Environmental Biotechnology
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Lecture – 09
Microbial Ecology and Environmental Biotechnology - Part B

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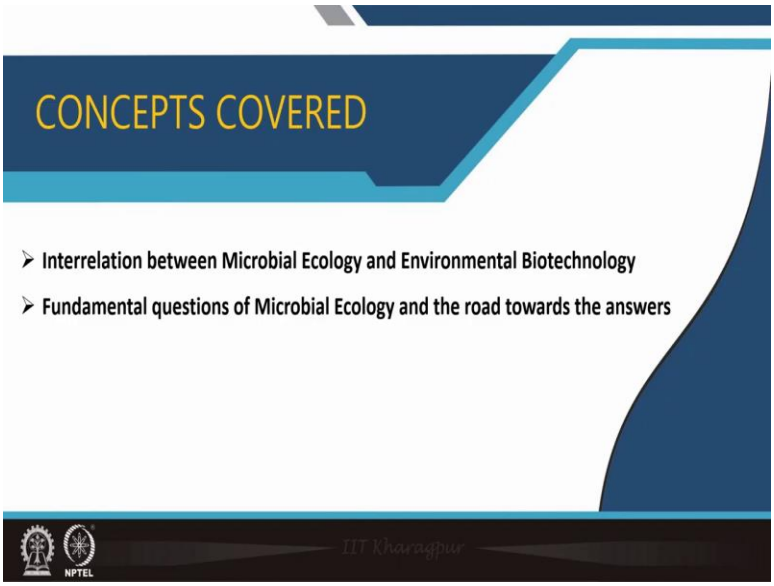

Welcome to the next lecture on Microbial Ecology and Environmental Biotechnology and in this lecture the following topics will be covered. Firstly the interrelation between microbial ecology and environmental biotechnology will be explained in more details. And fundamental questions of microbial ecology and the road towards the answers of those questions will be discussed.

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Now as we all understand that environmental biotechnology is gaining its arena and scope with which started with the abatement of pollutions and concepts of bioremediation etcetera into diverse environment to harnessing the energy from the waste water or from the resources agricultural residues etcetera. And in all such environments wherever microbial ecological concepts are being used as the core concept of the application process it has been understood that the basic processes of microbial community function need to be very clearly followed.

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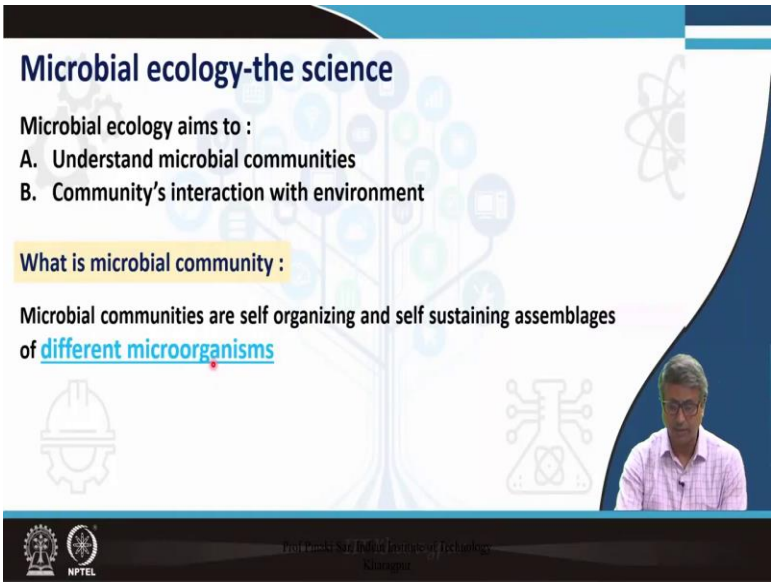

Now the science of microbial ecology as we understand it tries to answer 4 fundamental question because in earlier lecture we have discussed that microbial ecology is a scientific discipline it provides the scientific basis of community function community properties with respect to the

micro organisms which are present within it. So, this science of microbial ecology it tries to answer 4 fundamental questions.

So, let us see what are these questions? So, now before we enter into the questions I would like to briefly appreciate about the microbial ecology which is the science or the scientific of which provides the scientific foundation. And it aims to provide the understanding of the microbial communities and communities interaction with the environment. And what is microbial community? Microbial communities are self organizing and self sustaining assemblage of different microorganisms.

And these different microorganism means that it is up to the level of few 1000 different species of bacteria or bacteria and archaea and along with them maybe there are numerous fungi and other organisms together.

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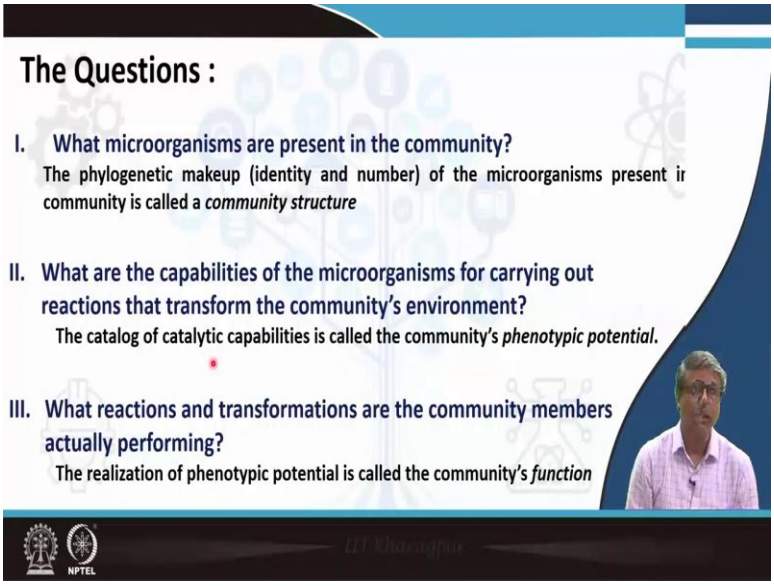

Now, the 4 fundamental questions that we ask during the microbial ecology research with any kind of environmental samples, the first question is very fundamental that what microorganisms are present in the community that is if I am working on an agricultural field or I am working on a waste water or a contaminated or polluted river water or river sediment. So, I would be asking the question that what microorganisms are present in that community.

So if it is a river water then what are the organisms present in that river water community. So, it basically means the phylogenetic makeup that means that phylogenetically if we want to explain how many organisms are there that is based on some molecular signature like 16s ribosomal RNA or similar kind of genes marker genes. So, basically we need to answer the question in a very quantifiable terms that how many different type of species are there we need the number, number of species.

So, number of species present there and identity of the individual species. So, if I say there are 100 species there in a particular environment based on the the phylogenetic makeup that is that means I get 100 different 16s ribosomal RNA gene or 100 different 18s ribosomal RNA gene for eukaryotic organisms. So, I would be able to see I say that there are 100 different organisms are there different species are there. Now what are their identities.

So, I need to establish their taxonomic identity. So, I need to know what are the species present there. So, basically this this first question which is trying to know what microorganisms. So, it is again expanded in terms of two points that is the identity of the organisms who are the organisms present there and what are their relative abundance. That is how many of a particular species are present there and that is referred as community structure.

So, with respect to any microbial community as I mentioned earlier be it a wastewater, river water, soil, ground water, forest system human gut any kind of environment wherever we are studying the microbial community structure. So, the microbial community structure refers to the phylogenetic makeup of the community that is the identity and the number of species, number of organisms present there.

The second question is what are the capabilities of the microorganisms for carrying out reactions that transform the community's environment? It is not about the general function necessarily we can study the general function of the organisms but it is more oriented towards describing the community's ability to transform its environment. For example if we are talking about a ground water where much not much of organic carbon is present but may be inorganic materials minerals are there.

May be some concentration of sulfate may be some concentration of low concentration of nitrate or some levels of ammonia could also be there sometimes in some of the aquifer we have found that. So, what are the capabilities of microbes who are present in the ground water who can transform who can carry out catalysis or transformation reactions which might alter the communities environment.

Like for example; in a sulphate rich environment we might be trying to know that; what is the ability of the organism to utilize sulfate or produce sulfate or in an ammonia or nitrate rich environment. We may try to investigate what are the capabilities of the microorganisms present there who can actually utilize nitrate. Either a dissimulatory or assimilatory nitrate reduction or utilize ammonia. So, answering this question the second question basically provides us a catalog of catalytic capabilities that what the microorganism can potentially do.

So, if we have a soil sample or if we have a water sample that represent the water community or the soil community because all the organisms are present in that sample then we need to understand that we will be doing some experiment. Or will be adopting some methodology through which the catalytic capabilities of the organisms all the organisms of course present in that sense that environment will be elucidated and that will be referred as communities phenotypic potential.

It is referred as phenotypic potential because they are capable it seems that they are capable of doing this. The third question is what reactions and transformations are the community members actually performing. Now it may appears that the question number three is just a repetition of question number two. Because in question number two already we try to answer what are the capabilities of the organisms which are which are going to help them to transform the environment around them and the question number three we are trying to know the reactions and transformations which are the which the community members are actually performing.

Because these two questions are actually not same why because in the third question we are trying to know the real scenario that is the actually what is being performed by the organisms it

is very difficult to answer this question. Because if we have a sample with us and from that sample we can prepare a catalog of catalytic abilities which is done in the question number two as part of question number two that these are the functions these are the genes these are the enzymes encoded by the organisms present in the sample that will answer the question number two.

But what about the realistic scenario that if we have maybe 1000 enzymes encoding genes in a set of in a community. How do we know that or we need to know that how many enzyme genes are actually expressing and the enzymes are actually producing within the community and the enzymes are able to carry out the necessary functions or the functions which they are they are made for.

So, however if we can adopt a method through which this realistic scenario the realistic performance of the organisms in terms of their catabolic abilities or their transformation reactions can be assessed then this question will provide us a very important aspect of the community which is called the communities function. So, the communities function is basically the realistic scenario what function the community is actually performing it is different from the potential.

Because potential is something which is highlighted in the question number two which is tested in the laboratory that for example one particular community might harvard genes for nitrogen fixation that will that will be listed within the catalogue of catalytic capabilities that nitrogen fixation gene like NIF gene is nitrogenase is there in the community that will be reflected as a community phenotypic potential.

But unless we establish that the NIF gene is actually expressing and the nitrogenous enzyme is actually producing and this nitrogenous enzyme is allowing gaseous nitrogen to be converted into ammonical nitrogen we will not be able to assess the community's function. So, the question number three is trying to answer the real function of the community member.

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And the fourth question what are the interrelationship among the communities members? Because as I mentioned earlier in the community there will be 1000s of members present minimum 1000 to 5000 species are there. So, these species must be interacting with each other. So, what are the pattern of interrelationship among these community members? And not only between the members alone but also with their environment that is the physical and chemical components which are present in the environment.

So, that has to be answered. Now this includes the interrelationship involves the special organization that is who is near whom and materials that are that the microorganisms exchange between themselves. Now it is very interesting that there have been continuous development of methods in terms of microbial ecology that which allows us to to answer these questions. And we will be discussing those on methods and approaches to answer these four questions very shortly.

But it is also important that understanding these interrelations is the ultimate goal of microbial ecology. Because once we are able to answer these four questions we would be more close towards applying this microbial ecology concept into environmental biotechnology.

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So, let us take this example of a forest or a paddy soil ecosystem where numerous microbial species are forming guild or consortium and they are continuously interacting with each other. And I can represent the numerous microbial species as this the color balls. So, these color balls basically represent different microbial species. As just this is just a representation that there are numerous or many microbial species could be there.

Now one way of answering this 4 question the particularly the first question is they try to identify these members who are present. So, if we can identify this member then it could actually provide us the answer to the first question that is the taxonomic identity and relative abundance. But at the same time we can also try to develop or implement some methods through which we can actually assess the community's phenotypic potential like what are the genes in present in these organisms.

What are the enzymes which are encoded by these organisms? We can we can make some assessment on them then it if we can make a catalog of that tests or that or enzymatic properties then it is going to give us the idea about the phenotypic potential. But again phenotypic potential is not the true reflection of the community's ability. True reflection of the community's ability will come only when we have some improved method through which the community function the realized potential is actually established.

And finally the relationship between all these tiny members among themselves and with the physical and chemical entities present in the particular environment will be elucidated.

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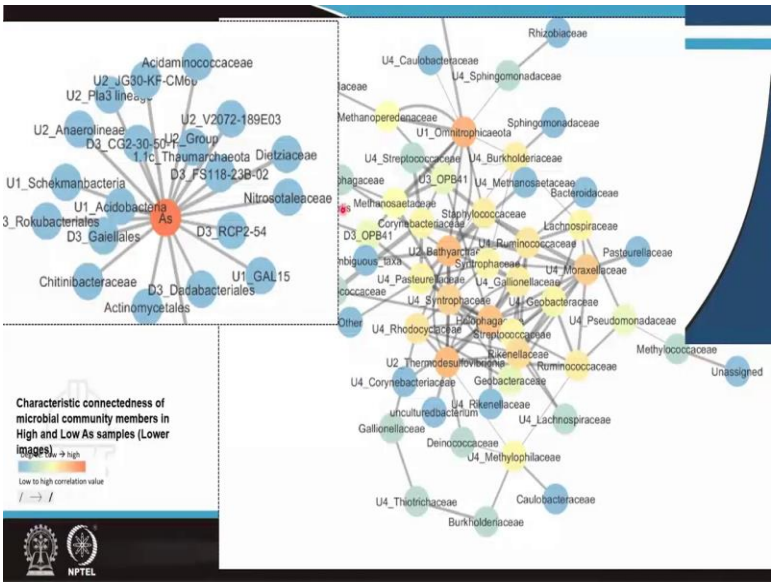

So, for example the inter relations. So, these are some kind of data that we have collected or we have obtained during our research from different environmental sample. So, as we can see when we are working on the arsenic contaminated groundwater system we are able to find out that what are the members of different bacteria who are actually more connected to arsenic? Or what are the bacteria who are connected among each other.

So, these are called networks. So, we could build such networks through which the interrelationships. Who is close to whom and how connected one species is with another species these can be elucidated through this kind of network based analysis.

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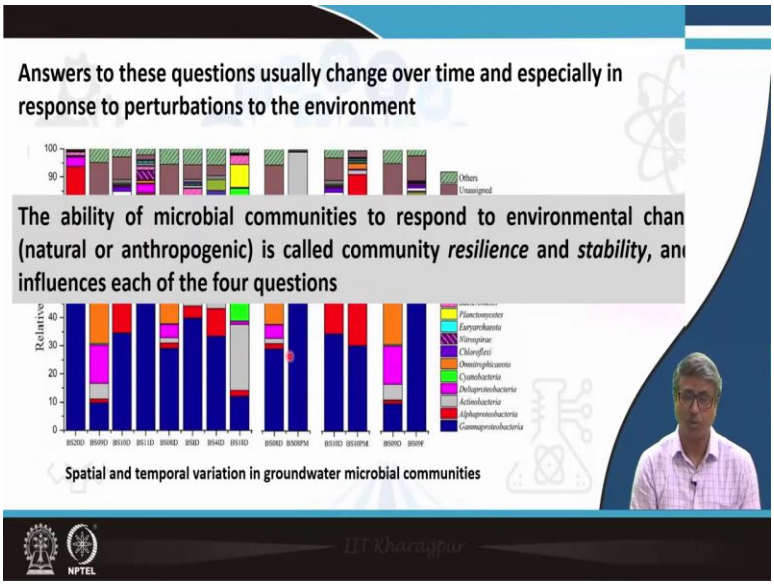

Now the next very important aspect is that after getting the answer to these questions that we will talk about the methodology through which these answer to these questions can be obtained. However it is important this answer to these questions usually change over time and especially in response to perturbation to the environment. This is not a kind of the one time process because the kind of organisms present there.

And the kind of functions they are capable of doing would be highly constrained by the environmental factors. So, as you can see that if we try to look into the community composition of ground water just ground water where the water samples can be used as a drinking water samples. So, these individual bars indicate the water sample microbial community that is the answer to the question number one that what are the organisms present there.

So, you can see that in the first water sample only few groups are there. So, each of the colors represent a distinct bacterial group whereas in sample number water sample 2 there are a number of large number of bacterial members are there. In sample number three relatively more number up there but in sample number four we can see that only one type of bacteria is more predominant and it means that if we have a couple of groundwater samples all are from a nearby localities we will be able to find out that each of the groundwater samples.

All of these ground waters are quite similar because they are from a similar same locality but

there may be a local variation in the geochemical or the geological environment that might have controlled or influenced the microbial community of the individual water and that is the question number one answer to the question number one if we are able to answer it will be seeing that the answer to this question is actually different with respect to different samples.

Now why the answer is different with respect to different samples it is because each of the water samples are taken from a different location. So, each of the location might have though it is a close by environment and the geochemical setting is nearly similar but there are minor variations minor fluctuations and those minor fluctuations might be having significant contribution on the microbial community composition.

The second is that with respect to time a particular water sample shows variation in its in its answer to the question number one. A particular water sample when it was analyzed during a dry season you can see more number of microbial species are there compared to the same water sample which is actually sampled and analyzed after maybe monsoon season. So, that means each of the microbial community is to be analyzed over a particular time and with respect to particular condition because it is going to be going to be affected by local regional or global factors including the environmental, physical and chemical as well as other biological entities.

The ability of microbial communities to respond to environmental changes because why in following monsoon some organisms are more abundant while some organisms completely disappeared because it is the effect of the environmental change which happened following monsoon. Now the ability of a microbial community to respond to the environmental change whether it is a natural or anthropogenic is called community resilience the ability to respond how it is responding and community stability.

So, a stop stable community will remain nearly insensitive. So, it will maintain its structure the answer to the question number one will be almost always similar for a particular sample particular community if it is sampled over a period of time only if the community is very stable. However if the community is not very stable we will see it is it is going to be changed as I have shown through this example and the community silence resilience means is ability to respond.

So, often it is going to be changing and it influences each of the four questions.

So, the community the silence and community stability will affect answer of each of these four questions.

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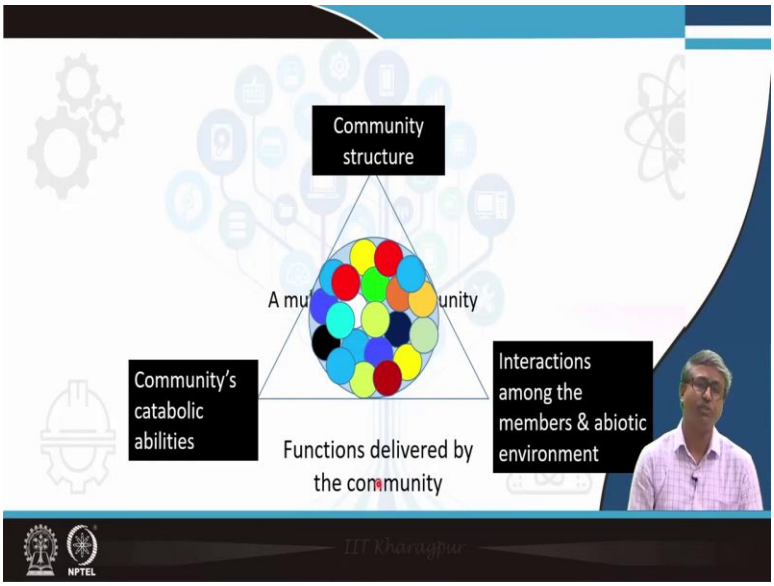

Now why it is so? It is so, because the function delivered by a particular microbial community is controlled by numerous factors. So, we will look into this in a graphical through a graphical representation that we can assume that the microbial community is composed of a number of microbial species and each of these colour balls are representing a different species. So, and together this is this is representing the community structure.

Now the community members present that is the microbial species present within community they represent their catabolic abilities because each of them will having will have a set of abilities. So, together they represent the community's catabolic abilities. And the interaction among the members and the abortic environment will always influence the overall function. So, if we try to understand the function delivered by the community.

We need to understand that it is actually controlled by three main factors. So, be it we can have a simple example that a particular soil collected from a from a given locality is found to be very fertile whereas a similar soil from adjacent field or adjacent locality is not. So, fertile same type

of crop if we cultivate will see without adding any extra fertilizer same variety of seeds etcetera are used even if even if that will see that the response of those are different.

Now if we trying to investigate that what is the control of the soil microbiota in controlling the soil fertility that is the function delivered by the community the soil community will be able to find out that one factor is the community structure who are the members present in the community. So, if we have two soils in the two soils we need to understand that the two different type or similar type communities are there and the catabolic abilities.

There may be same type of microbial species but their functional abilities might be different and the third is the interaction ability that how one species is interacting with another species and how a particular species or a particular set of species is capable of interacting with the geochemical environment that is also different. So, all these three factors together they control the function which is to be delivered or which are to be delivered by a particular community.

So, if we need to understand the details of the functions delivered we need to focus ourselves to elucidate these three things.

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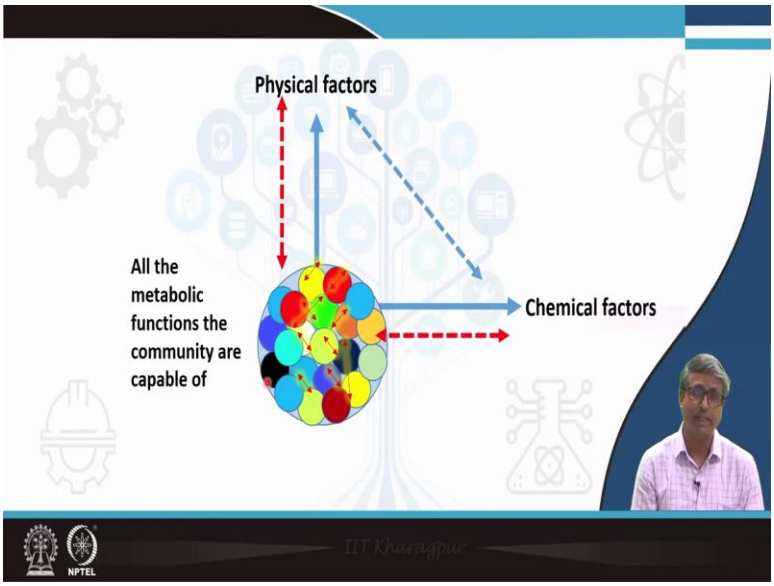

Now what controls the catabolic abilities of the microorganisms? Now a particular community might be composed of a set of species and those organisms are having genes and those genes are

responsible for encoding functions. So, that represent the catabolic abilities and together that represent the catabolic landscape of the community. However this is not a static entity it may appear that it is composed of a set of microorganisms.

So, it must be a static entity. So, we can elucidate this static entity very well which is called catabolic landscape but when we when it comes to the functional aspect of this catabolic landscape it is it is absolutely not a static entity because it is highly controlled by the physical and chemical factors. Because there are numerous species each of these species are having their genes and enzymes and these genes are enzymes are continuously affected by the physical factors and the chemical factors and the interaction between themselves.

And these microorganisms they not only be affected by the physical and chemical factors but also they can change their surrounding physical and chemical factors also. So, overall it is it is a more complex process or complex phenomenon that then we understand it.

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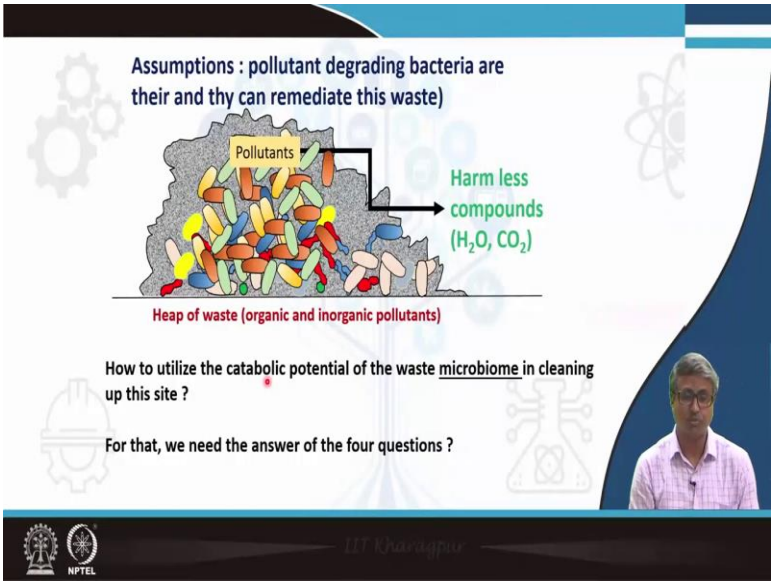

For example if we consider this heap of waste material which is full of organic and inorganic pollutants we can assume. So, this is just a dump site may be huge amount of organic inorganic waste are there. So, microorganisms will naturally start growing there and it is a very common event that in almost all such waste dump sites we have natural microorganisms growing there it is there is no surprise.

And it is also not surprising that many of these organisms who are naturally growing in that particular environment or any particular dump site are pollutant degrading. And so, they can remediate this waste or any kind of waste wherever microorganisms are there and these pollutants whatever pollutants are there they are supposedly converted into harmless compounds like water etcetera.

But how to utilize the catabolic potential of the waste microbiome in cleaning of this site because around the globe we see there is a huge problem with the dump sites and other contaminated waste. So, in everywhere on in most of the places natural microorganisms are there but how to utilize these natural microbial abilities catabolic potential to clean up these sites. Answer to this question is not. So, simple because it lies with the four questions that we initially posed.

So, if we can answer the four questions then we will be able to address this question that what we need to do in order to harness the ability of these natural microorganisms who are present in a particular site in terms of the pollution abatement for that particular environment.

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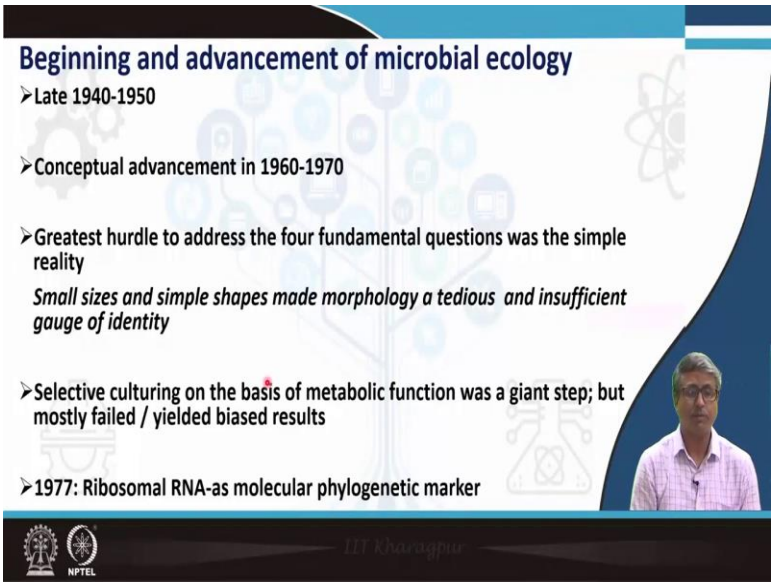

Now we will briefly talk about the beginning and advancement of the microbial ecology. It is started maybe in before 1840 or so, but it is it is discussed and researched during this course of time like 1940s, 50s based on mostly on the cultivation dependent or pure culture based

concepts. Conceptual advancements were made in 1960s and 70s. As we started realizing that many organisms are not cultivable. So, we are we are not able to cultivate all organisms.

So, one of the greatest huddle to address the four fundamental questions that we posed was the simple reality that is the small size and simple shape made morphology a tedious and insufficient gauge to identify. Like if we have a soil or a water sample and if we want to answer the question number one that how many organisms are there? And if we supposedly if we take the water sample and take it on a glass slide and see under the microscope we will not be able to find out more than 5 or 6 different morphological cell types or morpho types.

But that will not give us the true picture because microbiological cell morphology does not vary so, much with species variation and also the sizes are so, small that often many of the microbial species cannot be seen even properly through normal light microscope and for their cellular morphology we need to have electron microscopy. And even with electron microscope also we may not be able to discriminate the different species morphology in that way.

So, identification of the numerous species who are present in a particular microbial community remain a great challenge or great hurdle. Selective culturing on the basis of metabolic function was a giant step. So, during that time so, 60 to 70, 1960 to 1970 during that period of time and even little later also even till now also scientists are always trying to venture into developing new culturing method. So, that organism can be cultured successfully based on metabolic function.

Like nitrogen fixing organism can be cultured in a different way compared to an ammonia utilizing or a nitrate reducing microbe. So, utilizing this concept that metabolic functions could be connected to their growth and cultivation was a giant leap. But mostly failed why mostly failed? Because; apart from the major nutritional requirement there could be many other unknown requirements particularly when we aim to grow the microbes in the laboratory conditions.

We will discuss that part in not in today's lecture but in some other lecture but it was mostly failed the selective culturing. However we got some interesting information interesting outcome

of those studies where we can develop some methodology based on which based on metabolic functions we can actually grow certain organisms. 1977 was a very important time because ribosomal RNA gene or ribosomal RNA rather was identified as a molecular phylogenetic marker because that allows us to establish the identity the phylogenetic identity of the organism in a straightforward way.

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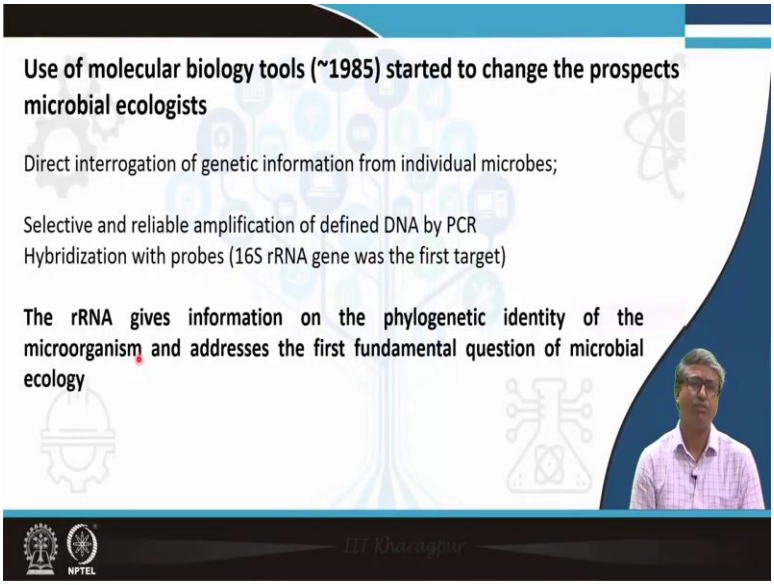

So, in the next 10 years or so, we see a kind of a remarkable change because use of molecular biology tools appeared including the PCR based amplification and the sequencing technologies. So, the direct interrogation of genetic information from the individual microbes where possible, so, if we have isolated the organism or even if we have not isolated the organism we can actually identify the organism directly through these genetic methods.

Selective and reliable amplification of defined DNA like a 16s ribosomal RNA gene or 18s ribosomal RNA gene or any kind of other genes or functional genes and also development of hybridization methods with probes the short oligonucleotide chains which are actually tagged with a fluorescent marker or a radio isotope particularly with 16s ribosomal gene was the first target. We were able to interrogate the community with respect to gaining the answer to the first question.

That is the our ribosomal RNA gene particularly gives information on the phylogenetic identity

of the microorganisms and addresses the first fundamental question. So, with respect to answering the first question that what are the organisms present in a particular community we are able to get a solid solution to that that if we have a sample we can extract the ribosomal RNA or ribosomal RNA containing gene and sequence it and then possibly we are able to identify the taxonomic or phylogenetic information of that.

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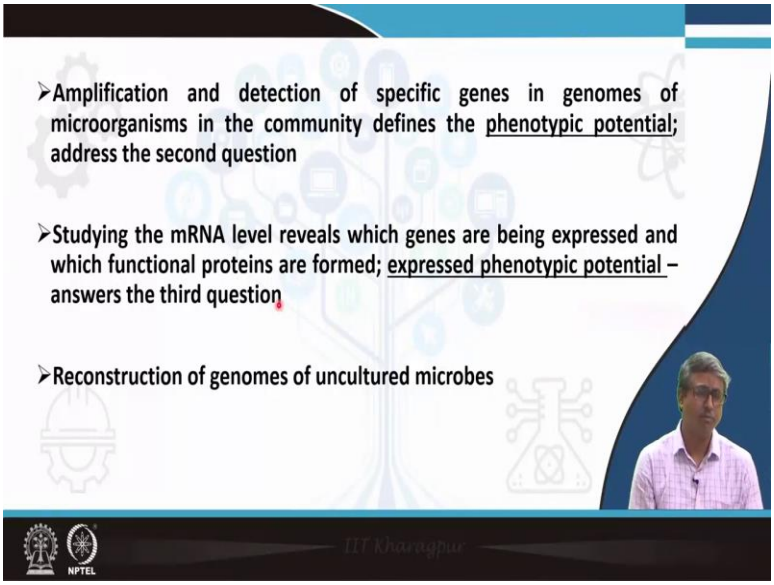

Now amplification and detection of specific genes not only the ribosomal RNA gene but also any kind of specific functional genes like genes related to nitrogen fixation. Gene related to phosphorus metabolism genes related to iron metabolism genes related to any other cellular activities or metabolic activities can be amplified using PCR reaction and when we try to catalog them or make an inventory of that that creates the phenotypic potential.

So, we can straight away identify that if these set of genes are there then this could be the phenotypic potential of this this community. So, again from a community directly we can we can answer this question. So, that is the answering the second question. Now about the third question; answering the third question, now studying the mRNA levels, so, since we know that the cellular process follow the central dogma where any gene which is going to be functionally active would produce the mRNA.

And then the mRNA will translate into protein, so, in order to understand if a particular

metabolic function is actually being performed or not? We need to target something which is essential for the function. Like if we have a gene like nitrogen fixation gene NIF gene if gene may be there in the DNA in the genome but that may not satisfy the answer that whether the NIF gene is actually functioning or the nitrogenase enzyme is actually functioning in the community.

But if we can target the NIF mRNA that could possibly satisfy us that if mRNA is produced that means the possibly enzyme is going to be produced the same thing could be applicable for getting the protein also. So, studying the mRNA level reveals which genes are being expressed and which functional proteins are formed. Expressed phenotypic potential will be answered. So, that is basically the third question is getting answered.

And then from that we can actually proceed towards the entire meta genome analysis and reconstruction of the genomes of even the uncultured organisms.

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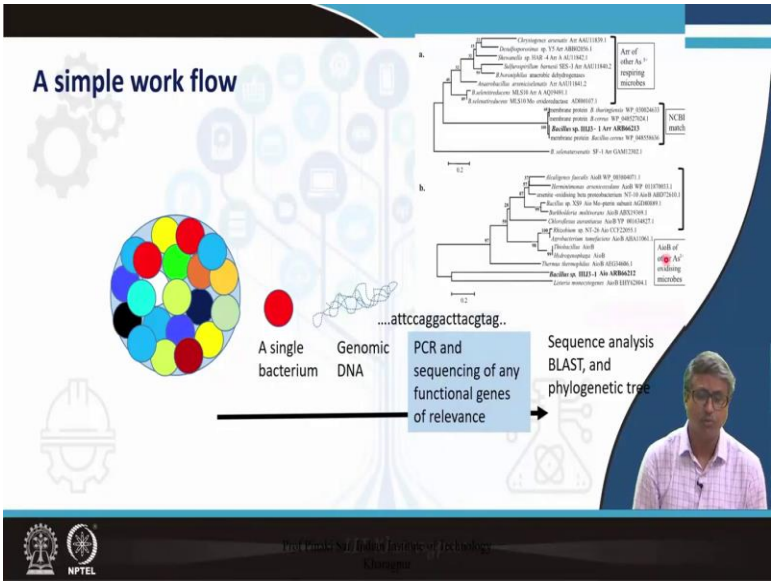

Now I will briefly show you the simple work flow. So, if we have a the community microbial community we can take a single bacterium out of it and getting get the genomic DNA extracted out of it and then do a PCR based reaction of and sequencing of the 16s ribosomal RNA gene which is basically the phylogenetic marker and then a sequence analysis like basically local alignment search tool based method and phylogenetic tree construction can help us to identify what is this species.

So, by constructing a phylogenetic tree we can very well identify that this is the bacillus species with closest relatives remain the bacillus areas and it is a type strain. So, we can actually go up to that. Similarly we can have any kind of functional genes this primer for functional genes and then those functional genes for example in this case we have ample try to look into some functional genes related to arsenic reduction or arsenic oxidation for this particular bacterial species.

And with a similar work strategy of sequencing and sequence analysis which we could identify what are the genes related to arsenic are present there. Similarly not only for arsenic rather for general metabolism also we can test any kind of relevant metabolic process which are relevant for the organism survival in that environment and create that inventory.

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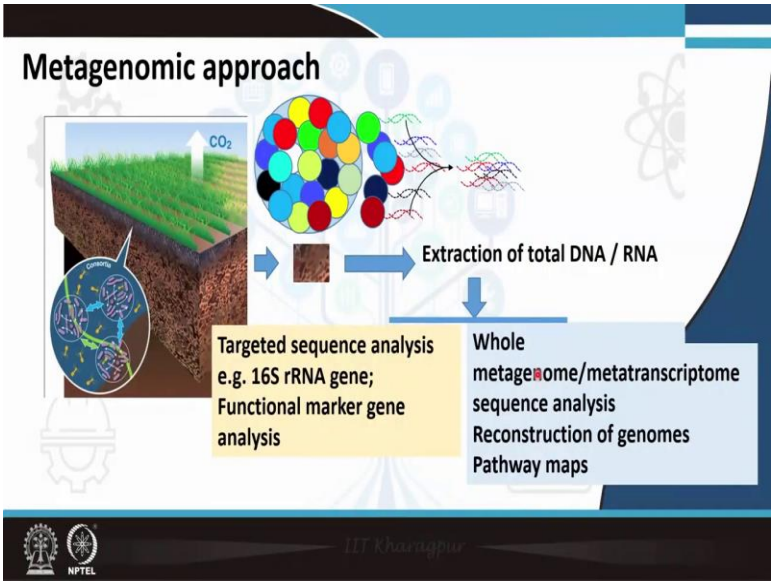

Now regarding the meta genomic approach. So, instead of individually getting the information for the organisms we can get a sample and from the sample we can extract the total DNA or the RNA. It is something like that individual instead of isolating individual cells we allow the lysis of the cells from the sample itself like in this case we have a soil sample. So, from the soil sample we take a piece of soil or some amount of soil and then do a DNA extraction or RNA extraction.

So, that it is expected that cells which are present there will be lysed and their DNA or RNA that is the nucleic acids will be released and we can. Now analyze this DNA or RNA. How we can analyze we can do it we can do the targeted sequence analysis like we can amplify 16s ribosomal RNA gene or any kind of functional marker gene. So, if you are working with mRNA or RNA then we can target any kind of specific set of mRNA encoding mRNA genes or mRNA primers for functional genes.

And we can actually ascertain whether the mRNAs for those functional genes are produced answering the third question again. Or we can actually sequence the entire genomic sample that is the meta genome. So, we can sequence the entire genome without going into any kind of PCR or targeted gene sequences. So, we can sequence the entire meta genome or we can sequence the entire meta transcriptome and then analyze.

So, that we can get the entire data set about the genes which are present there or the genes which are actually expressed under a given environmental condition or a given environment and then also we can also reconstruct the genomes of many organisms which are difficult to isolate or difficult to study otherwise. And finally we can also construct or reconstruct the metabolic pathways without going into the individual details of the metabolic reactions.

Because we have the entire inventory of genes which are relevant for a particular metabolic pathway in fact all the metabolic pathways which are possible for that particular community will be available to us and then we can build the metabolic pathway for any kind of specific metabolism that we want.

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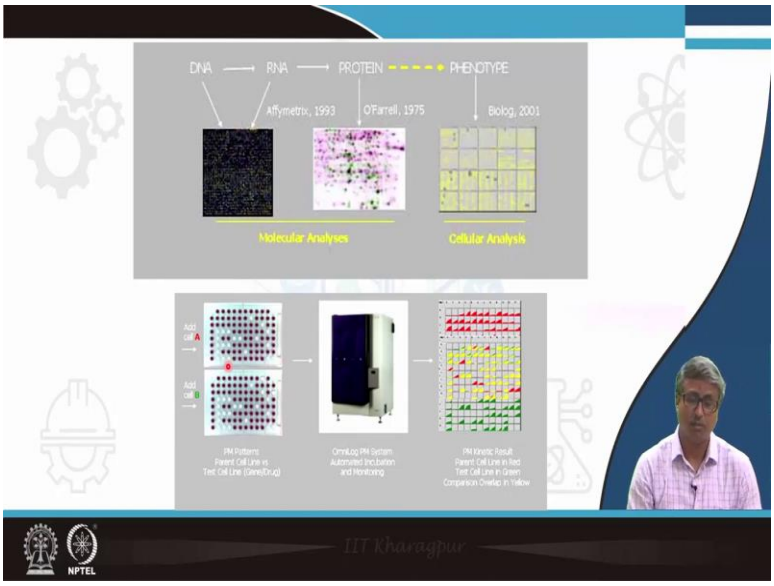

And this is further elaborated through different type of molecular analysis based on the DNA, RNA or the protein including different hybridization or using the different radioactive isotope based methods. And finally a very important and interesting method was developed which is called phenotypic microarray. So, apart from the normal microarray where we rely on the hybridization based technique. The phenotypic microarray is based on a set of substrates. So, this is a kind of a 96 well plate format. So, we have 200 micro liter volume 96 well plate.

So we have a set of substrates already provided there and we add the cell or add the community or the soil extract or the water sample directly into that. And let the cells present in the community utilize the substrate present there because in the 96 well plate format we can have more than 30 substrates in a triplicate format. So, we can have 30 substrates. So, based on the 30 substrate that we have provided we know that what are the substrate provided.

So, any kind of microbial sample that we provided, that the soil or the water can be can be tested about their abilities to provide to utilize those substrates and thereby we can develop a nice map about the community function.

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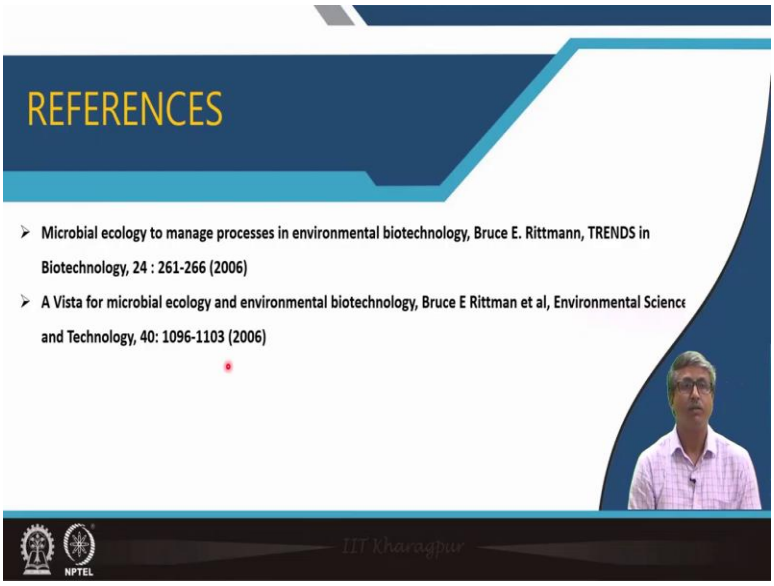

So, the reference for this part of my lecture is basically the Microbial Ecology to Manage Processes in Environmental Biotechnology by Bruce Rittman in trends in biotechnology and A Vista for microbial ecology and environmental biotechnology again by Bruce Rittman et all published in environmental science and technology in 2006.

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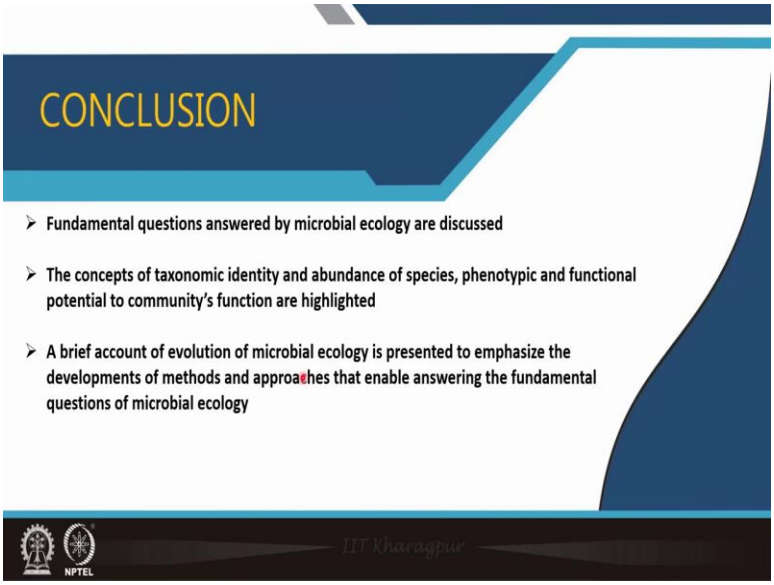

The in conclusion in this lecture we talked about the fundamental questions which are to be answered before we try to apply microbial ecology into any kind of biotechnological processes. The concept of taxonomic identity and abundance of species phenotypic and functional potential to communities function are highlighted. And a brief account of evolution of microbial ecology is presented. To emphasize the developments of methods and approaches that enable answering

the fundamental question of microbial ecology, thank you.