Applied Environmental Microbiology
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Lecture - 02
Introduction II

Hello, and welcome to the second lecture of Applied Environmental Microbiology. Today we are going to talk about the history of environmental microbiology, how microbiology first developed and we moved from very generic sanitation engineers to environmental microbiologist. So, let us take a look.

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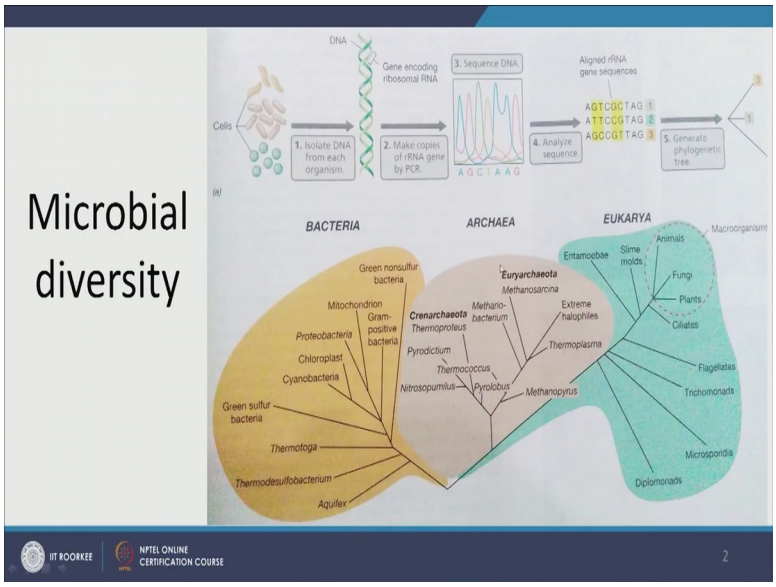

Initially, when we were trying to understand what microbiology is, the scientists and the engineers together were stuck by immense diversity that is present in the environment. It was very difficult to tell microbes apart from each other, because we did not have the right technology to do it. But as technology improved developed, we could start distinguishing between different kinds of microorganisms their activities start even making trees like this one. For example, where the oranges bacteria the green is Eukarya and the one in middle is Archaea, and start understanding how each of these branches on these trees are very unique microbes that have very different activities and behavior in environment.

The latest technology to do this is called sequencing and I would like to take a moment to explain to you that one of the intentions of this course is to acquaint to you with the latest technologies that are accessible and available to you in India that can be used to understand environmental microbiology better. So, will be focusing more on what is the current microbiology, but first we need to understand what the history of microbiology is.

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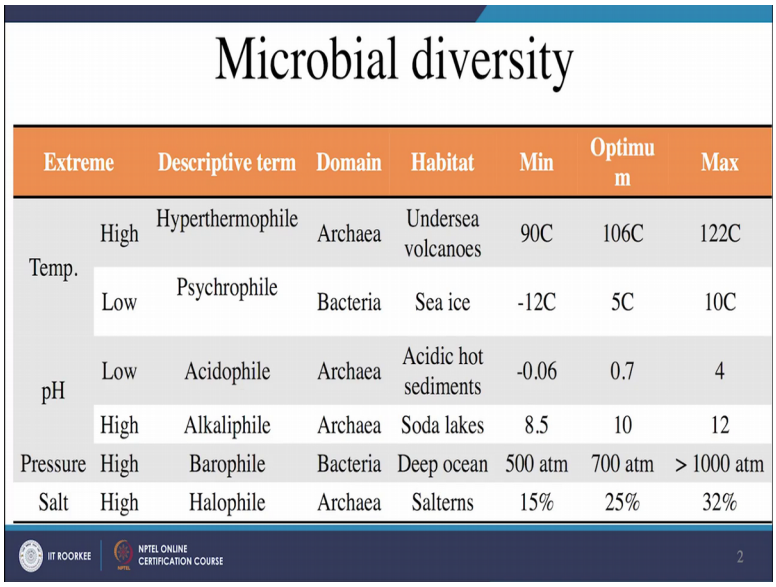

So, as I said before scientists were in awe that the microbes or in other words smaller forms of life were present in, very diverse and very different environments. And this table here shows to you some extreme environments in which we have seen microbes not only surviving, but also thriving.

For example hyperthermophiles microbes hyper very much thermophile loving who love high temperatures have existed in undersea volcanoes, where minimum temperature is 90C and maximum temperature can be boiling hot and more 122 Celsius.

So, these microbes actually survive and love this immense heat and this is very interesting story about how Archaea a very different domain in microbiology was discovered in these undersea volcanoes, and will talk about their story later , but imagine scalding hot water they had love it. And then we have extreme low temperature psychrophile one who love low temperatures, they have been found in deep sea ice

where temperatures range from minus 12 degree Celsius to 10 degree Celsius, and bacteria love living in this cold temperature.

So, if you see some psychrophile growing in your refrigerator do not be alarmed because they love it. Microbes have also been found in acidic sediments acidic hot sediments where the pH drops below 0, and rarely rises above for these are acidic ferric microbes acidophile means loving then we have alkaliphile the ones that love alkaline temperatures sorry alkaline pH, and these have been found in salt or lakes where the pH ranges from 8.5 to 12 microbes. When it comes to extreme pressure where will you find extreme pressure? Deep ocean obviously; so, in deep ocean where the atmospheric whether pressure ranges from 500 to more than 1000 atmosphere microbes are known to thrive and thus we know that they may have the aerofoil.

Similarly we have microbes that love in extreme that love and grow in extreme salty environment, and they are found in salt turns where salinity can range from 15 percent to 32 percent. Important to note that 32 percent is often the maximum salinity we can achieve on our earth the surface of our earth. Note here out of these extremophiles listed here only two categories are bacteria rest are Archaea, and when we talk of microbes you can talk about bacteria we talk about virus and fungus and maybe protozoa, but Archaea let us explore them.

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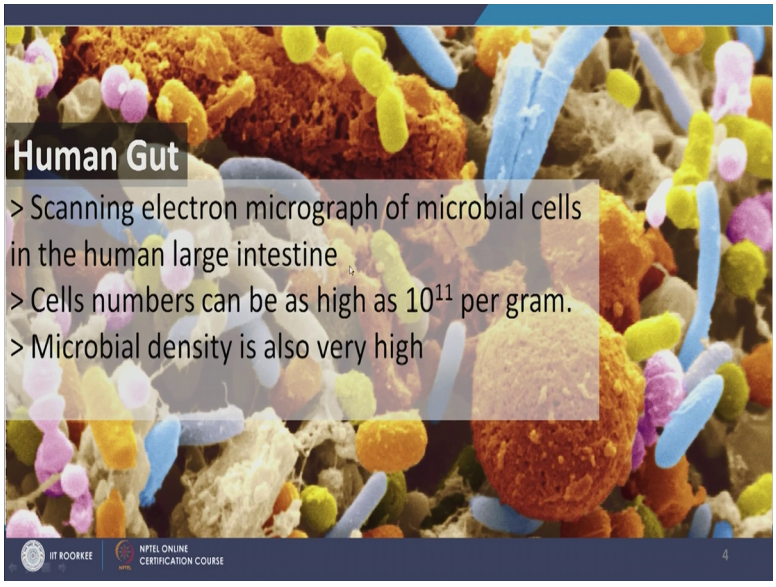

One of the first in discoveries in microbiology was mainly started poking into our intestine, and used some wonderful micro scopes to see what is going on in our gut we found that we have our gut was colonized by as much as 10 to power 11 microbes per gram of biomass in our gut.

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And we made these beautiful pictures again this is an artists impression and we had found out that these microbes affect us, but we did not know what kind of microbes are there.

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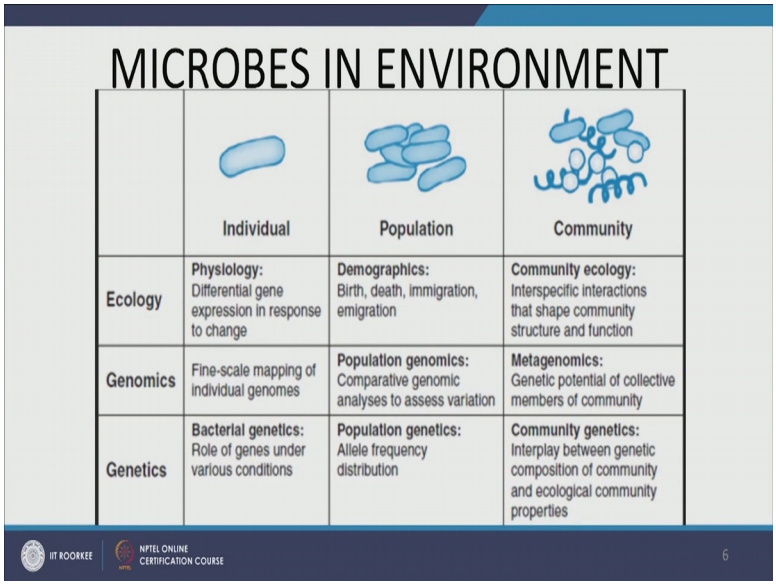

What we started finding out soon was that we can study microbes on individual level, population level and community level. Initially it was just easy to treat microbes as the black box, you have bad microbes in your gut take an antibiotic.

We need microbes throughout treat wastewater treatment; everything that is left is called sludge that is biomass. Without distinguish the different distinguishing different kinds of microbes, when we develop culturing techniques where which is that we can growth those microbes in our lab. So, we take microbes from this picture from our gut this has got microbiome by the way, we take these microbes let us say the blue rods and we grow them on plates. So, once we have grown them on plates we can study them individually.

We can see how they express different proteins, how they are built inside, what their genetic code is, how do they behave under different environment. Then what we can also do is we can put multiple microbes together and we can enrich them for a particular purpose this is when we study them on population level. So, this would imply please you want to know we have a mixed community or we have many microbes or the same type ;how what is their growth rate and this is some environmental engineers must be remembering about mono kinetics and kinetics of growth, and do not worry if you do not we will cover all of that again.

But on population level we study demographics of microbes and this is very important for environmental engineers where we are interested in the right food to mass ratio, you want to know exactly how much acetate to inject in a groundwater to sequester uranium right. And then in non population level we are also now recently interested in population genomics, where we are trying to understand how population of micro will microbes where is when then my metadaries. For example, let us say earlier we had clean groundwater. So, he had a particular microbiome in the groundwater, and then fluoride and arsenic levels increased exponentially let us say. How did the microbial community respond to it, and this is very important thing to understand, and this is important because this is exactly where we understand and we know that we are likely to find out microbes that are very unique for example, microbes that sequester chromium out of water.

Microbes that can survive up to 3500 ppm of chromium, we also look at population genetics to see how microbes of a similar kind its say I am interested in cellulose degradation, they say work with cow dung and I am very interested in seeing how to

degrade the cellulose. Now there is from the of micros very famous clostridia, many of the cellulose degraders are known to belong to crossed stadia. I want to see how within clostridia the genes that are responsible that help microbes to degrade cellulose, how they vary . So, this is allele frequency distribution will be talking about this as well and now because we have a tool called metagenomics. Metagenomics in genomics is a promising wonderful tool that has been recently developed, meta means big genomics means study of genes.

So, here in metagenomics what we can do is, we can sequence millions and millions of microbes. Either only a targeting gene that we are interested in or we can sequence the entire genome from as many microbes as possible, to get billions of sequences. So, in one go we can generate a tremendous amount of data, to know what different kinds of microbes are present in the community, that I am studying what are their function because now I can even go up to genome level and find out what genes they have, what genes are getting interest. Metagenomics is a big departure from the earlier microbiological techniques and since that not only is it wonderfully fast rapid, but also very cost effective? And its it would not be an exaggeration to say that might be are on verge of my metagenomic revolution.

In India I have seen that metagenomics is often referred to just amplifying high throughput amplification of a particular gene and sequencing it. Let us say I am interested in gene a; because gene a is related to methane formation methane methanogenesis. MCRA that is the gene name by the way, I am interested in gene which is MCRA and it is responsible for methane information in anaerobic environment. And what I can do is, I can take my environmental sample let us say I have a drill bit that goes into an petroleum oil field oil well and I get some sample from there, and I want to know how much methanogenesis is happening down in the well. So, I can extract the DNA of the microbes there very easily, all of them billions and billions of microbes I can extract the DNA, no not all billion microbes are same they very distinct. So, if you have billions of microbes we might have at least some million different kinds of microbes.

So, I extract all of their DNA, and then I can amplifier increase the concentration of the gene that I am interested in gene a or MCRA, and I can sequence only their gene and now you might ask if I already know the sequence of gene, then why is it important to sequence it. Because in microbes in environment we notice that the same gene might

have different may have some diversity. Some ATG see some codes might be different might vary and this is this is what is referred in India as metagenomics where I amplify one gene and see how it varies in the community.

And the most popular gene for this kind of metagenomics is 16 as RRNA which gives me information about what the microwaves in other words what name can I get to the micro not. What it is doing the example I shared with you earlier tells me what it is doing this microbe because they have MCR a gene is create generating with methane gas, but 16 as a RRNA will just tell me what is the name of microbe, and if I have the right knowledge I might be like oh I know this micro, it creates methane or this microbe eats cylinders, but it is not giving me functional information. Outside in many western countries this kind of sequencing is called as high throughput and click on sequencing. And metagenomics refers to whole sample metagenomics, which is I am not only interested in a gene I am interested in every single genetic element in the microbial community.

This is where the community genetics come into picture. As I promised let us dig into the historical roots of microbiology. Believe it or not many made a some centuries ago not long time ago some centuries ago the general level of hygiene in Europe the much developed Europe was pitiably worse people really wash themselves. Because they do not understand that not washing them will make them sick, and it was not until then Robert Hooke x invented a better microscope, that he actually could see oh look these are microorganisms these are small little microbes or micro kind of things in mold in fungus, and it is important to note that fungus the microbe fungus is much bigger than a bacteria.

So, fungal cell is much bigger than bacteria it is easier to see. So, Robert Hooke was the first person who actually used made a good microscope and used it well. The next gentleman who came into this picture was Anthony Wang Liu hawk. I do not know how to pronounce that name, but that is the best I can come up with. He invented a very simple microscope and he reported pictures of v animalcules. So, v means really small animalcules like molecules like very small animals and he was there want that actually popularized and showed that these v animolecules now we know microbes are found even in drinking water.

So, you can imagine the shock of an elegant English woman realizing that the water she is holding in her hand and drinking is full of microbes, but do not be alarmed even though our drinking water has tons of microbes, most of them are benign they would not hurt us. So, drink water and then science developed further scientists like Cohen they develop measures to prevent microbial contamination in lab, and this is where do I pasture came into picture the scientist whose quotation I quoted earlier in previous lecture.

So, Lui pasture coned they developed techniques, which allowed us to preserve food longer. To make sure that our milk does not go bad, this also led to medical understanding why we should disinfect our wounds in our hands every time they get dirty. Now let us take a moment and wonder, what kept scientists from developing microbiology before contribution of Hooke and Lynn hawk.

So, remember the v animolecules of Lynn hawk, and the simple microscope of hawk. So, maybe if you are watching this video pause it for a moment and think about it why did not microbiology develop before these two phenomenal scientists came into the picture, and yes perhaps you have guessed it right that we need the technology to see such small microbes, that are definitely invisible to our naked eyes to make sense out of them. To understand what they doing we did not even know a life existed beyond what we could see, and now as I shared in the previous lecture we know that most of the life forms are not what we can see.

Most of the life on earth is actually the in the nearly invisible to human eye microbial world, and thus a next question that I want you to think about this was invention of microscope the final frontier in microbiology. I can loosely rephrase this question and say that we had discovered microscope back then in time of hook and even hog different scientist, did we do everything that microbiology had to do have we discovered everything we need to know. Do we know about all the kinds of microbe that are present on the earth or is there a more scope for research, is there are there frontiers that lie unexplored.

So, again I suggest pause the video for a few seconds and think about it. And exactly as you might have noticed and hopefully guessed that no, invention of microscope is not the final flow frontier in microbiology.

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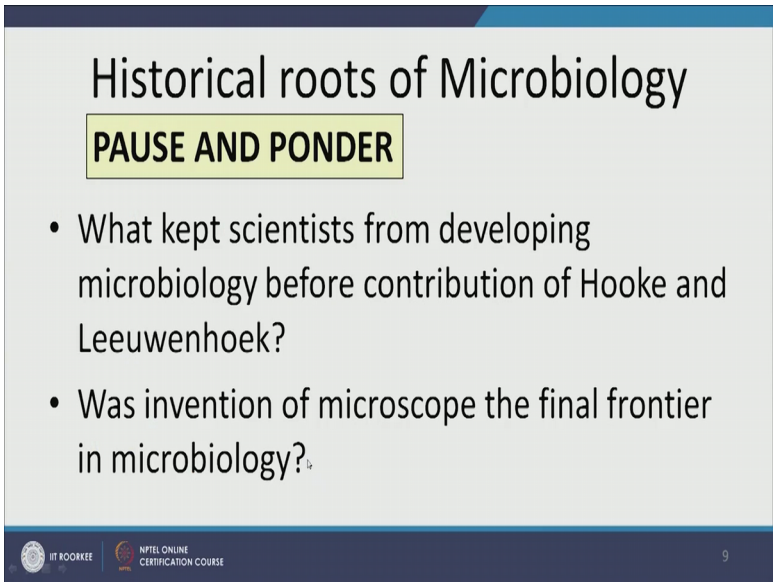

Every year we are developing new techniques and you are learning more and more about microwaves around us.

Now, how did this all begin? It began with ancient scientists and Anthony Lou hook one hook and Louis Pasteur corn, and Louis Pasteur was the first who demonstrated that spontaneous regeneration theory is wrong. Now what is theory of spontaneous regeneration? Earlier when people did not understand microbes, but thanks to hook and Lynn hook we had we knew that these microbes, these tiny any molecules actually exist all over all around us people believe that they spontaneously regenerate. This is a fancy way of saying that bowels first they never know microbes, and then suddenly they were lot of microbes this is all the food was clean.

Let us say the Chapati or the Dosa of was clean and few days later it fermented or fungus grew over it. This was spontaneous regeneration I do not go and put a fungus there. Because to human eye and mind for every plant for every living being there has to be a progenitor and them a progenitor for the microbes was not visible. So, people believe that they spontaneously regenerate and this is also in line with the creationist theory, we are not going to talk about that in this course though.

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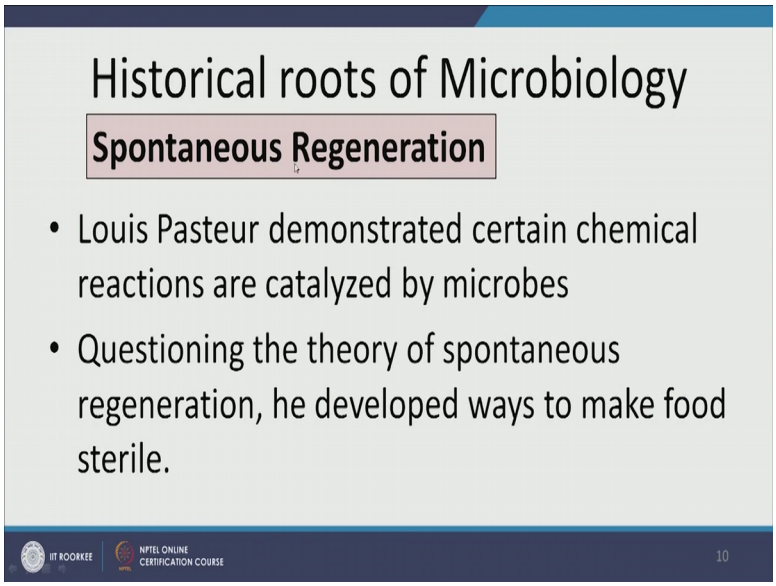

So, Louis Pasteur was the first one to demonstrate that certain chemicals are catalyzed by microbes and spontaneous regeneration is not correct. And then he understood that spontaneous regeneration is not right only then he could develop methods to stop food from going bad to stir from sterilizing and here is a cool thing we still use his techniques in love to make sure that our flasks are sterile. We still use his techniques to preserve our food for longer times. So, Louis Pasteur discovered two important things, first microbes are selective in what chemicals interact with and how they interact.

So, in the sense that they are not just too random things growing there, they have some program behind them or in some intention behind them. The other thing he found he proved was that they do not spontaneously regrow for a fungal growth to happen all over a Chapati or over Dosa, we need some fungal spores or fungal microbes already in the air and environment. And the way he did this was by modifying the existing laboratory setup to make a beautiful flask with a neck that bent in a unique shape.

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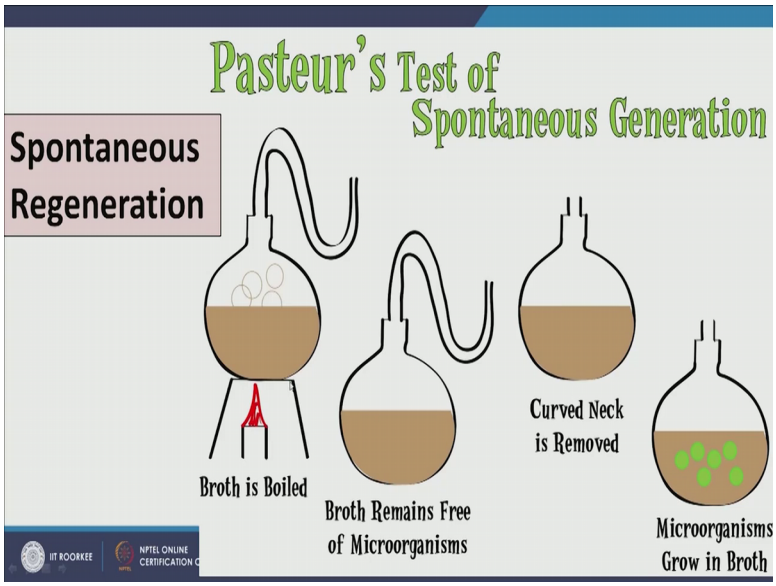

So, let us take a look at the slide and see here. This is a cartoon of his experimental setup where he had broth, broth is food for microbes they loved this food, very carbon rate nutrition rates and then in his glassware he had he got the special bent made. So, with this bent he prevented microbes from entering into the broth and first he boiled it. So, that all existing microbes would die, then he cooled it and he let it sit for week, a week 2 week, 3 weeks and it did not get contaminated.

So, this suggested that oh there is a possibility that microbes will not spontaneously regenerate or generate then he, but he, but this is not proof that they do not spontaneously grow. This just shows that in certain circumstances they do not spontaneously grow. So, what he did next was he removed the curved neck he removed this neck and he let it open. So, the air could freely move in and out, and he noticed at microorganisms grew in the broth and it became cloudy suggest in that if exposure of air is allowed then micros cavalry grow and will grow.

So, this says that for any life to form there has to be progenitor if you cannot see it does not mean it is not there.

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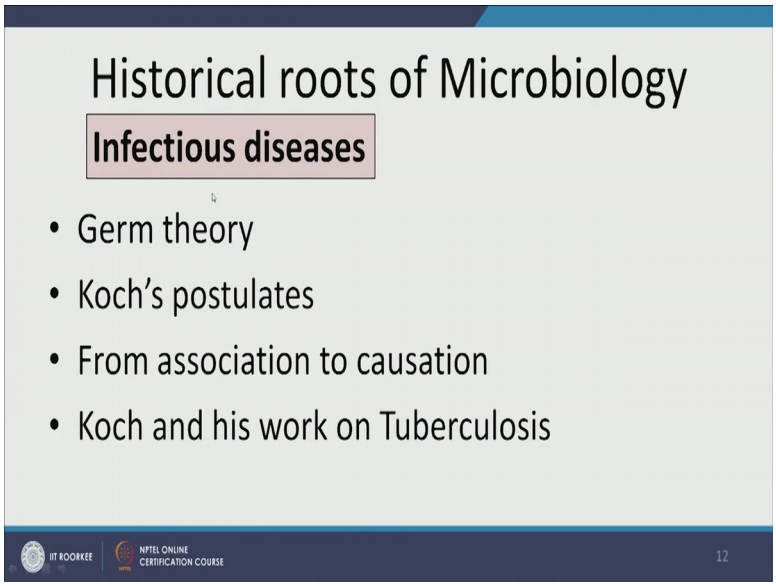

The next great scientist in microbiology was Koch's and this is his work on infectious diseases. He came up with the idea of germ theory, at this time people did not understand that there are infectious agents that we can call germs which are microbes and they are the ones that cause diseases. There were some really backward rudimentary and now senseless, now we believe their senseless ideologies about what diseases and what medicines are all about and what they should be.

So, Koch's was the first one to come with the germ theory, and he gave wonderful three postulates that will talk about very soon. Now what were these postulates and why did he give them? He wanted to associate the factors the disease agents with the cause of disease.

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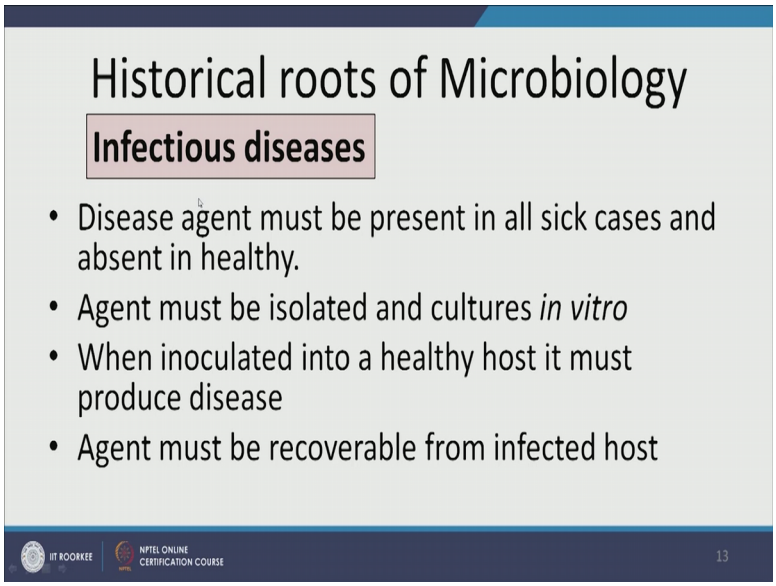

And let us look at this his postulate to understand the logic of Koch's. In order to identify disease agent, first we must find it in all sick individuals. So, this is what this wonderful scientist read. He took about if a disease, disease a and he took sick into sick mouse, sick mice that had that disease a took out their blood and looked at it under microscope to find what is this unique thing that is presented all sick animals and it is not present in healthy animals.

So, first Koch postulate is that the disease agent must be present in all sick cases and must be absent in healthy; that this does not prove that the disease agent or the unique agent that he has identified is actually the cause of the sickness. It just says well in a sick patient in a sick mice sick mouse we are likely we are we will find the diseased agent, but not in the healthy one.

So, the next thing he suggested that we need to do to prove that the disease agent is actually the disease agent, was that we need to isolate the agent and then grow it in the truth. So, what he suggests that we need to take the agent from the sick microbe and then make grow it in our lab. Once we have grown it on our lab we need to inject it in a healthy host and make sure that it ensures disease.

So, in other words there three steps to Koch's postulates, and to prove that a particular agent is a disease agent. Now remember this is the time of microbiology when we do not know they do not have germ theory, we do not know why and how people fall sick. So,

let us say there is a disease a and they are mice that are infect with disease a. So, he collected all of them as I said before he did the blood analysis. So, I looked at it under microscope and found out, that they are certain with certain animolecules for lack of other word that are unique and only present in sick animals not in healthy animals.

What then we then this next step of Koch's postulate is that we should remove these agents, these unique agents that are present only in sick animals and grow them in lab. Once you have grown in they grown them in lab, inject them to healthy animals and wait and watch. If healthy animals fall sick we can say that this particular thing that we had grown in lab and separated from sick animals is actually the cause of disease a is what we can call as germ. The disease agent and hence the germ theory was given, and a once he did this for the first time he went on and did it for tuberculosis, and this postulates are still used when we try to identify the agent for a particular disease, a wonderful example would be sars epidemic in china many years ago.

Some many years ago a new disease broke out in China a severe acute respiratory syndrome. If it mean sort of knew that it was viral by looking at its characteristics and the quick blood work, but we did not know what virus it is, and at any given time the multiple virus was a represent in human body. So, the scientist actually followed a slightly modified version of Koch's postulates two separate and isolate the infectious agent, and this is a beautiful diagram showing how Koch did it.

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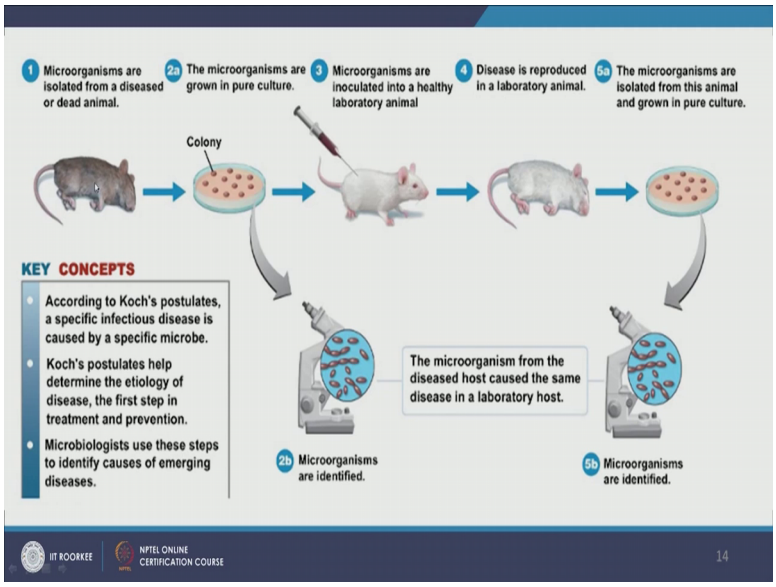

First he isolated the microbes from a sick or dead animal, he grew them in pure culture he injected it in a healthy mouse and then healthy mouse also started falling sick and the he removed he saw that the agent is also present in this sick mouse no it was not present earlier, but its present now, and he could grow it in the lab and both were same. So, it is this agent that makes a healthy mouse fall sick.

In modern era right now we do not need to rely on these multiple steps of colony formation, looking under microscope and maybe very slowly and very painfully characterizing the microbes and seeing what kills them were does not we can directly go and use these some wonderful techniques, it all started with sanger.

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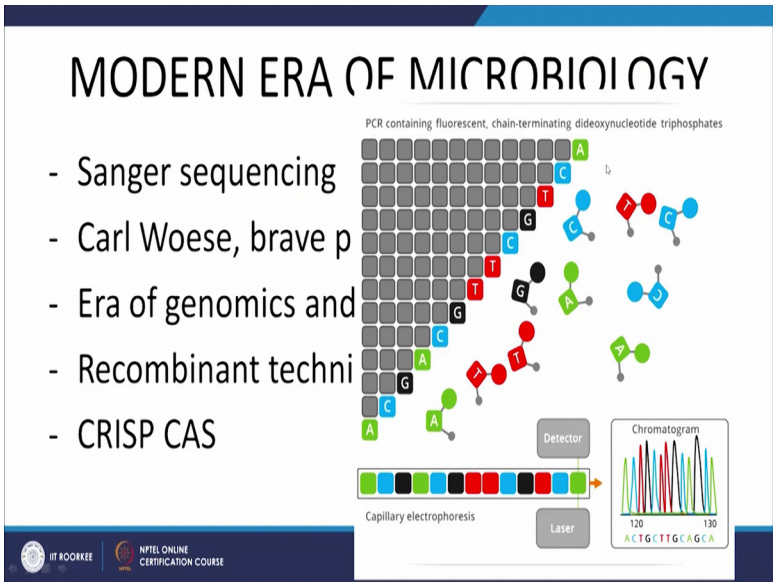

Sanger a scientist he is discovered invented this technique of sanger sequencing, wherein we could actually decode the genetic code.

So, the beauty of sanger sequencing is that it does sequencing by the method of synthesis and not by the method of removing the nucleotide, which were what people had earlier tried and failed. So, in sanger sequencing let us say you have a sequence here, and each of these gray blocks are your unknown nucleotides and what happens is instead of using the generic nucleotide that we have adenine guanine cytosine thymine, but in sanger sequencing we do is we use the dye doxy format of the nucleotide.

So, what happens in the first cycle the machine will allow a TGC, dyed oxy nucleotides to come and replicate to come and a start replicating. Now as the replication happens then the first correct complementary match will attach here. So, we can see here it says a, a has attached successfully a dyed oxy nucleotide it means that the complement t was present in the genome. So, when the a has attached here, then the replication cycle breaks because dioxyde due to its very nature will not allow the replication cycle to continue.

So, for the next replication cycle now we will have with the replication will end at a, n then the next great block here the unknown nucleotide they will again be exposed to different kinds of dioxin nucleotides, and the one that attaches here we know which one is attaching because you know we do not send them all together, we send them one by one and once c has attached we know that g is present here.

And so on and so forth until in each replication cycle we get to know, base by base what nucleotide is present in what sequence. And as a result sanger sequencing has very low error rate, but it is also time consuming and even though the costs have dropped a lot it in comparison to other sequencing methods it still remains to be very expensive.

So, earlier when we were still struggling with sequencing, we knew about bacteria we knew about virus we knew about eukaryotes. So, we knew something about protozoa about fungus there was a scientist German scientist Carl Woese. Now Carl Woese he had this passion to find out about life in extreme environments. So, he would find himself in places that no other scientist would be willing to go.

So, he would go to the hot springs that are on average at a temperature like 90 degree, minimum 80 degree 90 degree 95 degree, 100 degrees even bolding volcanic extremes and he would collect samples there and you can imagine stepping into really boiling water it is not something anyone would like to do. So, he definitely had a safety suit with him, he and his students would take sample from there and would try to reproduce these conditions in lab and one might wonder why would he do that, and who would fund such research and answer is very simple Carl Woese was trying to understand, how life developed when earth was still in infancy.

So, when earth was in infancy it was hot. So, fewer sulfur rich anaerobic environment definitely not the present earth we know now. So, would find these places on earth that

are still sulfur rich or hot, and have extreme pH extreme temperature and see if there is any life that is what he wanted to know.

So, even he would take samples from there and try to regrow the life forms or you know to detect life forms and those he found there is life. So, he knew that life can start here we knew it logically, but now we know it experimentally and then using these preliminary techniques of sequencing he actually sequenced the genomes of certain organisms that he could grow at extreme high temperature in sulfur rich conditions and funny thing he found was that these genomes looked very distinct very different from the genomes of bacteria.

So, he knew that perhaps they are not bacteria or maybe their different kind of bacteria there was some confusion there. So, what he did was, he tried culturing these organisms. So, he could see the morphology was same or not know and culturing them is an immensely difficult task because they need anaerobic extremely hot and unique environment which is hard to create. But finally, after many years of attempting this he succeeded and what he noticed was that not only are there genes very different from bacteria, their inner structure of the cell is also very different. Many things many features they actually share with eukaryotes and some features this share with bacteria.

So, they are neither bacteria nor or they eukaryotic the new cells, the cells with nucleus there is something in between them, but not quite between them because they are very unique in the way their cell membranes are made. And then he started asserting in the scientific community that this is a different domain of life, this is a different kingdom of life they should be given a separate name and he chose the name Archaea here.

So, but I call in this slide if you notice I say Carl Woese comma Brave pioneers. Carl Woese and other scientists were condemned for even thinking that there is a life form beyond bacteria, and that is why and they struggled a lot to make the point and eventually emerge true that yes now we have bacteria Archaea and eukarya three different forms of life.

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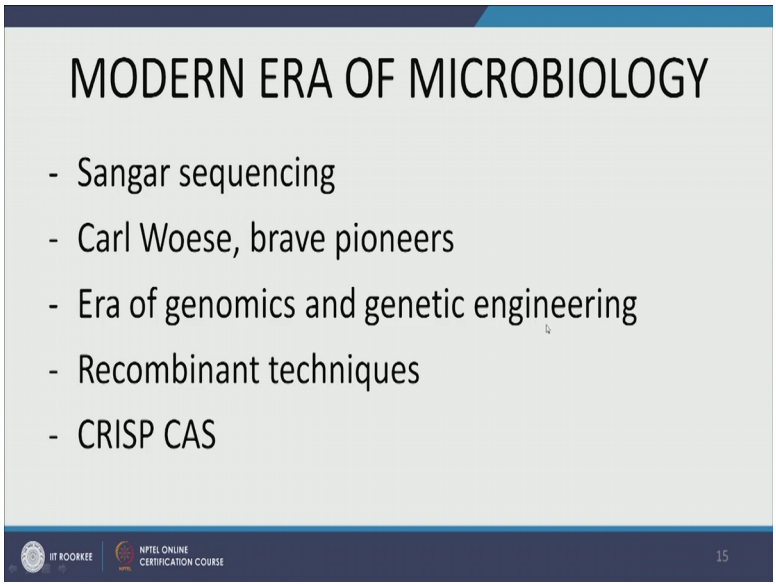

But now scientists do not have to struggle so much because now, is the era of genomics and genetic engineering Fancy words genomics, genomics is when we can actually look at the entire genes know what they are, quickly look them match them with existing database of gene set of microbes that we know and sort of and guess what actions they do what their qualities would be. Genetic engineering is when we can actually engineer the genes to suit our purpose this is something the biotechnology works with recombinant techniques and genetic engineering are definitely in domain and biotechnology will go very briefly over them, but not in depth, and now we have crisp cas. Crisp cas is a promising technique that actually.

So, genetic engineering and recombinant techniques have lot of limitations, we engineer genes in vitro not in vivo what it implies is that, we engineered genes outside a living cell and then we put it inside an ex living cell, and we let it multiply within that community and then we extract the gene or the protein that we are interested in. But crisp cas what crisp cas can do is, we can inject and we can operate we can engineer the genes of a living cell and this is very cost effective very accessible and promises treatment for many medical problems such as antibiotic resistance.

So, we can actually go inside a cell bacterial cell that is anti biotic christian tweak it. So, that it stops being resistant, we can oh it is also promising for cancer research crisp cas

that we can when cancer cells stop responding to treatment we can modify the cells, that they start behaving better.

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And respond to treatment better this is Carl Woese by the way just paying homage to wonderful scientists, and these are the preliminary sequencing sheets he had and these are very long very painstaking work to do and this is the result of his wonderful work.

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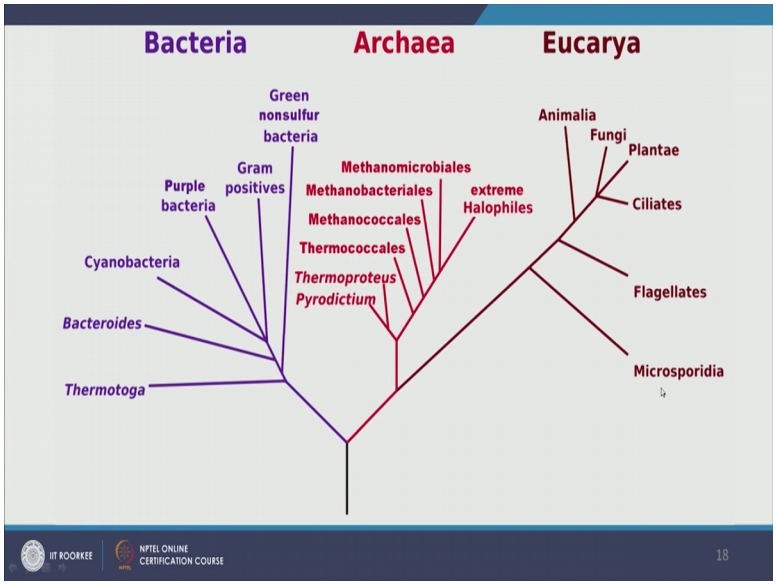

Now, we have a new kingdom Archaea and as I mentioned this is era of genomics.

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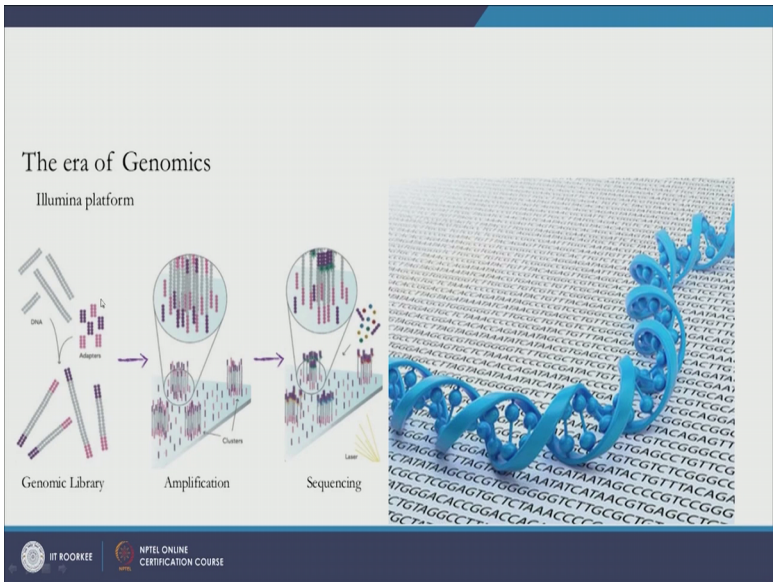

Now, and this is illumina platform, they are the one of the leaders in high throughput sequencing and I will go over this later in detail how this works and why its useful and where it is useful where it is not useful, for now I think this is a good time to revise this lecture and the introduction we had about earth being a microbial planet.

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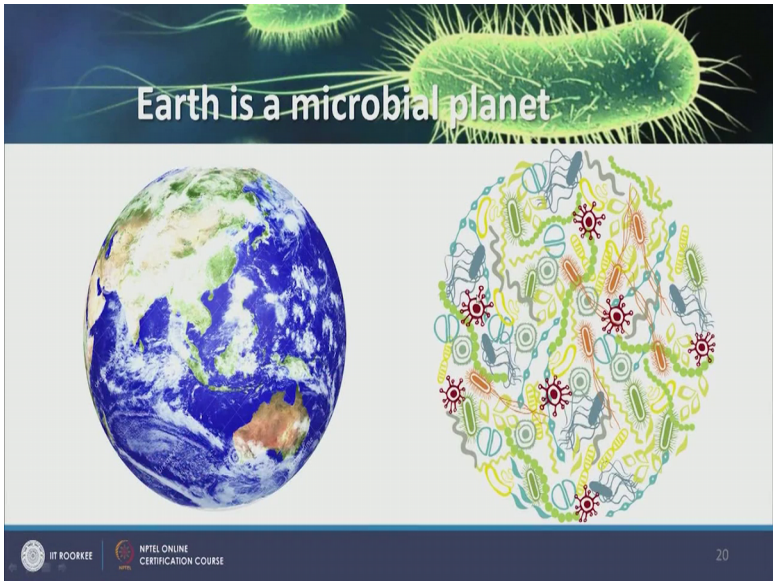

So, we this is where we started and lecture series from trying to understand that what we see is not all the disk there is more to life, there is more to our planet, then the big life forms that we see in notice and most of it is actually microbial.

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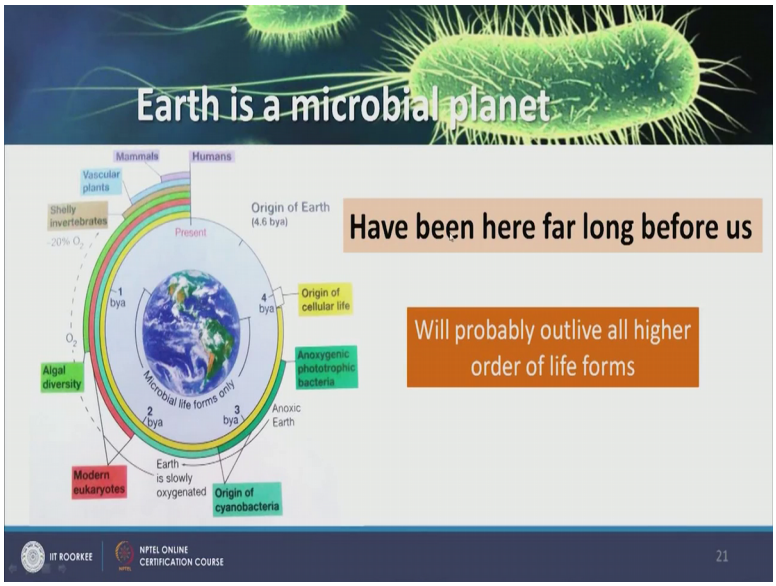

And we also noticed that the microbes star existed long before us, long long before us and will probably outlive all higher order of life forms because remember we talked about microbes that can survive and thrive in extreme environments.

So, if there is a catastrophe and higher orders of life are wiped out, and let us be very clear we are already in middle of an extinction phase on earth. So, species are going extinct very fast right now mass extinction, but microbes will probably outlive. Not necessarily in the way they are living right now and they will evolve because they were really fast, but they know.

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It is also important to remember that microbes are a majority in our human bodies, we have nearly 10 microbial cell for every one human cell, they are very important for our health and very few of them actually hurt us most of them help us and cyanobacteria.

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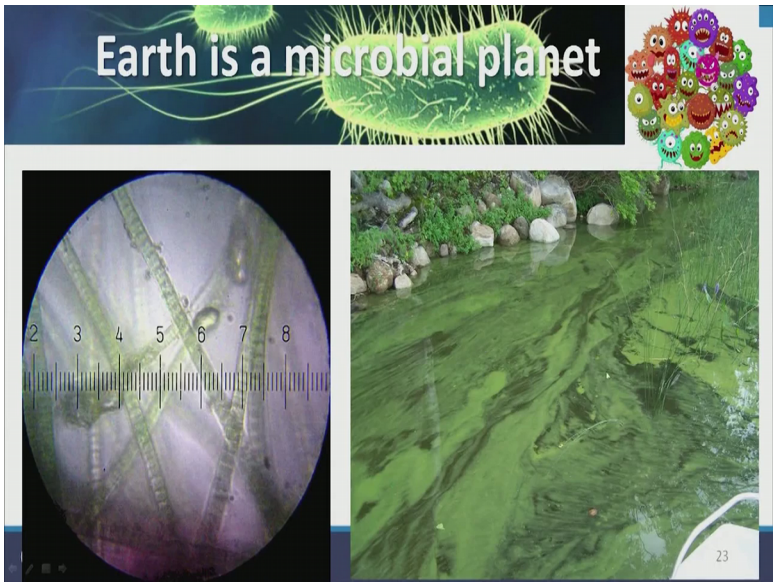

The green bacteria we see on our lakes, is often the bacteria that made earth's environment aerobic.

So, we really need to express and feel gratitude towards these tiny organisms, that whose microscopic picture is on the left side, that have allowed life forms such as ours to evolve

on earth. And let us look go spend a minute here to look at this left side of this slide; we noticed that we can see here that we see strands much like here. This is not one cyanobacteria if you look very carefully, you can see these small compartments each of these small compartment is one cell and they like to connect to each other in here like structures. Then we also learned that earth is a microbial planet, and the infinitely small might be playing an infinitely large role.

This is all for now. Thank you and I will see you in the next lecture.

Thank you.