Environmental Biotechnology
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Lecture – 30
Methods in Microbial Ecology with Relevance to Environmental Biotechnology (Contd.,)

Welcome to the next lecture of this course on environmental biotechnology in this particular lecture we will continue our discussion on methods in microbial ecology which are applied for different aspects of environmental biotechnology.

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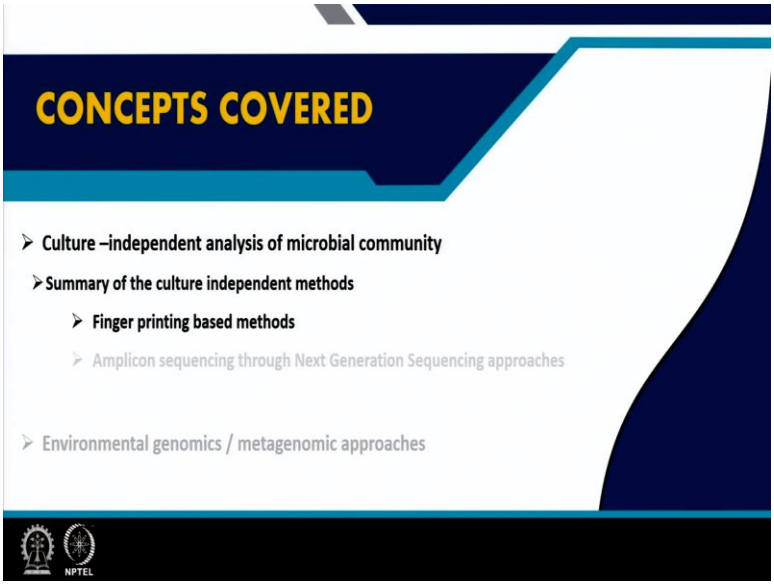

Now in this particular lecture we are going to cover some specific aspects of the community analysis methods. And particularly we will begin with summarizing the culture independent methods and the need for such methods. And then subsequently we will proceed to towards one of the the most important types of methods those are called community profiling or finger printing based methods.

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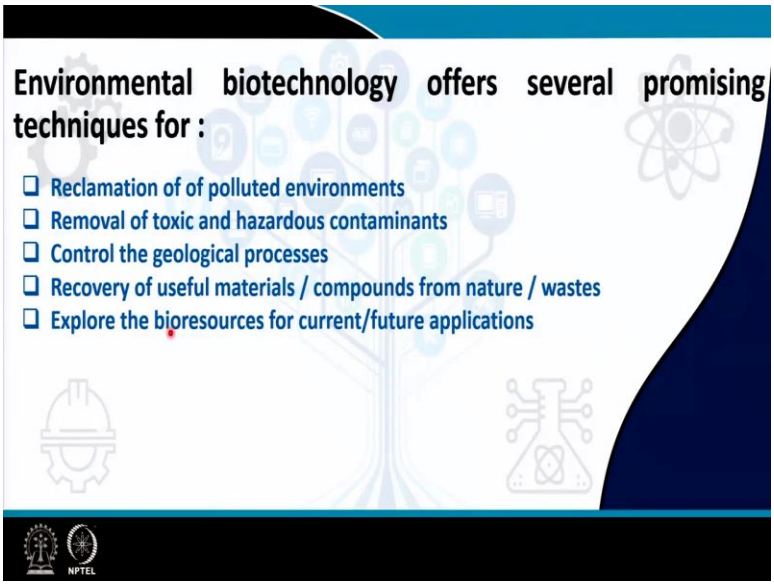

So, now firstly why do we need to adopt the culture independent methods of microbial ecology in environmental biotechnology. Now in order to answer to this question we must first appreciate that environmental biotechnology offers several promising techniques for reclamation of the polluted environments removal of toxic and hazardous contaminants control the geological processes recovery of useful materials compoundsfrom nature or different type of waste.

And explore the different type of bio resources particularly the microbial bioresources for current and future applications. Now when we try to develop these technologies we also get to know that for all these applications or technologies we necessarily rely heavily on the catalytic abilities of microorganisms particularly the prokaryotic organisms like the bacteria and archaea that naturally exists.

And although the fate of the pure culture strains which are obtained generally through different culture dependent methods as we discussed earlier remains uncertain as we expose them to real environment. Microbial catabolic function in environmental system are found to be strongly regulated through more complex yet coordinated processes than we generally perceive. This is true particularly when we try to compare our understanding on a particular environmental process maybe a wastewater treatment process maybe a contaminated river or a lake ecosystem or a bioremediation related program.

We tried if we try to compare the organisms based on the cultivable bacteria that we can isolate and characterize them. And then we look at into the culture independent method based analysis outcome we get to know that the communities are truly very complex and the way the organisms function within any environmental system and those functions which are relevant for any environmental biotechnology processes are really more intricate and more more coordinated.

And for that we need to have more advanced and more sophisticated methods we which will give us the deeper understanding about the community function which is necessarily required for developing any kind of environmental biotechnology processes some of which are listed above and many more may be included.

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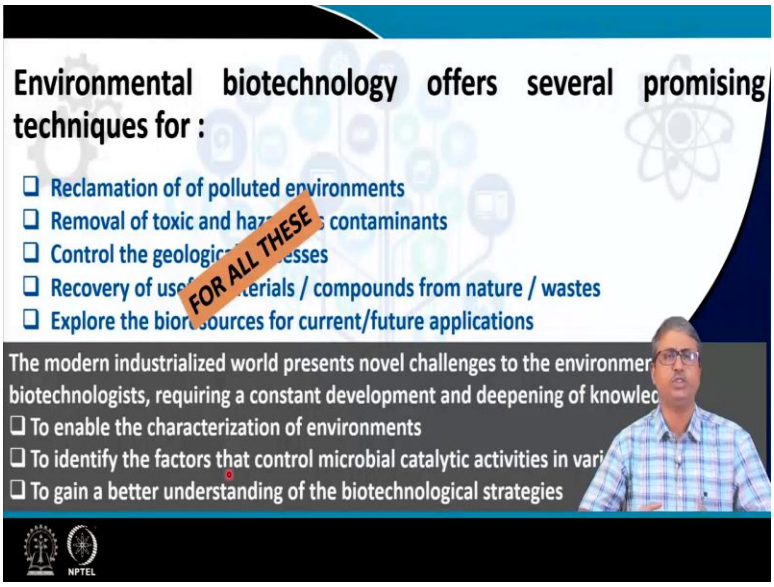

Now the modern industrialized world further more presents novel challenges to the environment particularly the environmental biotechnologists requiring a constant development and deepening of our knowledge. For example compared to the conventional wastewater treatment process or the sewage treatment process when we try to develop technologies which will enable us to sequester carbon dioxide or will evaluate the the chemical or the physical methods currently being used as carbon capture methods in industries in order to mitigate the climate change program or the climate change challenges.

So, we understand that the environmental biotechnologies need to always develop more robust

and more sensitive methods to cater the need of the society and the industries. Now to these are particularly to enable the characteristic characterization of the environments what is actually happening in the environments. So, microorganisms present in any environment are increasingly being considered as a very active and responsible component.

For example in a ground water or aquifer system hydro geological geological and geophysical parameters have been discussed and researched and we try to understand those physical or chemical parameters in order to characterize the ground water system. Now considering the huge catalytic role of microorganisms which naturally present in the aquifer system it is in increasingly being realized that not only the geological or the physical parameters but also the importance of the microbiological parameters must be incorporated in order to understand the the total picture of the aquifer conditions.

So, basically the characterization of any environment would be incomplete if we do not include microbiological aspect or environmental biotechnology aspects into that. The second could be the to identify the factors that control microbial catalytic activities in various environments. We are constantly trying to develop microbial systems for their environmental applications. For example in recent time there is a surge in plastic bioremediation or remediation of the emerging different other emerging pollutants like antibiotics and other molecules.

We have been trying to isolate bacteria or develop different type of microbial systems in order to treat the waste material like a hospital waste or other plastic containing waste. But what would be the fate of those organisms that we isolate and cultivate and characterize and then the potent strains are released into the environment or they are exposed to the contaminants. How they are going to respond when they are released or exposed to enormous split or plethora of factors which are there in any waste materials.

So, in order to understand many such factors it is necessary that we need to look beyond the conventional culture dependent methods and always it is required to gain a better understanding of the biotechnological strategies.

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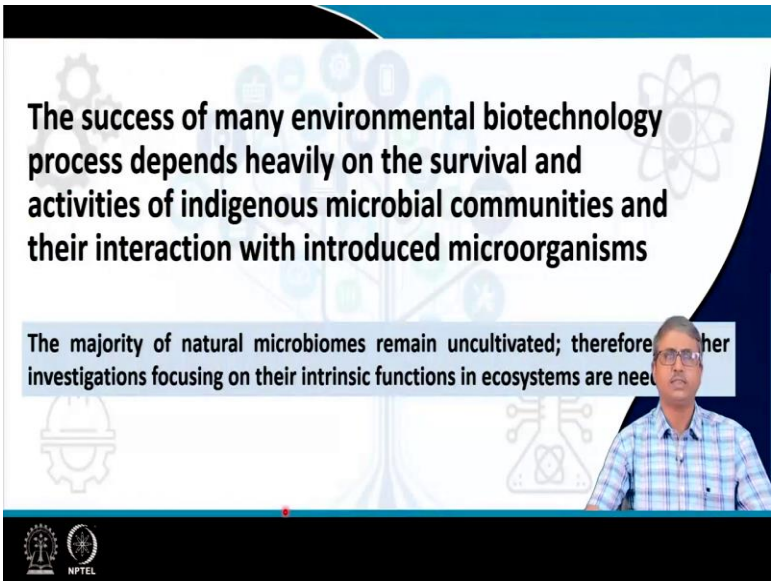

Now the success of many environmental biotechnology processes depends heavily on the survival and activities of indigenous microorganic microorganisms or the microbial communities and their interaction with introduced microorganisms. For example whether it is the petroleum degrading bacteria that those are being characterized isolated characterized and systems were developed for bioremediation.

But what happens to those organisms when they are released into the real petroleum contaminated environment. How the natural bacterial populations which are living already living in a contaminated sites? How they are going to react to this newly introduced species which is maybe may be better catalytically better equipped to achieve more degradation or maybe for different other purposes when we try to use improved microbial culture for different environmental processes like the production of gas or production of alcohol production of fuels or recovery of natural resources.

So, those interactions remain unnoticed or uncharacterized unless we include the culture independent methods. The majority of natural microbiomes remain uncultivated therefore further investigations focusing on their intrinsic functions in the ecosystems are always required.

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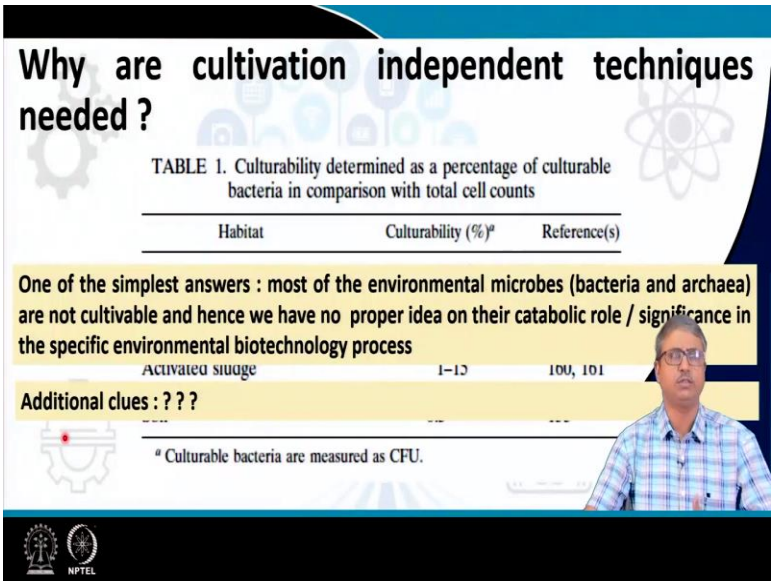

Now why then the cultivation dependent techniques are required. Now if we have we look at this particular data which is published several years ago that the cultivability of natural microorganisms are quite low. Except few in specific environments like the activated sludge we can see it is often less than 0.1% or so or in some of the environment like sea water environment it is even very low 0.001 or 0.01, 2.001.

Now so, basically the cultivability of the organisms is very low and in order to understand the true potential of the community or to function of the community for any biotechnology process we need to have cultivation independent method. So, therefore the answer to this question that why do we need cultivation independent techniques the see one of the simplest answer could be that the most of the environmental microbes that is the bacteria and archaea are not cultivable that means they are not been able to grown under laboratory condition.

And hence we do not we do not have any proper idea on their catabolic role or their significance with taking into consideration the specific environmental biotechnology processes. And of course there are additional clues like how these organisms actually interact with themselves how they are reacting to the different other environmental factors and what could be the other additional benefits that we can actually get from those organisms.

Particularly when we are we are discussing about waste to wealth concept in a circular economy.

So, waste degrading bacteria should be looked into resource producing bacteria as well that means the organisms which are capable of degrading some of the contaminants or some of the waste materials may produce some useful compounds but may not be in the laboratory condition always. So, that overall we need to have this kind of approaches which are cultivation independent.

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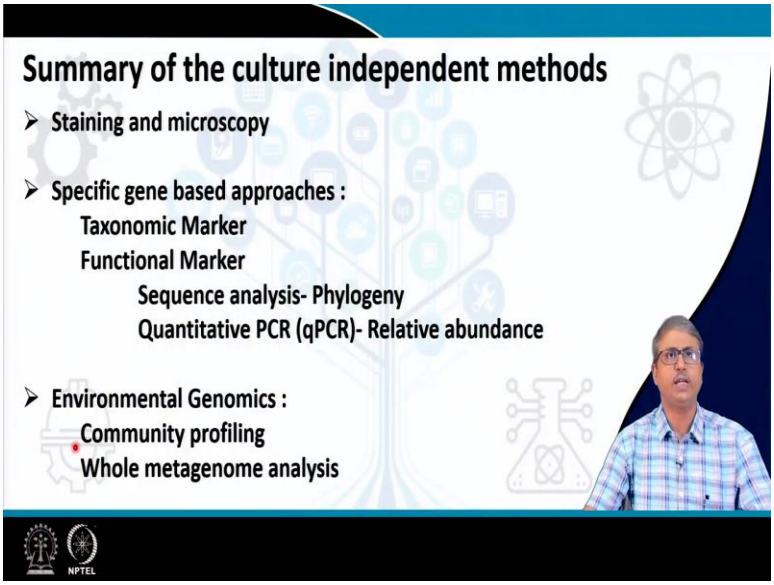

Now there are different types of cultivation independent methods as we already discussed. So, this training and microscopy remains one of the very simplest method but that has its own advantage and disadvantages like it cannot tell the functional properties in detail and it is restricted to the number of kind of the probes that we are going to use and it requires certain very specific type of microscopes for doing this assets and the sensitivity and the background chemical composition or the matrix the soil or the water or the sediment often interferes.

So, it is it is a good method but it is not as robust as it is it is expected. So, we our expectations from the culture independent methods are actually much higher. Now the second most important approach which is very, very common very popular widely used in environmental biotechnology or environmental engineering system is specific gene based approach or rather specific gene based approaches because there are multiple approaches.

These are basically achieved through using different genes these genes are the taxonomic marker

genes that means these genes are capable of identifying specific taxonomic group it may be general specific it may be family specific or it may be any other level of taxonomic hierarchy or functional marker genes that is the genes which are encoding the key enzymes of different processes of environmental environment relevance like ammonia oxidation or methane oxidation or methane production or sulfate reduction etcetera.

We will talk about these genes little detail after a while. Now within this specific gene based approaches we have sequence based analysis. So, we need to get the sequences and then analyze the sequences and derive the phylogeny taxonomic identity and also we can do the quantitative PCR based methods that will be discussing in another lecture. And that will provide us the relative abundance of these organisms or these functional genes thereby a kind of a the broad view of the community its potential and its functionalities will be available to us as we deploy these specific gene based approaches.

In addition to that there are of significant development in environmental genomics that we will be talking in some other lecture altogether.

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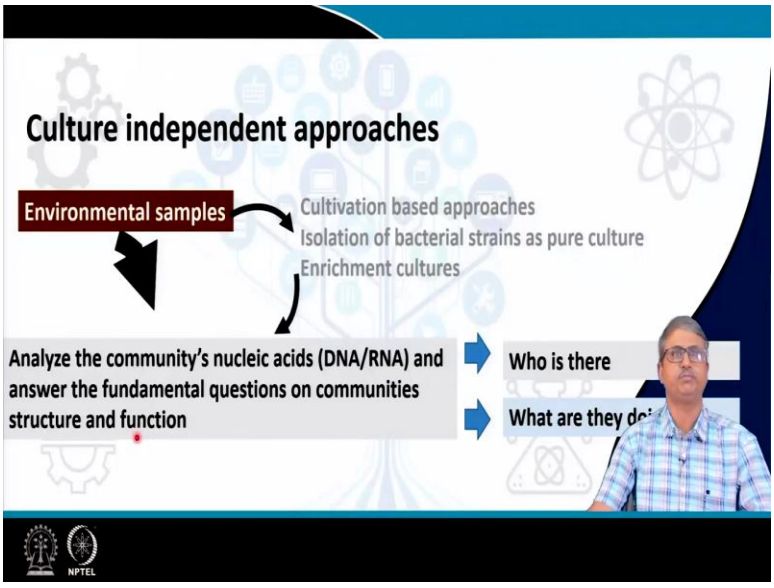

Now with respect to this culture independent approach earlier we have seen that environmental samples can be subjected for cultivation based approaches by direct isolation or enrichment methods. Today we are going to focus more on the direct methods which are basically cultivation

independent methods. So, they do not need any kind of bacterial bacteriological or microbiological medium to isolate or enrich any organisms.

So, we take the environmental sample and try to extract the community DNA or community RNA that is called the total DNA or total RNA from the from the sample and then analyze that in order to get the fundamental questions the answer of these fundamental questions that we ask on the community structure and community function like the questions can be converged into two very simple questions that is who is there and what are they doing.

And that can be actually a further led to the answering the bigger questions that how this could be useful for an environmental biotechnology process or how this could actually reflect this true state of affairs within an environmental biotechnology process may be a formation of useful gases within a wastewater treatment plant or remediation of plastic or remediation of a ground water pollution.

So, exactly what is happening inside this environmental process, so, answering those questions would require necessarily that we would analyze the total nucleic acids. Now here there is a possibility that we can actually go with two types of methods. So, let me just explain that in a very simple way. So, these environmental samples are assumed to be containing numerous bacterial or microbial cells.

So, there are many microbes which are present in any kind of environmental system. So, when you have a sample we expect that the DNA or the RNA that we are extracting we are basically extracting the DNA and RNA of this individual cells. So, actually we are not extracting the DNA or the RNA from the individual cells like picking one cell at a time but we are adopting a method that method will allow us to the extract the extract the total community DNA let us say we are trying to.

So, what it does basically the method. So, method will take the sample. So, method will take the sample and will have some reagent that will allow the lysis of the cells. So, all the cells will be lysed in situ in situ means the cells wherever the cells are there that are within the sample itself

they will they will be lysing. So, if we suppose we have the sample in a small tube. So, here we have the sample.

So, this is my sample, so, I can have multiple replicate of this and as I add my reagents into it. So, these are the cell lysis reagents. So, all the cells present within it will be lies. So, will what will happen we will have this lyse lysates cells and all the cells will be lysed. So, all cells will lies and they are DNA and RNA and all the protein etcetera will come DNA and RNA will be released.

Now this that DNA and RNA will be there in this solution. Now we can extract that we will take that and that will be used for in this particular case the nucleic acids that to answer the fundamental questions. Now there is another way of doing it that that actually will require isolation of cells. So, isolation of cells so, in this case it is not the cultivation of cells but rather segregate the cells from the soil or sediment or other chemical materials or other mineral particles etcetera.

Because often what happens the real samples the sediment may be oil sludge or a contaminated soil or a wastewater will have so, much of other materials the chemical materials or the physical materials conditions which are going to interfere with the further processes like the quality of the DNA will deteriorate the process that we apply with the DNA and sequencing will all be disturbed because of those chemicals.

So, sometimes some samples might require or based on the scientist choice the cells are actually separated out. So, this is not exactly growth of the cells but physically the soil or the sediment sample is suspended in some kind of liquid where the cells might come into the liquid phase and the the particulate matter will settle down then that liquid phase we will take and possibly will centrifuge and the cell some kind of pellet will be formed.

And that that pellet will be subjected to this reagent and so that we get to have this DNA or the RNA whatever we are trying to obtain. So, essentially our target is to get the total nucleic acid that is the DNA or RNA or both. So, generally for our simplicity or to keep the workflow simple

we will consider the DNA because working with RNA requires certain specialized settings and chemical reagents.

So, we will talk about RNA whenever it is applicable or required. So, coming to the main point that when we have an environmental sample we need to extract the total nucleic acid that is the total DNA in this case maybe and then use this DNA as a kind of a template or a kind of a starting material to analyze the the community composition community function and answer the questions like the fund of two fundamental questions that we have already discussed ok.

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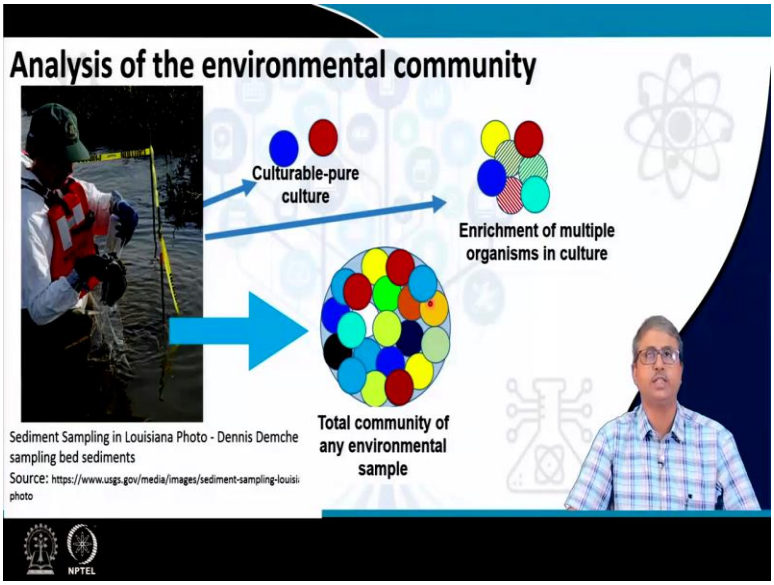

Now continuing the discussion with this line of thought. Now how do we analyze this environmental community then. So, earlier we thought that we can actually analyze through pure culture based methods. So, that could be either of these or both of these but in this case what I essentially mean that we will have the sample or the sediment and that sediment will be subjected. So, that all organisms present there will be represented in the sample no matter whether they are cultivable or not.

And then the nucleic acid which is extracted out of this sample is actually will cover all the cells which are which are present there.

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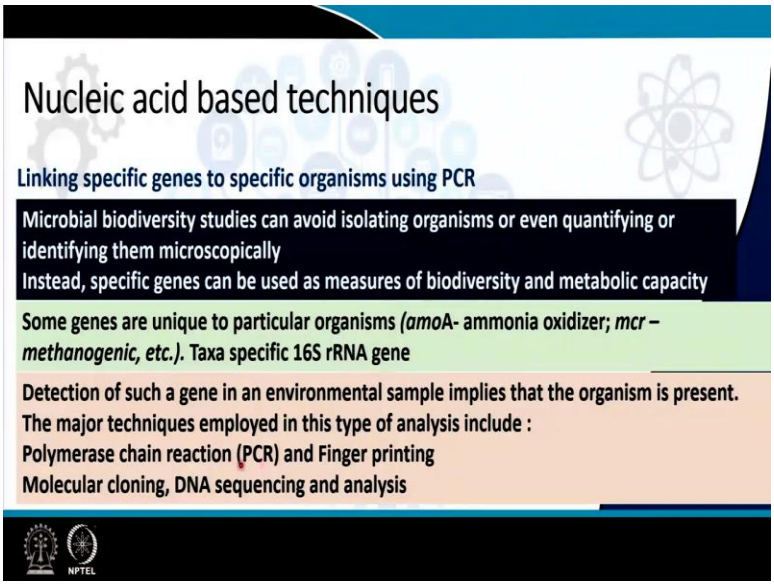

Now what are these nucleic acid based techniques. So, basically we are going to use the nucleic acid that is the total DNA or RNA. So, that means these are called nucleic acid based techniques. Now in this nucleic acid based techniques there are there are couple of techniques that will be discussing the one of the most important part of this nucleic acid based technique is that that this could be allowing us to linking a specific gene to a specific organism using the PCR.

So, fundamentally we will see that these are the two types of techniques one is the PCR based technique that is the polymerase chain reaction based technique where when we use the PCR to amplify a target gene the gene that we already know and we select that gene because that gene is specific for taxonomic reasons or maybe for some functional reasons and then use our further steps to analyze it.

So, microbial biodiversity studies can avoid isolating organisms or even quantifying or identifying them microscopically instead specific gene can be used as a measure of biodiversity and metabolic capacity. Now some of these genes that we generally use are unique to particular organisms these are the functional genes or maybe the taxonomic genes like for example ammonia oxidizers often have they contain the amog the ammonia monooxygenase.

Or the for the methanogenic archaea they contain the MCR gene. So, we can target or we often the environmental biotechnologies often use this kind of genes for as a functional marker and

characterize the environmental system otherwise the taxonomic group specific 16s ribosomal RNA gene or a broad or taxonomic marker gene like the using the universal primer for bacteria or archaea are also used.

Now detection of such a gene that is a particular gene in an environmental sample implies that the organism is present. So, if we are able to get a positive signal out of this PCR amplification using the total DNA of our environment environmental sample a positive response in terms of a PCR reaction would necessarily mean that that particular gene is there. So, that means the organism responsible for harbouring this gene might be there or likely to be there.

Now the major techniques employed in this type of analysis include the polymerase chain reaction that I have already mentioned and the different type of finger printing method and within this fingerprinting method also this might also include the molecular cloning DNA sequencing and further analysis.

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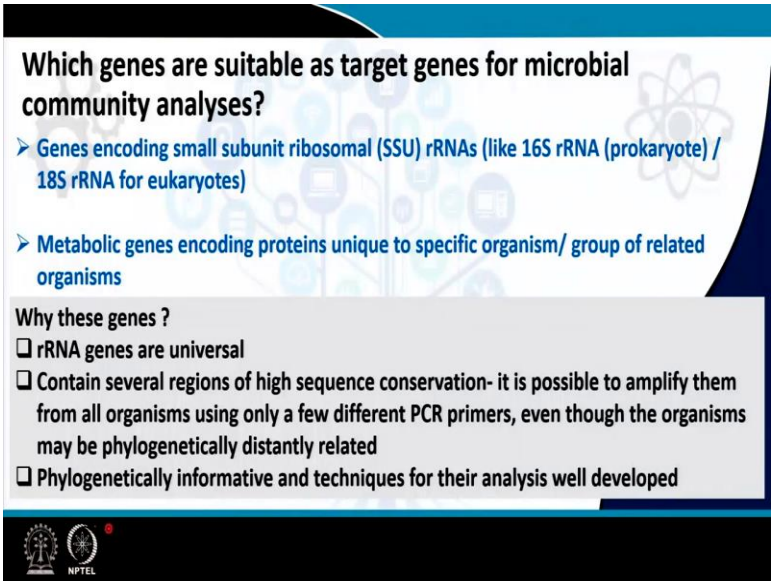

Now, coming to the; point that which genes are suitable as target genes for community analysis. So, two types of genes can be selected one is the genes encoding the small subunit ribosomal RNA or the RRNA genes like 16s ribosomal RNA for prokaryotes or 18s ribosomal RNA for eukaryotes alternatively or along with the taxonomic marker we can use the metabolic genes, genes encoding proteins unique to specific organism or groups in related groups of related

organisms.

Now why these genes why not other genes for example these genes are very specific like ribosomal RNA genes are universal and it contains several regions of high sequence conservation that is it is possible to amplify them from all organisms using only a few different set of primers even though the organisms may be phylogenetically distantly related. So, almost like all the community members even if you have 4000 to 5000 species in a community all of all these species members their 16s ribosomal RNA gene are going to be responded.

Or supposedly they will they will respond if the conditions are appropriately provided then the phylogenetically informative these genes that we are talking about the ribosomal RNA genes or the other functional marker genes these are phylogenetically informative. So, that we can derive more information about their phylogeny and their taxonomy and the techniques for their analysis are very well developed.

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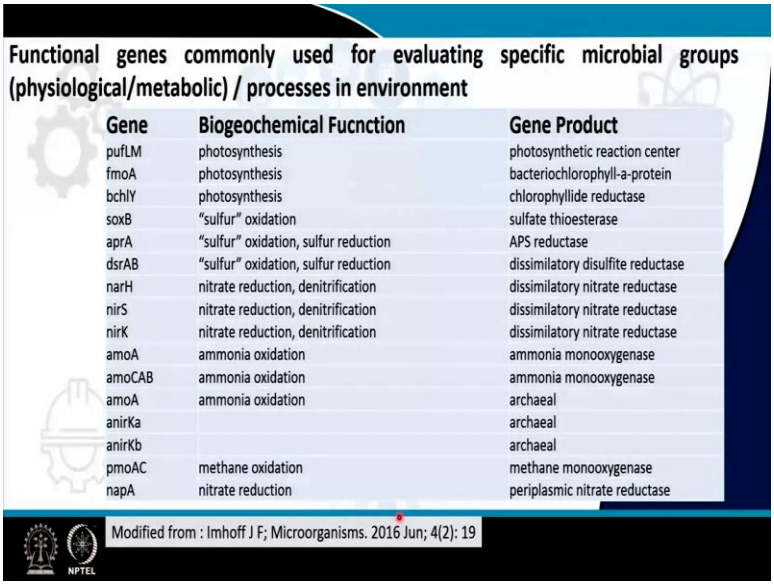

So, along with the ribosomal RNA gene that I have mentioned that we have both those 16s and 18s ribosomal RNA gene for prokaryotes and eukaryotes we have a large number of functional genes and these are very well known functional genes which are used to have different bio geochemical functions assays. So, these are the genes for the you can see the photosynthesis sulfur oxidation reduction nitrate reduction ammonia oxidation methanoxidation etcetera.

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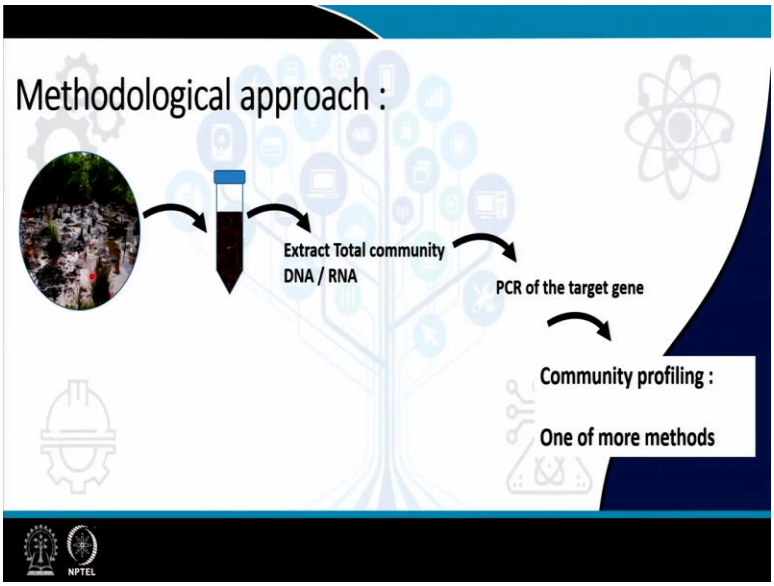

Now in a broad methodological approach: So, what we are going to do we have the sample. So, on the left side you can see a petroleum contaminated environmental sample. So, from there we take a subset of the sample during the sample collection and we can have actually multiple samples collected. So, that we have a overall broad survey or statistical support about the community analysis and extracted community DNA or the total DNA or the RNA from these samples again this can be done in a multiple replicates.

And then as we discussed earlier the PCR of this target gene like 16s ribosomal RNA gene in this case we are targeting or if we are targeting a functional gene we can opt for a functional gene as well. And then once we have the gene products with us that is the PCR is successful we can go for community profiling using one or more methods.

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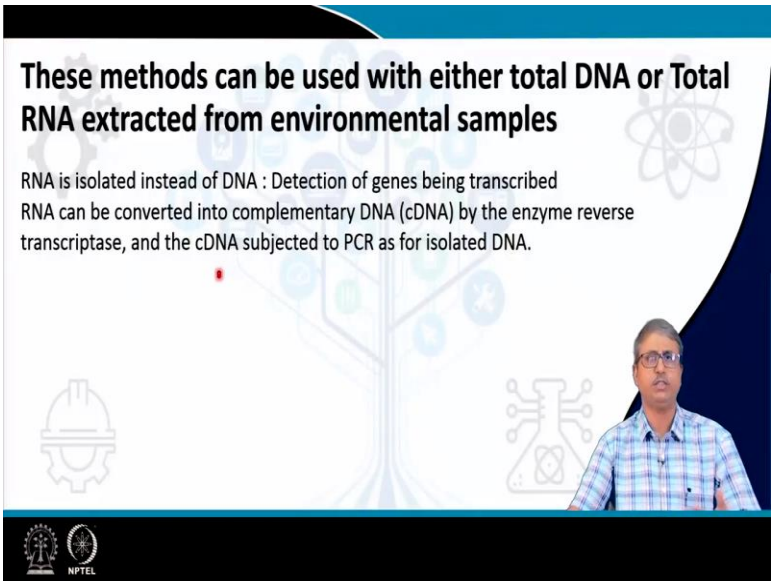

Now these methods that we are going to use generally can be used with either total DNA or total RNA as I mentioned extracted from the environmental samples we can extract DNA or we can extract RNA DNA is always a preferred because it is a very stable molecule. However extraction of RNA and its analysis will always be providing us some additional information. Like if we are able to extract RNA and analyze that it would provide us the genes which are being actually transcribed.

So, transcribe means this genes the cells which are metabolically active during the sampling point will be highlighted will be considered more rather than DNA because DNA is very stable compared to RNA, RNA comes from only the active cells RNA if we are working or planning to work on RNA from any environmental sample RNA has to be converted into complementary DNA or the cDNA by the enzyme reverse transcriptase.

So, one additional step will be incorporated before we go for the PCR. So, the cDNA will be subjected to the PCR and then the subsequent steps will be the similar.

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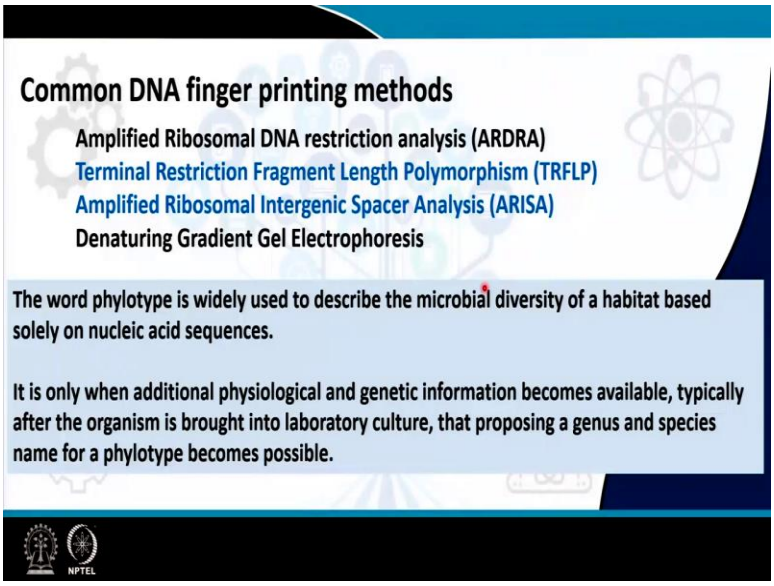

Now the common DNA fingerprint printing methods that are commonly available and widely used in environmental biotechnology sector for monitoring the community or exploring the community for different environmental biotechnology purposes are amplified ribosomal DNA restriction analysis ARDNA terminal restriction fragment length polyvon fissum or TRFLP, amplified ribosomal intergenic pressure analysis or ARISA and denaturing gradient gel electrophoresis.

Among these methods we will be discussing the two methods like the ARDNA and DGG in our subsequent lecture. Now each of these methods enable the detection of different phylotypes that is the phylogenetic type present in a community. Now we should understand that the genes such as those encoding the rRNA that have changed in sequence over the time as species have diverged and they are called orthologs.

Organisms that share the same or very closely related orthologs genes are called phylotypes. So, in general we do not bother much about this other details but different phylotypes actually in a very in a general sense refers to like different organisms. Like we can cannot call them as species unless we have additional clues on their physiology and genetic information because defining a species will require satisfying some other additional criteria. But unless we achieve that all those criteria we cannot say how many species are there but an approximation can be made looking at the phylotype.

So, phylotype could be a kind of a some kind of approximation about the kind of species present like if I get 1000 phylo types in an environment I may assume that there will be surely 1000 species but actual species could be little more even that.

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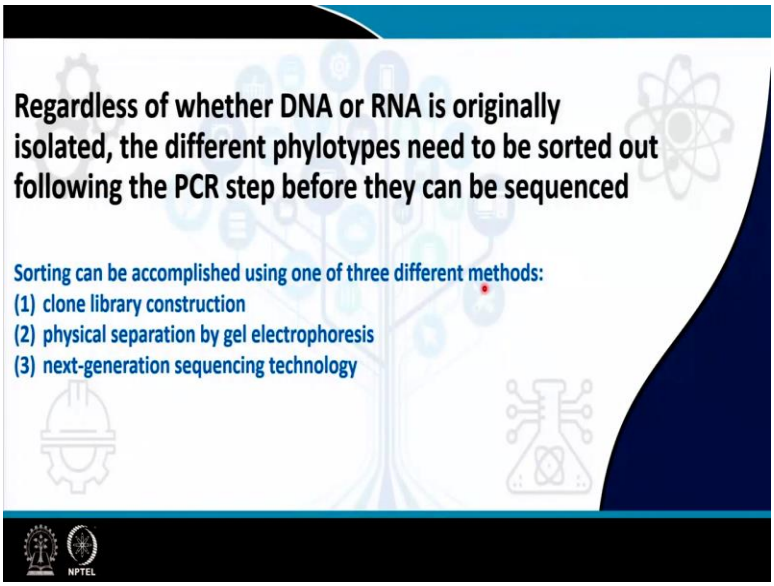

Now regardless of whether the DNA, RNA whatever is extracted and the different phylotypes need to be sorted out. So, since as I mentioned it could be possible that in an environment that we are working on there could be 1000 species that means that could be 1000 phyllo type in other way. Now when I extract the DNA all the DNA are together if I amplify all the 16s of 1000 phylotypes or species are together they are mixed.

And so, I cannot identify individually unless we segregate them. So, these different phylotypes which are actually represent in different species could need to be sorted out or they need to be segregated. So, this segregation is done by three methods one is the clone library method another is the physical separation by gel electrophoresis that is through the DGG method and the last one is the high throughput deep sequencing that is called the next generation sequencing technology based method.

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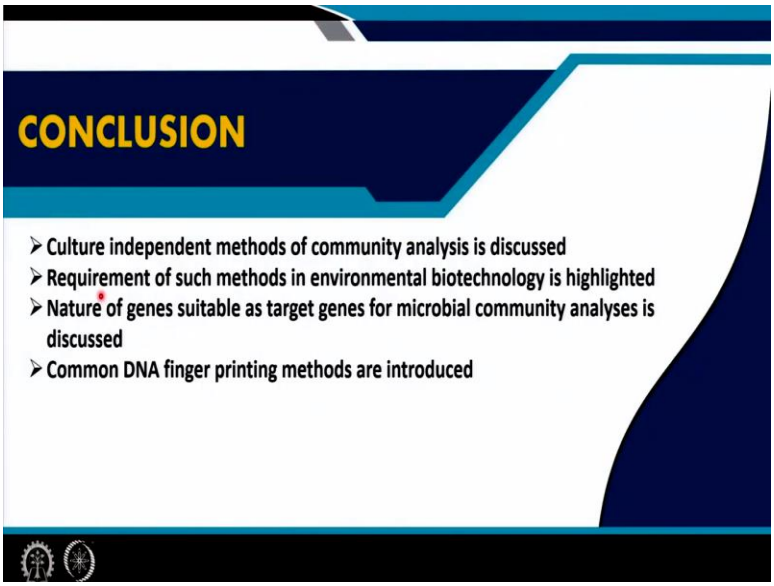

So, therefore in this lecture the following literature can be used. And in conclusion culture independent methods of community analysis is discussed. Requirement of such methods in environmental biotechnology is highlighted, nature of genes suitable as target genes for microbial community analysis is discussed and common DNA fingerprinting methods are introduced, thank you.