

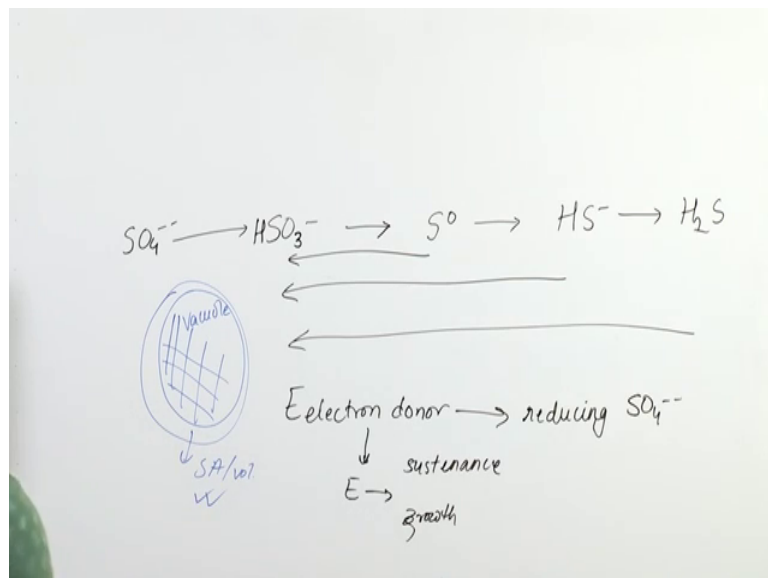
Applied Environmental Microbiology
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Lecture – 14
Functional Diversity of Bacteria II

Dear students, welcome to this lecture where we are going to continue our conversation on Functional Diversity and focus more on microbes that interact with elements that influence our nutrient cycles of earth. Of course, when it goes to carbon cycle in water cycle almost every microorganism we know has contribution to it, but today we are going to focus on sulfur and nitrogen.

So, first we will start with the sulfur cycle. Now instead of focusing sulfur cycle let us look at what microbes in do with sulfur and how they can use it. So, I have written here different forms of sulfur that are relevant to most microbes that involve with either sulfur oxidations sulfur reduction.

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So, first we have sulfate here it is the most oxidized form of sulfur that is most that is very relevant to most microbial communities, there is another higher form of oxidation for sulfur, but this is pretty good enough for now.

So, this sulfate it will when in it is reduced by microbes they get energy and they reduce it in a series of different enzymes and we will see about them very soon. So, microbes can reduce sulfate to sulfite and further to elemental sulfur and then to hydrogen sulfide and then hydrogen sulfide which is the most reduced form of sulfur. So, at each step as the microbes reduced this sulfur they generate energy which they can use as source of energy. There are few microbes that can actually go all the way from sulfate to H_2S , but usually it is a consortium of microbes it is a group of microbes that get together and participate in this reduction of sulfate.

So, the arrows show the reduction pathway for sulfur there is another parallel pathway which is the oxidation pathway. So, there are microbes that we have which oxidize elemental sulfur. So, this is oxidation pathway and as the oxidizer degenerate energy then we have microbes that oxidize HS^- and they generate energy and we have microbes that oxidize hydrogen sulfide to generate energy. Now earlier I mentioned that because of the principle of thermodynamics chemical reaction will be energetically favorable only in one direction.


So, an obvious question that must arise now is why the sulfur particularly lend itself to buy directional movement of electrons when energetically only one direction it should be favorable. Now this is a very good question and I want to mention why under normal circumstances this direction where reduced form of sulfur are getting oxidized is more energetically favorable; however, under extreme anaerobic conditions when no other electron acceptor is present then microbes in vast and energy to reduce sulfate. So, that they can use they can oxidize their electron donor and make up for the energy.

So, the energy that they get from the electron donor so part of this energy is used in reducing sulfate and still there is a pretty good chunk of energy left that microbes can use for their day to day sustenance and for their growth, and this is why this is how microbes make use of under extreme distress they can reduce sulfate and sulfide.

So, now let us take a look at the different kinds of microbes that have to do with Sulfate reduction or sulfur oxidation.

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GREEN SULFUR BACTERIA



- Phylogenetically coherent group of anoxygenic phototrophs (phylum – *Chlorobi*)
- Little metabolic diversity
- Typically nonmotile
- Strictly anaerobic anoxygenic phototrophic bacteria
- Morphologically short to long rods
- **Key genera** – *Chlorobium*, *Chlorobaculum*, *Chlorochromatium*
- Certain green sulphur bacteria form intimate two membered associations, called **consortium** with a chemoorganotrophic bacteria

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So, last class we talked about green sulfur bacteria this is where I ended the lecture now it is very important to note that green sulfur bacteria and other bacteria that we have talked about before like purple non. So, from purple sulfur bacteria they store sulfur as an energy reserve and then they cannot no longer carry on for the trophy, but their primary source of metabolism primary source of energy is not sulfur oxidation or sulfur reduction, the other bacteria such as green sulfur bacteria and green non sulfur bacteria these are similar examples of phototrophs recovered in the previous lecture and they do not metabolize sulfur as a primary source of energy.

So, green sulfur bacteria let us talk about them in a little bit in detail.


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GREEN SULFUR BACTERIA

- Thermophilic green sulphur bacteria. Note chlorosomes in the cell periphery
- Blue and green carotenoids
- Blue and brown carotenoids

GREEN NONSULFUR BACTERIA

- **Key genera** – *Chloroflexus*, *Heliothrix*, *Roseiflexus*
- Anoxygenic phototrophs of *Chloroflexi*
- Are widespread in most environments
- Most have not been cultured in isolation
- A member *Thermomicrobium* has unusual lipids; neither ester nor ether linked side chains are present. To form a bilayer the dialcohol molecules oppose each other
- *Chloroflexus* forms thick microbial mats with thermophilic cyanobacteria in alkaline hot springs



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This is a picture of green sulfur bacteria by the way they're thermophilic they love heat. So, where will you find them in hot water events in hot springs and undersea volcanic vents and if you see well in this particular place slide it is not very clear, but there are the chlorosomes, which contain their pigment, photosynthetic pigment, they are usually at the cell periphery and they can be blue and green carotenoids blue and brown carotenoids. Now green non sulfur bacteria they under they underlie in these particular 3 clay genera they are anoxygenic phototrophs of chloroflexi and they are widespread in most environments and I have sequenced many environments and I am more often than not always find some chloroflexi and most of them have not been cultured in isolation there. So, very little is known about them.

So, one of the initial ways in which we tried understanding the metabolism the function and the genetic fingerprint of a microbe was, we would grow them in lab, we that is called growing in culture or culturing them and once we have grown them in lab, we would study them how they look like under microscope what kind of food they eat, how they eat, what proteins they translate and how they utilize them and what their genetic signatures are, but many microbes we have not succeeded in growing them in culture and chloroflexi are them.

So, the only way we can know are chloroflexi exist is by without culturing them sequencing their genes and then finding it out there is a particular the chloroflexi thermo

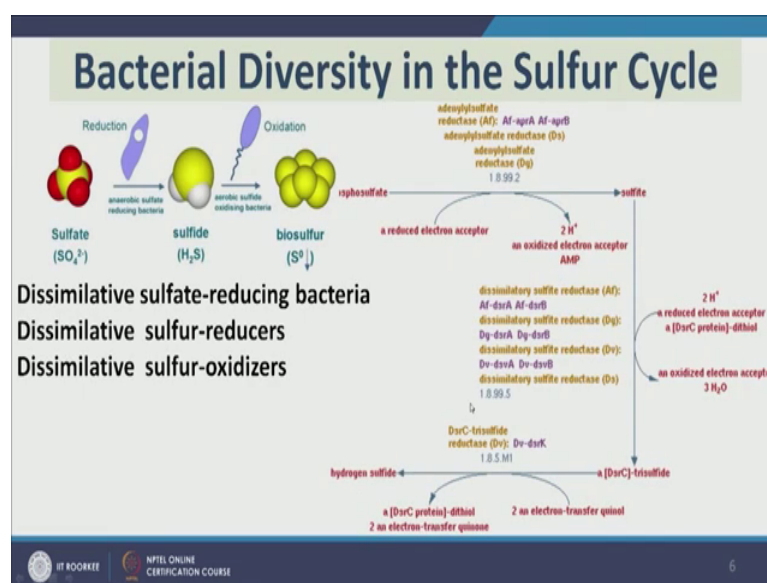
microbian, it has very unusual lipids, it has neither ester or ether linked chains like bacteria or archaea and when it has to make by layer it has to dialcohol molecules there oppose each other.

So, there is an alcohol molecule here this an alcohol molecule here and they have hydrophobic chains and they just oppose each other, there is how it makes by their chloroflexes were first found when in hard alkaline hot springs and they had made thick microbial mats. The other phototrophic bacteria that were similar in line with what we were talking about in previous lecture are helio bacteria in acido bacteria there is some information here that and that is go through it. So, helio bacteria the key general our helio bacterium and chloro acido bacterium their follow genetic like coherent again this implies that they are only found in certain freedom and that is firmicutes all of them are firmicutes and they are gram positive.

So, if your gram positive you lack that periplasmic cell membrane and you the first interaction of the gram stain would be will it would be video cellular membrane the anoxygenic phototrophs and they have a unique pigment bacterial chlorophyll G and that is the reason why we wonder to mention them separately, and they inter phylogenetically very restricted only 400 firmicutes and they can only utilize a narrow range of organic compounds as a carbon source and they are morphologically also very similar the either rod shaped or the filamentous strict anaerobes and if they do not have light then they can shift 2 chemo trophy.

So, they are not obligate phototrophs and then we had acid oh bacteria which are enough reasoning for the talks that were first discovered in Yellowstone National park. So, now, let us come to microbes that not only store sulfur for in in case they have to shift to chemotrophy, but they actually use sulfur compounds as their primary source of energy and that is where what I mentioned at the beginning of this class about sulfate reducing all the way to hydrogen sulfide and also hydrogen sulfide HS^- and sulphur elemental sulphur getting oxidized, and serving as a source of energy for microbes comes into play.

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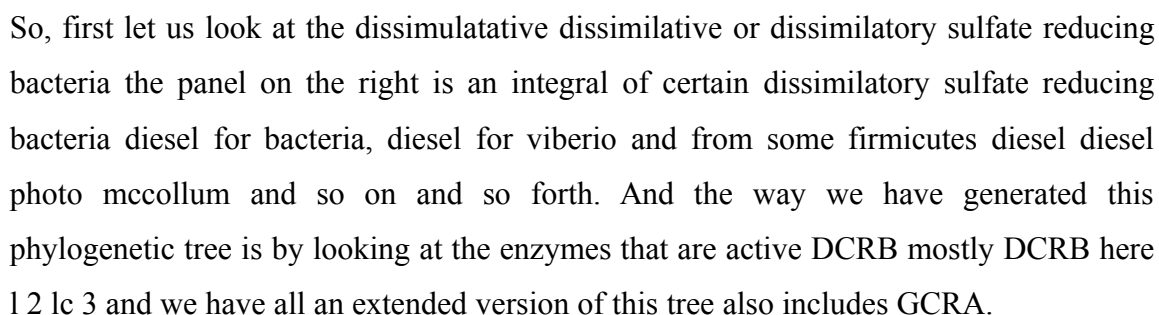
So, let us look at this slide we have sulfate here and they have 3 different enzymes mentioned here by the way they are multiple steps here that have been all generalized to one straight arrow they consume 1 ATP and releases our diphosphate. So, ADP and these 3 enzymes they at different steps they catalyzes reaction and eventually sulfate it gets in track it in track it gets attached with adenosine in 5 of the ATP and that is what diphosphate has been removed. And then with other series of enzymes it gets reduced to sulfite and then in this some sulfite to try sulfite and then hydrogen disulfide and in this process in sulfite reduction there is a series of enzymes that are dissimilatory sulfite reductase.

I want to talk about dissimilatory and assimilatory enzymes. So, dissimilatory process are the ones that do not assimilate the element that they are dealing with. So, in this case this dissimilatory sulfite reductase will reduce the sulfide, but not make sulfur in sulfide an integral part of the cell we are only carrying out this reaction. So, that sulfide can reduce and we can get the energy. So, the energy will become an integral part of the cell, but not solve for itself.

Now, one other the reason why I want to mention this DCRB DCRB DCRVB DCVA DCRA and DsrC, because these serve as markers for sulfate reduction remember I was telling you if I know the function I can know what kind of environment I am dealing with and then once sulfite has turned into trisulfide it, further can be reduced to hydrogen

So, sulfide is not very long lasting because the moment it comes in contact with any electron acceptor it is a very good electron donor. So, it comes in trust within an electron acceptor it is likely to turn into elemental sulfur. So, this reaction is pretty quick all right. So, we have 3 different kinds of microbes that use sulfur we had dissimilatory or dissimilated sulfate reducing bacteria we have dissimilated sulfur reducers and dissimilated sulfur oxidizers.

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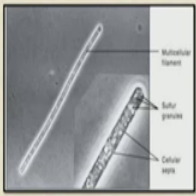
Now, this tree here is shown to express to you to drive the point home that dissimilatory sulfate reducing bacteria immensely diverse. They are found in per to bacteria, in nitrospira, in firmicutes, thermodesulobacterium and euryarchaeota.

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Bacterial Diversity in the Sulfur Cycle


Aerobic Chemolithotrophs- Sulfur oxidizers

- Sulfur-oxidizing bacteria are Gram-negative rods or spirals
- Grow in filaments
- Obtain energy through oxidation of reduced sulfur
 - Including hydrogen sulfide, elemental sulfur and thiosulfate
- Molecular oxygen serves as terminal electron acceptor
 - This produces sulfuric acid




- Thioploca



- Large, filamentous sulfur-oxidizing bacteria that form cell bundles surrounded by a common sheath
- Thick mats found on ocean floor off Chile and Peru
- Couple anoxic oxidation of H_2S with reduction of NO_3^- to NH_4^+



- Thiothrix

- Filamentous sulfur-oxidizing bacteria in which filaments group together at their ends by a holdfast to form cellular arrangements called rosettes
- Obligate aerobic mixotrophs





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Now, let us look at sulfur oxidizers. So, these will oxidize your sulfur to higher forms of more oxidized forms of sulfur such as sulfite or sulfate and sulfur oxidizing bacteria gram negative rods or spirals. So, you can see morphologically they are pretty limited.

So, and they grow in filaments and they obtain energy through oxidation of reduced sulfur, which includes hydrogen sulfide elemental sulphur and thio sulfate. So, they can reduce all these forms of sulfur. So, these are sulfur oxidizers and sulfur not necessary elemental sulfur, molecular oxygen serves as terminal oxygen electron acceptor and this produces sulfuric acid.

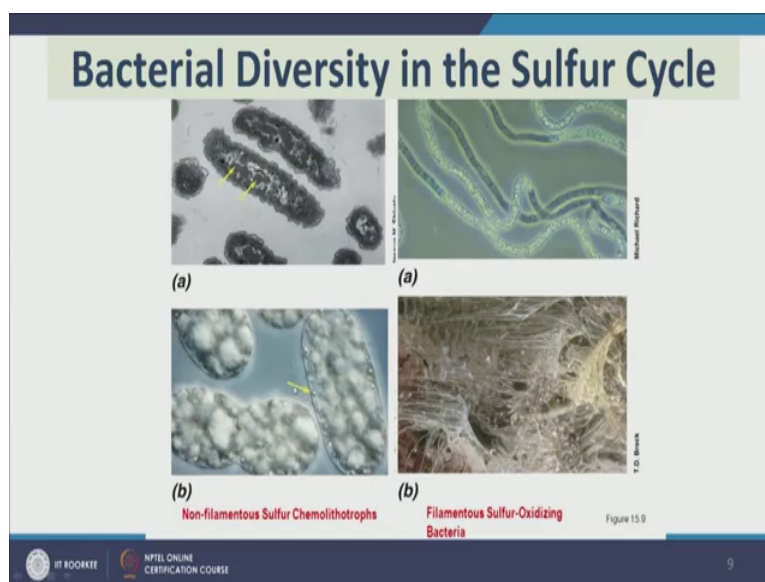
So, if oxygen is present and the reduced forms of sulfur are being oxidized then we will get sulfite and sulfate and if there is water present definitely this is sulfuric acid, as I mentioned before there are some compounds that are more oxidized form of sulfur and there are some that are more reduced form of sulfur, but this is the general chain. Then we have thioploca these are 2 examples thiothrix thioploca thioploca is the picture is here their large filamentous sulfur oxidizing bacteria their form cell bundles surrounded by a common shape.

So, if you know very carefully in this particular picture you notice that there is a filament a green cylinder kind of filament that is going diagonally through this picture. Now this is not one self-do not think that if you see a long thread of thiotrocha it is not all one microbe.

But in fact, these this is one common sheet that is that surrounds multiple cells. So, cells grow on one axis at top of each other and there is a common sheet their turns. So, morphologically we might get an impression that the cell is very long, but it is just a common sheet that covers these cells these thick mats of this thiotrocha are found on ocean floor near Chile and Peru, South America, and they often coupled with anoxic oxidation of H_2S with reduction of nitrate to ammonia.

So, their electron acceptor is often nitrate not oxygen thiotrocha not this is thiotrocha these are filament of sulphur oxidation bacteria and here the filaments hold on one end. So, in one end they hold on to each other and they form cellular arrangements called rosettes. So, these are like a bouquet. So, at one end they attach to each other and then from that and the filaments come out these are obligate aerobic mix of troughs all righty.

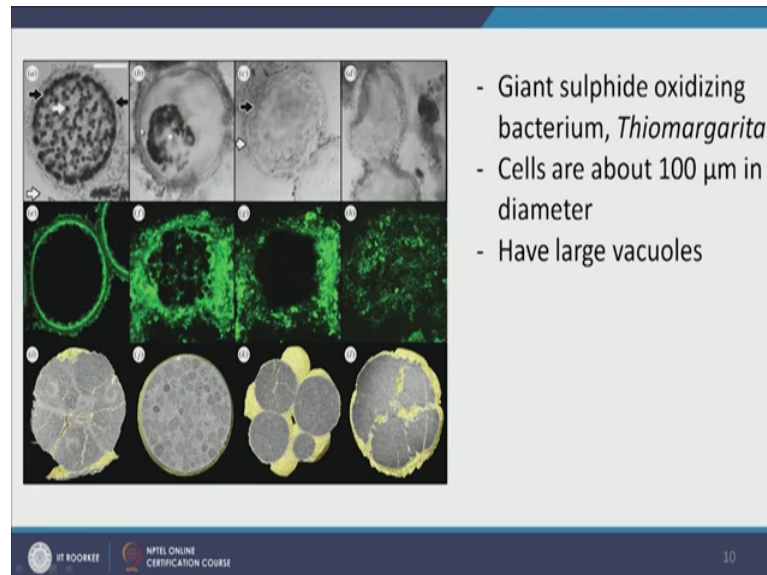
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Now these are non-filamentous sulfur chemolithotrophs. So, they are non-filamentous they do not make filament and their sulfur chemolithotrophs and you can notice here this small vacuoles, where sulfur is either being stored and these are filamentous sulfur

oxidizing bacteria. Now by the way if you see a name mentioned to the right side of the picture it is the name of the scientist who took the picture all righty.

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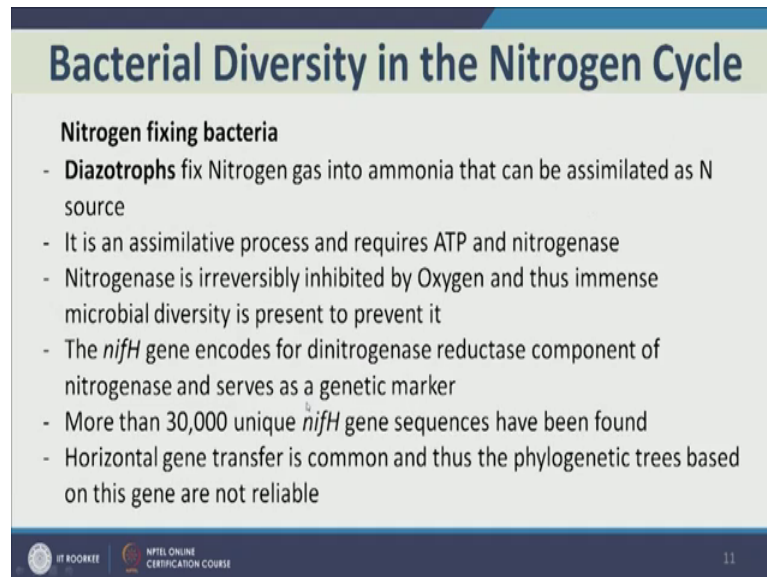
Now, this is a picture of giant sulphide oxidizing bacteria thiomargarita. Now these are very bad the each diameter here would be 100 micrometer, now think about it why (Refer Time: 14:51) 100 micrometer serve as a good diameter for cell to live one of the first concepts that I covered. In this class was there is a suitable size range and the higher surface area to volume ratio microbe has the more evolutionary fit it would be. So, why would a microbe like thiomargarita go to such high size such as 100 micrometer.

The simple reason is the answer is given to us by these pictures if we look at these pictures here we have stained the cytoplasm, in the one that is black and green and what you note here is that in the first panel in the middle row you know that the cytoplasm is available only at the periphery and inside what is going on inside it is a big vacuole.

So, basically this is a cell with a big vacuole inside it and this is a vacuole and thus it is surface area to volume ratio is still evolutionary from evolutionary perspective it is still fret and this vacuole is where it stores it is sulfur because it has 2 oxidized sulfide. So, it stores and all the it is it stores it is electron donors here and a this is one of the situations were getting and getting a morphological insights by a microscopy help us understand the metabolism of the of the microbe.

Now, if you look here this is the vacuole the gray portion the yellow portion is the cytoplasm and then the vacuole is breaking into 3 different vacuoles 4 different vacuoles and they are showing different processes of life in thiomargarita.

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Bacterial Diversity in the Nitrogen Cycle

Nitrogen fixing bacteria

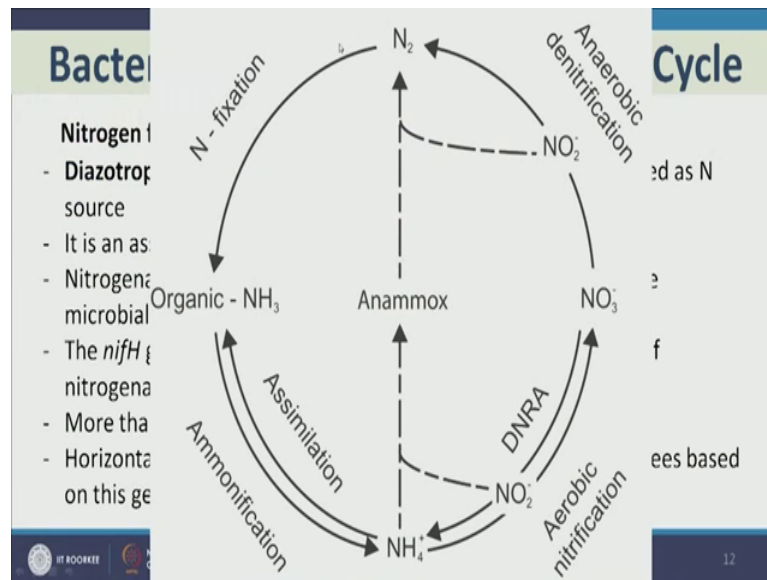
- **Diazotrophs** fix Nitrogen gas into ammonia that can be assimilated as N source
- It is an assimilative process and requires ATP and nitrogenase
- Nitrogenase is irreversibly inhibited by Oxygen and thus immense microbial diversity is present to prevent it
- The *nifH* gene encodes for dinitrogenase reductase component of nitrogenase and serves as a genetic marker
- More than 30,000 unique *nifH* gene sequences have been found
- Horizontal gene transfer is common and thus the phylogenetic trees based on this gene are not reliable

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Now let us come to nitrogen cycle 9, nitrogen cycle we have meant you have talked about a nitrogen cycle in details. So, you should remember some things about nitrogen cycle and one of the first thing that we will talk about a nitrogen cycle is nitrogen fixing bacteria, because I sphere mention nitrogen fixing is the most energy consuming part of nitrogen cycle.

So, let us revise nitrogen cycle first. So, as you mentioned in one of the previous lectures nitrogen cycle preceded this way. So, let us start with nitrogen fixation the most energy consuming. So, nitrogen gas that is abundant in atmosphere.

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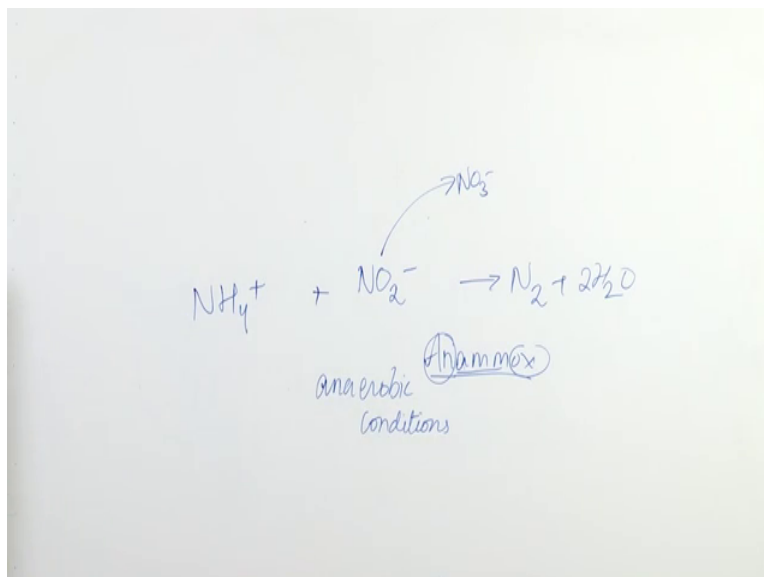
Where is this fixed into organic ammonia and this happens via 2 routes either it can be through lightning. So, extreme electrical charge or it can happen through nitrogen fixing bacteria and we are going to talk about these bacteria. So, that is a microbial contribution in fixing nitrogen and this organic nitrogen can undergo ammonification very degrades into ammonia and this ammonia can be reassimilated into organic nitrogen.

So, this this side cycle goes both ways. So, this is cell cell decay a ammonification and then when ammonia is assimilated into organic ammonia this is cell growth. Now this ammonia usually like undergoes nitrification aerobic nitrification to nitrate, now the reason is why we are skipping nitrite is because this is not stable if oxygen is present it would not stocks arise this all the way to nitrate oxygen is a very good electron acceptor sometimes; however, we will have some nitrite and this nitrite is the reason why people get blue baby syndrome my children get blue baby syndrome disease and once it has made nitrate the nitrate can undergo anaerobic denitrification. So, there is no oxygen present it is getting reduced all the way to a nitrogen which escapes us in that atmosphere or it can undo and it can continue the nitrogen fixation.

Now, few years ago there was a new discovery where we found out about anammox which is anaerobic ammonium oxidation an anaerobic ammonium oxidation, we found it ammonia can be anaerobically oxidized right away to nitrogen and not and skipping the steps that our nitrate and nitrite and nitrate can also be anaerobically reduced to nitrogen.

So, at the way anammox works is it might be ammonia interacts with nitrite and then it forms the nitrogen. No, this reaction is very simple. So, let us write it down.

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So, the way it works is we have ammonia ammonium versus and it interacts with nitrite. So, this is not the most oxidized form of nitrogen and this is not very stable if oxygen is present it will automatically go to nitrate very quickly it is only in rare situations when we still get nitrite present in water or in environment. Now this nitrite in under anaerobic conditions, under anaerobic conditions it will it can combine with ammonia to form nitrogen gas and release water. Now this is not a chemical reaction this is done by microbes and these microbes are called Anammox and aerobic ammonia oxidizing bacteria anammox this is correct anaerobic ammonium oxidizing bacteria and archaea both.

So, this is a biological process and this is the reaction that happens in anammox. Now if you look here most of these cycles have microbial components even nitrogen fixing is carried out by microbes nitrogen assimilation into organic net ammonia from ammonium is also a microbial process, its decay is also a microbial output then when ammonium degrades into nitrate this can be in chemical because it is energetically very favorable. So, it does not require the enzymatic activities or microbes, but among which is when nitrate and ammonia combine to form hydrogen is microbial process all right.

So, let us look at nitrogen fixing bacteria the one that we are going to talk first our dies or troughs. So, diazotrophs as zo is an old name for nitrogenous compound die means 2. So, di azo means di nitrogen which is nitrogen gas. So, diazotrophs fixed nitrogen gas into ammonia that can be assimilated as a nitrogen source it is an assimilated process and requires ATP and nitrogenous.

Now this is energy intensive process and that is the reason why it requires such high electric charge as is available only in thunderbolts. So, it requires plenty of energy input and it requires specialized enzymes that will lower down the energy barrier and thus we do not require one million ATP we recover only is few ATP and we are able to diazotrophs are able to assimilate nitrogen.

This nitrogenous is a very interesting enzyme because it is very sensitive to presence of oxygen it is irreversibly inhibited by oxygen. So, if it interacts with oxygen if there is any oxygen present in the environment and nitrogen is ex nitrogenases exposed to it will be irreversibly inhibited and they will have no more nitrogen fixing and thus there is immense microbial diversity that to prevent it what it implies what the sentence implies is that microbes have evolved in a very diverse forms to protect nitrogenous, one of the simplest way to protect neurogenesis do not carry nitrogen fixing activity when there is oxygen present.

Now, this must give you an idea what happens when we take plants when we uproot plants that have nitrogen nitrogen fixing bacteria sticking on their roots root not (Refer Time: 22:40) they get exposed to oxygen to some degree and then nitrogen fixing is hampered. So, do not do that I do not approve nitrogen fixing legumes this nitrogenous is encoded by nifH gene. So, my dear friends if you want to find out whether there is a nitrogen fixing happening or not just look for this gene the chemical reactions that will help you amplify this gene and help you get a detection for this gene you can even quantify it very easily.

So, it in quotes for die nitrogenous reductase which is a component of nitrogenous nitrogenous is a very complex structure one of it is domain is dinitrogen nees reductase, which is specific to nitrogen fixing and it serves as a genetic marker more than 30 000 unique nifH gene sequences have been found. So, just imagine we admit every time and we have admitted this at the beginning of this lecture that microbiology is still in it is

infancy, in sense that their diversity of microbes has not been tapped yet and even then we have such a high number of different kinds of nif genes that we have noticed.

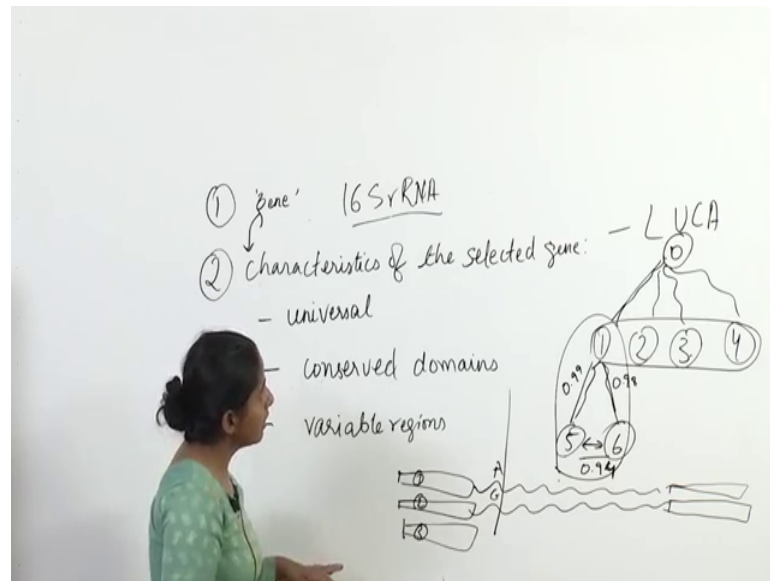
So, note here nitrogenous is a very very important activity for life every cell requires nitrogen. In fact, each of our nucleoside is nitrogen rich. So, our genetic material requires nitrogen are proteins require nitrogen life requires nitrogen and how do we get nitrogen from the abundant nitrogen in the air by nitrogen fixing this nitrogen fixing is a very very important part of ecosystems and it.

So, happens that the nitrogen fixing protein nitrogenous is sensitive to oxygen and thus it is essential for life to continue on earth that this protein is protected from exposure to oxygen and no wonder life has in it is panic mode come up with. So, many diverse forms of nifH genes and nitrogenous and ways in which it can protect nitrogen is, Horizontal gene transfer is common and thus phylogenetic tree is based on the genes are not reliable. This is one time when I need to talk about how phylogenetic trees are made and why it is important because nifH gene is commonly transferred by horizontal gene transfer and now let us look at how phylogenetic trees are made and (Refer Time: 24:55) meant by the sentence here that the phylogenetic tree is based on these genes are not reliable.

So, dear student's phylogenetic trees are trees that we make and they are basically clustered and diagrams that we make on basis of genetic similarity. Now if you remember the entire genome of a microbe is very long and it does not make sense for us to classify and the similar the microbes and their similarity and the dissimilarity on basis of the entire genome because there is. So, much diversity and so much different kinds of genes and genetic elements present in (Refer Time: 25:33) for any microbe.

So, the way we go about making phylogenetic trees is we choose a gene of interest that is present in all microbes that we are interested in. Let us say I am interested in all bacteria. So, I will choose a gene that is present in all bacteria. So, first point is when we make my phylogenetic trees, we do not go for entire genome instead we go for a celeb gene we choose a gene and we go for it.

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The second point is that what should be the characteristics of this gene. So, let us look at the characteristics of the selected gene first it should be present in all microbes that I am interested in that is it should be universal second it should not be. So, different in microbes that I cannot even align them I cannot even see words I cannot even start measuring their similarity and dissimilarity. So, there must be some conserved domains to serve as the starting point for my alignment and my analysis and the next is there should be some variable regions and as microbes evolve these variable regions undergo change, but conserved domains remain same and I think this is a very good point time to mention that the phylogenetic trees are very helpful and very useful because they give us an idea of the evolution.

So, the reason why we started making phylogenetic trees was because we wanted to know who was our last universal common ancestor LUCA and we assumed that our last common universal ancestor or LUCA had certain genetic fingerprint and as it evolved it is daughter lineages, his daughter lineages had slightly different genetic fingerprint and, but there were some and if we could really track these genetic fingerprints and how they changed over time we can get an idea of which microbes evolved first came first and which came later.

So, let us say let us say we have sampled 1 2 3 4 5 6 we had known their genetic fingerprint, but we do not know how which came first which evolved later, but now if we

make that cluster (Refer Time: 28:06) we noticed that 1 5 and 6 are more similar to each other 5 and 6 are more different from each other, but they are more similar to 1. So, in this case the similarity between 1 and 5 would be really high, but the similarity between 5 and 6 would be comparatively low let us say 0.9 4 these are just arbitrary numbers 0.9 9 similarity between, 1 and 5, 0.9 8 similarity between 1 and 6, and 0.9 4 similarity between 5 and 6.

So, this way we can say because they are more similar to one we assumed we know that they came from one. So, they branched out from one and they are not different from each other. So, similarly we notice that the dissimilarity dissimilarity between 1 2 3 and 4 is less than their similarity they share from some other organism 0. And then we can have we can make our evolutionary tree and now we know that 0 is our last universal common ancestor.

So, science is still finding out what our last common universal last viewers the common ancestor is who it was what kind of microbe it was a bacteria archaea eukarya we do not know eukaryote we do not know, but we are still making these trees. So, how do we make these trees we look at certain genes of interest? In these genes should have certain characteristics as evolution goes on there should be parts of these genes that do not undergo any change. So, that is the marker for the gene I am seeing this conserved domain of this particular that is specific to gene a. So, this should be part of gene a and then there should be variable domains as 0 branches out into 1 2 3 4 lineages in one branch around into 5 and 6 lineages I should be able to say there these differences.

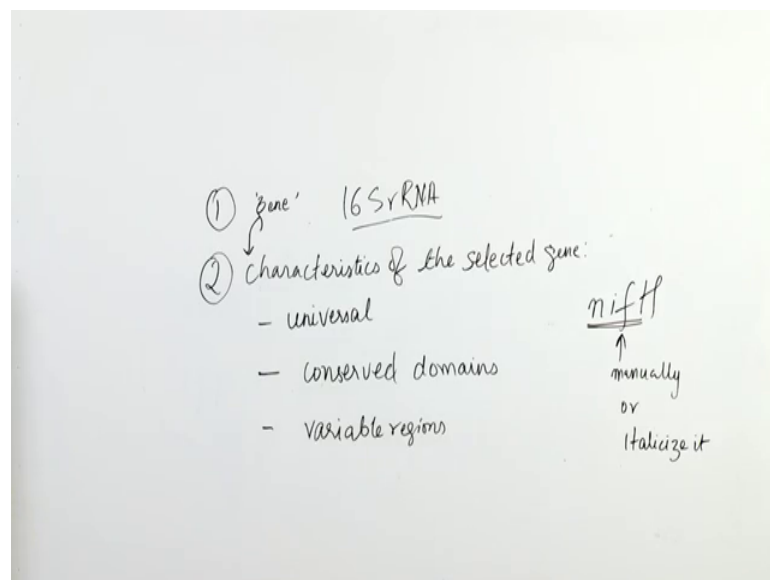
So, it looks like this these are universal domains and then there are hyper variable domains where there are 4 variable domains or hyper variable domains where variations happen. So, what we do is when we are seeing the similarity between 1 2 3 4 5 and 6 we align them. So, this is 1 2 3 4 5 and 6. So, for we align their conserved domains and we notice the differences in there, we notice the difference here is a here is G. So, we know that there is a difference here and then we count how many differences are and that is how we get our similarity.

So, this our evolutionary tree and these are the 3 properties that our gene of interest should have universality kinds of domains and variable regions typically early on and it is quite a typical practice even now we found out that there is one gene that satisfies all

these 3 qualities for all bacteria and that gene is 16 S rRNA it is not gene in that encodes for a protein it is part of the ribosome that participates in translation and it is 16 S ribosomal rRNA. So, RNA it is RNA. So, it has u instead of t and 15 S this is when we centrifuge it depending on where how far it has very precipitated we have given it this name there are actually 4 components of rRNA 16 S is 1 of them .

So, this particular rRNA we know has conserved domains and has hyper variable domains. So, people have made beautiful big elaborate trees of all microorganisms that we know on basis of 16 S rRNA gene in this particular slide we were talking of another gene *nifH*.

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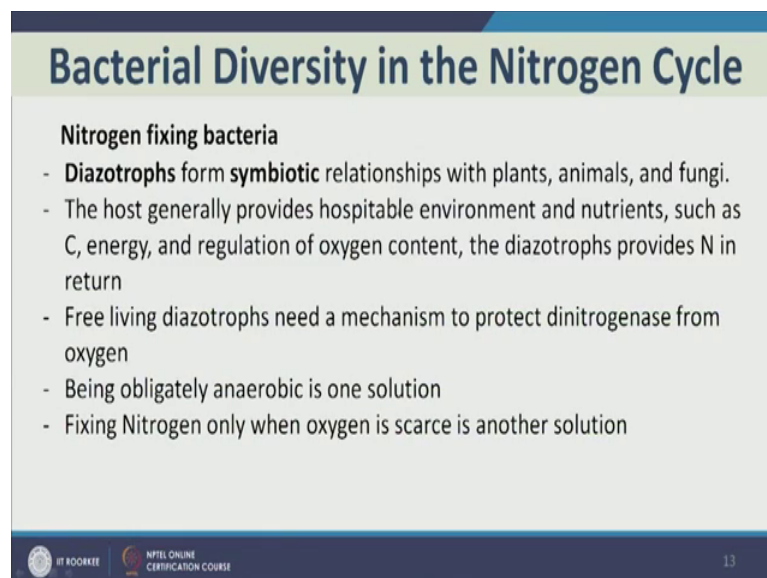
Now this gene encodes for nitrogenous and I think this is you should note this that when we manually write name of a gene we make sure that we underline it and when we type it on a computer or typewriter we always italicize it. So, either underline it if you are manually writing it or it will italicize it.

So, we were talking about *nifH* gene which encodes for a particular part of nitrogenase which is very important for nitrogen fixation is essential. Now it is said that if when people were investigating different parameters that affect agricultural output and the nitrogen assimilation in plants and they were looking at how nitrogen assimilation and fixation happens in environment microbiologically they found out about this gene what they did was they started making trees according to this gene.

So, instead of using 16 S rRNA they use nifH in and what we very soon found out was that this is not a very good gene for making phylogenetic trees, because even though it is universal to all nitrogen fixing bacteria it has more variable regions and conserved domains there is such variation in different nifH genes that we cannot make meaningful evolutionary trees.

So, even though we use this as a marker for nitrogen fixation if we want to take all nitrogen fixing bacteria and make the evolutionary tree to find out what their last universal common ancestor was then we need to go back to 16 S rRNA gene all righty.

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Bacterial Diversity in the Nitrogen Cycle

Nitrogen fixing bacteria

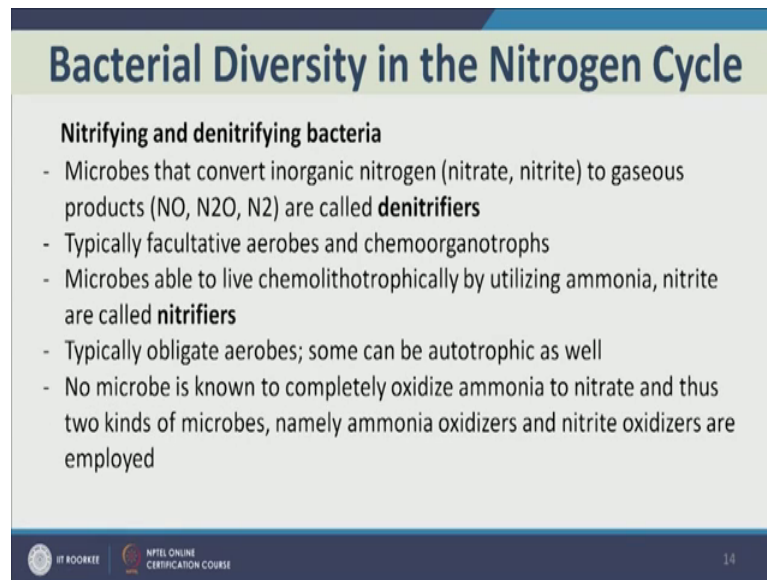
- **Diazotrophs** form **symbiotic** relationships with plants, animals, and fungi.
- The host generally provides hospitable environment and nutrients, such as C, energy, and regulation of oxygen content, the diazotrophs provides N in return
- Free living diazotrophs need a mechanism to protect dinitrogenase from oxygen
- Being obligately anaerobic is one solution
- Fixing Nitrogen only when oxygen is scarce is another solution

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Now sometimes diazotrophs which forms symbiotic relationship with plants animals and fungi, which is very important for plants like legum legumes and in this case the host will give them a nice environment live it will give them all nutrients such as carbon source energy it will also regulate the oxygen content. So, making sure that they are not exposed to excessive of oxygen and diazotrophs in turn will provide the fix nitrogen to the host.

Now, the free living diazotrophs all of them form symbiotic relationship by the way they need a mechanism to protect the dinitrogenase from oxygen and the easiest ways do not grow when there is oxygen present. So, that is fixing nitrogen only when oxygen is scarces and the other solution is be obligatory obligatory anaerobic. So, only grow in under strictly anaerobic conditions.

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Bacterial Diversity in the Nitrogen Cycle

Nitrifying and denitrifying bacteria

- Microbes that convert inorganic nitrogen (nitrate, nitrite) to gaseous products (NO, N₂O, N₂) are called **denitrifiers**
- Typically facultative aerobes and chemoorganotrophs
- Microbes able to live chemolithotrophically by utilizing ammonia, nitrite are called **nitrifiers**
- Typically obligate aerobes; some can be autotrophic as well
- No microbe is known to completely oxidize ammonia to nitrate and thus two kinds of microbes, namely ammonia oxidizers and nitrite oxidizers are employed

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So, next we have nitrifying and denitrifying bacteria now microbes that convert inorganic nitrogen nitrate and nitrite to guess his products are called denitrifiers, they are typically facultative aerobes, they are oxidizing and their chemoorganotrophs microbes able to live chemolithotrophically by utilizing ammonia nitrite are called nitrifiers, they are typically typically obligate aerobes some can be autotrophs as well no microbe is known to completely oxidize ammonia to nitrate.

And thus 2 kinds of microbe's ammonia oxidizer and nitrate nitrite oxidizers are employed to oxidize ammonia to nitrate and that is all for today. In the next class are you talking about functional diversity of strange microbes? So, we will be talking about microbes that are bioluminescent microbes that are magnetic and a very strange in many different ways. So, wait for the mystery of mysterious microbes and this is all for today.

Thank you very much.