MICROBIAL BIOTECHNOLOGY

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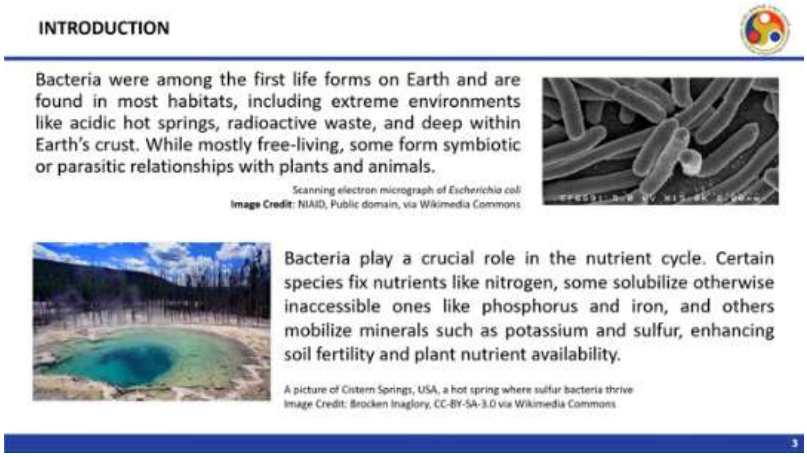
Lecture06

Lec 6: Structure & life cycle of representative groups of prokaryotic microbes

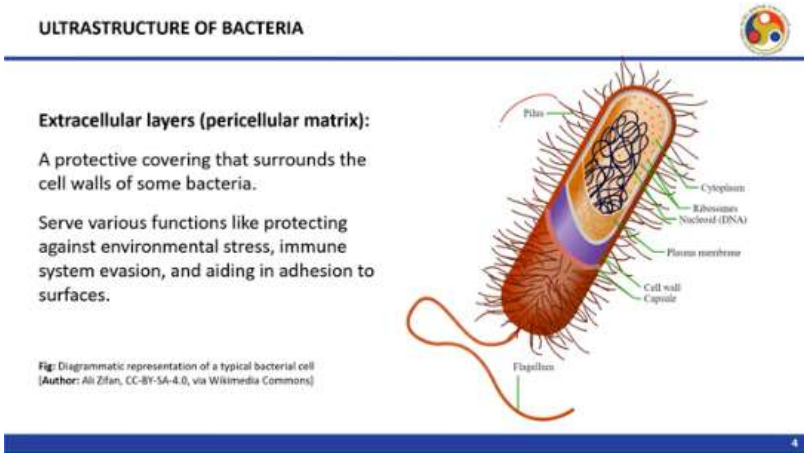
Welcome to my course on microbial biotechnology. We are in module 2. Currently, we are discussing the structure and life cycle of representative groups of prokaryotic microbes. In this lecture, we will focus on bacteria, particularly the ultrastructure of bacteria, the different organelles they contain, and also bacterial endospores. So, you already know that bacteria are very tiny organisms, unicellular, and also ubiquitous in nature.

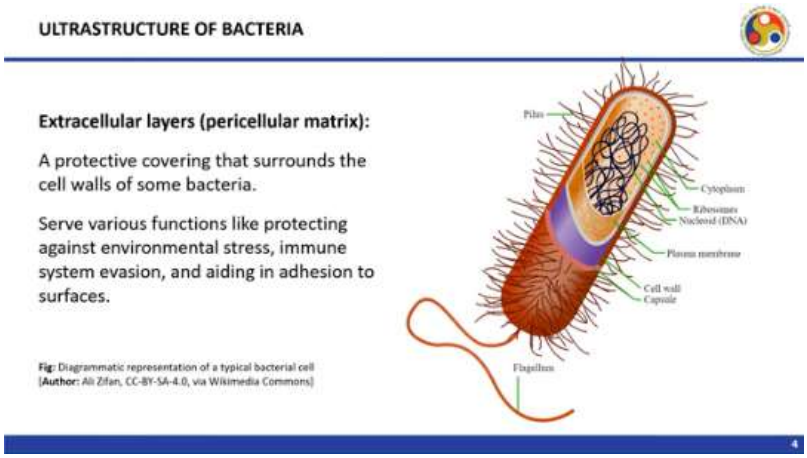


They were among the first life forms on Earth and are found in most habitats, including extreme environments like acidic hot springs and radioactive waste. and also deep within Earth's crust. While mostly free-living, some form symbiotic or parasitic relationships with plants and animals. Bacteria play a crucial role in nutrient cycles. Certain species of bacteria fix nutrients like nitrogen; some solubilize otherwise inaccessible ones like phosphorus and iron.

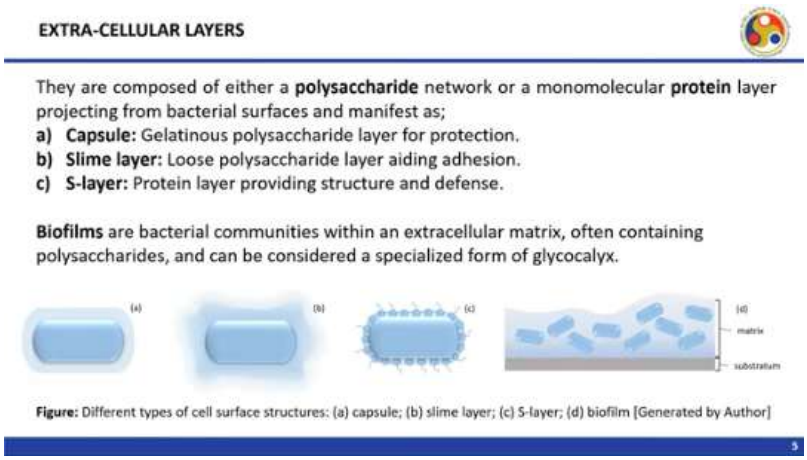
Others mobilize minerals such as potassium and sulfur, thereby enhancing soil fertility and resulting in plant nutrient availability. If you look at the ultrastructure of bacteria in this picture, you can see a diagrammatic representation of a bacterial cell with many interesting structures. For example, the pilus, then you have ribosomes, nucleoid, plasma membrane,

cell wall, capsule, and flagellum, and there are many more. We will discuss these in detail today.



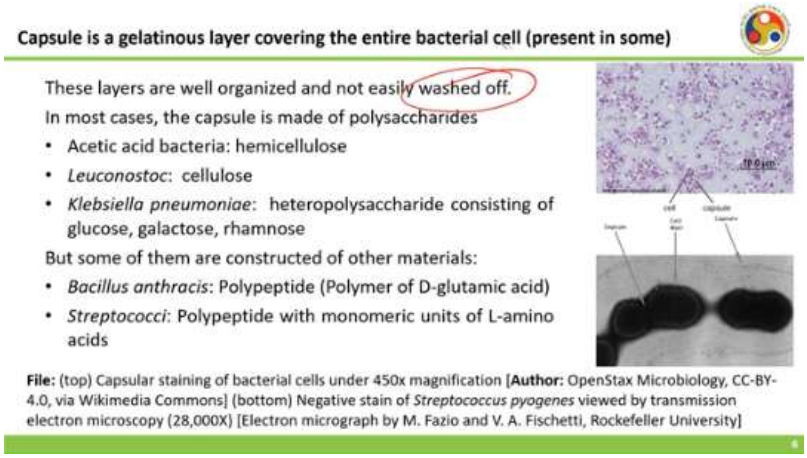


So, one of the important components of a bacterial cell is the extracellular layers which is also known as the pericellular matrix. It is a protective covering that surrounds the wall of some of the bacterial species. It serves various functions like protecting against environmental stress, immune system evasion and aiding in adhesion to surfaces. Extracellular layers are composed of either a polysaccharide network or a monomolecular protein layer projecting from bacterial surfaces and they manifest as either as a capsule or slime layer or ass layer and also as biofilms. So capsule is basically a gelatinous polysaccharide layer for protection.



Slime layers are loose polysaccharide layer adding in the adhesion and S layers are protein layers providing structure as well as defense. Biofilins are bacterial communities within a extracellular matrix often containing polysaccharides and can be considered as a specialized form of glycocalyx. Capsule is a gelatinous layer covering the entire bacterial cell and not present in all bacteria. These layers are well organized and not easily washed off. This is an important point to remember.

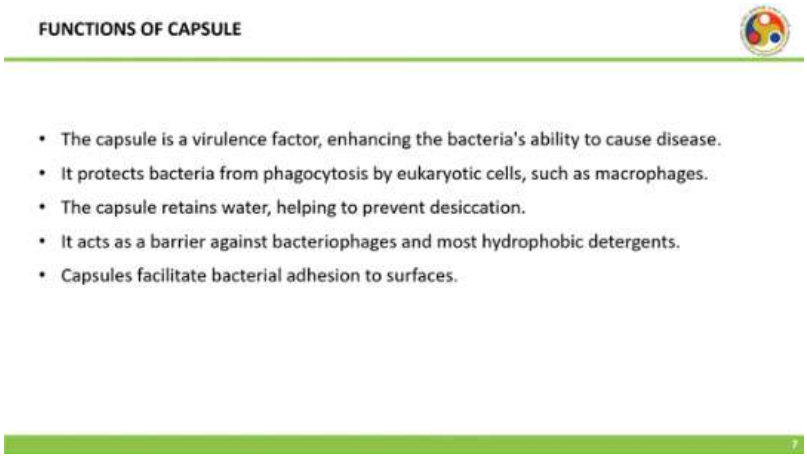
In most cases, the capsule is made of polysaccharides. However, some variations are there. For example, in acetic acid bacteria, it is composed of hemicellulose. In leukonostoc we have cellulose. In klebsiella pneumoniae we have heteropolysaccharide consisting of glucose, galactose and remnose.



And some of them are also constructed of other materials, like Bacillus anthracis, which has a polypeptide that is a polymer of D-glutamic acid. Then, streptococci have polypeptides with monomeric units of L-amino acids. So, in this picture, you can see a capsular staining of bacterial cells under 450x magnification and the negative stain of

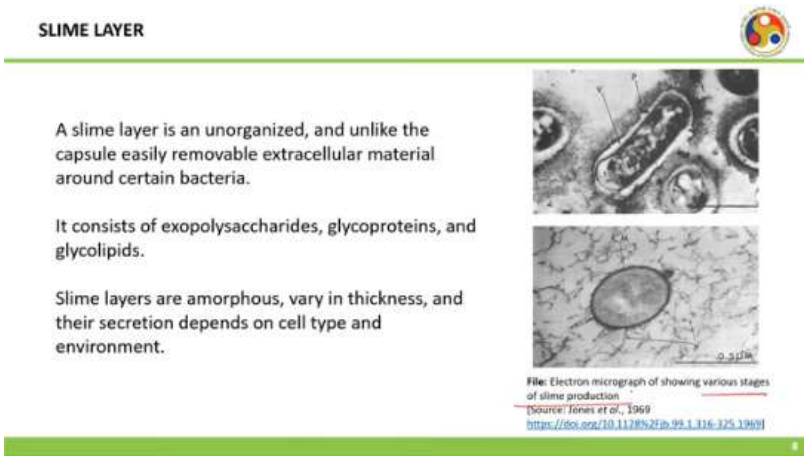
Streptococcus pyogenes under transmission electron microscopy. What are the functions of a capsule? The capsule is a virulence factor.

It enhances the bacteria's ability to cause disease. It protects bacteria from phagocytosis by eukaryotic cells, such as macrophages. The capsule retains water, helping to prevent desiccation of the bacterial cell. It acts as a barrier against bacteriophages and most hydrophobic detergents. The capsule facilitates bacterial adhesion to surfaces.

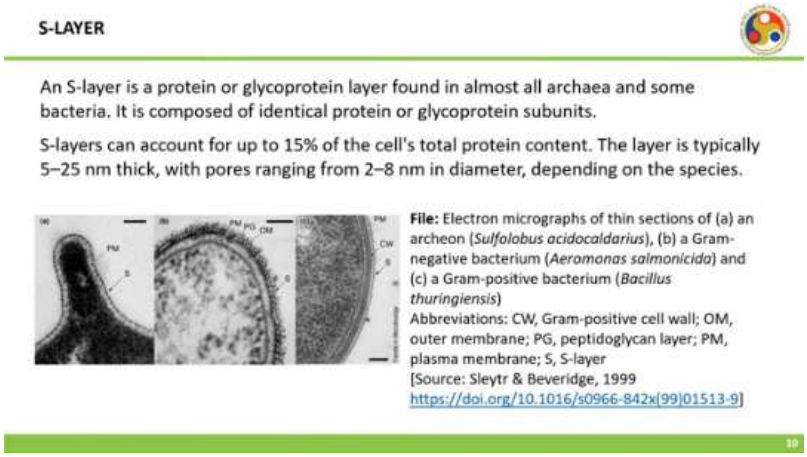


What is a slime layer? A slime layer is unorganized and, unlike the capsule, it is easily removable extracellular material found around certain bacterial species. It consists of exopolysaccharides, glycoproteins, and glycolipids. Slime layers are amorphous, vary in thickness, and their secretion depends on cell type and environment. In this figure, you can see the various stages of slime production.

We are not going into details of these descriptions, which are beyond the scope of this particular course. What are the functions of the slime layer? The slime layer primarily protects bacteria from environmental threats like antibiotics and desiccation. It aids in surface adhesion, for example, in the case of implants and catheters, serves as a food reserve, and provides resistance to chemical sterilization such as chlorine and iodine. Some bacteria, like Bacillus anthracis, use a slime layer to absorb antibodies and evade immune attacks.



What is an S-layer? An S-layer is a protein or glycoprotein layer found in almost all archaea and some bacteria. It is composed of identical protein or glycoprotein subunits. S-layers can account for up to 15% of the cell's total protein content. The layers are typically 5 to 25 nanometers thick, with pores ranging from 2 to 8 nanometers in diameter, depending on the species.



In this picture, we can see the electron micrograph of thin sections of an archaeon in figure A, a Gram-negative bacterium in figure B, and a Gram-positive bacterium in figure C. We can see the various S-layers in these different types of bacterial species. What are the functions of an S-layer? The S-layer protects cells from bacteriophages, Bdellovibrio (which are predatory bacteria that consume other bacteria), phagocytosis, and high molecular weight substances like lytic enzymes. In acidophiles, it helps resist low pH. Glycosylated S-layers facilitate adhesion.

And the S layer may also provide protection against ionizing radiation and high temperatures. Let us now discuss about one of the important component of a bacterial cell,

the cell wall. The bacterial cell wall maintains the structural integrity of the cell. Its primary role is to protect against internal target pressure. caused by higher concentration of proteins and other molecules inside the cell compared to the external environment.

FUNCTION OF S-LAYER

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In acidophiles, it helps resist low pH.

Glycosylated S-layers facilitate adhesion, and the S-layer may also provide protection against ionizing radiation and high temperatures.

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The bacterial cell wall is distinguished by the presence of peptidoglycan located just outside the cell membrane. Here you can see three conditions of bacteria, one in hypertonic solution, another in isotonic and the last one is in hypotonic. And here you can see the flow of water as shown by the dark red arrows. And in the isodinic solution, the inflow and outflow of water is balanced. Gram staining, one of the important techniques which was developed by a Danish bacterialist, Hans Christian Gram in 1884.

CELL WALL

The bacterial cell wall maintains the structural integrity of the cell.

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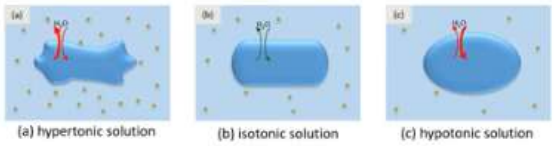

(a) hypertonic solution (b) isotonic solution (c) hypotonic solution

Fig: Cells under different tonicity, and the direction of the flow of water molecules:
: Cell walls protect cells from internal turgor pressure [Generated by Author]

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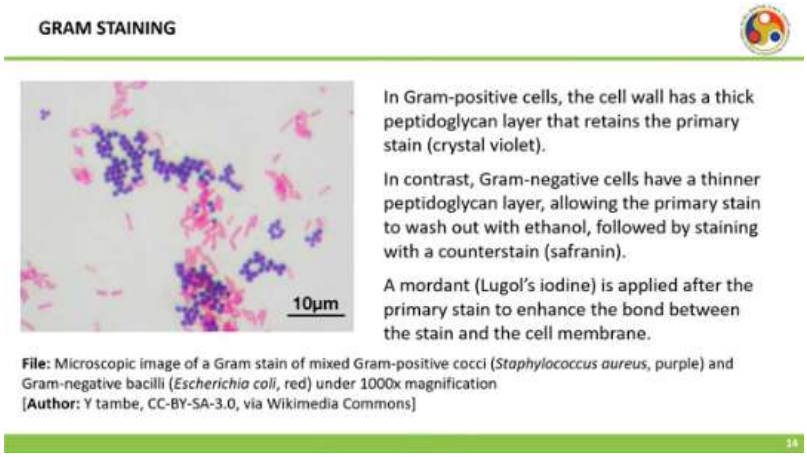
It was used to classify bacteria into two broad groups, the gram-positive and gram-negative, which you already know. In the earlier module, we have discussed at length about this. So in brief, gram staining differentiates bacterial cells based on their chemical and physical properties of their cell walls. In gram-positive cells, the cell wall has a thick peptidoglycan layer that retains the primary stain, the crystal violet. In contrast, gram-negative cells have

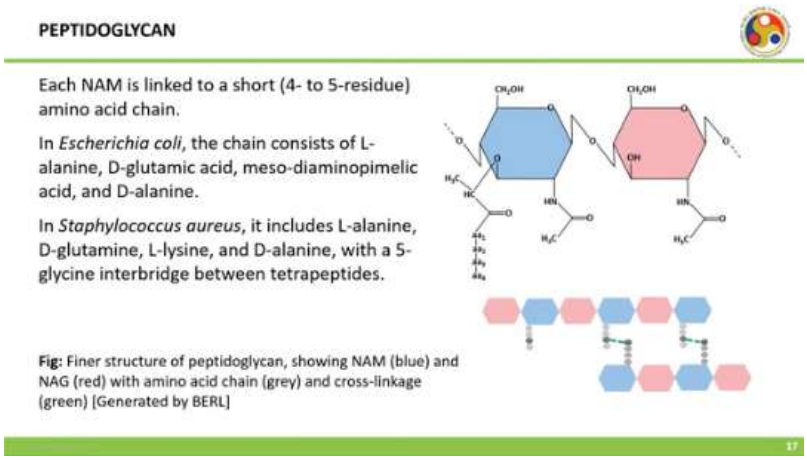
a thinner peptidoglycan layer, allowing the primary stain to wash out with ethanol, followed by staining with a counter stain, safranin.

A mordant is applied after the primary stain to enhance the bond between the stain and the cell membrane. These are routinely practiced in bacterial laboratories, particularly in clinical contexts. The gram-positive cell wall is mainly composed of peptidoglycan, which accounts for 90% of its structure. The cross-linked peptidoglycan chains form a rigid structure with minimal periplasmic space. The cell wall also contains teichoic acids and lipids, which combine to form lipoteichoic acids that act as chelating agents.



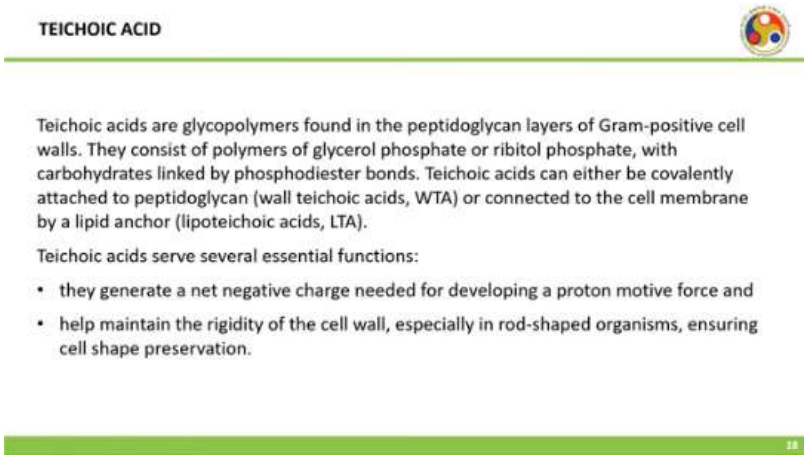
Despite the rigidity of the peptidoglycan layer, its porosity allows many substances to pass through the gram-positive cell wall. Let us discuss the peptidoglycan in a little more detail. This consists of linear chains of two alternating amino sugars. The first is NAG or N-acetylglucosamine, and the other is NAM or N-acetylmuramic acid, which are linked by beta-1,4 glycosidic bonds. Peptide chains cross-link these sugars, enhancing the overall strength of the cell wall.

In this picture, you can see the fine structure of peptidoglycan, showing the NAM and the NAG within the amino acid chain, which is gray here, as you can see. These are the cross-linkages in this whole structure. Each NAM is linked to a short 4- to 5-residue amino acid chain. You can see here four amino acid residues in E. coli. The chain consists of L-alanine, D-glutamic acid, meso-diaminopimelic acid, and D-alanine.



In Staphylococcus aureus, it includes L-alanine, D-glutamine, L-lysine, and D-alanine with a 5-glycine interbridge between tetrapeptides. So these are some of the final structures of peptidoglycan, which we have already discussed in the earlier slide. Teichoic acid. Teichoic acids are glycopolymers found in the peptidoglycan layers of gram-positive cell walls. They consist of polymers of glycerol phosphate or ribitol phosphate with carbohydrates linked by phosphodiester bonds.

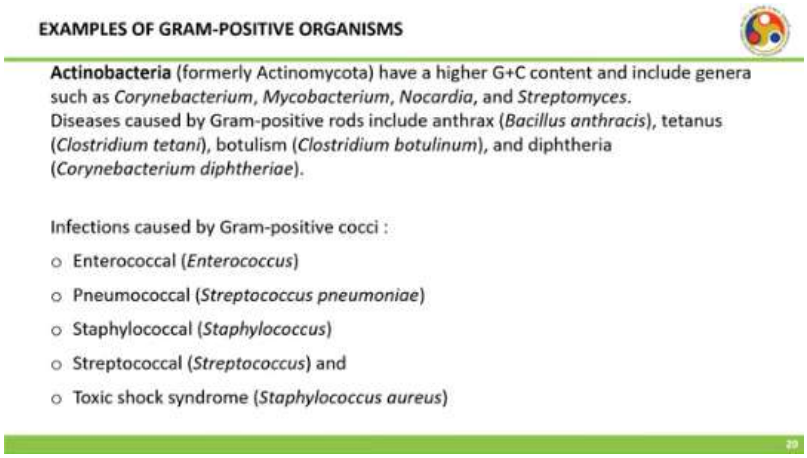
Teichoic acids can either be covalently attached to peptidoglycan or connected to the cell membrane by a lipid anchor. Teichoic acids serve several essential functions. For example, they generate a net negative charge. needed for developing a proton motive force and help maintain the rigidity of the cell wall, especially in rod-shaped organisms ensuring cell shape preservation. Let us look into some of the examples of gram-positive organisms.



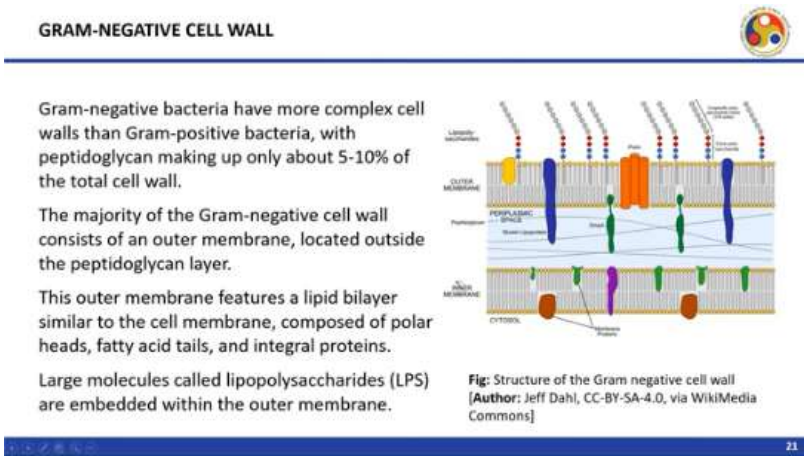
which are typically a single membrane surrounded by a thick peptidoglycan layer. For example, in the phyla Bacillota, excluding the classes Mollicutes and Negativicutes and, you know, Mesotota. We have in these Bacillus and Firmicutes a very low GC content, and

this can be divided into anaerobic Clostridia and aerobic Bacilli. The anaerobic Clostridia contain genera like Clostridium, Acetobacterium, Alkalibacter, and Eubacterium. And the aerobic Bacilli contain genera like Bacillus, Listeria, Staphylococcus, Streptococcus, Lactobacillus, Leuconostoc, and so on.

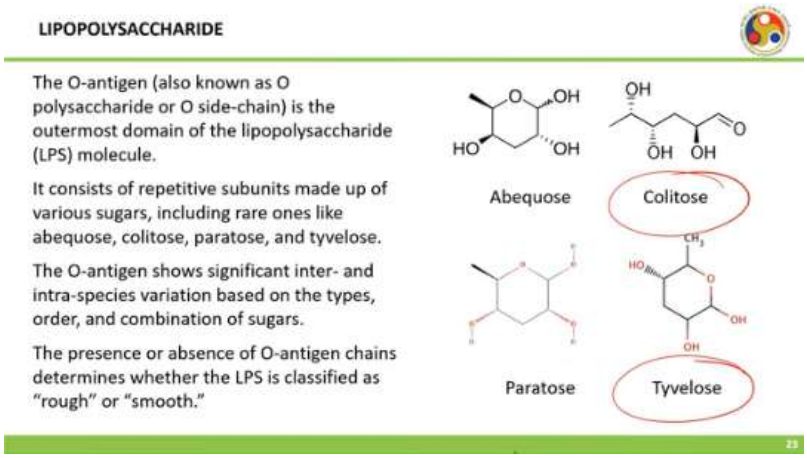
Actinobacteria, which was formerly known as Actinomycota, have a higher GC content and include genera such as Corynebacterium, Mycobacterium, Nocardia, and Streptomyces. Diseases caused by gram-positive rods include anthrax (Bacillus anthracis), tetanus (Clostridium tetani), botulism (Clostridium botulinum), and diphtheria (Corynebacterium diphtheriae). Infections caused by gram-positive cocci can be summed up as enterococcal, pneumococcal, staphylococcal, streptococcal, and also toxic shock syndrome caused by Staphylococcus aureus. Let us look into the gram-negative cell wall.



It is more complex than the gram-positive bacteria's, with peptidoglycan making up only about 5 to 10% of the cell wall. The majority of the gram-negative cell wall consists of an outer membrane located outside the peptidoglycan layer. This outer membrane features a lipid bilayer similar to the cell membrane, composed of polar heads, fatty acid tails, and integral proteins. Large molecules called lipopolysaccharides are embedded within the outer membrane. These lipopolysaccharides comprise three distinct components.

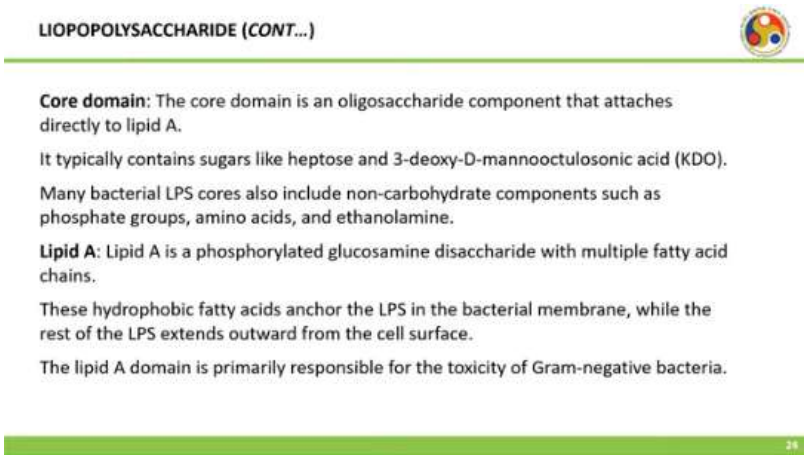


The O antigen (or O polysaccharide), the core polysaccharide, and lipid A. The core polysaccharide can be divided further into outer core and inner core. The O antigen, also known as O-polysaccharide or O side chain, is the outermost domain of the lipopolysaccharide molecule. It consists of repetitive subunits made up of various sugars, including rare ones like abequose, colitose, and paratose, and tyvelose. The O antigen shows significant inter- and intraspecies variation based on the types, order, and combination of sugars.

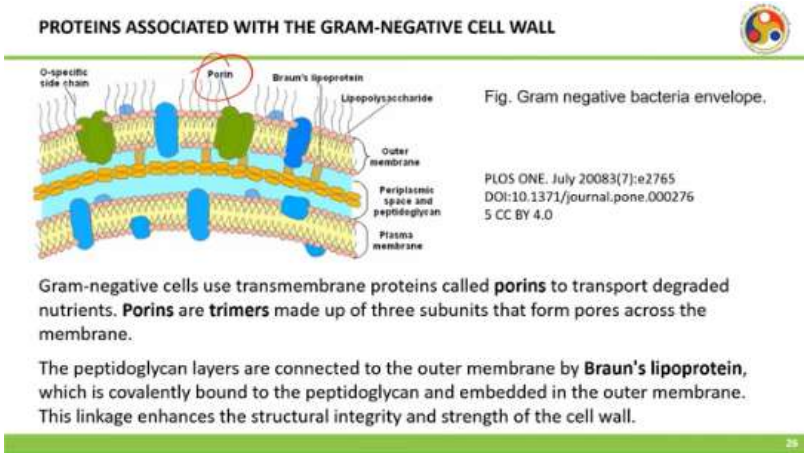


The presence or absence of O-antigen chains determines whether the LPS is classified as rough or smooth. The core domain. The core domain is an oligosaccharide component that attaches directly to lipid A, as we have seen in the diagram earlier. It typically contains sugars like heptose and 3-deoxy-D-manno-octulosonic acid, also known as KDO. Many bacterial LPS cores also include non-carbohydrate components such as phosphate groups, amino acids, and ethanolamine.

Lipid A is a phosphorylated glucosamine disaccharide with multiple fatty acid chains. These hydrophobic fatty acids anchor the LPS in the bacterial membrane, while the rest of the LPS extends outward from the cell surface. The lipid A domain is primarily responsible for the toxicity of Gram-negative bacteria. What are the proteins associated with the Gram-negative cell wall? Unlike Gram-positive bacteria that use exoenzymes, Gram-negative bacteria employ periplasmic enzymes stored in the periplasm.



to break down essential nutrients. The periplasm is the space between the cell membrane and the outer membrane, as you can see in this picture. Gram-negative cells use transmembrane proteins called porins to transport degraded nutrients. Porins are trimers—you can see here, one, two, three—made up of three subunits that form pores across the membrane. So there will actually be a pore in the center of these trimers.

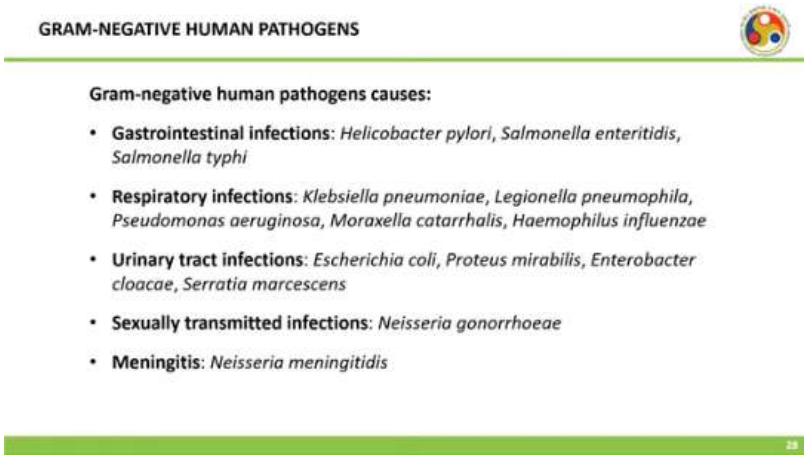


The peptidoglycan layers are connected to the outer membrane by Braun's lipoprotein, which is covalently bound to the peptidoglycan and embedded in the outer membrane. This linkage enhances the structural integrity and strength of the cell wall. Some examples of

gram-negative organisms include: Most bacterial phyla are gram-negative, with Proteobacteria being a major group. These include Escherichia coli, Salmonella, Shigella, and other Enterobacteriaceae, as well as Pseudomonas, Moraxella, Helicobacter, and Delovibrio, which we just mentioned in the earlier slide, acetic acid bacteria, and Legionella.

Other notable groups of gram-negative bacteria include Cyanobacteria, spirochetes, green sulfur bacteria, and green non-sulfur bacteria. Gram-negative bacteria include many human pathogens. Some of these cause gastrointestinal infections, for example, Helicobacter pylori and Salmonella enteritidis, then Salmonella typhi. Others cause respiratory infections like Klebsiella pneumoniae, Some cause urinary tract infections, for example, E. coli, Proteus mirabilis, Enterobacter, then Pseudomonas.

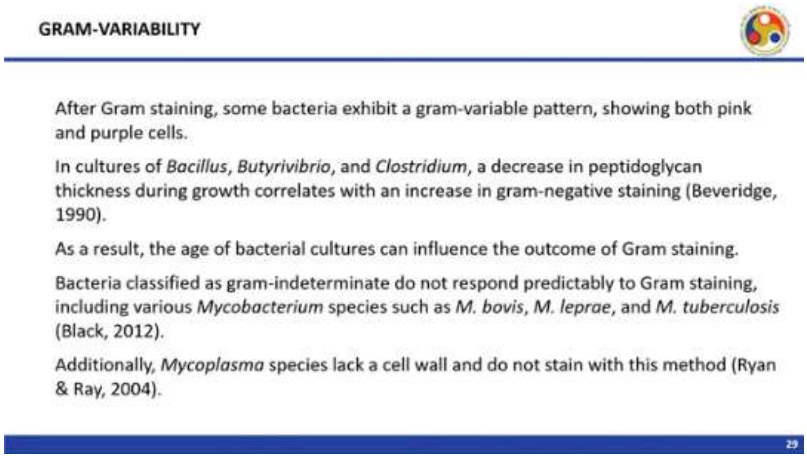
Some are also involved in sexually transmitted infections like Neisseria gonorrhoeae. Meningitis is caused by Neisseria meningitidis. Let us discuss gram variability. We know broadly that there are two types of bacteria based on Gram staining. One is gram-positive, another is gram-negative.



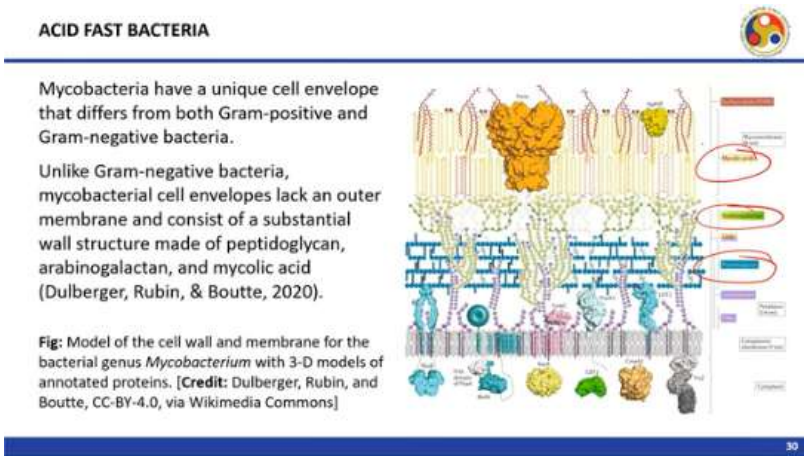
But there are certain variations. After Gram staining, some bacteria exhibit a Gram-variable pattern, showing both pink and purple cells in cultures of Bacillus, Butyri, Vibrio, and Clostridium. A decrease in peptidoglycan thickness during growth correlates with an increase in Gram-negative staining. As a result, the age of bacterial cultures can influence the outcome of Gram staining. Bacteria classified as Gram-indeterminate do not respond predictably

to Gram staining, including various Mycobacterium species such as M. bovis, M. leprae, and M. tuberculosis. Additionally, Mycoplasma species lack a cell wall and do not stain

with this method. Acid-fast bacteria. Mycobacteria have a unique cell envelope that differs from both Gram-positive and Gram-negative bacteria. Unlike Gram-negative bacteria, mycobacterial cell envelopes lack an outer membrane and consist of a substantial wall structure made of peptidoglycan, arabinogalactan,



and mycolic acids. This figure shows the model of the cell-wall membrane for the bacterial genus Mycobacterium and displays the different proteins present, including those in the cell membrane and cytoplasmic membranes. Mycobacteria resist decolorization by acids during staining due to their high mycolic acid content. The Ziehl-Neelsen stain, or acid-fast stain, is the most commonly used method to identify acid-fast bacteria. Let us discuss the bacterial cell membrane.



The bacterial cell membrane is a delicate lipid bilayer approximately 6 to 8 nanometers thick that surrounds the cell and separates its internal contents from the external environment. It is selectively permeable. Allowing certain substances to be retained within the cell while preventing diffusion into the periplasm. You can see various components of

the cell membrane, such as transmembrane proteins. Then we have peripheral proteins and glycoproteins, the lipid bilayer, which is the most important component.

Structural molecules over here, and also we can see certain carbohydrate chains embedded on the cell membrane. The primary structural element in the bacterial cell membrane is the Lipid bilayer, which is composed of a double layer of phospholipids. This is one layer, and this is the second layer. Numerous proteins are dispersed throughout this bilayer and remain associated with it.

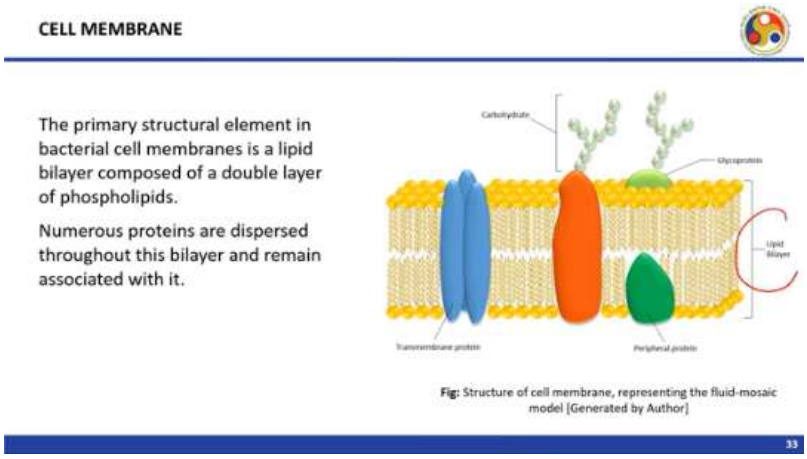


Fig: Structure of cell membrane, representing the fluid-mosaic model [Generated by Author]

For example, the phospholipids have a polar head. There is a polar head and a non-polar tail, which contains a negatively charged phosphate group, is hydrophilic, while the non-polar lipid tail, composed of two fatty acid chains, is hydrophobic. It is insoluble in water. These three carbons form a glycerol molecule, which is a three-carbon alcohol that acts as the backbone of the phospholipid. Linking the phosphate group to the two fatty acid chains, glycerol hydroxyl groups form ester linkages with the fatty acids, connecting them to the molecule as you can see in the picture.

Then, the fatty acid chains—these are two long hydrocarbon tails attached to the glycerol via ester linkages. The length and saturation of these fatty acid chains influence the fluidity and other properties of the phospholipid bilayer. Another important structure of a bacterial cell is the nucleoid. The nucleoid means nucleus-like. It is not a true nucleus.

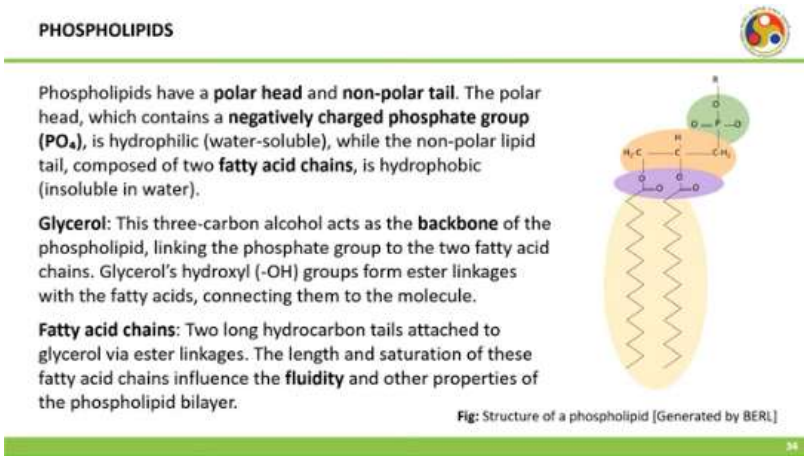


Fig: Structure of a phospholipid [Generated by BERL]

This is a region in prokaryotic cells that contains most or all of the genetic material of a bacterial cell. Due to the chromosome's length exceeding the cell's dimensions, compaction is necessary. The nucleoid forms through condensation and organization, aided by chromosomal architectural proteins, RNA molecules, and DNA supercoiling. For example, here you can see the circular E. coli genome. Which is quite large in diameter when it is open.

So, there will be, you know, random coiling of the DNA, and finally, this will be organized into a very compact structure. and giving rise to the nucleoid. So, you can see the random coil volume, for example, is something around 523 cubic microns, and then the nucleoid volume is something about 1 cubic micron. So, that is the level of reduction, which is a 1000-fold condensation. The genome length varies from bacterial species to species, typically spanning several million base pairs, and a cell may contain multiple copies of such a genome.

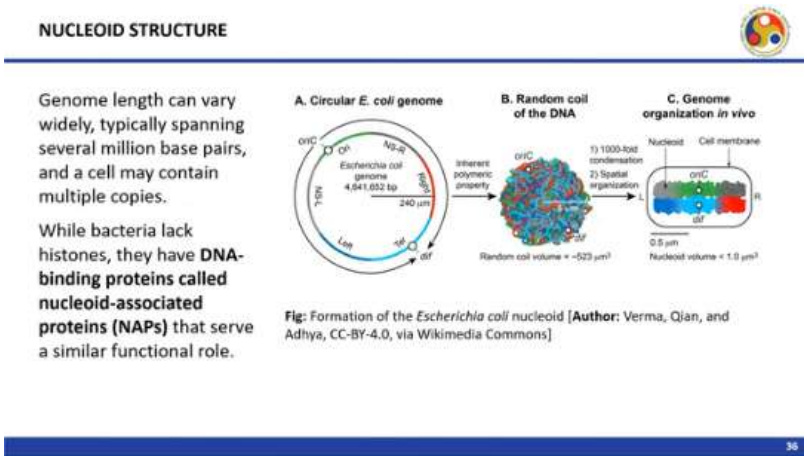


Fig: Formation of the *Escherichia coli* nucleoid [**Author:** Verma, Qian, and Adhya, CC-BY-4.0, via Wikimedia Commons]

While bacteria lack histones, they have DNA-binding proteins called nucleoid-associated proteins or NAPs that serve similar functional roles to histones. There is another type of genetic material or nucleic acid inside the bacterial cell known as plasmids. This is in addition to the nucleoid, and these are known as extra-chromosomal DNA. These, referred to simply as plasmids, are small circular double-stranded DNA molecules that exist independently of chromosomal DNA and can replicate autonomously. Plasmids often carry genes that enhance the organism's survival, such as those conferring antibiotic resistance.

Here, you can see the main bacterial DNA and these extra-chromosomal DNA molecules known as plasmids. When a cell divides into two daughter cells, just as the main DNA is divided among the two daughter cells, the plasmid DNA also undergoes replication and is divided into the two daughter cells. These plasmids can vary in size from 1 to over 400 kilobase pairs and can number from 1 to several thousand copies within a single cell under certain conditions. In certain cases, there is plasmid integration into the main chromosome. In this case, the plasmid DNA replication becomes integrated along with the main DNA replication.

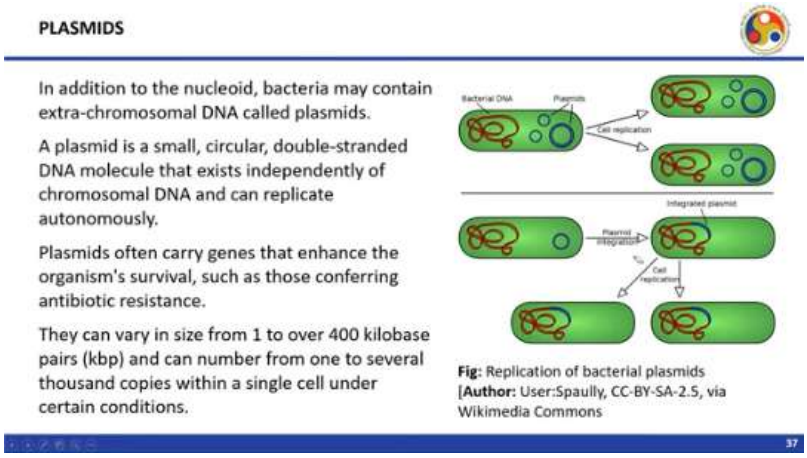
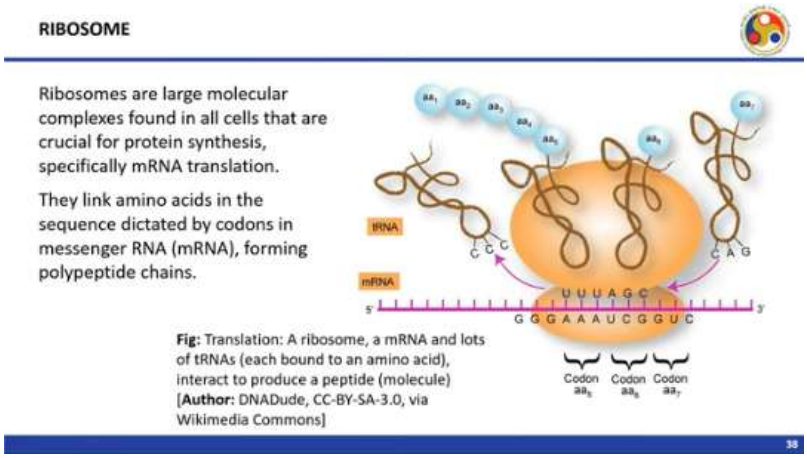


Fig: Replication of bacterial plasmids [**Author:** User:Spaully, CC-BY-SA-2.5, via Wikimedia Commons

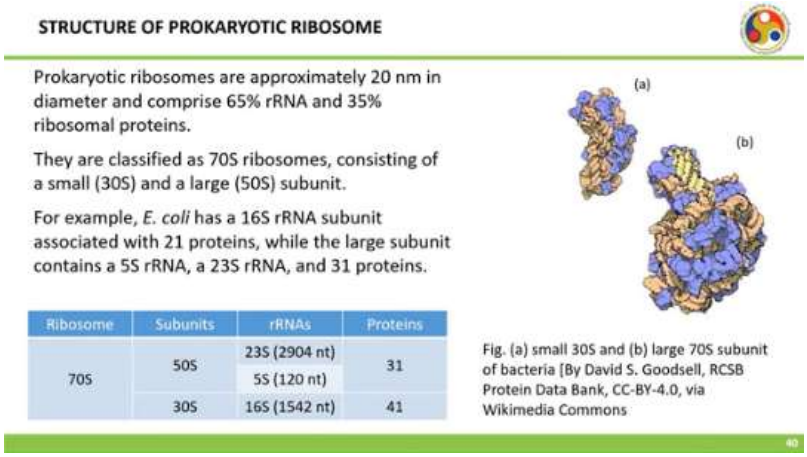
This can be a special case or exception. Let us now discuss another interesting structure: the ribosome. Ribosomes are large molecular complexes found in all cells that are crucial for protein synthesis, specifically in mRNA translation. These ribosomes link amino acids in the sequence dictated by codons in messenger RNA, forming polypeptide chains. Here, you can see the codons, this is the mRNA, and overall, this is the ribosome with two subunits—the small and the large subunit—and these are the tRNAs that bring in amino acids loaded into them.

And the codon dictates the sequence of these amino acids to be formed into polypeptides inside this polymerizing machinery. We will discuss it a little bit in detail. These ribosomes

consist of two main components, as I already mentioned, the small and large ribosomal units. They are made up of ribosomal RNA and ribosomal proteins. Together, they form the translational apparatus responsible for protein production in cells.



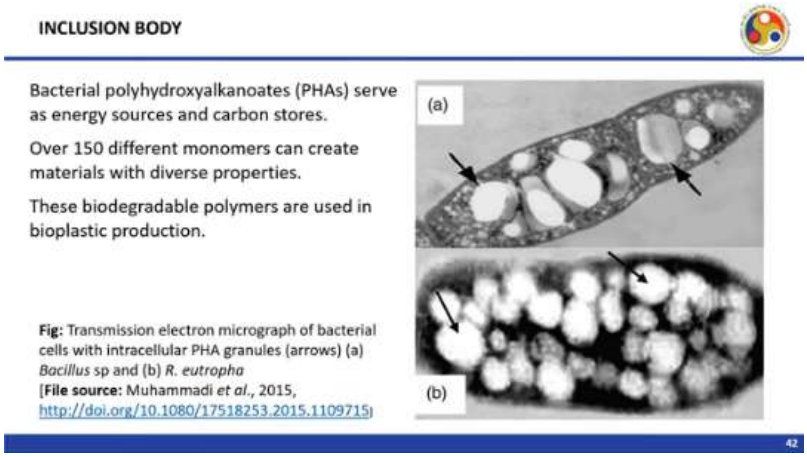
Prokaryotic ribosomes are approximately 20 nanometers in diameter and comprise 65% rRNA and 35% ribosomal proteins. They are classified as 70S ribosomes. They consist of a small 30S and a large 50S subunit. For example, E. coli has a 16S rRNA subunit associated with 21 proteins, while the large subunit contains a 5S rRNA, a 23S rRNA, and 31 proteins. So, this whole ribosome is size 70S and has two subunits,



50S and 30S, and then you can see the rRNAs: 23S, which is around 2,904 nucleotides; 5S, which is around 120 nucleotides; and 16S, which is around 1,542 nucleotides. The large subunit has 31 proteins, and the small subunit has 21 protein molecules. Another important component of bacteria is the inclusion bodies. These bacterial inclusion bodies, of course, are less abundant than those found in eukaryotic cells. One important type is

polyhydroxyalkanoates, or PHAs, which are polyesters produced by various microorganisms through the fermentation of sugars or lipids.

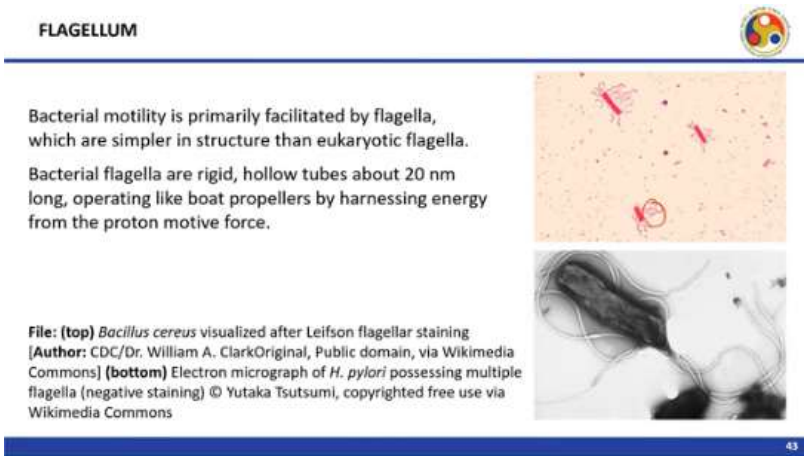
So this is a TEM micrograph of bacterial cells with intracellular PHA granules. In A, we can see these granules present in Bacillus species, and in B, we can see the granules present in R. eutropha. Bacterial polyhydroxyalkanoates serve as an energy source and carbon store. Over 100 different monomers can create materials with diverse properties. These biodegradable polymers are used in bioplastic production, and today they are gaining a lot of importance because of concerns about plastic pollution.



And these can probably be used as renewable, sustainable sources of polymers for our future use. Another structure that is important in bacteria is the flagellum. So we know that bacteria are unicellular organisms. They do not have any macrostructures like higher organisms for moving around, such as feet or hands. But they have a very special structure called a flagellum.

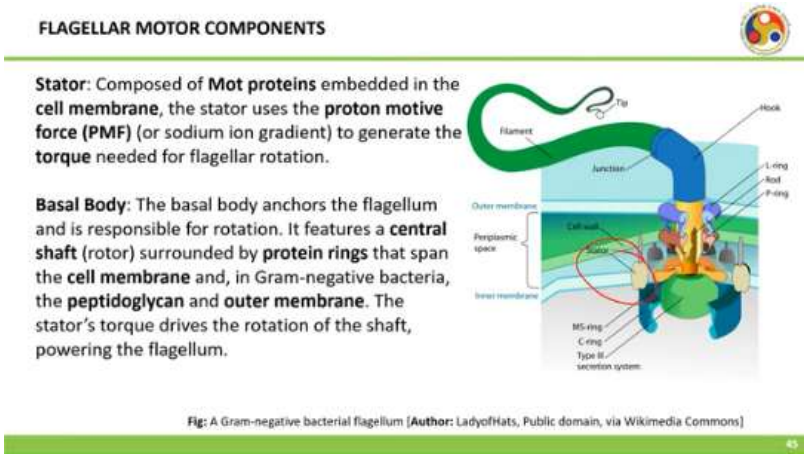
which provides motility. Flagella are simple in structure, particularly bacterial flagella, and compared to eukaryotic flagella, some eukaryotes also have similar structures. Bacterial flagella are rigid, hollow tubes, about 20 nanometers long, operating like propellers by harnessing energy from the proton motive force. So you can see these small structures here and also the magnified structures over here.

In these two pictures. Bacillus on the top and H. pyrale, which possesses multiple flagella, as you can see by negative staining over here. Let us look into the structure of flagella. It consists of three main parts: the filament, the hook, and the motor. The filament is a long, hollow structure extending from the cell surface, made of protein flagellin.



So, this is a hollow structure. The hook is a curved, flexible structure that connects the filament to the flagellar motor, allowing the filament to rotate. The motor is a rotary engine that spins the cell membrane, powered by ion gradients—proton or sodium—and responsible for driving the rotation of the flagella. What are the components of the flagellar motor? It basically contains a stator, as you can see over here.

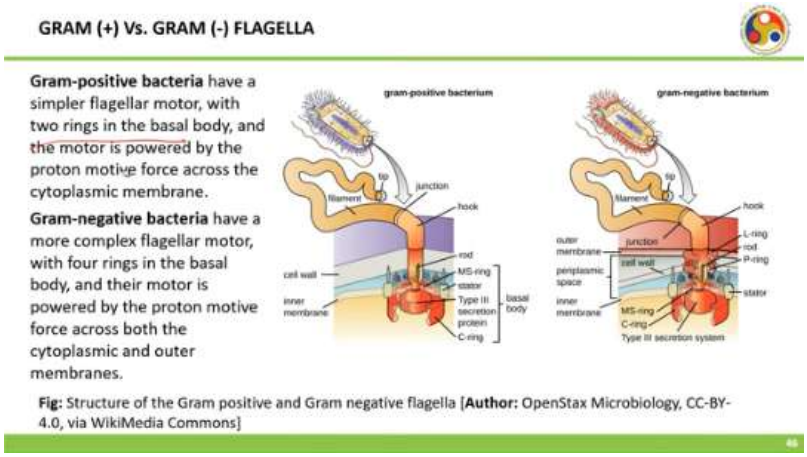
This is composed of Mot proteins embedded in the cell membrane. The stator uses the proton motive force to generate the torque needed for flagellar rotation. Then we have the basal body. The basal body anchors the flagellum and is responsible for rotation. It features a central shaft, or the rotor, surrounded by protein rings that span the cell membrane.



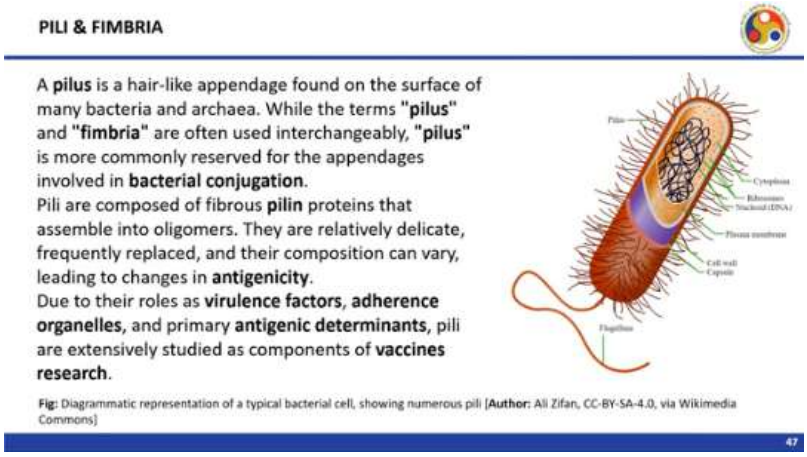
And in gram-negative bacteria, the peptidoglycan and outer membrane, the stator stalk drives the rotation of the shaft, powering the flagellum. However, there are differences in the gram-positive and gram-negative flagella structure. The gram-positive bacterium flagella you can see here and the gram-negative flagellum structure you can see here. So if you look into it keenly, you can see many of the differences, but we will now simply sum

them up. Gram-positive bacteria have a simpler flagellum motor with two rings in the basal body.

The motor is powered by the proton motive force across the cytoplasmic membrane. Gram-negative bacteria have a more complex flagellar motor, as you can see here, with four rings in the basal body, and the motor is powered by the proton motive force across both the cytoplasmic and outer membrane. Let us now discuss pili and fimbriae. A pilus is a hair-like appendage found on the surface of many bacteria and archaea. While the terms pilus and fimbria are often used interchangeably,

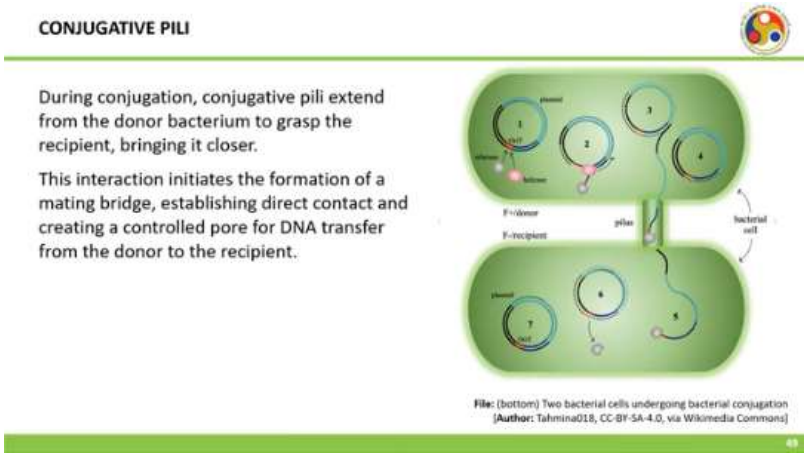


Pilus is more commonly reserved for the appendages involved in bacterial conjugation. Pili are composed of fibrous proteins called pilin that assemble into polymers. They are relatively delicate, frequently replaced, and their composition can vary, leading to changes in antigenicity. Due to their roles as virulence factors, adherence organelles, and primary antigenic determinants, pili are extensively studied as components of vaccine research. Conjugative pili.

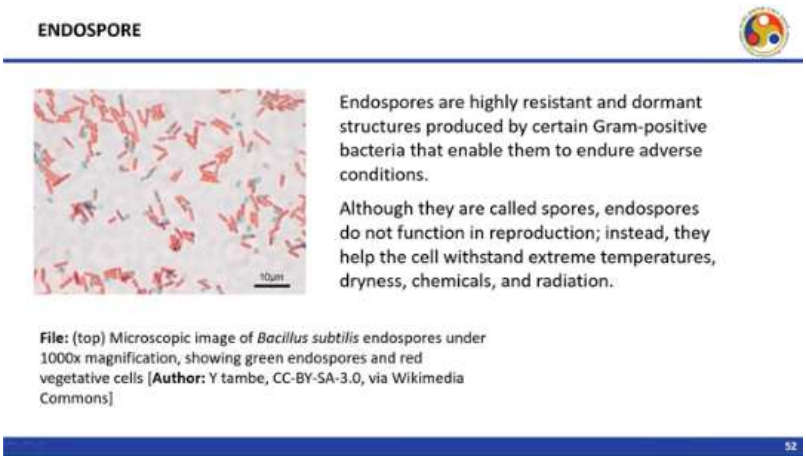


These are also known as the sex pili and are crucial for DNA transfer between bacteria during bacterial conjugation, which is a process that resembles sexual reproduction and involves the formation of mating pairs. A well-studied example is the F-pilus of E. coli, which is encoded by the F sex factor. These pili typically have a diameter of 6 to 7 nanometers. Conjugative pili. During conjugation, conjugative pili

extend from the donor bacterium to grasp the recipient, bringing it closer. This interaction initiates the formation of a mating bridge, establishing direct contact and creating a controlled pore for DNA transfer from the donor to the recipient. And you can see here the pore through which the DNA is being transferred. An attachment pilus is a short pilus that allows bacteria to adhere to other bacterial cells, animal cells, or even inanimate objects. These appendages typically measure 3 to 10 nanometers in diameter and several micrometers in length, with a single bacterium having up to 1,000 pili.

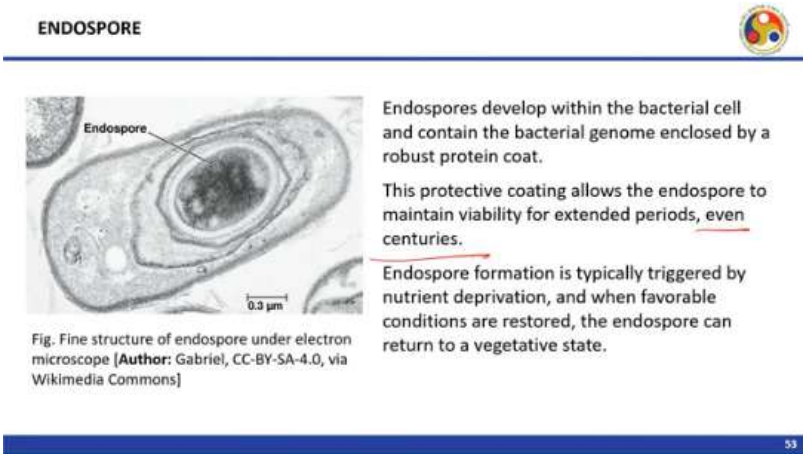


Fimbriae contain adhesins that enable bacteria to adhere to various surfaces, helping them withstand shear forces and acquire nutrients. They also play a key role in forming pellicles on the surface of liquid cultures, particularly in aerobic bacteria, allowing them to remain near the air while accessing nutrients. Let us now discuss one important topic in bacteria: endospores. These endospores are highly resistant and dormant structures produced by certain Gram-positive bacteria, enabling them to endure adverse conditions. Although they are called spores, endospores do not function in reproduction.



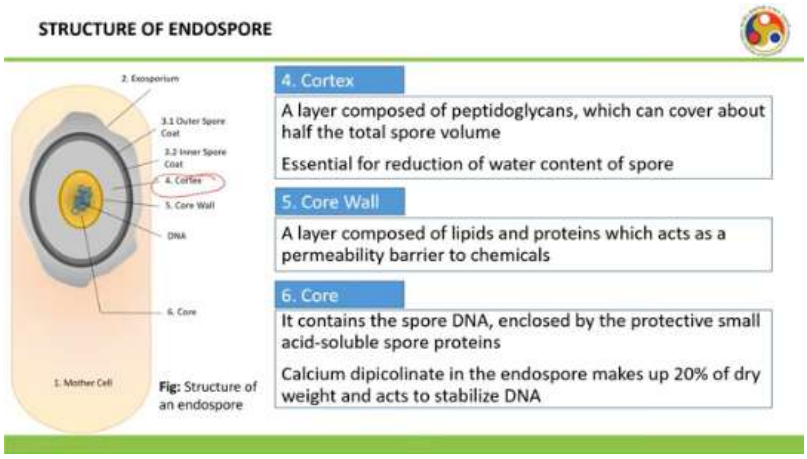
Instead, they help the cell withstand extreme temperatures, dryness, chemicals, and radiation. So, in this microscopic image of Bacillus subtilis, we can see endospores under 1000x magnification. So, you can see here an endospore, and there are certain structures surrounding it. We will try to understand what these endospores are and how they are produced. So, this is an image under an electron microscope.

Endospores develop within the bacterial cell. And contain the bacterial genome enclosed by a robust protein coat. This protective coating allows the endospore to maintain viability for extended periods, even Endospore formation is typically triggered by nutrient deprivation, and when favorable conditions are restored, the endospore can return to a vegetative state. What are the structural components of an endospore?



So, we can see here the mother cell, which is the anti-cell you can see. This is the cell within which the endospore is formed. Then we have Exosporium—this is a thin covering composed of proteins and glycocalyx, which sometimes covers the spore. Then we have a spore coat, which may have inner and outer layers.

This is a proteinaceous layer which acts like a sieve that excludes large toxic molecules from entering inside. It may also contain enzymes that are involved in germination. Then we have a cortex. This is a layer of peptidoglycans which can cover about half the total spore volume. This is essential reduction of water content of the spore.



Then we have the core wall. This is a layer composed of lipids and proteins which act as a permeability barrier to chemicals. And then we have the core which contains the spore DNA enclosed by the protective small acid soluble spore proteins. Calcium dipicolineate in the endospore makes up 20% of dry weight and act to stabilize the DNA inside. Now let us have a look at the sporulation process.

So here we can see the vegetative phase of a bacteria. Now, how these vegetative phase transforms into a endospore and back into the vegetative phase, we will try to examine. This entire process known as sporulation is actually very complex and it is tightly regulated developmental pathway that allows the bacterium to form endospores. In response to environmental stress, for example, nutrient depletion or scarcity of nutrients of food. One thing we need to focus here is the formation of the Z ring.

This Z ring is formed by FTS-Z protein that assembles into a ring and its depolymerization causes inward constriction forming the septum that divides the cell into two daughter cells. So, we can simply divide it as stage 1, stage 2, stage 3, stage 4, stage 5, stage 6 and 7 and then the germination occurring and we will now discuss all these various stages in little bit of detail. So, the first stage or the initiation is when the sporulation begins, when the bacterial cell comes under unfavorable conditions or gets exposed to unfavorable conditions like nutrient limitation. which triggers the activation of sporulation genes. So, this is a vegetative stage under unfavorable conditions.

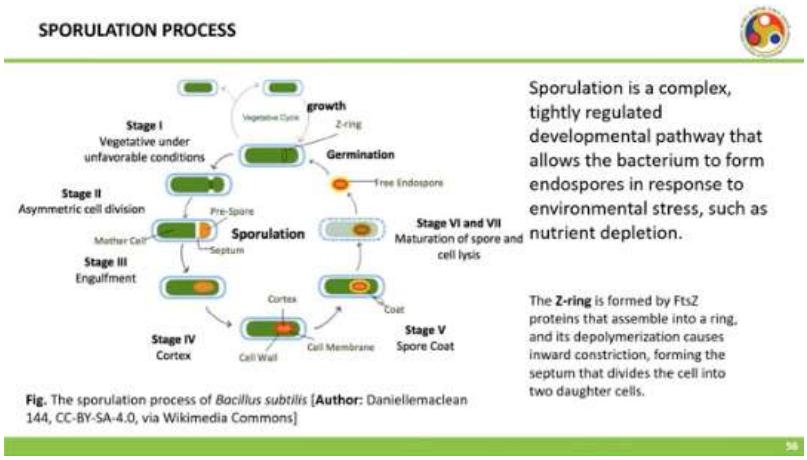


Fig. The sporulation process of *Bacillus subtilis* [**Author:** Daniellemaclean 144, CC-BY-SA-4.0, via Wikimedia Commons]

So, initiation will occur over there. Then it moves to the next phase, the stage 2 or the asymmetric division. Here the cell divides You can see here one cell is bigger and another cell is smaller. So, this is the cell bigger one and this is the cell which is a smaller one.

So, in stage 2 we have asymmetric cell division. The larger mother cell and the small mother cell. The next stage or stage 3 is the engulfment stage where the mother cell will engulf the prespore and thereby it will form a protective double mundane. So, this is the mother cell and this is the prespore. So finally, it goes into stage 4 and subsequently stage 5 which is the cortex and spore coat formation stage.

The pre-spore will synthesize a peptidoglycan cortex, and the mother cell adds a protein coat, enhancing resistance in these stages. Then, it proceeds to the maceration stage, where the endospore dehydrates, gaining resistance to heat, UV, and chemicals before the mother cell releases the mature spore. Then, it enters the dormancy stage, and this can remain dormant for centuries, as already mentioned. The endospore enters a dormant state, surviving harsh conditions until it can germinate when favorable conditions return. Thus, the endospore will exist in free form between dormancy and the germination stage.

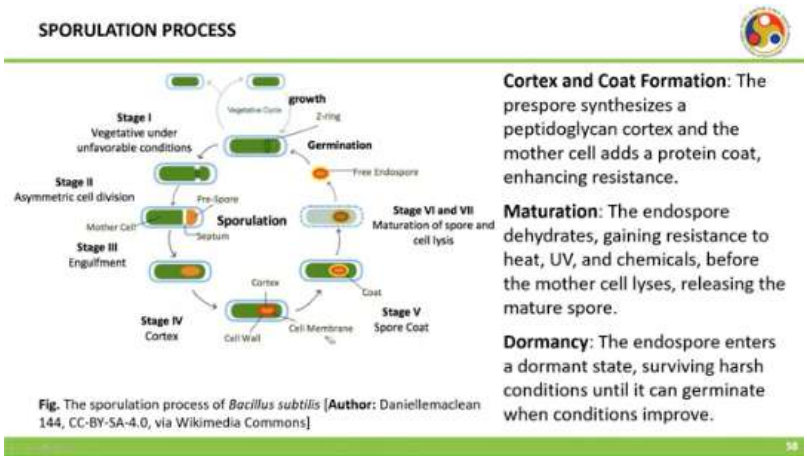


Fig. The sporulation process of *Bacillus subtilis* [**Author:** Daniellemaclean 144, CC-BY-SA-4.0, via Wikimedia Commons]

So, with this, we come to the end of this lecture. Next, we will discuss other organisms in the following lecture.