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Cell wall

- Most prokaryotes have a rigid **cell wall**. The cell wall is an essential structure that protects the cell protoplast from mechanical damage and from osmotic rupture or **lysis**. Prokaryotes usually live in relatively dilute environments such that the accumulation of solutes inside the prokaryotic cell cytoplasm greatly exceeds the total solute concentration in the outside environment. Thus, the osmotic pressure against the inside of the plasma membrane may be the equivalent of 10-25 atm. Since the membrane is a delicate, plastic structure, it must be restrained by an outside wall made of porous, rigid material that has high tensile strength. Such a material is **murein**, the ubiquitous component of bacterial cell walls.

- Murein is a unique type of **peptidoglycan**, a polymer of disaccharides (glycan) cross-linked by short chains of amino acids (peptide). Many types of peptidoglycan exist. All Bacterial peptidoglycans contain N-acetylmuramic acid, which is the definitive component of murein. The cell walls of Archaea may be composed of protein, polysaccharides, or peptidoglycan-like molecules, but never do they contain murein. This feature distinguishes the Bacteria from the Archaea.

Gram-positive cell wall

- Gram-positive cell wall is 20-80 nm thick and consisting of numerous interconnecting layers of peptidoglycan.
- Chemically, 60 to 90% of the Gram-positive cell wall is peptidoglycan.
- In Gram-positive bacteria it is thought that the peptidoglycan is laid down in cables of several cross-linked glycan strands approximately. These cables then themselves become cross-linked for further cell wall strength.
- Interwoven in the cell wall of Gram-positive are teichoic acids and lipoteichoic acids. Teichoic acids extend through and beyond the rest of the cell wall and are polyalcohols composed of polymers of glycerol, phosphates, and the sugar alcohol ribitol and are covalently bound to the peptidoglycan. Teichoic acids covalently bound to cytoplasmic membrane lipids are called lipoteichoic acids.
- The outer surface of the peptidoglycan is studded with surface proteins that differ with the strain and species of the bacterium.
- The periplasm is the gelatinous material between the peptidoglycan and the cytoplasmic membrane.
- The peptidoglycan in the Gram-positive cell wall prevents osmotic lysis.
- The teichoic acids probably help make the cell wall stronger.
- The surface proteins in the bacterial peptidoglycan, depending on the strain and species, carry out a variety of activities. Some surface proteins function as enzymes. Other proteins serve as adhesins. Adhesins enable the bacterium to adhere intimately to host calls and other surfaces in order to colonize those cells and resist flushing

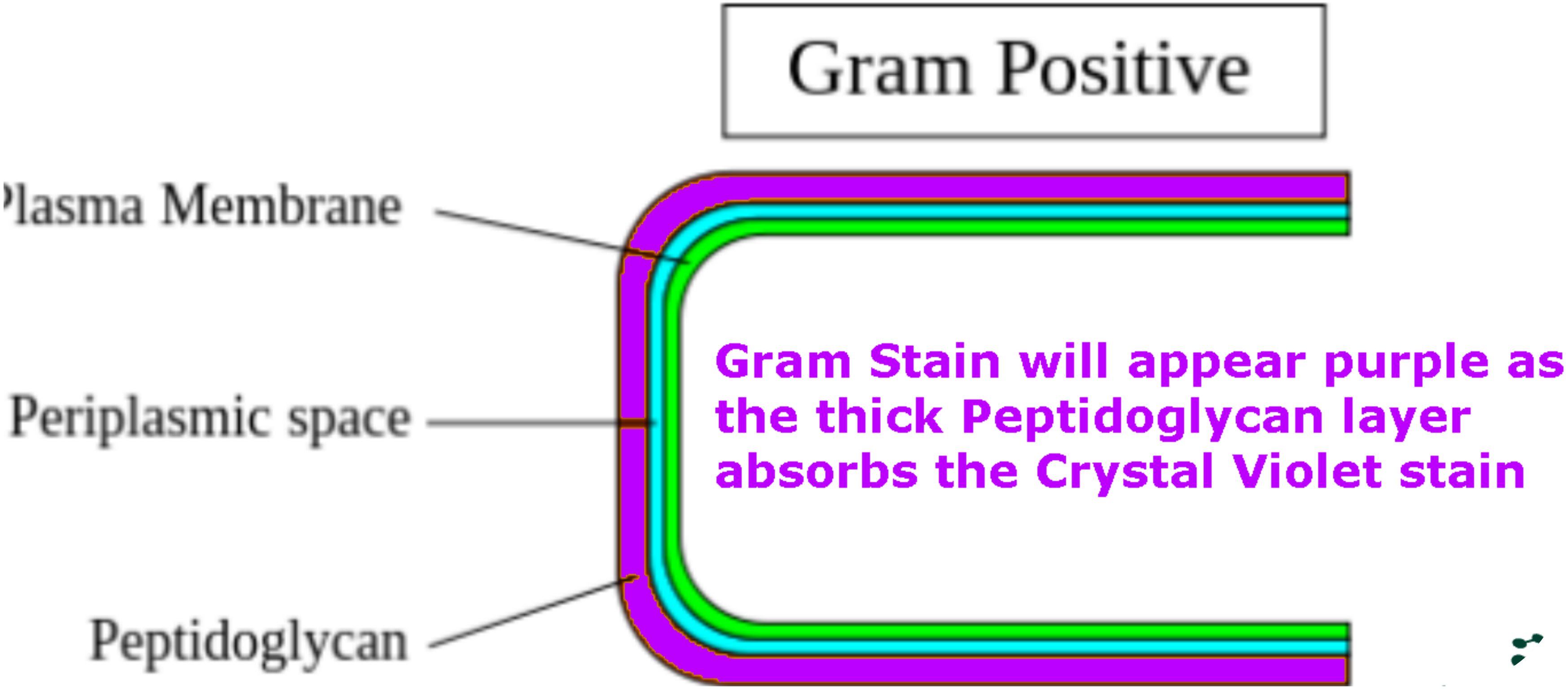


Figure: Structure of Gram positive cell wall

Gram negative cell wall

- In the Gram-negative bacteria the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane.
- The gram-negative bacteria do not retain crystal violet but are able to retain a counterstain, commonly safranin, which is added after the crystal violet. The safranin is responsible for the red or pink color seen with a gram-negative bacteria.
- The Gram-negative's cell wall is thinner (10 nanometers thick) and less compact than that of Gram-positive bacteria, but remains strong, tough, and elastic to give them shape and protect them against extreme environmental conditions.
- The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS) in addition to proteins and phospholipids. The LPS molecule is toxic and is classified as an endotoxin that elicits a strong immune response when the bacteria infect animals.
- In Gram-negative bacteria the outer membrane is usually thought of as part of the outer leaflet of the membrane structure and is relatively permeable. It contains structures that help bacteria adhere to animal cells and cause disease.
- The peptidoglycan layer is non-covalently anchored to lipoprotein molecules called Braun's lipoproteins through their hydrophobic head. Sandwiched between the outer membrane and the plasma membrane, a concentrated gel-like matrix (the periplasm) is found in the periplasmic space. It is in fact an integral compartment of the gram-negative cell wall and contains binding proteins for amino acids, sugars, vitamins, iron, and enzymes essential for bacterial nutrition.
- The periplasm space can act as reservoir for virulence factors and a dynamic flux of macromolecules representing the cell's metabolic status and its response to environmental factors.
- Together, the plasma membrane and the cell wall (outer membrane, peptidoglycan layer, and periplasm) constitute the gram-negative envelope.

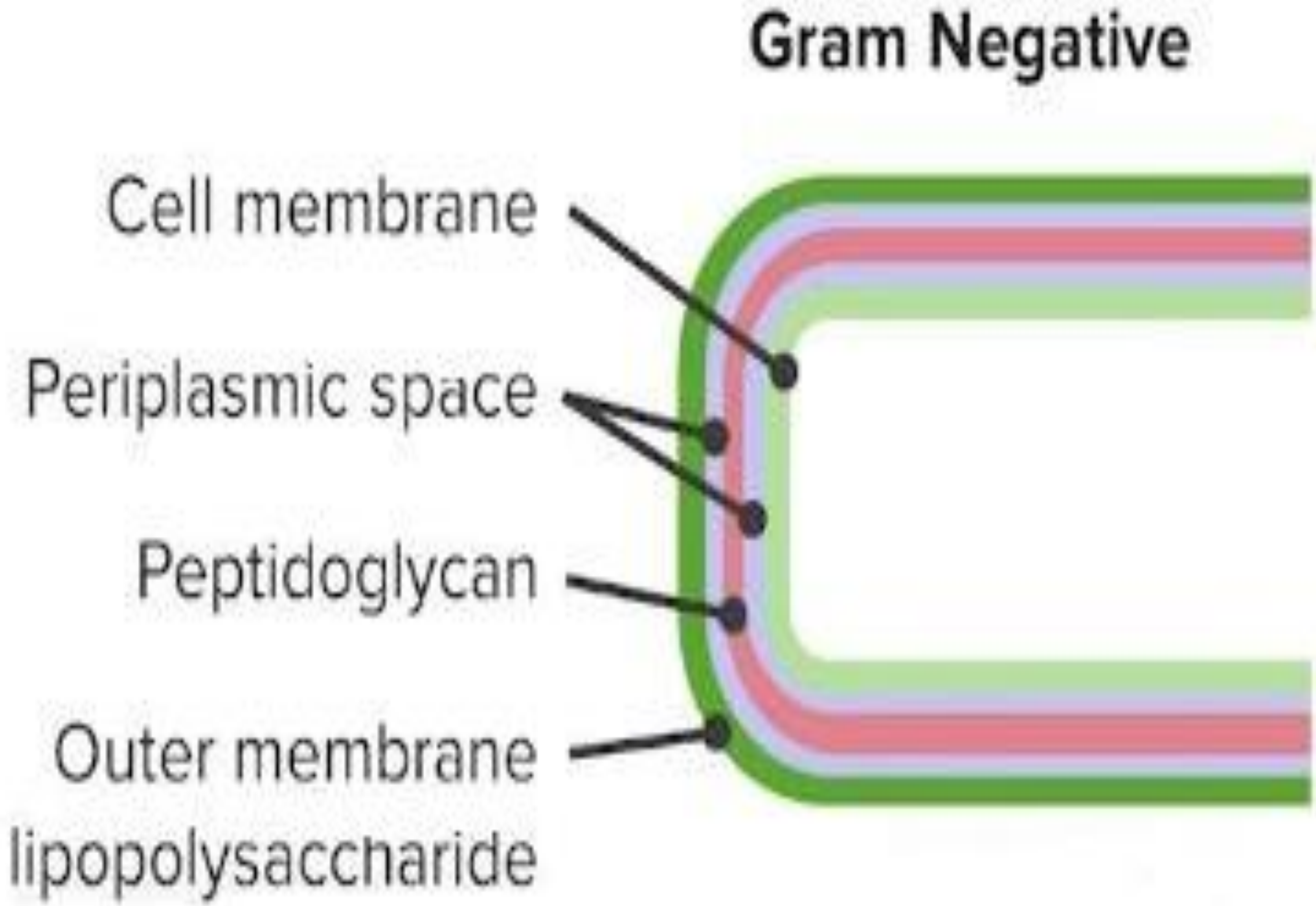


Figure: Structure of Gram negative cell wall

GRAM POSITIVE BACTERIA

VERSUS

GRAM NEGATIVE BACTERIA

Gram positive bacteria retain the crystal violet stain during gram staining	Gram negative bacteria do not retain the crystal violet stain during gram staining
Appear in purple color under the microscope	Appear in pink color under the microscope
Outer membrane is present	Outer membrane is absent
Peptidoglycan layer is thick and multilayered	Peptidoglycan layer is thin and single-layered
Periplasmic space is absent	Periplasmic space is present
Cell wall is around 20-80 nm	Cell wall is around 5-10 nm
Cell wall is smooth	Cell wall is wavy
Cell wall contains virtually non lipopolysaccharide content	Cell wall high lipopolysaccharide content
Lipid and lipoprotein content is low in the cell wall	Lipid and lipoprotein content is high in the cell wall
More susceptible to anionic detergents	Less susceptible to anionic detergents
Examples include actobacillus, Actinomyces, Bacillus, Streptococci, Clostridium & Corynebacterium	Examples include Acetobacter, Chlamydia, Borrelia, Bortadella, Burkholderia, and Enterobacter

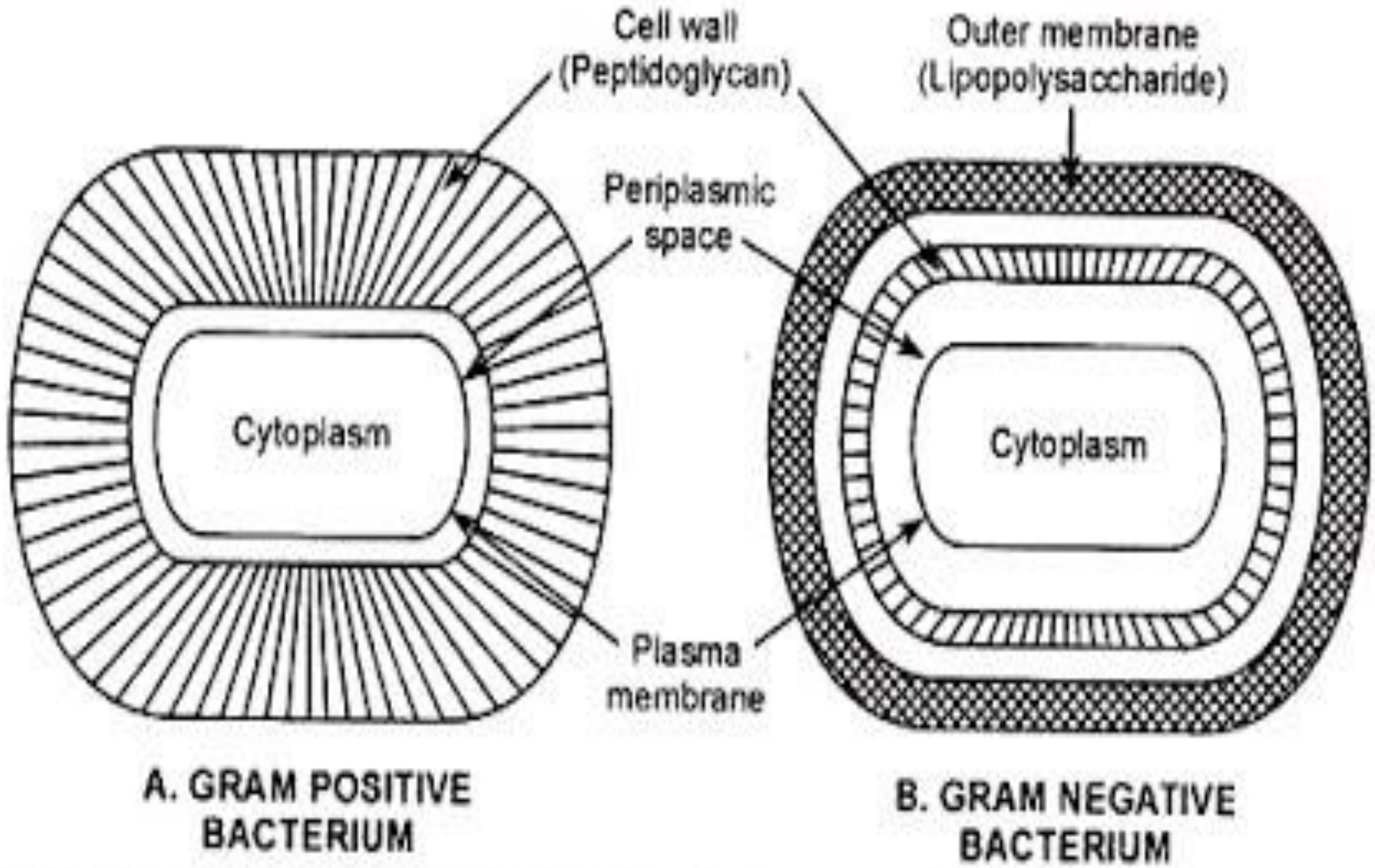


Fig. 2.3. Diagrammatic representation of cell wall of gram positive and gram negative bacteria.

Archaeal cell wall

- Archaea are single-celled microorganisms that lack a cell nucleus and membrane -bound organelles.
- Like other living organisms, archaea have a semi-rigid cell wall that protects them from the environment.
- The cell wall of archaea is composed of S-layers and lack peptidoglycan.
- Four types of archaeobacterial wall have been defined:
- Those made of pseudomurein, a heteropolymer that resembles peptidoglycan
- Those made of methanochondroitin, a sulfated heteropolysaccharide similar to that found in animal connective tissue
- Those made of glycoprotein (S-layer)
- Those that completely lack a wall and are surrounded exclusively by the plasma membrane.

Cell membrane

- The **plasma membrane**, also called the **cytoplasmic membrane**, is the most dynamic structure of a procaryotic cell. Its main function is a s a **selective permeability barrier** that regulates the passage of substances into and out of the cell.
- The plasma membrane is the definitive structure of a cell since it sequesters the molecules of life in a unit, separating it from the environment. The bacterial membrane allows passage of water and uncharged molecules up to mw of about 100 daltons, but does not allow passage of larger molecules or any charged substances except by means special membrane **transport processes** and **transport systems**.

  Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The phospholipids are amphiphilic molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments. Dispersed within the bilayer are various structural and enzymatic proteins which carry out most membrane functions.

- The membranes of **Bacteria** are structurally similar to the cell membranes of eukaryotes, except that bacterial membranes consist of saturated or monounsaturated fatty acids (rarely, polyunsaturated fatty acids) and do not normally contain sterols.
- The membranes of **Archaea** form bilayers functionally equivalent to bacterial membranes, but archaeal lipids are saturated, branched, repeating isoprenoid subunits that attach to glycerol via an ether linkage as opposed to the ester linkage found in glycerides of eukaryotic and bacterial membrane lipids.
- The structure of archaeal membranes is thought to be an adaptation to their existence and survival in extreme environments.

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

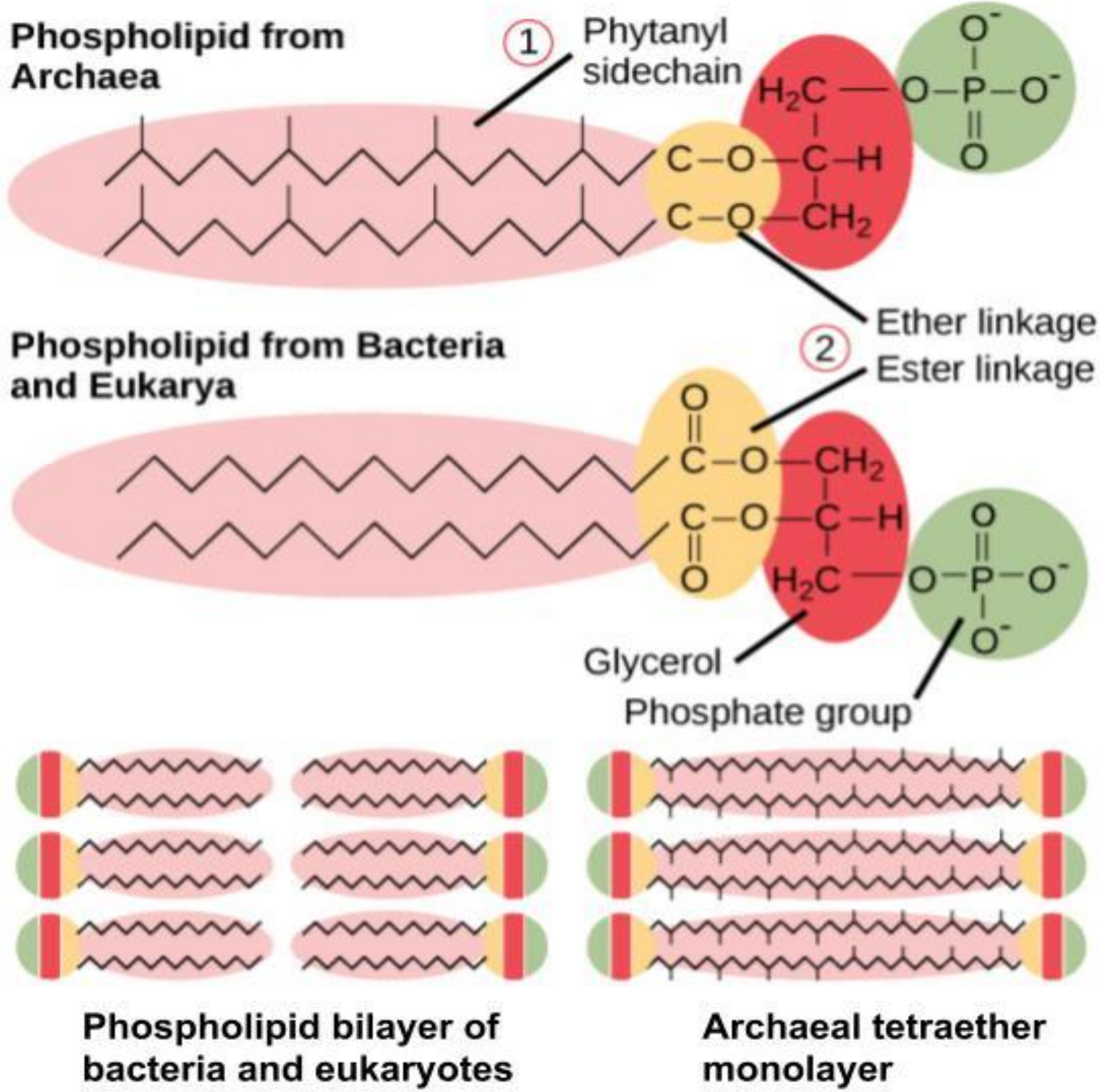


Figure: showing Structure and difference between of bacterial and arcahaeal cell membrane

Ribosomes

- Prokaryotic ribosomes are around 20 nm (200 Å) in diameter and are composed of 65% rRNA and 35% ribosomal proteins.
- The unit of measurement used to describe the ribosomal subunits and the rRNA fragments is the Svedberg unit, a measure of the rate of sedimentation in centrifugation rather than size. This accounts for why fragment names do not add up: for example, bacterial 70S ribosomes are made of 50S and 30S subunits.
- Bacteria have 70S ribosomes, each consisting of a small (30S) and a large (50S) subunit. *E. coli*, for example, has a 16S RNA subunit (consisting of 1540 nucleotides) that is bound to 21 proteins.
- The large subunit is composed of a 5S RNA subunit (120 nucleotides), a 23S RNA subunit (2900 nucleotides) and 31 proteins
- Ribosomes are minute particles consisting of RNA and associated proteins that function to synthesize proteins. Proteins are needed for many cellular functions such as repairing damage or directing chemical processes. Ribosomes can be found floating within the cytoplasm or attached to the endoplasmic reticulum. Basically, their main function is to convert genetic code into an amino acid sequence and to build protein polymers from amino acid monomers.

- Ribosomes act as catalysts in two extremely important biological processes called peptidyl transfer and peptidyl hydrolysis.

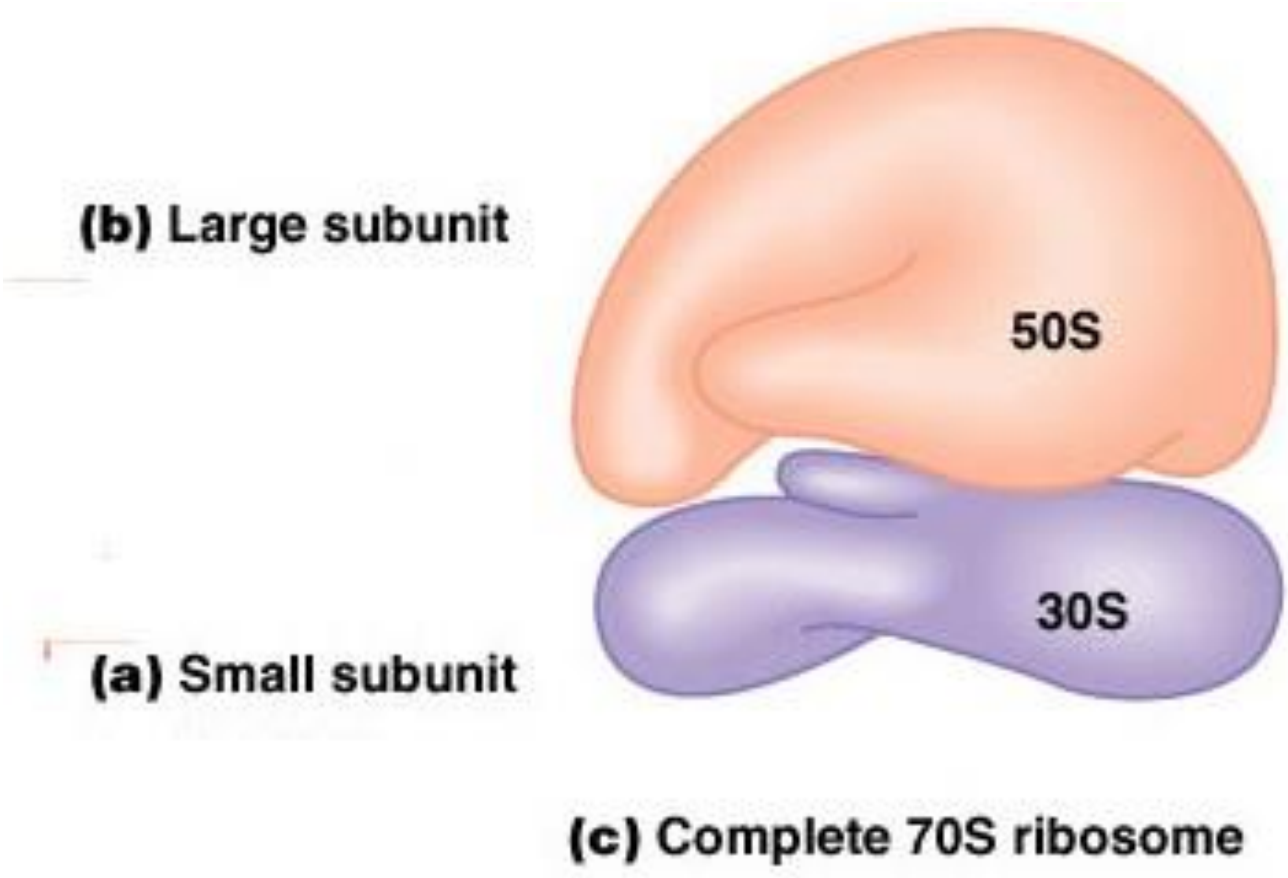


Figure: Structure of bacterial ribosomes

Nucleoid

- The **nucleoid** (meaning *nucleus-like*) is an irregularly shaped region within the cell of a prokaryote that contains all or most of the genetic material.
- The length of the DNA chromosome is very large compared to the dimensions of the cell, and so must be compacted to fit.
- In contrast to the nucleus of a eukaryotic cell, it is not surrounded by a nuclear membrane. Instead, the nucleoid forms by condensation and functional arrangement with the help of chromosomal architectural proteins and RNA molecules as well as DNA supercoiling. The length of a genome widely varies (generally at least a few million base pairs) and a cell may contain multiple copies of it.
- The nucleoid is essential for controlling the activity of the cell and reproduction.
- It is where transcription and replication of DNA take place. Within it, we can expect to find *enzymes* that serve as biological catalysts and help with replication, as well as other proteins that have other functional and structural roles, including assisting the formation of DNA, facilitating cell growth, and regulating the genetic material of the cell.

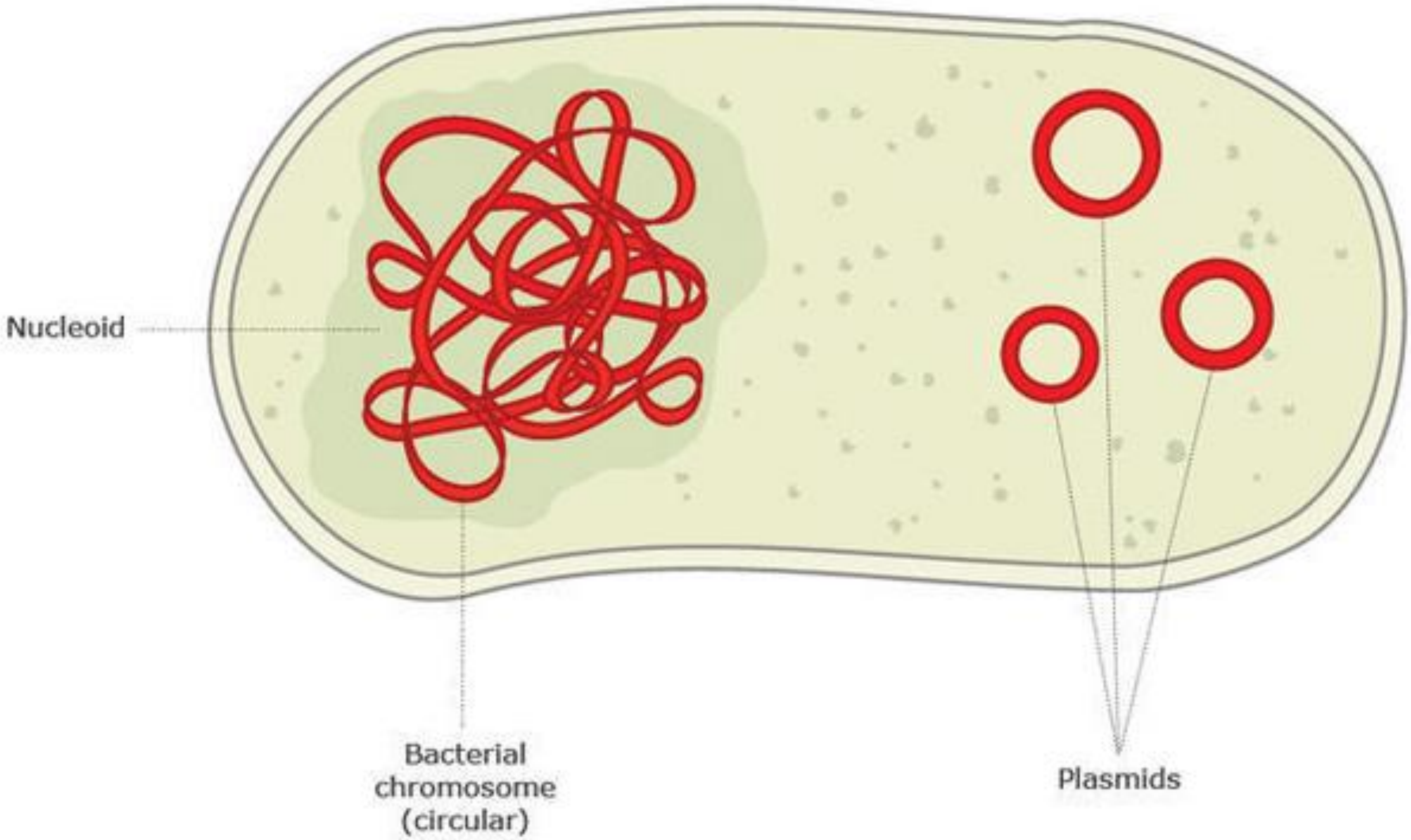


Figure: showing nucleoid and plasmid in bacterial cell

Plasmids

- A **plasmid** is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria; however, plasmids are sometimes present in archaea and eukaryotic organisms.
- In nature, plasmids often carry genes that benefit the survival of the organism and confer selective advantage such as antibiotic resistance. While chromosomes are large and contain all the essential genetic information for living under normal conditions, plasmids are usually very small and contain only additional genes that may be useful in certain situations or conditions. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms. In the laboratory, plasmids may be introduced into a cell via transformation.
- Plasmids are considered *replicons*, units of DNA capable of replicating autonomously within a suitable host. However, plasmids, like viruses, are not generally classified as life. Plasmids are transmitted from one bacterium to another (even of another species) mostly through conjugation.
- This host-to-host transfer of genetic material is one mechanism of horizontal gene transfer, and plasmids are considered part of the mobilome. Unlike viruses, which encase their genetic material in a protective protein coat called a capsid, plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host; however, some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer.
- The size of the plasmid varies from 1 to over 200 kbp, and the number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances.

Classification of plasmids

Plasmids can be broadly classified into conjugative plasmids and non-conjugative plasmids.

Conjugative plasmids contain a set of transfer genes which promote sexual conjugation between different cells. In the complex process of conjugation, plasmids may be transferred from one bacterium to another via sex pili encoded by some of the transfer genes (see figure). Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids. An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence.

- Plasmids can also be classified into incompatibility groups. A microbe can harbour different types of plasmids, but different plasmids can only exist in a single bacterial cell if they are compatible. If two plasmids are not compatible, one or the other will be rapidly lost from the cell. Different plasmids may therefore be assigned to different incompatibility groups depending on whether they can coexist together. Incompatible plasmids (belonging to the same incompatibility group) normally share the same replication or partition mechanisms and can thus not be kept together in a single cell.

Another way to classify plasmids is by function. There are five main classes:

- Fertility F-plasmids, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili.
- Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
- Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen. e.g. Ti plasmid in *Agrobacterium tumefaciens*

Plasmid Structure

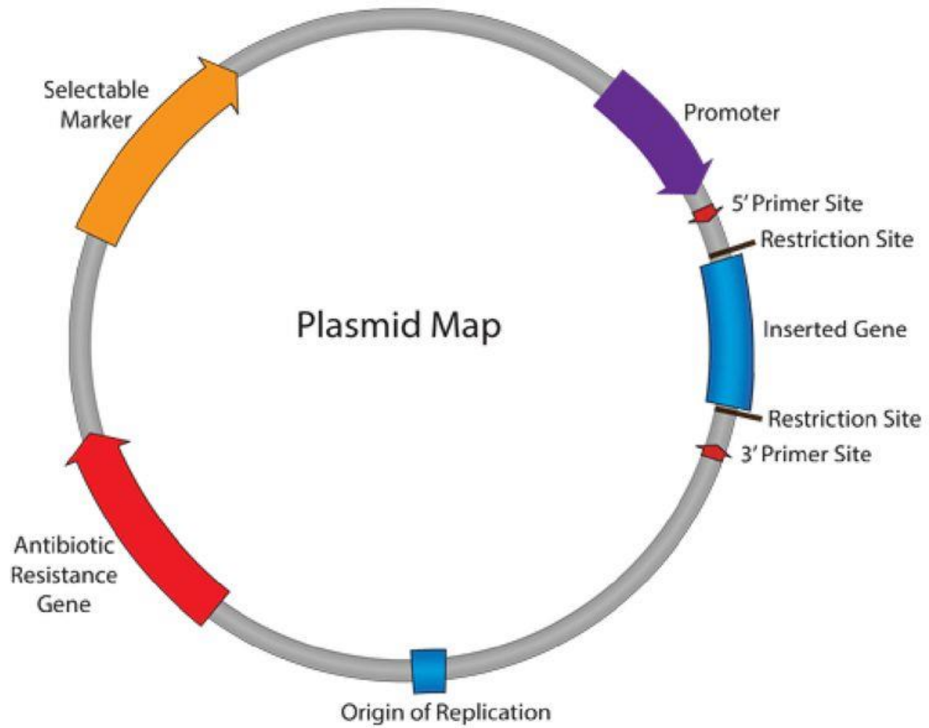


Figure: Structure of bacterial plasmid

Spores and sporulation

- Spore is a unicellular structure. • Spores form a life cycle in many plants, algae, bacteria, fungi and protozoa. • Spores are formed during unfavorable conditions. • They can survive without nutrients. • it forms highly resistant structure.
- Essentially, sporulation refers to the formation of spores from vegetative cells during unfavorable environmental conditions. As such, it may be described as an adaptive response that allows the organism to survive given adverse conditions (radiation, extreme heat or cold, lack of nutrition etc).
- Compared to vegetative cells, spores (formed during sporulation) are multilayered structures that tend to be dormant (or relatively dormant). These characteristics make it possible for some of the spores to preserve the genetic content of the organism during harsh environmental conditions.

- During certain unfavorable conditions (depending on the organism), some of the vegetative cells go through a series of morphological changes (and some level of programmed gene expression) that ultimately produce spores. Apart from genetic material, spores also contain some cytoplasm, specific acids, ribosome, and the appropriate enzymes among others that allow the spore to germinate during favorable environmental conditions.

- A majority of spore-forming bacteria are Gram-negative bacilli (rod-shaped). These include aerobic Bacillus and anaerobic Clostridium species. Although some Gram-negative bacteria have been shown to be capable of producing spores, it is only a few species found in a few genera.

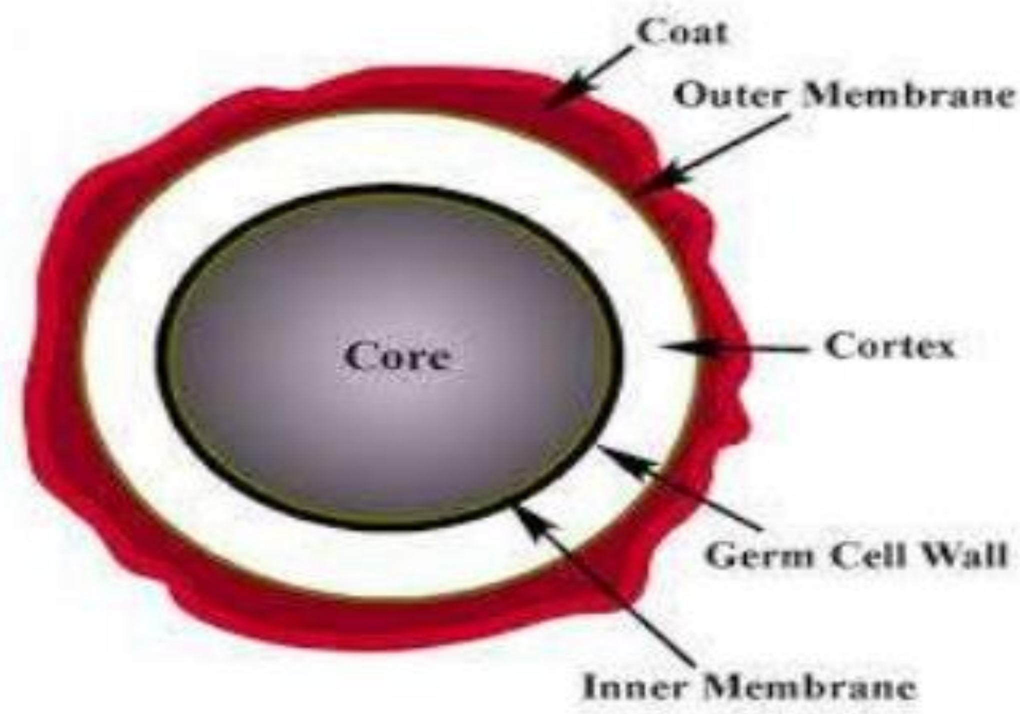
  Some filamentous cocci have also been shown to be capable of sporulation (producing endospores)

Endospores: Endospores are the most common types of spores. They are typically produced by Clostridium (e.g. Clostridium botulinum), Bacillus (e.g. Bacillus anthracis) and Sporosarcina bacteria (e.g. Sporosarcina ureae).

Endospore Structure

- The resilience of an endospore can be explained in part by its unique cellular structure. The outer proteinaceous coat surrounding the spore provides much of the chemical and enzymatic resistance. Beneath the coat resides a very thick layer of specialized peptidoglycan called the cortex. Proper cortex formation is needed for dehydration of the spore core, which aids in resistance to high temperature. A germ cell wall resides under the cortex. This layer of peptidoglycan will become the cell wall of the bacterium after the endospore germinates. The inner membrane, under the germ cell wall, is a major permeability barrier against several potentially damaging chemicals. The center of the endospore, the core, exists in a very dehydrated state and houses the cell's DNA, ribosomes and large amounts of dipicolinic acid. This endospore-specific chemical can comprise up to 10% of the spore's dry weight and appears to play a role in maintaining spore dormancy. Small acid-soluble proteins (SASPs) are also only found in endospores. These proteins tightly bind and condense the DNA, and are in part responsible for resistance to UV light and DNA-damaging chemicals. Other species-specific structures and chemicals associated with endospores include stalks, toxin crystals, or an additional outer glycoprotein layer called the exosporium.

Structure of spore



- sporulation • The process of production of spores is called sporulation or sporogenesis. The one vegetative cell forms a single spore, which, after germination, develops into a new cell. It takes 8hrs-19 hrs to complete.
- The process of forming an endospore is complex.
- The model organism used to study endospore formation is *Bacillus subtilis*. Endospore development requires several hours to complete. Key morphological changes in the process have been used as markers to define stages of development.
- As a cell begins the process of forming an endospore, it divides asymmetrically (Stage II). This results in the creation of two compartments, the larger mother cell and the smaller forespore. These two cells have different developmental fates. Intercellular communication systems coordinate cell-specific gene expression through the sequential activation of specialized sigma factors in each of the cells. Next (Stage III), the peptidoglycan in the septum is degraded and the forespore is engulfed by the mother cell, forming a cell within a cell. The activities of the mother cell and forespore lead to the synthesis of the endospore-specific compounds, formation of the cortex and deposition of the coat (Stages IV+V). This is followed by the final dehydration and maturation of the endospore (Stages VI+VII). Finally, the mother cell is destroyed in a programmed cell death, and the endospore is released into the environment.
- The endospore will remain dormant until it senses the return of more favorable conditions. [A sigma factor is a small protein that directs RNA polymerase to specific cites on DNA to initiate gene expression.]

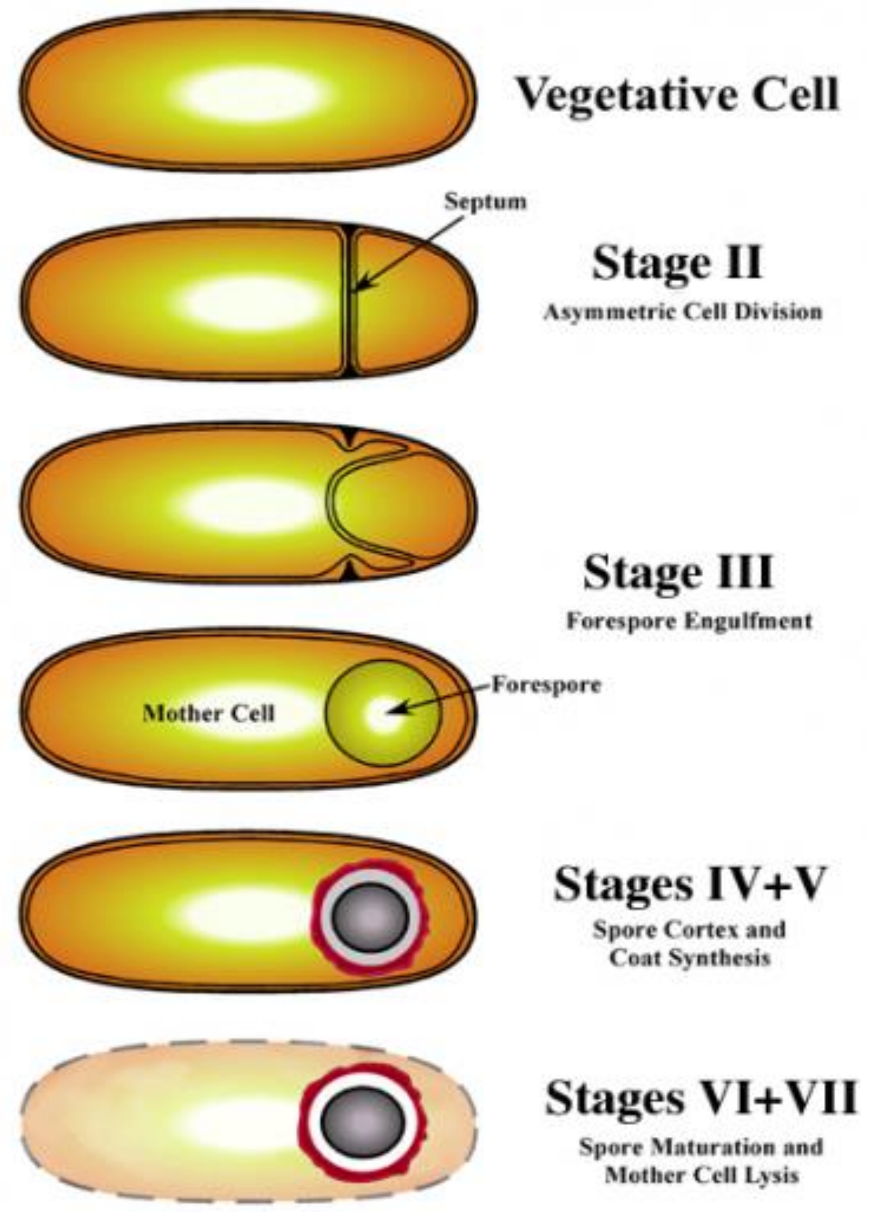


Figure: Stages of sporulation (endospore formation)