Environmental Chemistry and Microbiology

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Module - 8
Lecture - 40
Cell Biology - III

Welcome everyone to the third part of Cell Biology.

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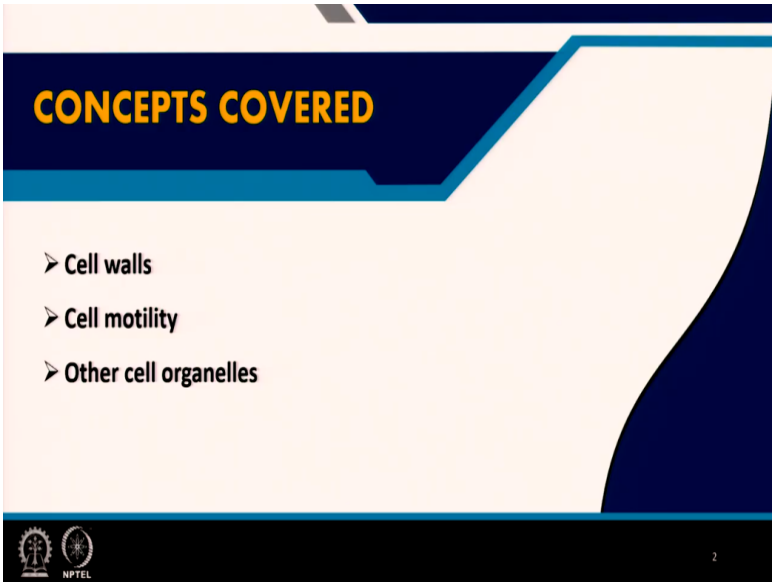

We are going to cover a few other cell organelles in this topic and that is cell walls; how do cells move; what are the different organelles that they use for movement; and other cell organelles.

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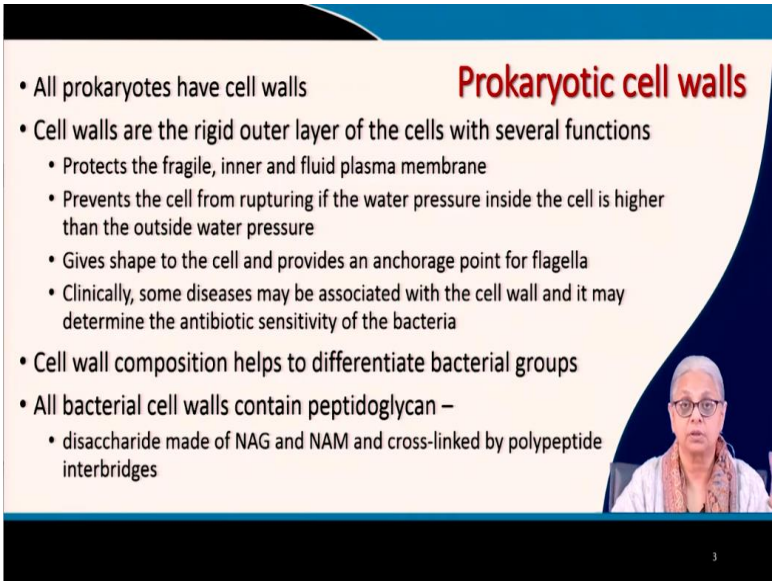

So, let us start with the most important part of the bacterial cell or the prokaryotic cell; and that is the cell wall. So, we know that for living as independent organisms, the first thing that allows them to live independently is the cell wall. So, all prokaryotes have a rigid cell wall. So, these cell walls, like I said, are rigid outer layers of the cell. And they have several functions to perform.

You have already seen in the previous topic that the plasma membrane which is basically the site which determines how nutrients go in and out of the solution; how waste is excreted. That is a very fragile inner membrane and it is highly fluid and it is not bonded at all. So, it is very easy to disturb it. It is an extremely fragile membrane. So, without the cell wall to protect it, this membrane cannot exist.

And the membrane, the plasma membrane is the one that does all the useful work of determining what goes in and out. So, the first thing is protection for the plasma membrane. That is the first function of the cell wall. The second is to maintain the water pressure at the level that is required for the cell to function. So, it prevents the cell from rupturing, if the water pressure inside the cell becomes higher than the outside pressure. So, that equilibrium is maintained by the cell wall.

As I said, the cell wall itself is a rigid membrane, unlike the plasma membrane. It gives shape to the cell and it provides an anchorage point for the flagella. And last, but not the least, the cell wall has been found to determine whether a particular infectious agent, microorganism will cause a disease or not. So, from a clinical standpoint, some diseases are associated with

the cell wall of that organism. And in many cases, it is the cell wall that determines the antibiotic sensitivity of that bacteria or prokaryote. I can also mention over here, something that has been mentioned in some textbooks, that it is only the modern bacteria that are pathogenic to us. Archaebacteria have never been found to be pathogenic to human beings.

That itself is an interesting observation. Again, it has something to do with the evolution of life and so on. So, remember that a pathogen likes the same conditions as the host, which means that the infected person or organism has to have the same environmental conditions as the pathogenic organism. So, these modern bacteria and human beings have had a long association and it is the archaebacteria which have no association with human beings.

Then we come to cell wall composition. This cell wall has different chemical composition in different bacterial groups or species. So, that is another marker for differentiating different types of bacteria. What is common to all these modern bacteria is that all bacterial cell walls contain a chemical called peptidoglycan. So, this peptidoglycan has 2 parts to it. The glycan part; if you remember in cell chemistry, in the previous topic, we looked at NAG and NAM. So, N-Acetylglucosamine and N-Acetylmuramic acid are the building blocks of the cell walls. So, these are disaccharides that form the glycan part of it. These building blocks, they form strand and these strands are linked, cross-linked to each other by polypeptide interbridges. So, some interbridges are shorter and others are longer, that gives identity to the bacterial species. So, I would ask you to look into the textbook and look at the detailed diagrams of the peptidoglycan wall that is there in different bacterial species. So, gram negative, gram positive bacteria have different levels of peptidoglycan in their cell wall. And the structure is different based on these polypeptide interbridges but the building blocks are the same, NAG, NAM.

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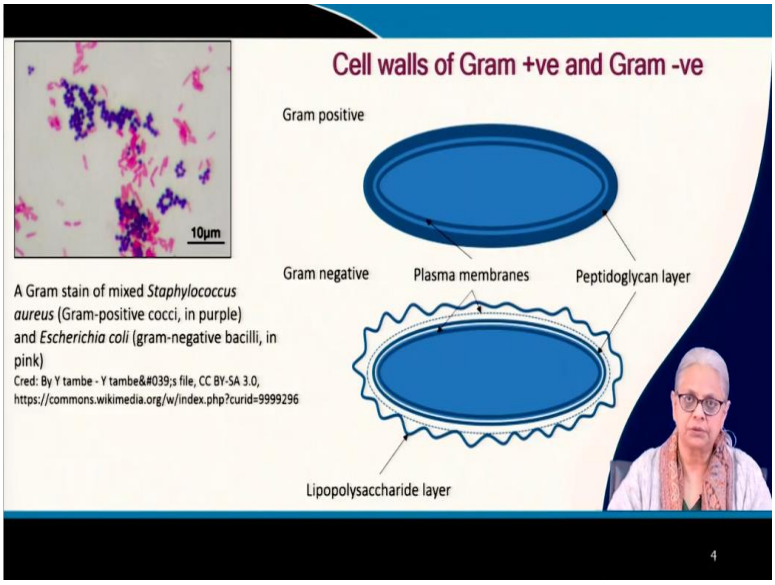

So, as I said, I think at some point in the past, there are 2 major categories of the modern bacteria. So, that is gram positive and gram negative. You can see how these gram positive and gram negative bacteria are differentiated. There is a gram staining procedure which we will look at in the next topic on microscopy. Because, when you want to look at a mixed population of bacteria, the first thing that most people would do is to put it through a gram staining procedure.

In the gram staining procedure, you will have two outcomes. Some cells will be coloured a dark purple; and other cells will be found to be pinkish or reddish in colour. This difference in colour between two groups of bacteria, that is mainly because of the difference in composition of the gram positive and gram negative cells. In the gram positive cells, the cell wall is very simple. There is a plasma membrane around the cytoplasm, which is followed by a peptidoglycan layer. This peptidoglycan layer is extremely thick in gram positive cells. In gram negative cells, you have a plasma membrane. Then you have three different layers around the plasma membrane. The first thing is the inner plasma membrane. That is followed by a very thin peptidoglycan layer. This thin peptidoglycan layer is followed by an outer plasma membrane. So, there are 2 plasma membranes, an inner membrane and an outer membrane. Outside all of this is what is called a lipopolysaccharide layer. So, this is kind of like a slime layer. It is also called an exopolysaccharide layer, EPS or LPS. So, that is the difference between gram positive and gram negative cell walls.

So, when all the cells in a mixed population are subjected to the same staining procedure; there are several steps in that procedure. We will go into the details under microscopy. But

for now, it is important for you to know that the first stain is crystal violet. Crystal violet gives a purple colour to all the bacteria. Then it is decolourized by alcohol and then counter stained by safranin. Safranin has a red colour. Now, the cells that have a thin peptidoglycan layer are unable to hold on to the crystal violet stain. So, they get decolourized by alcohol. And then when safranin is added, they turn red. That is the counter stain. The other gram positive cells have a thick peptidoglycan layer. So, when they are stained by crystal violet, they retain that stain. And when alcohol is applied, they do not get decolourized and when the safranin is added, they remain purple. So, purple is a darker colour, and the colour remains the same. So, here we have *Staphylococcus aureus*. It is gram positive. It is purple. And we have *E. coli* or *Escherichia coli*, which is gram negative; and it is pink in colour. So, this is one of the oldest staining procedures perhaps that we know of that has been used and it continues to be used even today to differentiate bacteria into two groups, based on the nature of the cell wall.

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Characteristics	Gram positive	Gram negative
Morphology	Cocci or spore-forming rods	Non-spore forming rods
Outer membrane	Absent	Present
Peptidoglycan layer	Thick (Multi-layered)	Thin (Single-layered)
Periplasmic space	Absent	Present
Teichoic Acids	Present	Absent
Toxins produced	Exotoxins	Exotoxins and Endotoxins
Flagellar Stucture	2 rings in basal body	4 ring in basal body
Gram reaction	Retains crystal violet dye and stains blue or purple	Decolorised by alcohol to accept counterstain (safranin) and stains pink or red
Lipopolysachharide (LPS) content	Absent	Present
Lipid and Lipoprotein content	Low	High
Lysozyme disrupted cell wall	High	Low
Resistance to physical disruption	High	Low
Inhibition by basic dyes	High	Low
Resistance to drying	High	Low

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So, here we have the characteristics of gram positive and gram negative bacteria. The morphology may be cocci or spore forming rods. They are non-spore forming rods in the gram negative group. The outer membrane is absent in gram positive bacteria. It is present in gram negative bacteria. The peptidoglycan layer is thick; it is multi-layered. In gram negative, it is thin, it is single-layered. Periplasmic space is absent. Let me show you some more diagrams.

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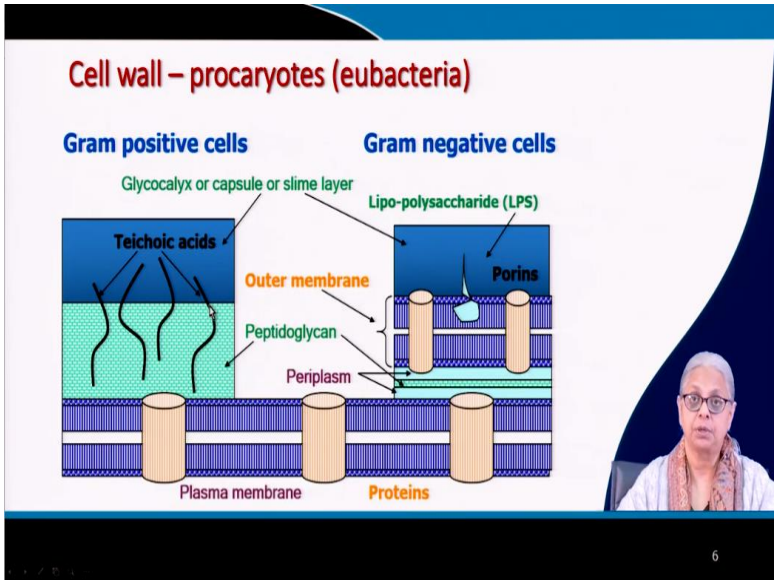

So, the plasma membrane is common to all bacteria. It is there in archaebacteria; it is there in bacteria. And it is there in gram positive, gram negative. All of them have a plasma membrane. It is the outer layer, the layer outside the plasma membrane, which has differences. The gram positive cells have a thick peptidoglycan layer. And the gram negative cells have this thin peptidoglycan layer, which is actually a monolayer.

And there are spaces between the plasma membrane and the peptidoglycan layer. You have periplasmic space. And there is an outer layer, an outer membrane, which is again formed by phospholipid. There is a bilayer, which is formed by phospholipids again. And you have other transmembrane proteins, including the porins, which will determine what comes in to the periplasmic space and then the plasma membrane. So, and outside this, you have a lipopolysaccharide layer. So, this is all about gram negative cells.

In gram positive cells, after the thick peptidoglycan layer, you also have what are called teichoic acids. These teichoic acids further have glycocalyx or capsule or slime layer. And this slime layer allows these bacteria to stick to surfaces. So, these are some of the things that are common to prokaryotes, the eubacteria; the only thing common between bacteria and archaebacteria is the plasma membrane; the rest of it (cell wall) is all different.

So, these teichoic acids are present in gram positive, but not in gram negative. Are toxins produced by these bacteria? Gram positive bacteria exude exotoxins. So, exotoxins is what is extruded by the cell. And endotoxins and exotoxins are generated by gram negative bacteria.

And many of them are pathogenic. Flagellar structure is different in gram positive and gram negative bacteria. I have already mentioned the gram stain.

I have also mentioned that lipopolysaccharide is absent in gram positive bacteria and present in gram negative. There are several other differences between lipids, lipoproteins, lysozyme, their resistance to physical disruption, the inhibition by dyes and the resistance to drying. Here, you can see that because of the nature of the peptidoglycan layer, all these differences are a consequence of that difference.

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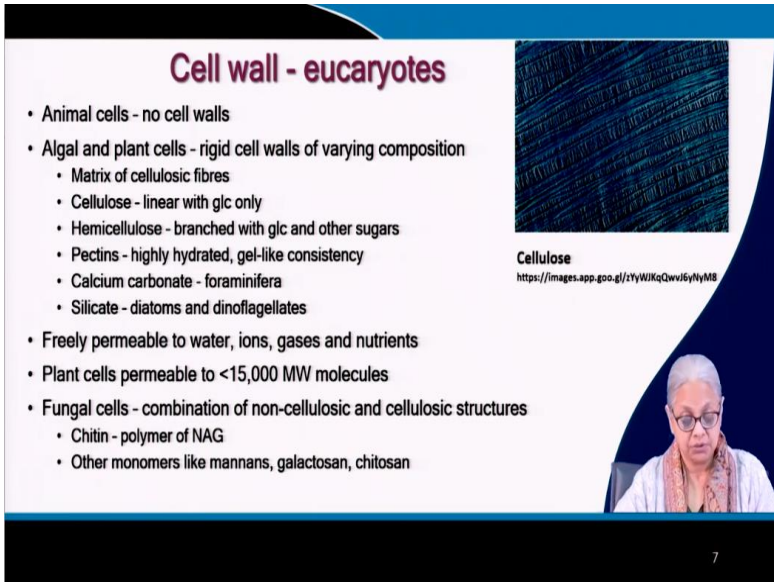

In terms of the cell wall of eukaryotes; so, putting the prokaryotes aside, we have to remember that there are other microorganisms that are not bacteria. So, let us take a look at the eukaryotes. Animal cells, we know do not have cell walls. Algal and plant cells have rigid cell walls of different chemical composition. In general, algal and plant cells have a matrix of cellulose fibre. So, just like clothes, you can see that in clothes you have a warp and a weft, but nature does not do that. It just places fibres in alternate layers. So, you can see very similar structure to clothes, where you have woven clothes, but not exactly the same. So, you have these fibres that are sitting on top of each other and cellulose is very similar to what we have in cotton, yes. So, we have these alternating fibres which are made out of cellulose and they are seated on each other in alternate arrangement. So, it is a matrix of cellulosic fibres. This cellulose, we know is a linear strand of glucose only. And it is a beta-1,4-glycosidic bond. Then we have hemicellulose. So, hemicellulose is branched. It has glucose and other sugars and perhaps other bonds. You also have pectins. Pectins are highly hydrated. They have a gel like consistency.

You can have calcium carbonate in a particular organism called foraminifera. And you have diatoms and dinoflagellates. These are all types of algae. And they have; their shells are made out of silicate.

Then we come to the fact that the cell walls of eukaryotes are freely permeable to water, ions, gases and nutrients. That is how these organisms are able to survive in their environment. So, that is very different from the prokaryotes. Plant cells are able to allow large molecules, even up to 15,000 molecular weight. So, even large molecules which have any size less than 15,000 molecular weight, can permeate through these plant cell walls. That is how plant cells are able to absorb nutrients from their environment through the root zone.

And then we come to fungal cells. Fungal cells have a combination of non-cellulosic and cellulosic structures in their cell walls. So, we have chitin. Chitin is a polymer of NAG. And we have other monomers like mannans, galactosan, chitosan. All these are monomers that can be part of the fungal cells.

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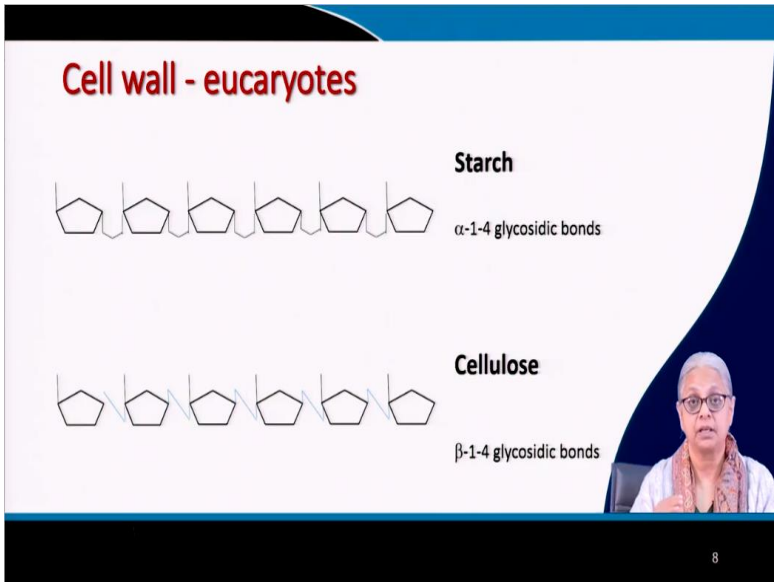

I have already shown you the structure of starch and cellulose. And when we talk about cellulose, remember what I said in a previous lecture; I said that plant cells and especially the plant structure is made out of lignocellulosic compounds. So, the actual rigidity of the plant, its ability to stand erect and maximise harvesting of sunlight and converting it to chemical

energy and so on; all of that is based on the rigidity imparted by lignocellulosic structures within the cell wall.

So, all of that is very important for plants as well as algal cells. Starch on the other hand, is a highly biodegradable material. It provides no rigidity at all. It is in contrast to cellulose, which provides some level of rigidity to the structure; and much of it comes from lignin as well.

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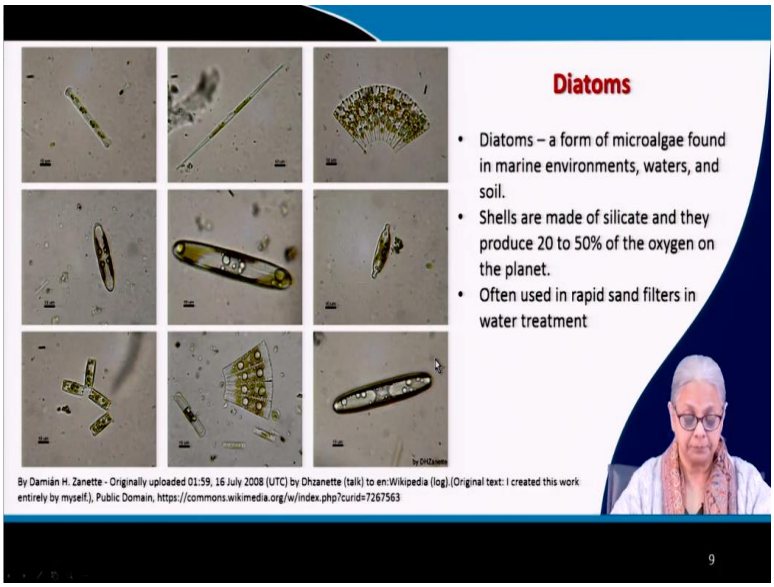

Then we come to diatoms. Diatoms are, like I said, silicate shells. They are a form of microalgae that have silicate shells. They are found in marine environments; they are found in many freshwater environments as well as soil. Like I said, the shells are made out of silicate and they are considered to produce 20 to 50% of the oxygen on the planet is attributed to this form of microalgae. And we use them. In environmental engineering, the biggest application of diatoms is in rapid sand filters that are used in water treatment plants. So, in many water treatment plants, you will find sand. And in combination with sand, you will find fine sand as well as diatomaceous earth. So, this diatomaceous earth is finer than perhaps the finest sand particles. And it provides a high degree of filtration in water treatment. And you can see over here, several examples from Wikipedia; all of them. And these are all different types of microalgae.

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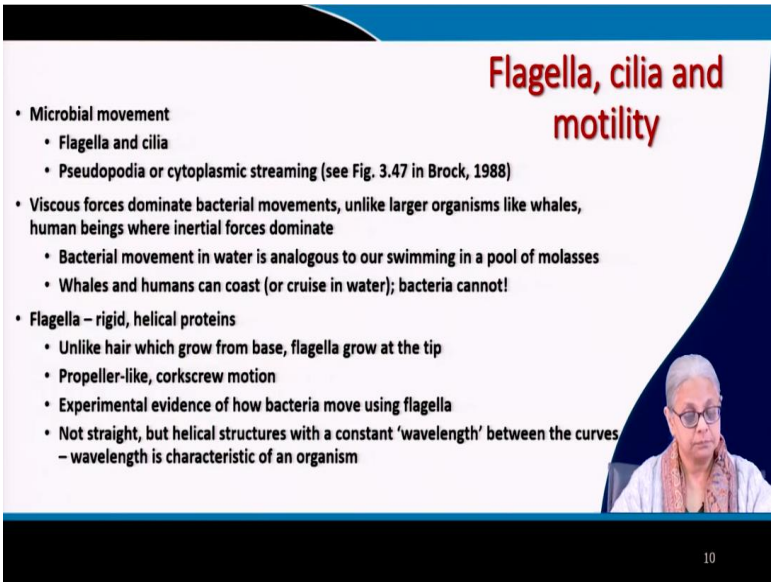

Then we come to some other cell organelles, flagella, cilia and motility. Flagella are used for cell motility as well as cilia. So, these 2 are the cell organelles that are used by several microorganisms for moving. We also have another example of pseudopodia or cytoplasmic streaming. You can refer to some of the older textbooks and there are examples of how amoeba creates a false foot and literally streams its cytoplasm in a particular direction to ingest food.

So, we are going to look at all these different ways of movement within microorganisms. I have already mentioned that viscous forces dominate bacterial movements and large organisms like whales and human beings are dominated by inertial movement. So, like I said, we know how difficult it would be for us to swim in a pool of sugar syrup or molasses. And bacterial movement in water is no different. So, they are going to expend a huge amount of energy in moving from one point to another; and they generally do it in search of either nutrients or light or oxygen. So, we will take a look at some of these examples. Let us start with flagella. Flagella are rigid helical proteins. Unlike hair which grows from the base; you know that our own hair grows from the base, not from the tip. Flagella grow at the tip. Even though they look like hairy appendages; let me show you.

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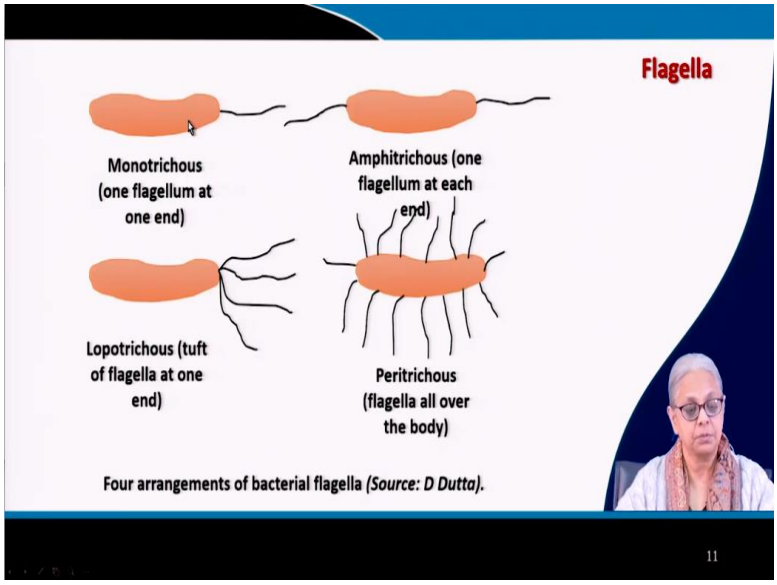

So, these are; this is a bacterial cell which has a flagella attached to its surface, to the cell wall. And unlike hair, it grows at the tip. It moves through water in a propeller-like corkscrew motion. So, if you know how a propeller on a boat works, you know that it is moving, it is rotating and it pushes the boat forward in water. So, it is the same principle that the flagella uses to push the bacterial cell in the water.

And I will show you some examples. The textbook has several examples, experimental evidence of how bacteria move using flagella. They are not straight. They are helical structures with a constant wavelength and that wavelength is used to define the species of a particular bacteria. So, let us take a look at different types of arrangements of bacterial flagella. We have the first one. Monotrichous means, one flagella at one end. So, this is one flagella attached at one end. Amphitrichous means, both ends so, one flagella at each end of the cell. These are for bacilli type cells. Here we have Lophotrichous. Lophotrichous is a tuft of flagella at one end and that means multiple flagella attached at one end. Then we have Peritrichous. Peritrichous means, all the flagella are distributed throughout the surface area of the bacterial body or the cell.

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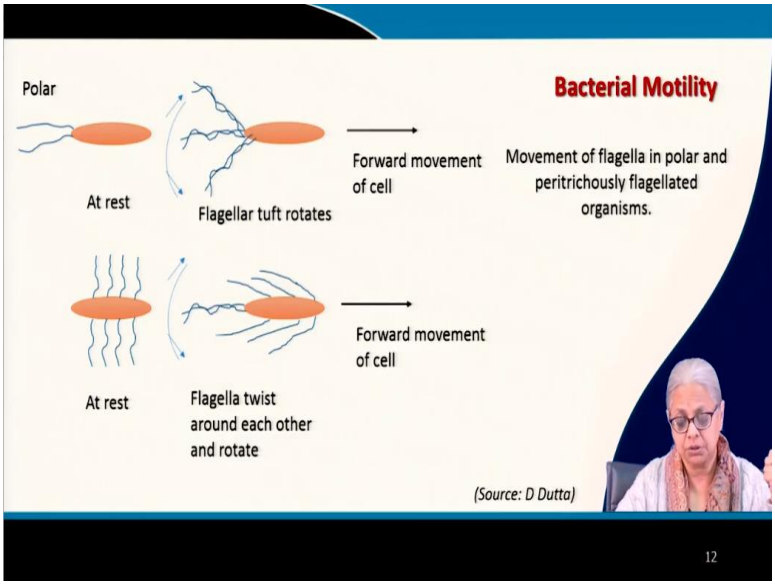

How does the bacteria move in water? So, here we have a polar arrangement of the flagella. So, here is a bacterial cell. It has only 2 flagella. Now, these 2 flagella will sort of braid around each other. They will form a structure. This structure will move to and fro literally. So, this is the nature of the movement of the flagellar tuft. This will push the cell in a forward direction.

Now, if we have a Peritrichous arrangement of flagella, this is the position of the flagella when the bacterial cell is at rest. When it moves, it will all arrange itself so that the flagella are very close to the body of the cell. And they will all again move to and fro in the same dimension. And that will push the cell forward. How does the cell move? Does it move in a straight line? The answer is no. Every time the cell has to move; and if it has to change direction; so, what I just showed you here is a run and that run is in a straight line direction.

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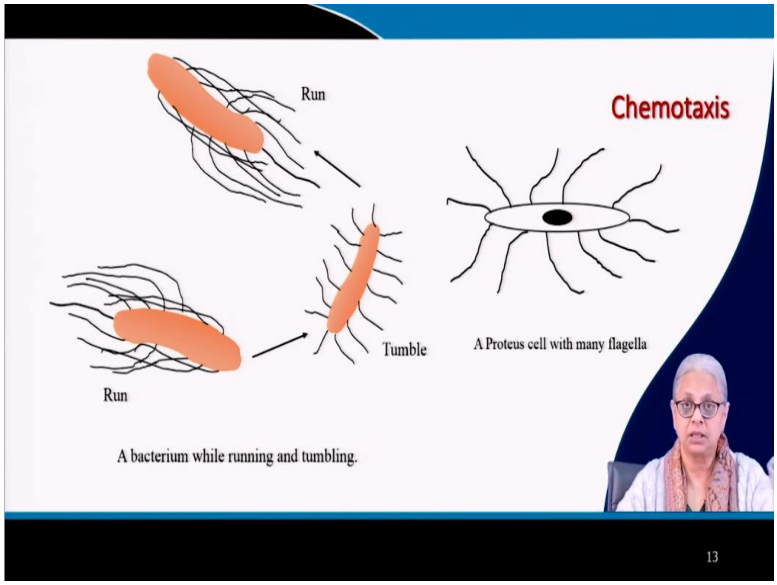

But when it wants to change direction, how does it change direction? So, the first thing it does is, it comes into its resting position. It tumbles over, so that it is facing a new direction. And it starts the new run. So, it has a run, a tumble and a run. The tumble is simply for changing direction.

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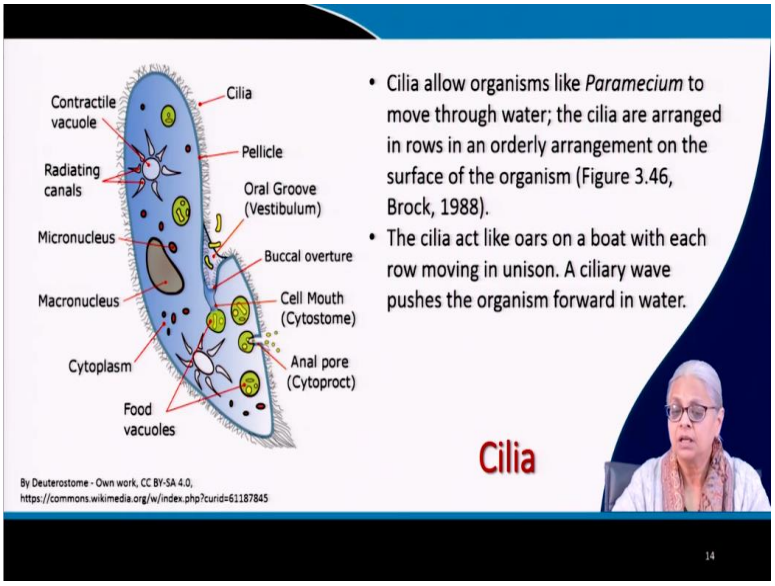

Then we come to paramecium. Paramecium is a protozoa. It is a higher microorganism. It is still free living, it is still independent but it is much bigger than the bacteria. It has what are called cilia. So, this cilia are like small hairy appendages that are spread over the entire surface of the paramecium. These cilia are arranged in rows. So, over the body; they are spread all over the body, but they are arranged in rows.

It is almost like hair, but because they are arranged in orderly form, they work like oars on a boat. So, if you think about the old style big boats which had hundreds of boatmen pushing it, pushing the boat or rather the ship through the water. So, you have that kind of arrangement here. So, each row of cilia is going to move at the same time. So, you have a ciliary wave. So, the ciliary wave is where each row of the cilia is moving together, pushing in one direction. And then, the next row starts doing the same thing; and so on and so forth. So, there is a ciliary wave over the entire body of the protozoa, that pushes the paramecium through the water. It is very interesting. You can take a look at the nature of these cilia on the surface of the paramecium.

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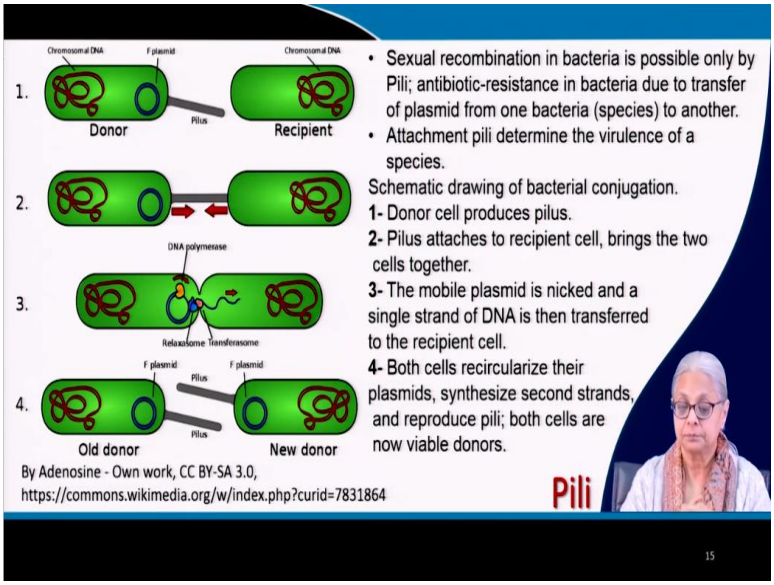

And then we come to pili. So, pili are again not always present in all bacteria. They may be present in certain bacteria. And I said initially that bacteria divide by binary fission. By and large, bacteria will not have sexual recombination, for the simple reason, they divide by binary fission, and the DNA of the bacterial cell is replicated exactly, and then it is transmitted to the two daughter cells that each bacterial cell produces.

So, there is generally no sexual recombination. However, under certain conditions, it has been observed that the bacteria will create an organelle called a pilus and this pilus is going to attach itself to a recipient cell. So, in this particular case, we have a donor cell. It has the initial chromosomal DNA and a certain plasmid called the F plasmid. It may be any type of plasmid. I will come to why that is so important.

Now, this plasmid is considered to provide antibiotic resistance to this particular bacteria. So, let us imagine a situation where different bacteria of the same species are being exposed to a particular antibiotic. Some of these bacteria are resistant to that antibiotic and that is because of this plasmid; not because of the original DNA. There are some cells which are not resistant; and survival requires that they also become resistant by getting this plasmid. So, how is that possible? This is considered to be possible because of this organelle called the pilus. So, when this pilus from the resistant bacteria attaches itself to the non-plasmid containing bacteria, you get this kind of attachment. The plasmid which is another smaller strand of DNA, which gives the bacteria its resistance to that particular antibiotic, that is cut and it is transmitted to the recipient cell through this, because of this pilus.

And at the end of the process, both the bacteria have a pilus and a plasmid. Both of them have become resistant to the antibiotic to which only one was resistant. Similarly, this new cell and the old cell, both will continue to provide this plasmid to other bacteria in its environment, giving antibiotic-resistance to all the cells in their environment. So, even though sexual recombination does not happen, this is the only way that bacteria are capable of transmitting or transferring some of their DNA to other bacteria of the same species. These attachment pili will determine the virulence of a particular species, because once it becomes resistant to a particular antibiotic, they become more virulent. So, that is all of it.

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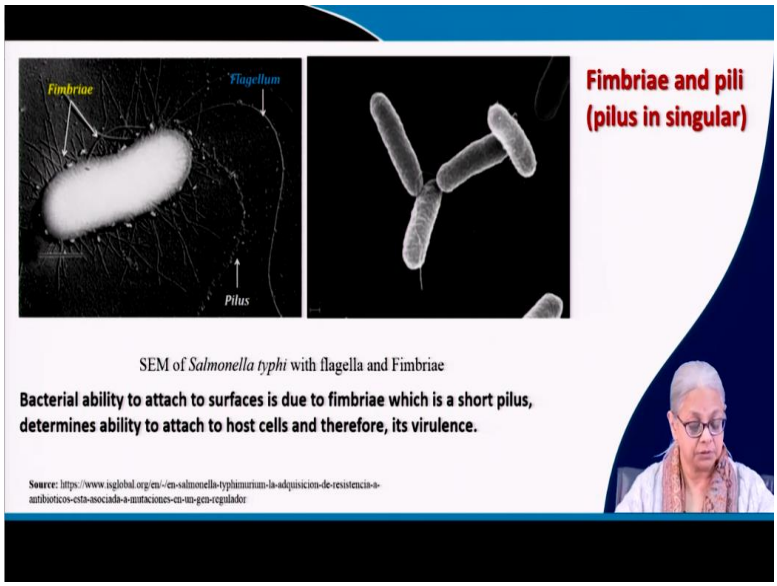

So, these are some SEMs, Scanning Electron Micrographs of fimbriae as well as pili. And these fimbriae are used for the bacteria to attach themselves to surfaces. So, you can see these

fimbriae. They are also hairy appendages that allow the bacteria to attach to surfaces. And then, the flagella are extremely long tail-like structures; and they are the ones that allow the bacteria to move within water. And the pilus is; you can see it over here. The pilus is a very different type of organelle. It does not resemble either the fimbriae or the flagella. And it has a very different nature, which is used for attaching itself to bacteria of the same species or even a cross-species. So, the fimbriae and the pili are together responsible for determining the virulence of the bacteria. Because, remember, if it attaches itself to a surface and then creates a pilus, then first step of the process is attachment. And then, by transferring the plasmid that gives it resistance and other functions, then it determines the virulence of that particular bacteria.

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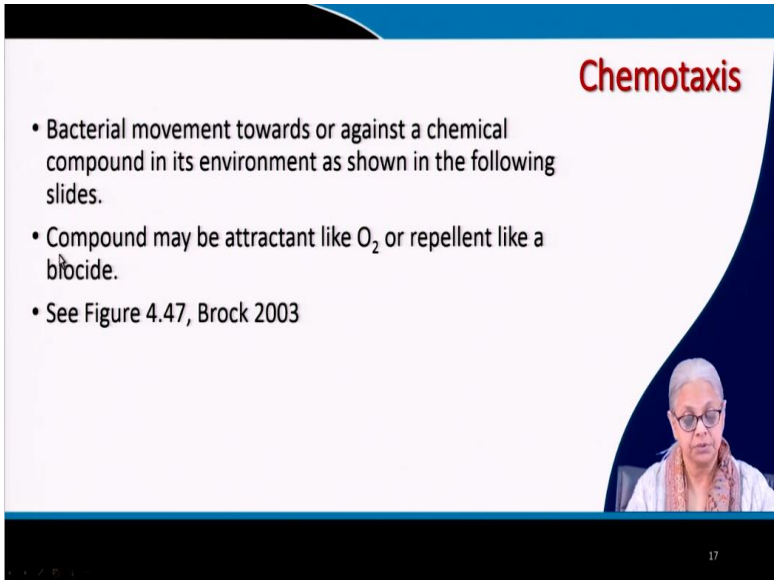

Then, there are examples of the movement of bacteria. Now, you can have chemotaxis; you can have phototaxis; you can have aerotaxis. So, chemo means, the bacterial colony will move in response to a chemical concentration gradient. So, the attractant may be oxygen or the repellent may be a biocide. The entire group or colony of bacteria can be found to move towards the food it wants or towards an attractant like oxygen; or it will move in the opposite direction towards a pesticide or a biocidal chemical. So, it will move away from that. This kind of signalling has been observed in bacteria, depending on the environment. So, chemotaxis is one thing. You can refer to these figures.

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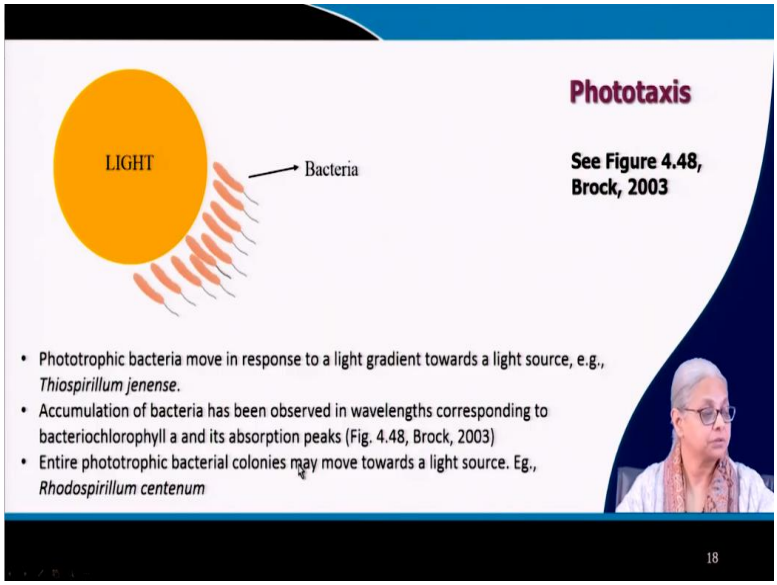

Here I have an example of phototaxis. So, there are certain photosynthetic pigmented bacteria which need light. So, if you have them on a surface and you provide a light source, you will find that the entire colony of some of these bacteria are capable of moving towards the light source. That is one example of phototaxis. Another example that is very interesting is based on the chlorophyll. We know that chlorophyll has absorption peaks that are at certain wavelengths. So, it was found in one particular experiment that if you have a glass slide and you have it exposed to different wavelengths of the visible light spectrum; it was found that, particular species were congregating literally in those wavelengths which were corresponding to the wavelengths of the bacteriochlorophyll absorption peaks. So, these are examples of phototaxis. That those wavelengths or the light source are useful for the bacteria for photosynthesis. So, these phototrophic bacteria were moving in response to a light gradient towards the light source. And I have already mentioned this point. And then we come to phototrophic bacterial colonies moving. So, there are several examples of the entire colony or even single cells moving in response to a light gradient, phototaxis.

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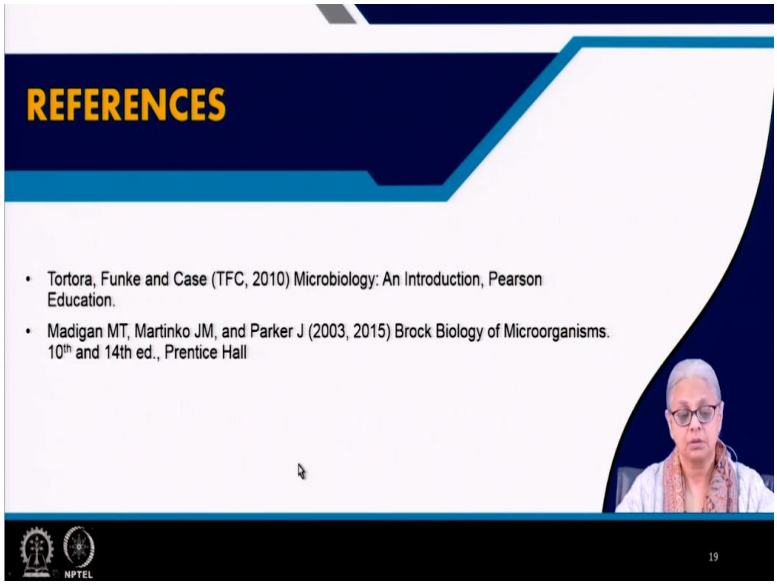

I will end this part of the lecture here. Thank you.