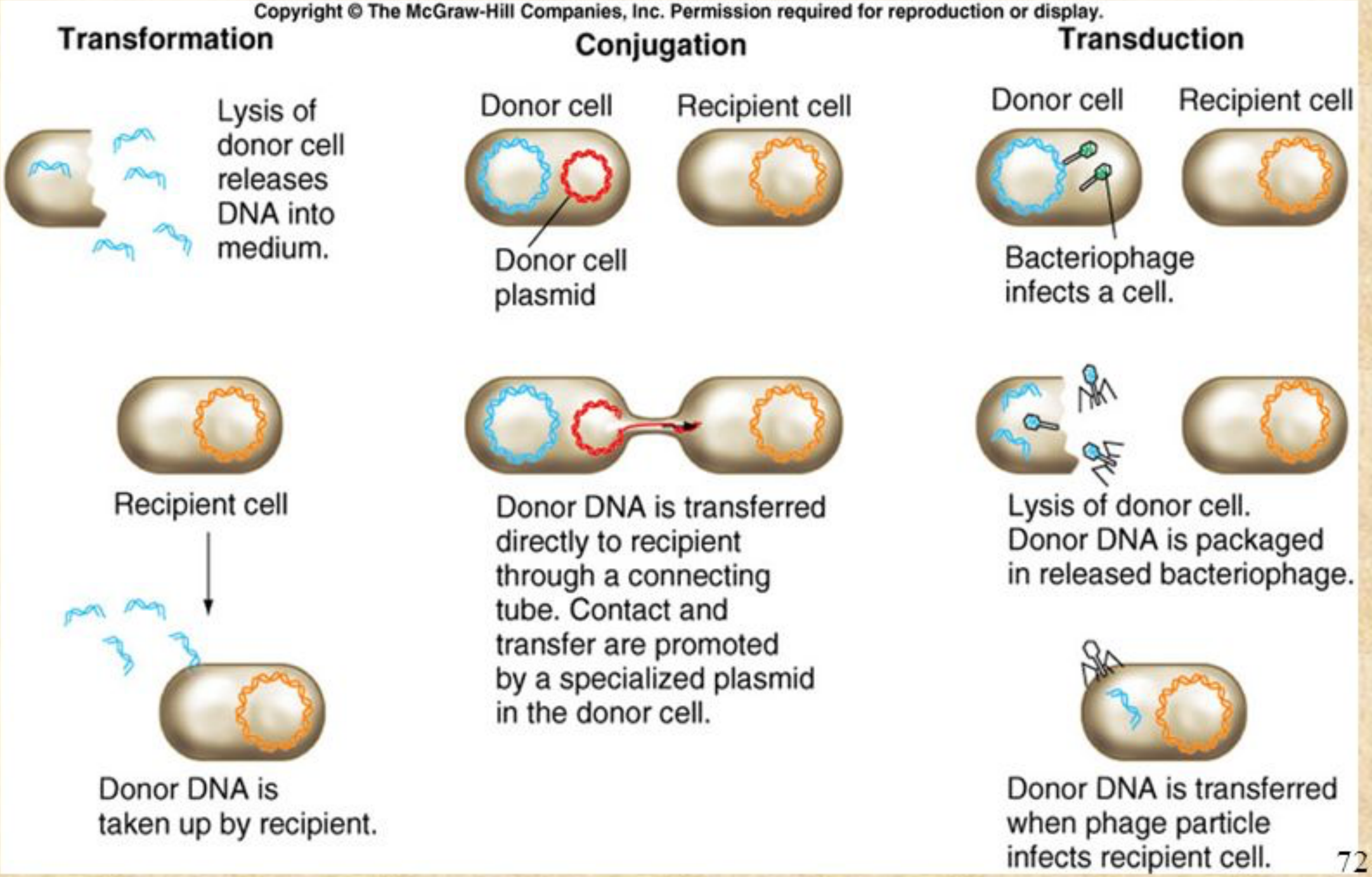
- Cell division = reproduction in prokaryotes
- Binary fission – simplified form of cell division used by bacteria
- Steps:
  - DNA is replicated
  - Cell increases in size, splits in two
  - Plasma membrane pinches together and a new cell plate forms
  - Control of the bacterial cell cycle: growth rates, biochemical signals, and environmental conditions

Exponential Growth by Binary Fission

1. DNA replication
2. Cell elongation
3. Septum formation
4. Septum completion leads to separation or further division
5. Process repeats



Three types of gene transfer



Factors required for bacterial growth

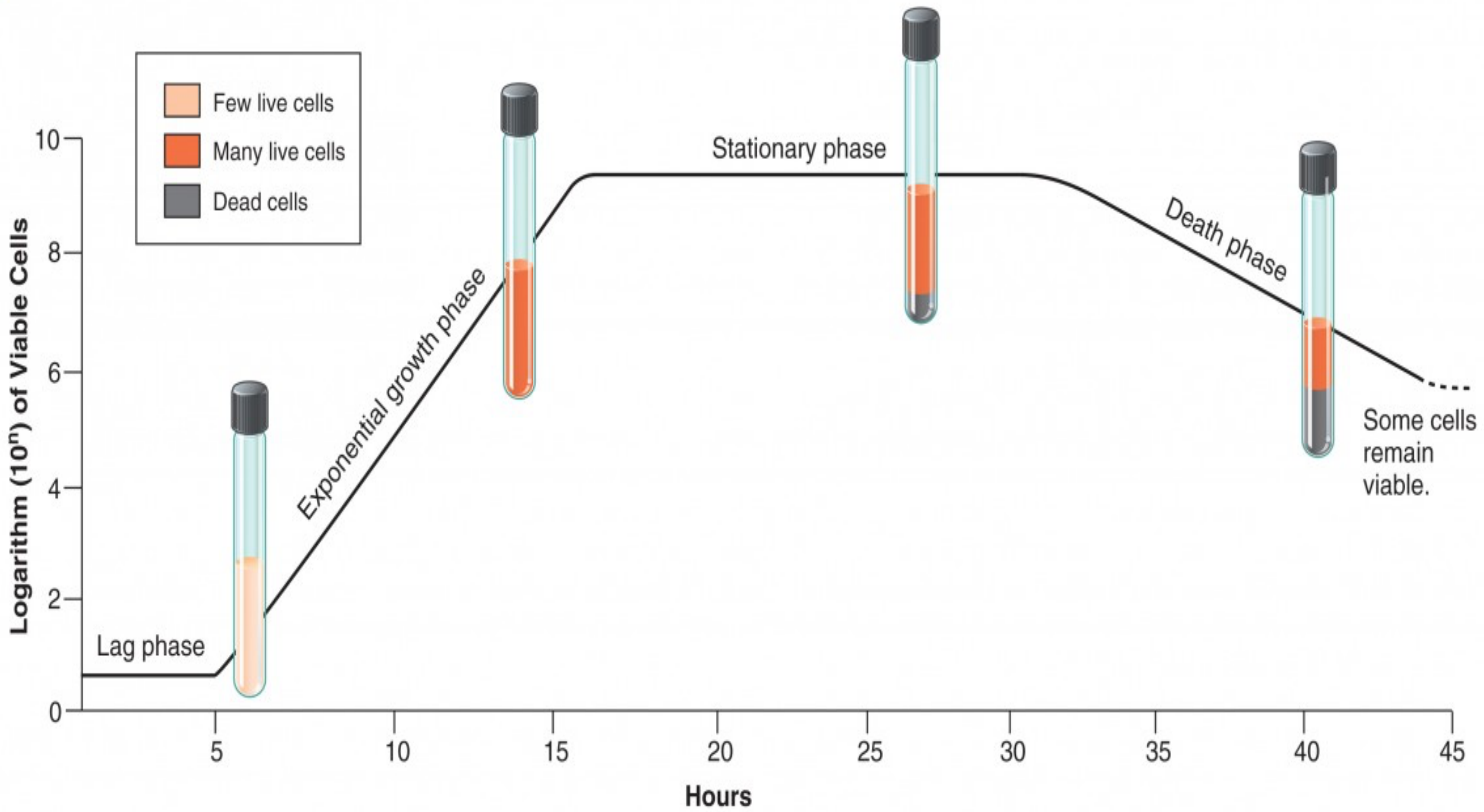
1. Source of Carbon
2. Source of energy
3. Source of basic chemicals such as Amino acids, lipids etc
4. Water
5. Trace elements

Factors determining growth conditions

1. Temperature
2. pH
3. Oxygen requirement

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Bacterial Growth Curve



Total cells in population, live and dead, at each phase.

Lag phase:

1. 1st phase
2. Cells adjust to new environment
3. Unlimited nutrient supply
4. Negligible toxic metabolites
5. Low cell count
6. Length of phase depends on organism

Log/ exponential phase:

1. Exponential cell division
2. Exhibits classic properties such as susceptibility to antibiotics, Biochemical reactions, staining properties etc.
3. High cell counts
4. Nutrient supply slowly reduces, toxic compounds accumulates

Stationary phase:

1. Accumulation of toxic compounds
2. Lack of nutrients
3. Cell division ceases, maintains in number sufficient enough to replace dying cells
4. The count remains roughly constant
5. Highly resistant. Begins to form spores

Death Phase

1. Reduction in number of cells
2. Too little nutrients, too much toxic compounds
3. Subculture can rescue in early part

Physical conditions

- Growth atmosphere
- Growth temperature
- pH

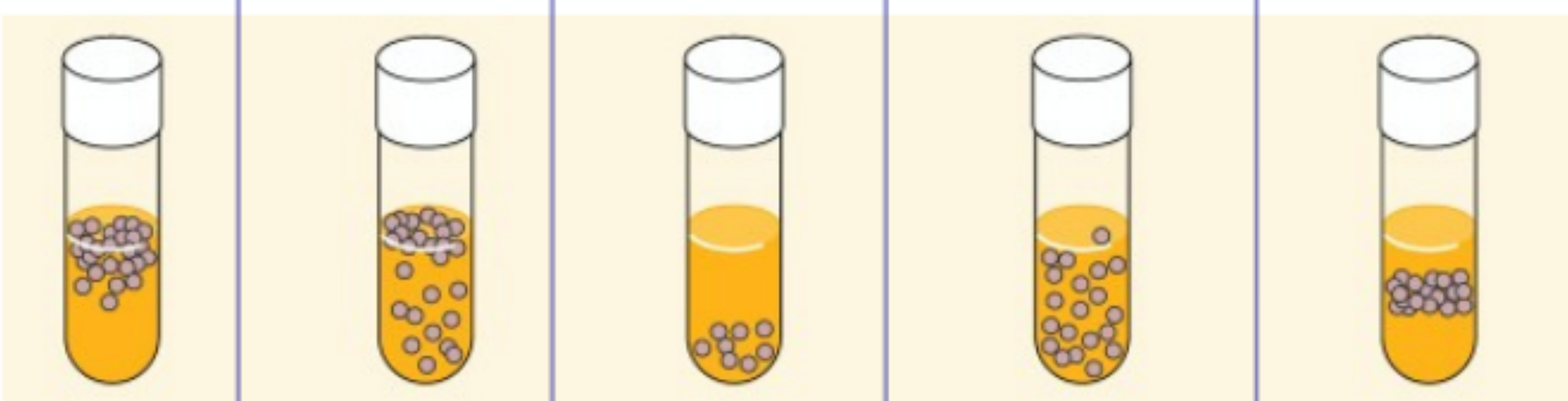
a. Growth atmosphere

Descriptive term	Property	Example
Growth atmosphere		
Strict (Obligate) aerobe	Requires O_2	*Pseudomonas aeruginosa*
Strict (Obligate) anaerobe	Will not tolerate O_2	*Bacteroides fragilis*
Facultative anaerobe	Aerobe, can grow anaerobically	Staphylococci, *Escherichia coli*
Aerotolerant anaerobe	Anaerobe, can tolerate O_2	*Clostridium perfringens*
Micro-aerophilic	Prefers reduced O_2	Campylobacter spp, Helicobacter spp
Capnophilic	Prefers increased CO_2	Neisseria spp

Classification of Organisms Based on Oxygen Requirements

- **Microbial Growth is affected by Oxygen Concentration**

Obligate aerobes	Facultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles



b. Growth temperature

Descriptive term	Property	Example
Growth temperature		
Psychrophilic	Low temp $<10^{0}$C	Flavobacterium spp
Thermophilic	High temp $>60^{0}$C	*B. stearothermophilus*
Mesophilic	20-40^{0}C	Most bacterial pathogens

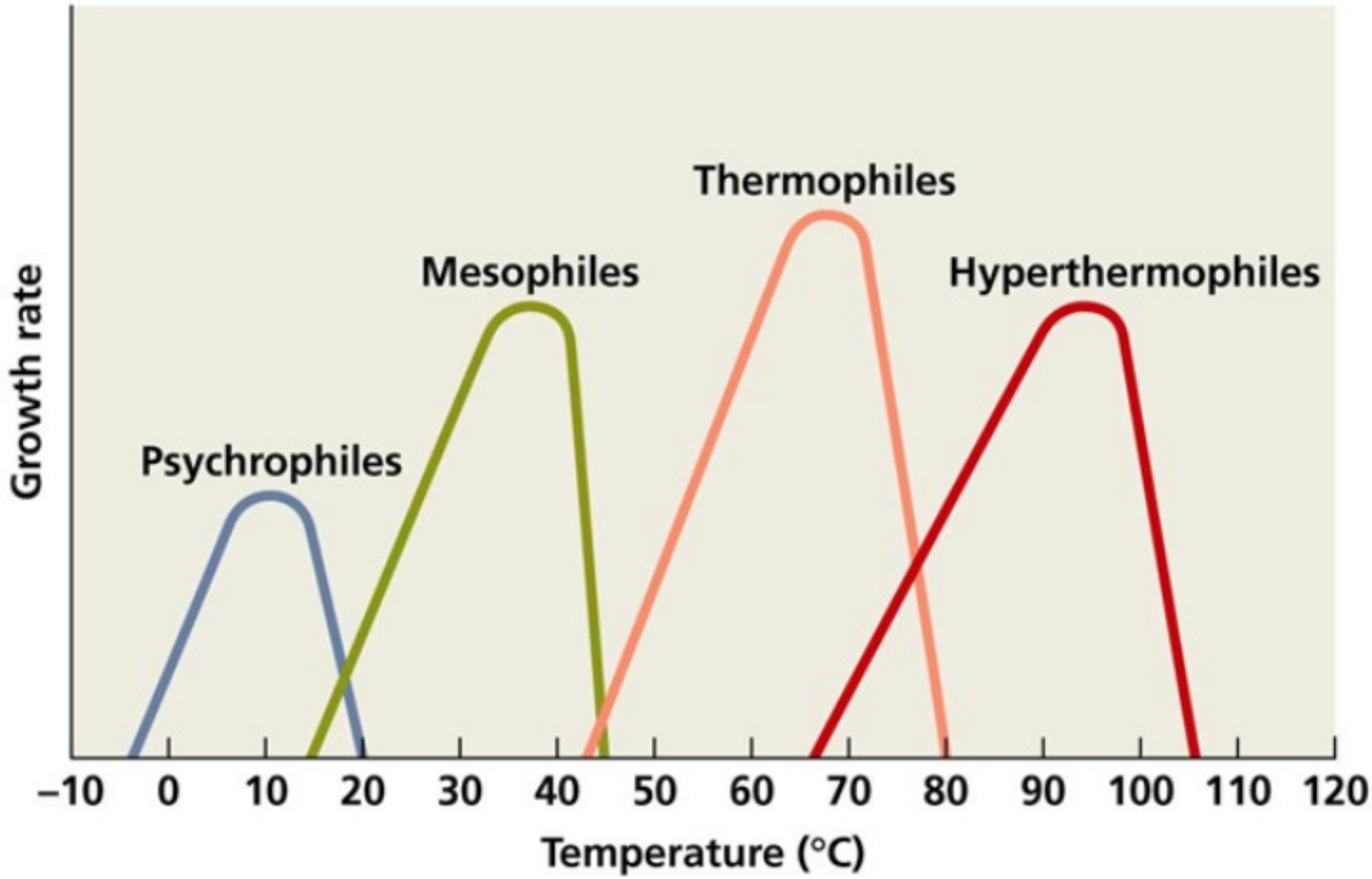
Factors that Affect Microbial Growth

<u>Temperature</u> –

- Affects proteins and lipid membranes
  - If too low, membranes become rigid and fragile
  - If too high, membranes become too fluid

- Categories based on Optimum Temperature
  - **Psychrophile** – optimum below 15^{o}C
  - **Mesophile** – optimum between 20^{o}C – 40^{o}C
  - **Thermophile** – optimum higher than 45^{o}C
  - **Hyperthermophiles** – optimum above 80^{o}C

Catagories of Microbes Based on Temperature Range



Effects of pH

- **Classification of Microbes based on pH**
  - Organisms sensitive to changes in acidity
    - H^+ and OH^- interfere with H bonding

  - **Acidophiles** – prefer below 7
  - **Neutrophiles** – prefer 7
  - **Alkalinophiles** – prefer above 7

  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6