

Applied Environmental Microbiology
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Lecture – 13
Functional Diversity of Bacteria I

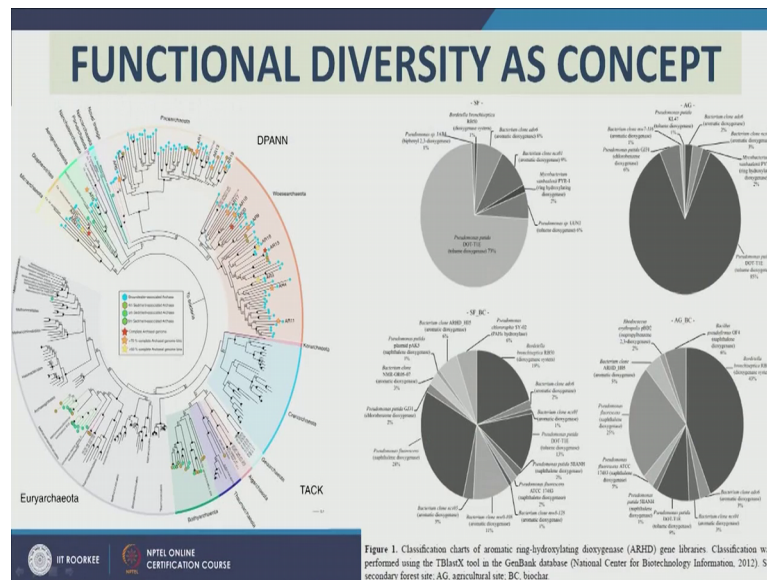
Dear students, welcome to the lecture on Functional Diversity of Bacteria. So, far I have been talking about metabolic diversity and explaining to you how under different environments different compounds can serve as electron donors and electron acceptors. And how microbes can harness this chemical energy for the sustenance and growth, in this lecture and in next two lectures; we will be talking about functional diversity. For how does this metabolic diversity translate into functional diversity?

Now, metabolism is one aspect of function for a microbe; there are multiple other functions for microbes such as how does it grow? Most of microbes we know grouped binary diffusion, but we will notice subsequently that some microbes are slightly different. And then apart from growth, how do they move their motility? How do they look like? What is their morphology? And why do they look like in a particular way? Do they attach to surfaces around them? Do they sink? Or do they float in aquatic systems?

All of these are encompassed within the word functional characteristics of microbes. And thus in this lecture, we will start with functional diversity of bacteria. Subsequently, we will also talk about functional diversity of Archaea and even eukaryotes briefly. The bacteria are very important for two reasons; one we really understand how they operate under conditions that are relevant to human environment and human microbiome.

And the other reason is that they are really very relevant and very abundant in environments that directly affect us and directly interact with. Now, coming to functional diversity; this slide shows you here.

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The phylogenetic or taxonomic diversity of certain samples, so, on the left panel we have this beautiful dendrogram. And I want to mention here that even though it appears that these dendrogram have. So, many branches and thus imply that there is immense diversity in this particular sample; note that this is a very shallow sequencing, a very shallow analysis of the sample.

When we do high throughput sequencing or in other words we sequence and we sample the sediment or the microbial sample that we have more in depth, we notice that the diversity is much more than this. So, we get a much more number of species than what we are noticing in this and this is Archaeal by the way. So, we have euryarchaeota in light blue then we have crenarchaeota in sky blue, we have a (Refer Time: 02:47) Archaeota in red, pressure Archaeota in light pink and then other kinds of Archaea.

Now, taxonomic diversity does not inform us a lot for example, we know that there are organisms that might be very similar to each other taxonomically. So, let us say these two branches are very close to each other and you will have to really squint your eyes and focus to notice the movement of arrow here.

These blue dots are very close to each other, so this microbes are supposed to be very similar. But we can note that sometimes they have very different functions and very different morphology. So, we can observe that they are very different; sometimes as

different as algae is to human beings, but genetically they are very similar. I have think we should take a segue here and let me explain to you what taxonomic diversity is.

So, remember I would talked about genes; how each gene encodes for a particular protein. Now we know that each gene encodes for a particular protein, now in different microbes they might require same protein or a similar protein for their day to day functioning. Now what happens in this instance is that; it is very likely that the same gene exists in other microbes, but it has some sequences that are different or some nucleotides are different; where there should be an a now we have a, g or c or t or something like that some combination like that.

And thus even though the genes are serving the same purpose that they encoding for protein for a particular function; they might have some dissimilarity in their organetic makeup. And there are many reasons why this happens, but in short we want to know that; we can classify how close or far away two microbes are by noticing how similar their genetic fingerprint is.

So, the more similar genetic fingerprint is we say that on an evolutionary basis they are similar to each other. We will talk about this more in detail when I talk about taxonomical classification of bacteria. And when I try to explain to you the latest advances in microbial ecology and applied environmental microbiology.

But for now I think it is enough for you to understand the taxonomic diversity is when we get the sequences, we give them names. For example, we matched them with existing sequences that we know very well often we say this is just clusteria thermocellum, this is e coli, this is some other by microbe, this is euryarchaeota some. And then we know how far away they are from each other, from the previous analysis we have done.

And depending on our previous knowledge and the current sequences that we have generated we make the dendograms; which is what you are seeing here on the left panel. And the distance between two branches of dendogram ideally should represent the dissimilarity between the two species or two types of microbes; this is taxonomic dendogram, this is taxonomic representation.

Then I will comes to phylogeny; we look at the sequences directly how similar are the sequences to each other? And how dissimilar they are? So, if this were to be a

phylogenetic map; you would note that the distance between the two branches tells you about how dissimilar two genetic sequences are.

Now, as I mentioned before taxonomic or phylogenetic diversity does not necessarily imply functional diversity. In a very simple example I would go give you is let us say we are talking about oil fields; let us say very interested in petroleum exploration and I know wherever there is petrol; there would be microbial communities around it that are degrading the petrol. Because petrol is a very rich source of electrons, it is a very rich source of carbon; so, perfect carbon electron source and microbes will degrade.

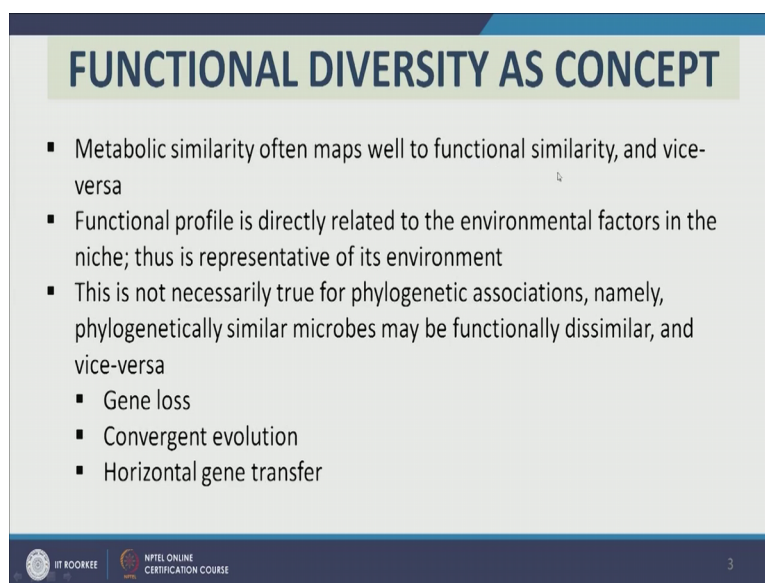
There are some issues with petrol when it comes to a degradation and thus bacteria usually have a bacteria archaea; microbes have very tough time degrading these petroleum compound, this hydrophobic compounds. And we will talk about that later when I talk about bio degradation, but if I know that there are microbes around petroleum; I can assume that all of them have similar function. Function a; they need to get some way around the hydrophobicity of petroleum.

So, that they can interact with the petroleum and they can start degrading it, b all of them are degrading hydrocarbons, c; apparently all of them should be in under similar energetic conditions that is they must have same electron acceptor and thus if they are under fermentative condition and all must be fermenting hydrocarbons. If they are under sulfate reducing conditions then all must be sulfate reduces or most of them must be sulfate reducers.

This way we can note that even if we have a big diversity, when we are talking in terms of genetic signature or when we are talking in terms of what kind of microbe they are. So, its taxonomy or phylogeny functionally they might be very similar. On the other hand we might have functionally dissimilar microbes who are very closely related to each other in terms of genes.

And in terms of how we have classified bacteria and that is my introduction to functional diversity as concept. And that is why it is very important to deal with these three things very separately, to deal with phylogenetic diversity separately, taxonomic diversity separately and to deal with functional diversity separately. And since we have been talking about metabolic diversity, I think it makes sense to jump into functional diversity because metabolism is one very essential component of function for any microbe.

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FUNCTIONAL DIVERSITY AS CONCEPT

- Metabolic similarity often maps well to functional similarity, and vice-versa
- Functional profile is directly related to the environmental factors in the niche; thus is representative of its environment
- This is not necessarily true for phylogenetic associations, namely, phylogenetically similar microbes may be functionally dissimilar, and vice-versa
 - Gene loss
 - Convergent evolution
 - Horizontal gene transfer

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So, let us look here metabolic similarity often maps well to functional similarity and vice versa; why would this be? So, let us think about it this way when I talk about metabolic similarity, I am talking about how does a microbe utilize the energy available from redox come reactions happening within itself or outside itself to sustain itself and to grow?

Now, depending on what environment a microbe is in; we would know what kind of metabolism the microbe will undergo. For example, if you do not have oxygen; we do not have nitrate, we do not have more electron loving a compounds left except for sulfate. We know that the environment wants that microbe should or it environment is ideal for microbes; that can reduce sulfate to low other compounds, sulfur compounds more reduced sulfur compounds.

Then we can assume that the microbial community that are thriving in this environment will be sulfate reducers. And because they are sulfate reducers, they will have all the proteins, all the enzymes and morphologies that are ideal for living in environment. For example, people living in a community and we know to know this from our demographical studies; that in ancient world or as the human life progressed and evolved communities that were isolated from other parts of the world developed characteristics physical characteristics that were unique to their environment and that were perfect for them to survive in that environment to sustain on the food that can grow in the environment.

So, if we know what is the uniqueness of the environment we can sort of get an idea of the functional characteristics of the inhabitants of that environment. And thus metabolic similarity often ends up into functional similarity. So, all sulfate reducers will have some commonalities and how they behave; all iron reducers or oxidizers will have certain similarities or in fact, all metal and metalloid reducers and oxidized oxidizers will have certain characteristics; that will come under one umbrella of functional characteristics.

So, but; however, it is important to note that this is not necessarily true for phylogenetic association. What it is saying is that; as I have already mentioned this just a while ago the two microbes might be phylogenetically very similar to each other, but functionally very diverse. And an example I love to give E Coile hylogenetically very similar to each other two strains of E Coile; one is benine; you can eat it. Another if you get infected you will die within a day; so think about it phylogenetically so similar; but functionally; so different.

Especially when it comes to public health; second one it says functional profile is directly related to environmental factors in the niche; thus is representative of its environment. So, if I know the functional profile of a microbe for example, I know that this particular microbe has these appendages that help it attach to surfaces and this microbial community has a capacity to reduce sulfate.

So, I already know now what the environment is the environment is probably aquatic where there is a flow of water and it is important for the microbe to attach to some surface. So, that it can generate a small niche where it has ideal conditions for growing like biofilms and it is an aerobic environment because it was a sulfate reducer.

So, knowing the functional characteristic of microbe; I can predict the environment of the microbe. Coming back to our oil well one typical example would be, I want to explore petroleum oil fields and I want to know where is petrol is. Now I can go digging every 500 meters of earth surface and it is not very good for environment; digging every 500 hundred meters of earth surface and then taking samples and measuring hydrocarbon content. The one way to go about is that whenever I dig; I can take the sample and I can do microbial analyses.

If my microbial analysis suggests that we have microbes that are degrading hydrocarbon, I can say well somewhere nearby there must be a good source of hydrocarbon; it does

not have to be right where I am, where my drill was; where my sample was taken from, but it has to be nearby. And why does it not have to be exactly where I took the sample from?

Because you know in underground, we have ground water flowing; so, some hydrocarbons might have dissolved in it and undergone dissolution and moved to that place from where I took my sample. It is also possible that there were some gaseous elements in hydrocarbon and the microbial community there was exposed to the gaseous element which was flowing freely; through the soil and or through the aquifer and microbes were happily degrading it.

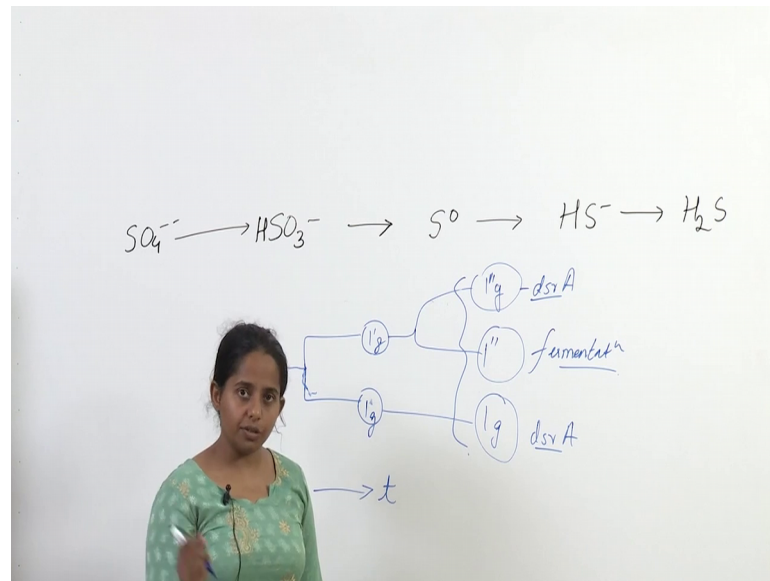
So, but that gives me information and this is when why my microbes act like a marker for what kind of environment it is. And I will give you an example again is there are certain genes that if we can amplify and detect them, we can get an idea of what kind of environment we are talking about.

For example, like you give me an unknown sample and I test what kind of genes are present? What kind of functions are present? What kind of proteins these communities can make? And I get an idea they have capacity to do it sulfate reduction and they have capacity to degrade hydrocarbon; I know right away that this is a sulfate reducing environment and it is hydrocarbon rich.

Now, coming to the third bullet point; functional diversity need not necessarily correlate very well with phylogenetic association. Because some phylogenetically similar microbes may be functionally dissimilar and vice versa; which is phylogenetic genetically dissimilar microbes may be functionally similar. Now there are three reasons for it; gene loss, convergent evolution and horizontal gene transfer.

So, let us look at gene loss; what is gene loss? So, let us say there is an original microbe.

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Let us say there is an original microbe in an environment here 1 and as 1 grows and it has its prejudices; they eventually split because with each replication there is a chance for slight mutations being inherited. So, we break down this into two slightly different microbes; 1 prime and 1.

So, one stays as it is, but one particular line of its descendants are slightly different genetically. So, this is our genetic dendrogram it is so, showing me how the ancestor is different from the progenies of ancestors have evolved over time; so, this is time. Now, let us say 1 in 1 prime; both of them have a particular gene g; now why will they have particular gene g, because they are living in the same environment.

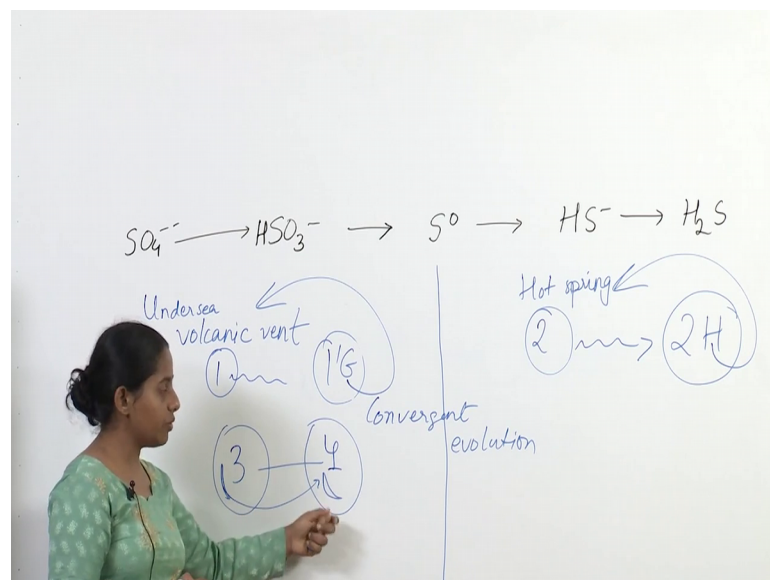
Remember the same logic then living in same environment, they are exposed to similar conditions. Similar conditions and similar reactions and metabolism would be perfect for them and most profitable for them to succeed evolutionary. So, they carry similar potential to translate proteins that do the job; so, they both have gene g.

But over course of evolution; let us say this state as such 1 g; over course of evolution one progeny of; so, one progeny of 1 prime g drop the gene; something happen and the gene was lost; so this is a case of cell gene loss. Now, even though all these three are phylogenetically similar to each other, they are not exactly same. So, in some sense there are some dissimilarities, but they are still pretty close to each other; there is one particular member that does not have this gene.

Now, let us say this gene encodes for sulfate reduction; let us say this is a very popular gene *dsrA* which encodes for sulfate reduction. So, this microbe now does not have the functional capacity to reduce sulfate through this pathway. It might do other things, it might go for fermentation or it might go for some other reduction of some other component that is present.

But may never be able to undergo this particular process through which sulfate is reduced and this is one reason why phylogenetically very similar microbes can be functionally diverse. The second reason that is given here is convergent evolution; so now, let us look at convergent evolution.

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Now, in convergent evolution let us imagine that two environments that we are interested in. One is undersea volcanic vent and the other environment we are talking about is a hot spring. So, this is early in earth's environment plenty of hot springs and plenty of undersea volcanic vents. Let us imagine they have similar temperatures, they both are aquatic environment and let us say they have similar pH. And in this particular extreme case they have similar salinity. So, they will select from microbes that have similar functions remember similar environments select for similar functions.

So, both of them should have similar functions; however, undersea volcanic vents started with microbe 1 and hot springs started with microbe 2. So, different microbes 1 and 2

and they might be very different from each other in terms of their genes, in terms of their classification and also in terms of their morphology.

But as they were exposed to this environment and they were the ones that survived. So, they eventually mutated me until they developed a particular genes g. Now this has particular gene g and this gene g allowed them to live in this environment. So, microbe one evolved; this community involved until it developed the capacity given to it by capital G; which allow it encoded for the protein that allowed them to live in undersea volcanic vent and thrive there.

Now, let us come to microbe 2 hot spring; let us say the hot spring up in Himachal Pradesh. Now in this hot spring the initial microbe was microbe 2 and its community evolved over long period of time to develop gene H. And gene H encoded for a particular protein that allowed it to survive in hot spring. Now functionally, the protein encoded by gene capital G and the protein encoded by capital gene in H are similar. Because they allow the microbes to thrive in similar environments and we notice that their metabolism is similar their electronic acceptors donors everything is very similar, but the g's are very different and this is a possibility.

So, the sequences are very different; so, they might be phylogenetically very diverse, but functionally they are same this is what is referred to as convergent evolution. So, why is it convergent evolution? Because these microbial communities one and two evolved independently and separately geographically, distantly from each other, but they converge to similar function; even though they have different genes they converge stood similar things.

Now, one example that is typically given are the wings of birds and the wings of insects. Structurally they are very dissimilar from each other, but functionally they are same and they have evolved to do similar function which shows flying; thus that also is an example of convergent evolution. The third reason why phylogenetically dissimilar microbes can have same functions or similar microbes can have different function is called horizontal gene transfer. And this we will talk in depth when we talk about environmental challenges such as antibiotic resistance.

Now, horizontal gene transfer is the capacity from microbes to transfer their genes; just by mere contact. There are three different ways in which this can happen and we will talk

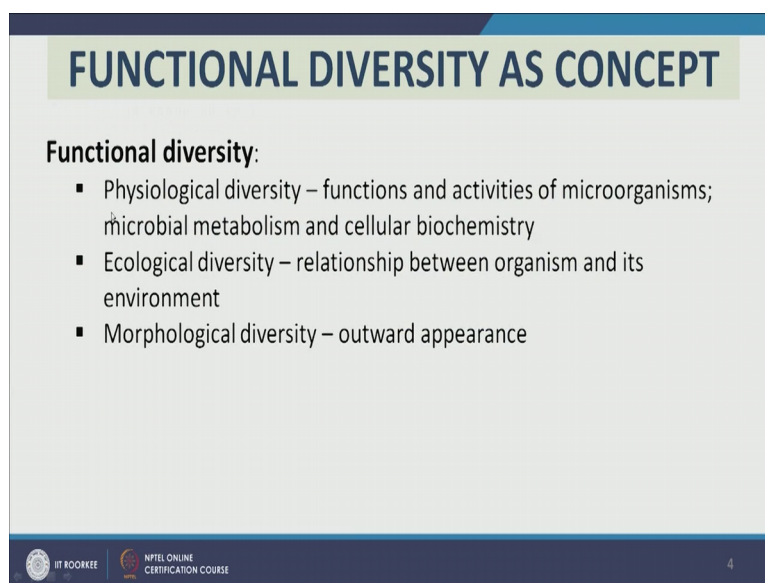
about those three different ways later, but for now it is important to know that if this is microbe 3 and this is microbe 4. And microbe 3; has a special gene line here that allows gives it a competitive H or maybe does not give it a competitive H either way it has a capacity to come in contact with micro 4 and give it this gene.

So, initially we only had this capacity in the microbial community of 3, but now we also have this capacity in microbial community of 4. One example I want to give here and we will talk about it in detail later is how soil microbial communities naturally have a background level of antibiotic resistance; for most antibiotics, in fact, all antibiotics to my knowledge or at least most of them that we know.

And many pathogens initially did not have this capacity to resist antibiotics and that is the reason why we use antibiotics on them. Because they could not resist they died when we expose them to antibiotics by consuming antibiotics. However over time these antibiotic resistant microbes in the environment; let us say in soils they came in contact with pathogens and they could transfer the genes that gave pathogens the ability to resist antibiotics.

And thus now we have pathogens that are antibiotic resistant; so now, the pathogens initially did not have this capacity, but via horizontal gene transfer they have gained this functional capacity to resist antibiotic. And now this is why phylogenetically very diverse organisms pathogen that lives inside a host like human body at 37 degree celsius or similar warm temperatures versus soil microbes that live on soil and a very different conditions. They are far more genetically and economically very different they otherwise also very different, but they have one functional similarity thanks to horizontal gene transfer.

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FUNCTIONAL DIVERSITY AS CONCEPT

Functional diversity:

- Physiological diversity – functions and activities of microorganisms; microbial metabolism and cellular biochemistry
- Ecological diversity – relationship between organism and its environment
- Morphological diversity – outward appearance

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So, functional diversity can be there are three aspects to functional diversity that apart from metabolism. One is physiological diversity; the other is ecological and the third is morphological diversity. So, physiological diversity implies what are the functions and activities of microorganism? And this includes the metabolism and activities would be how it interacts with other community members? Does it enter into complicated interactions with the other community members, which we will talk about in subsequent lectures and metabolism and cellular biochemistry.

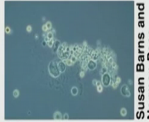
Ecological diversity talks about the relationship of microbes with its environment; so, this is when how is the microbe changing the environment? For example, we know there are certain types of geo bacter, when they interact with iron and they change its valency and in turn they gain energy from it; they also alter the minerals around them.

So, initially let us say we had pyrite, but now we will have some other kind of structure of the mineral. So, not only are they changing as such in themselves and they are gaining energy and they are changing directly the mineral that they interacting with, but they also change the geochemistry of the environment. So, microbes change the environment and then we have morphological diversity outward appearance how do they look like.

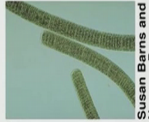
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PHOTOTROPHIC BACTERIA

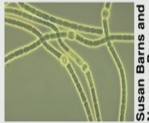
- Conserve energy from light
- Evolved early when earth was anoxic
- The first ones were anoxygenic phototrophs
- H_2 , H_2S , Fe_{2+} as electron donor
- Present in 5 phyla: *Proteobacteria*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, and *Acidobacteria*
- All use chlorophyll like pigments and other accessory pigments to harvest energy from light and transfer this energy to membrane bound reaction center, where ATP is synthesized
- Many phototrophs can fix CO_2 , others need or prefer other C sources



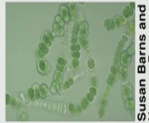
(b)



(c)





(d)



(e)

Susan Barnes and Norman Pace

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So, first we are going to talk about phototrophic bacteria if you remember phototrophic they use light as their source of energy. So, they conserve energy from light; they convert it into chemical energy which they use for driving their biochemical reactions. And this phototrophic bacteria we want to start with them because they evolved first when earth was still anoxic.

And the first among phototrophic bacteria were anoxygenic phototrophs; so anoxygenic phototrophs, if you remember in the previous lectures we talked about there the phototrophs that use light as source of energy because a phototroph and they do not generate oxygen. So, they do not use water as electron donor they might use other electron donor, such as hydrogen, hydrogen sulfide and ferrous. They are distributed in 5 phyla proteobacteria, chlorobi, chloroflexi, firmicutes and acidobacteria.

As I promised you in the introduction lecture; you do not need to remember the names. But here is the catch; if you really listen to the lectures with pay attention and go through the slides and do the homework sincerely eventually we will start remembering the names. And why would you start remembering the name because in their homework you will notice how immensely diverse proteobacteria are.

Not only do their phototrophic bacteria, but they are also have heterotrophic bacteria in that too of different kinds. And then you will also notice how far make itself can you phototrophic; they can be parasites and they can also be cellulose degrading bacteria and

when you hear these names over and over again; you will remember them and there are only few phyla to talk about; so, there is not remember, but do not focus on cramming them just listen to them and let the name register in your mind.

Now, all of these phototrophic bacteria they use chlorophyll or chlorophyll like pigments and other accessory pigments to harvest energy from light and transfer this energy to membrane bound reaction center where ATP is synthesized.

Now, I hope you remember from the previous lectures; we talked about how the light can be used to generate PMF and the PMF can then turn the ATP synthase motor, which we will synthesize ATP. Many phototrophs can fix carbon dioxide others need or prefer other carbon sources. So, this bullet is highlighting there are three different kinds of phototrophs; one who can fix carbon dioxide directly, so they are autotrophs.

And others they need; so, they are obligate trait to use other carbon sources and some prefer other carbon sources. So, some have dual capacity they can also fix carbon dioxide and they can also use other electric carbon sources.

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Domain and Evolutionary Group	Groups Based on Structure and Physiology	Characteristics and Significance
<i>Bacteria</i> Purple bacteria and relatives	Purple photosynthetic bacteria	Some can form a purple layer in anaerobic zone of lakes; mitochondria arose from ancient purple bacteria similar to <i>Rhodospirillum</i>
	Pseudomonads	Versatile bacteria in the soil; useful in cleaning up industrial pollutants
	Enteric bacteria	Inhabit intestinal tracts, aiding digestion (<i>Escherichia coli</i>) or causing disease (<i>Salmonella</i>)
	Myxobacteria	Gliding bacteria whose colonies make complex multicellular spore-forming stalks
	Nitrogen fixers	Free in soil or associated with plants (<i>Rhizobium</i>); fix nitrogen in biological form
	Rickettsias	Live only inside other cells; cause typhus fever and Rocky Mountain spotted fever
Cyanobacteria	Cyanobacteria	Have photosynthetic system like a plant and are nitrogen fixing; most are self-sufficient organisms; earliest organism to release oxygen; precursors of chloroplasts

Now this table we have on the left side purple bacteria and relatives; we will talk about purple bacteria very soon. And here we have cyanobacteria; which is a phototroph it has a photosynthetic system like plant, but it releases oxygen; so, its oxygen a phototroph.

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Cyanobacteria

- Large, morphologically and ecologically heterogeneous group of oxygenic, phototrophic bacteria
- First oxygen evolving photosynthetic organisms
- Both unicellular and filamentous, with more diversity
- Range from 0.5 μm to 100 μm in diameter
- Five morphological group:
 - *Chroococcales* – unicellular, binary fission
 - *Pleurocapsales* – unicellular, multiple fission
 - *Oscillatoriales* – filamentous, nonheterocystous forms
 - *Nonstolcales* – filamentous, divide along a single axis
 - *Stigonematales* – filamentous, divide along multiple planes forming branching filaments

The slide includes five microscopic images of cyanobacteria: (a) shows a dense cluster of small, spherical cells; (b) shows a long, thin filament of cells; (c) shows a cluster of cells with some larger, more complex structures; (d) shows a long, thin filament of cells; and (e) shows a branching filament of cells. Each image is credited to 'Susan Barnard and Norman Pace'.

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Now, let us look at this sign cyanobacteria more in detail; these are large morphologically and ecologically heterogeneous group of oxygenic phototrophic bacteria. So, many terms in this bullet; so let us take a look at each of them, they are phototrophic bacteria pretty evident, they are oxygenic; they are generate oxygen, so therefore, they use water as their electron donor. They are heterogeneous; how are the heterogeneous morphology and ecologically.

So, cyanobacteria might come in different shapes; they might make long filaments, they might exists as cone, as spheres and they might make different kinds of filaments and we will see some pictures of how morphologically they are different. And ecologically they are heterogeneous to they exist in different kinds of environment; mostly aquatic for different kinds of aquatic environment. And by the word large here, we implied that not only are they heterogeneous, but the range of the different kinds of morphological and ecologically distinct cyanobacteria that we know is large.

So, after we know that initial microbes were perhaps and oxygenate phototrophs; then came oxygenic phototrophs. And the first among them we believe our cyanobacteria; they are both unicellular and filamentous with more diversity. So, they can be unicellular and some of them are filamentous and have more diversity. They range from anywhere from 0.5 micrometer to 100 micrometer in diameter, you can imagine 100 micrometer is when they make filaments.

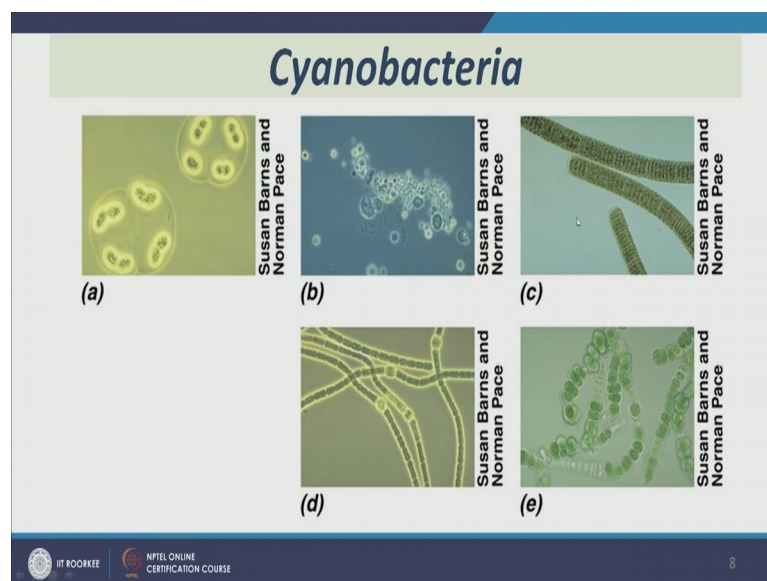
There are five morphological groups for cyanobacteria; this first one is unicellular. So, they exist a single cell and they divide by binary fission, which is one parent cell divides equally to form two daughter cells. Then we have pleurocapsales; where again they live senior cellularly, but they have multiple fashion. So, one may divide into more than one and this is very interesting; this is also the key for extremely fast replication rate of certain bacteria.

Then we have oscillatoriales; their filament; so, they make long filamentous change and usually the way it works for filamentous bacteria is that when then they divide; the daughter cells stick to each other. Then we have non stall colors there again filamentous and they divide a long single axis. So, you can expect filaments long filaments in only in one direction.

And then the fifth one of filamentous and they divide along multiple planes. So, we get like a branch a tree structure where they can they not only branch into one direction, but into multiple direction in each of these branches we will have multiple branches. So, makes a very beautiful web; now let us look at the pictures.

The first one is an example of the first type which is unicellular undergoing binary fission, this is unicellular undergoing multiple fissions. This is example of filamentous and the first form and the next is filamentous who divided along single axis.

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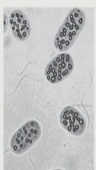


So, here you are dividing along single axis and here it is where this picture is not very clear, but there are multi branches; multiple axis of division.


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PURPLE SULFUR BACTERIA

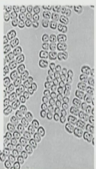
- Purple Phototrophic *Bacteria*
- Carry out anoxygenic photosynthesis; no O₂ evolved
- Morphologically diverse group
- Genera fall within the *Alpha*-, *Beta*-, or *Gammaproteobacteria*
- Contain bacteriochlorophylls and carotenoid pigments
- Produce intracytoplasmic photosynthetic membranes with varying morphologies




a)



b)



c)



d)

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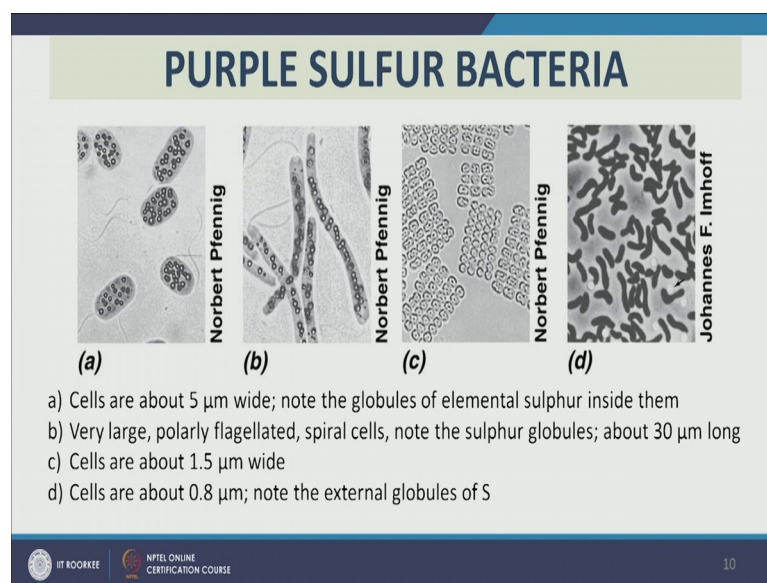
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Now, next we come to purple sulfur bacteria; these are phototrophic bacteria, they are purple in color as is evident and myself or we mean the store sulfur within themselves. So, why would the store sulfur within themselves? Well they carry out anoxygenic photosynthesis so; that means, no oxygen is generated; this might seem like a difficult and technical term for you guys; some of you. So, I will break it down easily an means no; oxy is for oxygen and genic means generation.

So, no oxygen generation they do not use water as your electron donor and no oxygen is evolved; they morphologically very diverse. They generally fall under alpha, beta, gamma proteobacteria; so all of these come under proteobacteria. These are classes within phyla pro to bacteria by the way and they contain bacteriochlorophylls and carotenoid pigments; this carotenoid pigments that give them various colors and they produce intracytoplasmic photosynthetic membranes with may varying morphologies. And these photosynthetic membranes is where these pigments are and there photosynthesis happens.

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So, let us look at these pictures for purple sulfur non bacteria; unfortunately these are not colored, but you can imagine all of them in purple tone instead of black and white. Cells in the first picture are typically 5 micrometer wide and no did these little circles inside; these dark circles is bare sulfur in store. Now simple question why would a phototrophic bacteria like to store sulfur? Think about it maybe pause this video here and think about why would purple sulfur bacteria store or any bacteria store sulfur?

So, I hope you got the answer they store sulfur as a source of energy that they can store for use when there is no light; when they can no longer carry on photo trophy, they can get energy from phototrophy then they should have an alternate mechanism and they use of. So, if you see a lot of sulfur globules it implies that the microbe is very successful in storing energy.


Look here now these are very large microbes and they are polarly flagellated which means to on their pause they have a flagellum. So, they have this whip like structure which I have some move; these are spiral cells, so it is not very clear in this picture because this is a bright field microscopy picture.

And if you remember it gives 2D images; so, these are spiral cells. So, they are they spiral around their own axis and again they have sulfur globules that you can note and they are very long about 30 micrometer long.

Look here; see here their cells are about 1.5 micrometer wide and here they are 0.8 micrometer wide and note there is a difference; this one are the most different. Here if you look very carefully; the blackmuns are your purple sulfur bacteria and then there are these white globules outside the bacteria. So, this is a particular example when the sulfur is stored in an external globule and not in internal globule and no wonder this is a smaller than all of these bacterial.

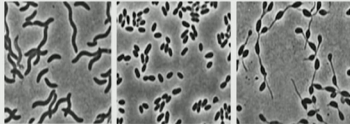
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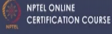
PURPLE NONSULFUR BACTERIA



- Metabolically most versatile
- Not always purple and produce an array of carotenoids
- Typically photoheterotrophs with wide range of carbon sources and electron donors
- Are morphologically and phylogenetically diverse

The blue culture is a carotenoidless mutant strain showing that bacteriochlorophyll *a* is actually blue. The bottle on far right lacks one of the carotenoids of the wild type and thus is greener





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Now, purple non sulfur refectory; so, this bacteria does not store sulfur. So, first let us handle the color, but what about these purple bacteria? Why their color? So, the blue culture here these are the different cultures of similar purple non sulfur bacteria; they are similar, but they are different types as in some genes have been knocked out.

So, in the first one the gene that encodes for carotenoid has been knocked out. So, the original bacterial chlorophyll is blue in color and because the carotenoid is not there this mutant does not have carotenoid. So, it does not have a reddish or yellowish color on it and we notice the simple blue. And this picture on the far right is the one that does not have a wild type carotenoid and thus is slightly more greener than blue.

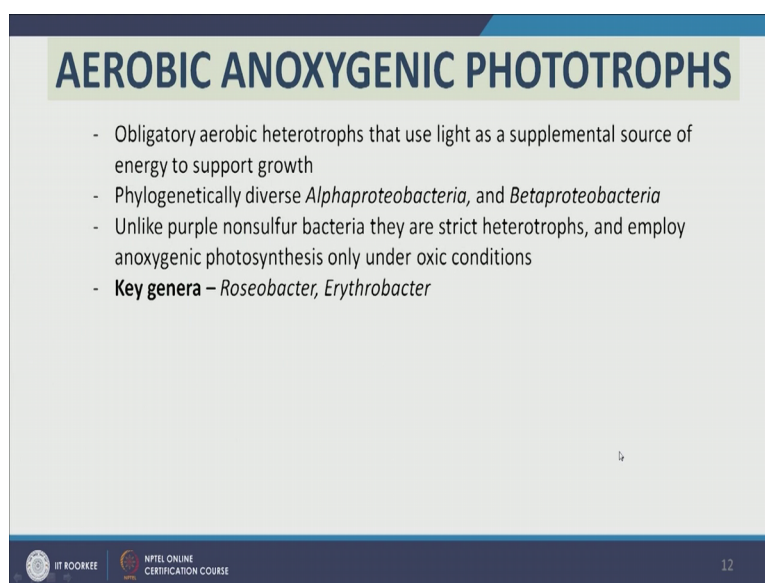
Now it might not make sense why is the yellow more green than blue? But this is more green because it does not have the one type carotenoid. So, purple non sulfur bacteria they metabolically moles towards versatile; so think of it all kinds of metabolism you can

think they can do it. They are not always purple and produce an array of carotenoids as is evident from the picture on the left panel.

They are typically photo heterotrophs with wide range of carbon source and electron donors; this is very important, if I ask you simple questions which are metabolically most versatile bacteria? You should know purple non sulfur bacteria. These are typically photo heterotrophy; so, they use light a source of energy and some other carbon source not carbon dioxide, but some other.

But they are metabolically very diverse; so some of them might actually use carbon dioxide and might be autotrophs. And also a wide range of electron donors; so, they are not obligate phototrophs, they do not need light to survive. They are morphologically and phylogenetically very diverse and there are three bright field microscopy pictures here trying to give you an idea of morphological diversity of purple non sulfur bacteria.

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AEROBIC ANOXYGENIC PHOTOTROPHS

- Obligatory aerobic heterotrophs that use light as a supplemental source of energy to support growth
- Phylogenetically diverse *Alphaproteobacteria*, and *Betaproteobacteria*
- Unlike purple nonsulfur bacteria they are strict heterotrophs, and employ anoxygenic photosynthesis only under oxic conditions
- **Key genera** – *Roseobacter*, *Erythrobacter*

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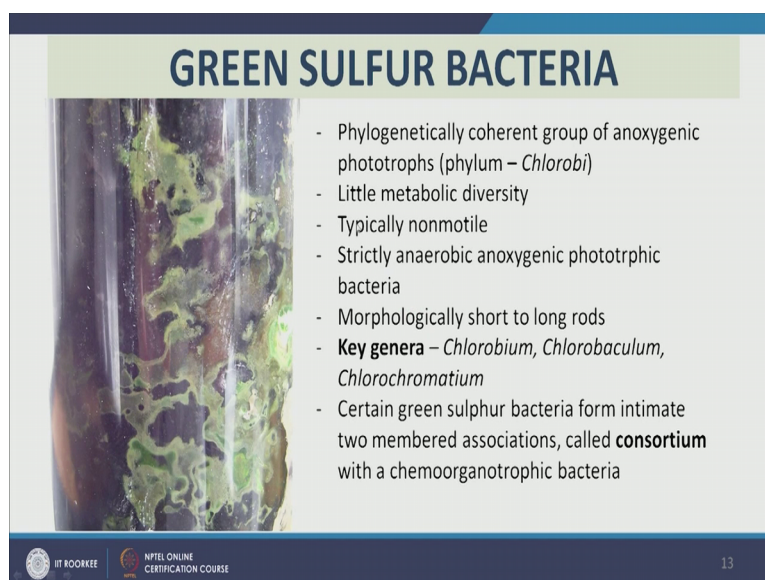
And then we have aerobic and oxygenic phototrophs; these are aerobic. So, they live in air, but the stone produced in oxygen, but they do not produce oxygen and they are phototrophs.

Now, most of them are obligates and the ones that are obligates; they use obligatory aerobic. So, when we talk about obligate it means they need oxygen to live; the heterotrophs, so they have multiple sources of carbon apart from carbon dioxide and they

use light as a supplemental source of energy to support growth. So, they are not obligatory dependent on light a source of energy, they are phylogenetically diverse with in alpha and put beta proteobacteria.

Unlike purple non sulfur bacteria they are strict heterotrophs. So, no autotrophy for them and they employ an oxygenic photosynthesis only under oxi conditions. So, when a oxygen is present in their environment only then they will undergo photosynthesis that does not generate oxygen and they are mostly found in under roseobacter and erythrobacter genre.

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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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And now we have green sulfur bacteria here. So, this picture does not look as green as I wish it did. But these are green sulfur bacteria growing here in this bottle; they are phylogenetically coherent group of anoxygenic phototrophs; what this implies is that they fall under single phylum Chlorobi. Now green sulfur bacteria, if someone says you know what is Chlorobi and they are an oxygenic phototrophs. So, they do not generate oxygen, but therefore, phototrophs they have little metabolic diversity.

So, this is one example where phylogenetic diversity functional diversity and metabolic diversity all agree with each other the same they fall under similar phyla; same phyla, they have little metabolic diversity and they have similar morphology similar function. They are strictly anaerobic and they are an oxygen in phototrophic bacteria;

morphologically they are either short or long rod. So, they have same morphology and their key genera are chlorobium, chlorobaculum, chlorochromatium.

And certain green sulfur bacteria form intimate to membered association called consortium with the chemoorganotrophic bacteria and they share nutrients with each other.

So, this is all for today and in the next lecture; we will talk more about the sulfur cycle and different kinds of bacteria that participate in sulfur cycle. This is where photo trophy ends and in the next lecture look forward to more sulfur and nitrogen cycle.

Thank you very much.