

Applied Environmental Microbiology
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Lecture - 01
Introduction I

Hello. Welcome to our course applied environmental microbiology. I am your instructor for this course and my name is Gargi Singh, by training I am an environmental engineer in the department of civil engineering and this is very important to highlight to suggest that environmental engineering was not always called as environmental engineering. When we started this field, where we understood that taking care of environment is also on a responsibility of a civil engineer. It was conferred to a sanitation engineering where the interest was just to take the waste material and put it away from our side or maybe put it in a black; black box system and ensure that our surface water bodies are clean.

However as science developed further we understood the biological and chemical mechanisms that allow us to keep our environment clean and sustainable still by when it came to biology of the environment, very little was known and it was often assumed that biology works in predictable ways because it has members that act like little black boxes and I will give you an example some of you might have background in civil engineering or chemical engineering and might be familiar with wastewater treatment and for those who are not very briefly wastewater treatment process are processes that use sewage and the domestic wastewater we generate put microbes in it and allow the microbes to degrade all the biodegradable organics.

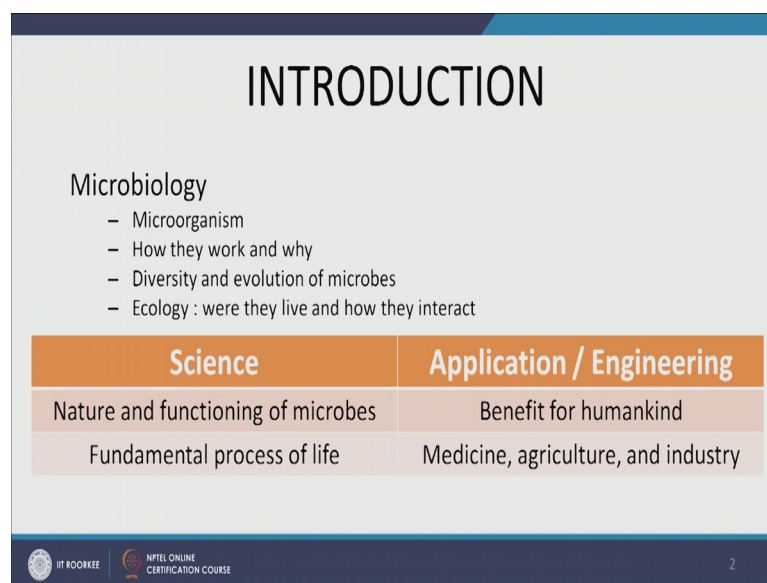
And then what we are left with is clean water for many many decades, civil engineers and other professionals who worked with sanitation engineering considered the microbiology of wastewater treatment as a group of heterotrophs who simply degrade the organics, but when science developed further we understood that they are there is not just one kind of microbe or microorganism that is at work. So, what used to be collectively referred to as biomass when we talked about sludge now started being respected in a way and distinguished in sense that now we had heterotrophic bacteria autotrophic bacteria we had anoxic and oxic microorganisms and this is how we started respecting that not all microbes are similar.

As the science of microbiology developed even further we understood that even within these broad classifications such as heterotrophic microbes there is a huge diversity, there are many different kinds of microbes in past 2 decades, past 3 decades. Now microbiology has given us techniques such as a genetic sequencing where we can actually sequence the genome and the genes of bacteria and understand how similar or how far apart they are from each other and with onset and discovery of these wonderful techniques what we have; now is a much broader and more clear vision of applied environmental microbiology.

Unfortunately in many regions of the world India included the microbiology of environment and its applications are not really well understood and I mostly confined to the domain of pure research and is ascribed as something that perhaps a bio technician would do or somebody with a biological background would do and this is where I want to give you a small introduction that you do not have to be familiar with biology. Let alone microbiology in order to understand this course really well what is important in this course for you from you is that you pay attention towards start covered in the lectures and honestly sincerely do the assignments and the question answer that I will share with you during the course of during this course.

Now, today being the first lecture, I would like to take some time to make you more familiar with what we are going to discover together in this course and how it might be applicable for you. Now in microbiology in general, we talk about microorganisms.

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INTRODUCTION

Microbiology

- Microorganism
- How they work and why
- Diversity and evolution of microbes
- Ecology : where they live and how they interact

Science	Application / Engineering
Nature and functioning of microbes	Benefit for humankind
Fundamental process of life	Medicine, agriculture, and industry

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We want to know who they are and this is where micro microbiology and biology get very tricky and put off many engineers and scientist and science students because there is a perception in microbiology we need to memorize so many things by wrote and I assure you in this course you do not need to memorize different names; you do not need to know how creano or coyote is different from you or coyote.

But you do need to understand how the microbiology in itself works and if you what information do you need in order to understand why microbes are doing what they are doing and in the first place how what they are doing and how you can tweak that in order to serve your environmental purposes which brings us to the second bullet here the first aspect is to understand what microorganisms are present how they work and why they do what they do and, but it results in is an understanding of the immense diversity of microbes and how they evolve from one kind of microorganism to another microorganism.

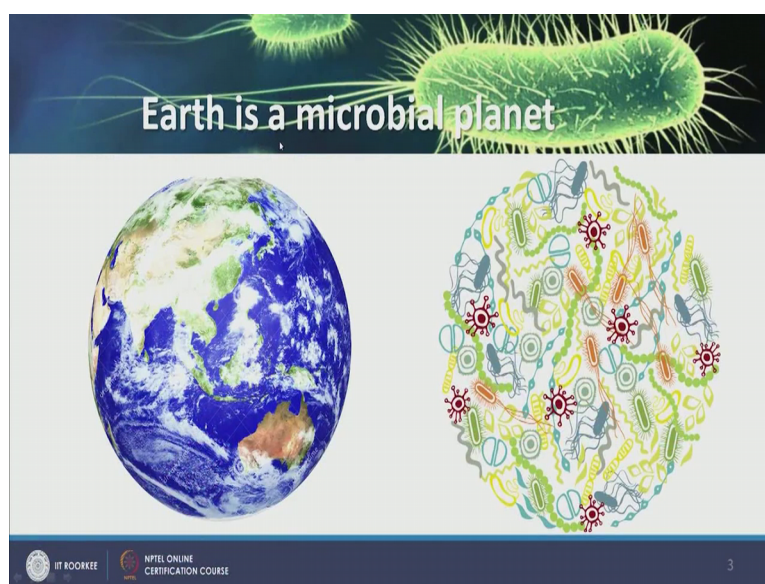
And speaking of diversity of microorganisms its really beautiful to note that most microbiologist including myself believe that we have barely scratched the surface, we believe that we know less than one percent of microbes that are there on our Earth and the fourth bullet here talks about ecology which is where do they live and how do they interact with each other and this is particularly important for environmental engineers environmental scientists and technicians we want to understand, how micros behave with

each other and how they behave with the environment how they are influenced by the environment and how do they influence the environment in return.

Microbiology in itself has 2 legs on which it stands one is the scientific aspect and the other is the application or the engineering aspect the science wants to know the nature of microbes how the microbes function what is the fundamental process of life what is the biochemistry of life where as the application aspect and the engineering aspect wants to know; how can I use this information that I have to improve our living standard to benefit our humanity to make our civilizations more sustainable. So, that we can live happily on this earth for a longer time and the application aspect also looks into medicine engineering and agriculture.

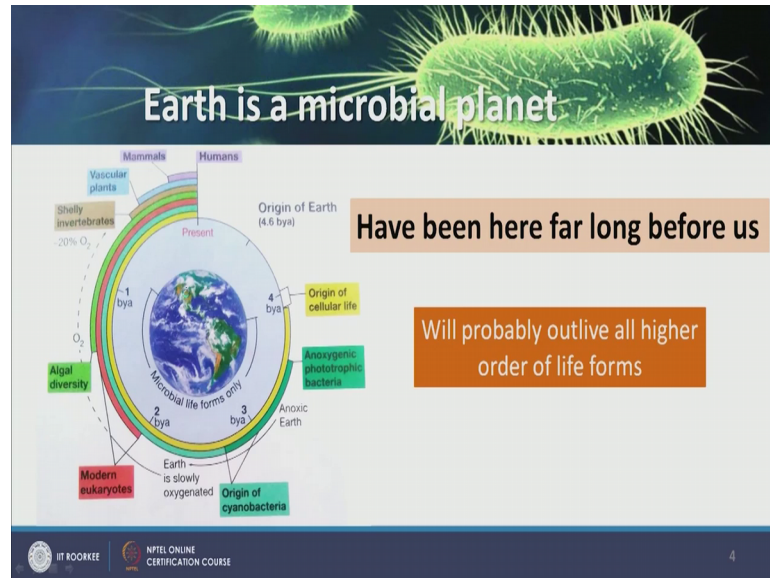
And now we have an emerging field of industrial microbiology and lot of what you will study in this course will actually help you address any of these applications in this course we are not focusing as much on science and that is why you get a good riddance from all those scientific names and techno long enumerations of microbes and you can directly jump into how we can use this information that we have to better our world to make better medicines to have cleaner surface water to have cleaner groundwater cleaner air now and just how to be healthier.

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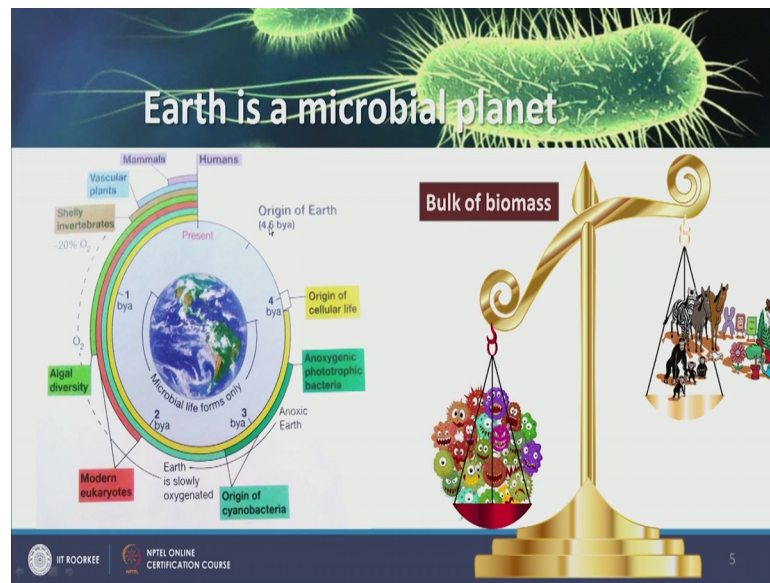
Now, this is very important to understand that why applied in our inter microbiology its very relevant today we think of earth in terms of lithosphere in terms of lithosphere it has lot of blue in it.

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So, all the maps, there is a lot of water and then there is land what is hiding in this picture are the tons of microbes that actually inhabit the earth and if we look at from biomass perspective the percentage of carbon that storing microbes, we can say that the life on earth is mostly in microbial form not in the higher forms of life that we see and let us look at the origin of earth and this will cement further our understanding that earth is a microbial planet.

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It is said that earth is nearly 4.6 billion years old and the life started some four billion years ago that is origin of cellular life.

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Now, we are in understanding that non-cellular forms of life started way before a four billion years, but definitely the cellular life started four billion years that is where the microbes came and then somewhere between four and 3 billion years we got anoxic phototrophic bacteria and this is very beautiful to understand at this time for all these

billions of years earth was anoxic which is another way of saying there was no oxygen on earth.

Earth was an anaerobic environment and humans as now we are could not have existed survived at all in this on this earth then; however, a miracle started happening sometime around 3 billion years then between 3 billion and 2 billion years ago, cyanobacteria; a green beautiful bacteria beautiful under microscope by the way a green cyanobacteria came over evolved on earth and what this cyanobacteria started doing it started using the light to produce food which is something, they are familiar with as photosynthesis in the case of plants and started generating oxygen.

So, over the course of next billion years the earth was slowly oxygenated. So, from this primitive form of life and we I am using primitive instants of time not necessarily in sense of complexity definitely not in sense of complexity I want to be clear, but in terms of time the life started also incorporating and exploring the aerobic forms of existence. So, around 2 billion years ago modern eukaryotes now this is a technical term eukaryote prokaryote EU means true Karyon means nucleus.

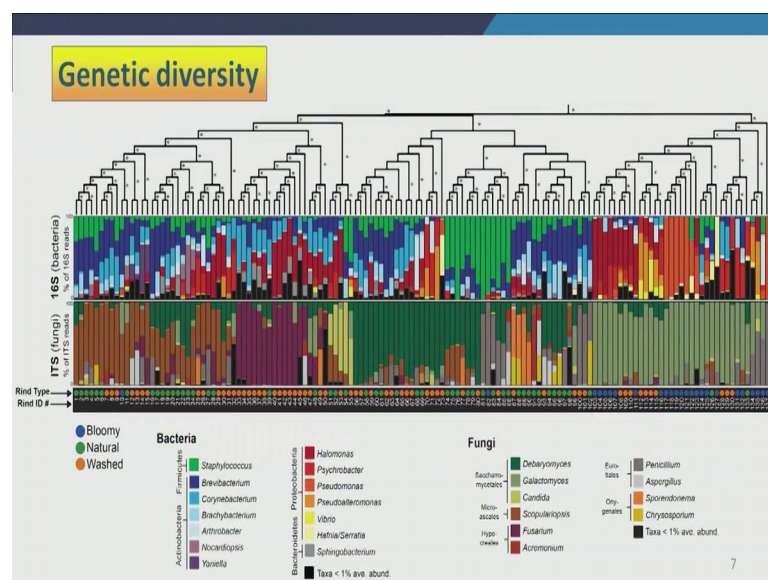
So, something that has a particular kind of cell which we call is are called as eukaryotes and it might be helpful to mention that humans are eukaryotes all the higher order of life have eukaryotic cell and the uniqueness in eukaryotic cell are we will see later is that they have a nuclear membrane in which they have destroy the genetic material for some 2 billion years ago, these higher forms of life the modern eukaryotes came into picture and then low and behold very soon we had algae we had this photo synthetic eukaryotic organisms and then much actually much near the present some five hundred million years ago shelly invertebrates came that is called a plants mammals and humans are relatively a recent phenomenon very recent phenomena.

So, we can see for nearly 4.6 billion years of earths existence we have at least 3 and half years of only microbial existence and if we look at in terms of biomass more organic carbon is stored in microbes than in all higher forms of life combined and thus we are assured as microbiologist that earth is a microbial planet now in this in this slide, we have 2 beautiful pictures on left we have a scientist showing off a plate with different microbes growing.

Now, in this plate, it is very beautiful to note that each of these colonies have emerged from a single bacterial cell these are bacterial colonies by the way and on the right side we have a picture and this is an artist's impression of a 3 dimensional picture of microbes of different morphologies different shapes. So, if you look at this visually on the left in our plate we have microbes that look very similar their colonies that they make somewhat circular and start with red and as they spread out, they get more yellow and then even white.

On the right side, we have this beautiful strange and very distinct geometries some of them are coca shaped or round shapes some of them are dot shaped some of them look like these galaxies and some of them look like dots that are clustered together and what this wants to highlight is that on a physiological level there is immense diversity in nature as represented by the right picture and what we humans have been able to capture in lab is a small fraction only one kind of microbe and that is the reasoning behind the popular saying that we have only scratched the surface of environmental microbiology this is in terms of physiology. So, this is how microbes look.

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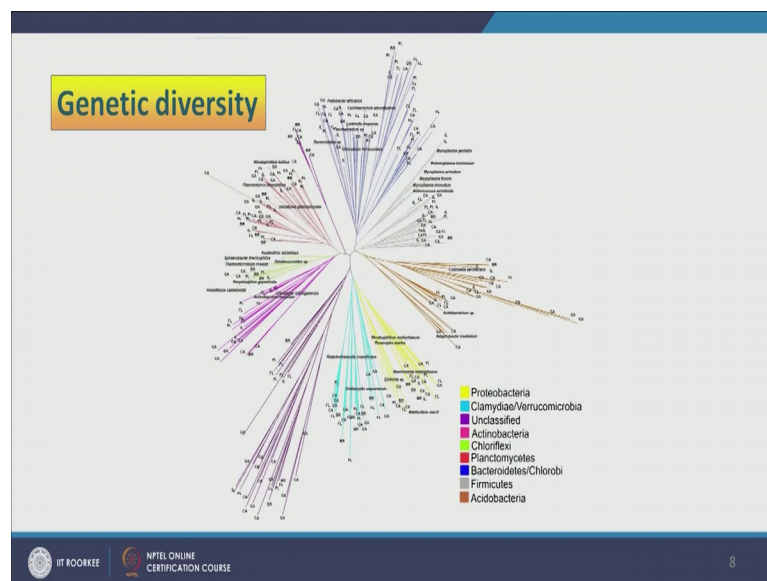


Now, what microbes are beneath their looks is even more complicated and more interesting. So, in higher order of life we see 2 mammals who have to stand upright on 2 legs with 2 hands an upright body and a head and we assume they are humans they must be similar to each other and we do know that genetically we humans are very similar to

each other in physiology we are very similar as well; however, in terms of in case of microbes we cannot have similar-similar ideology does not work for microbes 2 microbes that are perfectly spherical and maybe give a response similarly to different light that we throw on them might be very distinct and very unique in their behavior.

For example e coli 2 strains of e coli have exactly similar morphology one might be benign and might actually help when it is in my gut the other might kill me in couple of hours. So, same string same species of microbes that look very similar have similar morphologies might be very different in their activity and lets even if they are similar in their activity there might be very very different than their genetics. So, we will get through what these genes are what the genetics are is, but for now just know that genetic diversity tells me the essential differences that are there in the make up in the; a code of life in these my microbes.

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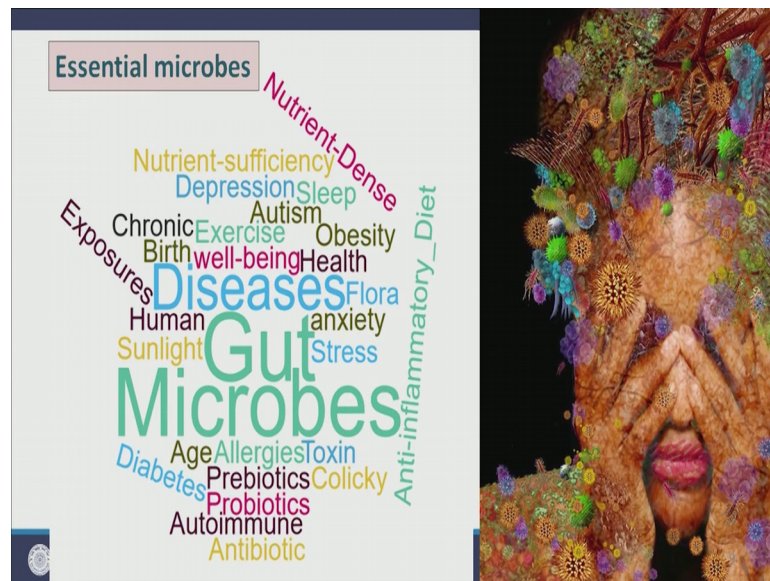


So, in here you have a Dendrogram which has been represented in a tree of life format showing each of these streaks represent each of these streaks represent a particular kind of microbe a particular very distinct from each other the closer to microbes are to each other for example, all the blue ones that have clustered here together and then the brown ones have clustered the closer they are to each other we can say that more similar these microbes are to each other the color represents the broad phylum that they belong to and I can speak with good level of certainty that even though if they are 2 clustered the 2

bacteria are clustered closer for example, the yellow are proteo-bacteria and even if 2 lines are very close to each other we might come under the impression that overall they might behave similarly.

But there might be as different as we are from algae. So, in this particular picture here which says genetic diversity 2 lines that are very close to each other and have same color might be very different in their behavior and in their genes.

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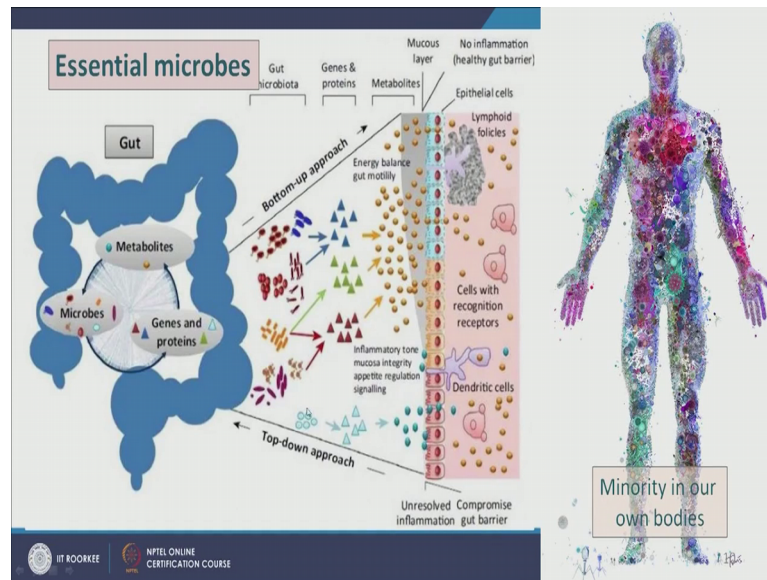


Now, this slide talks about a very important aspect of essential microbes why is this important to know why do I want to know about tree of life I do want to know about genetic and physiological diversity because there are microbes that directly impact our health that impact our environment impact our industry such as agriculture and other industries and this is on right; there is a picture from I think time magazine this was the cover of that magazine showing how human beings are inhabited by microbes and I think this is a great time to share this in case you did not already know that in our human bodies we are a minority and what this means is for every single human cell in your body, we have at least ten non-human cells ten microbial cells.

So, just take a moment to ponder about this we are a minority in our own body now are we really humans then and this is the picture that time used to illustrate this novel finding that we have and now we know that microbes affect us in terms of nutrition they actually will allow us and you will see this very soon that given the right microbes in the gut we

in in the gut we might know never face a nutrition deficiency or if you do not have those good microbes you might really suffer a problem and coming back to the essential microbe.

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This picture shows some wonderful research that is going on right now to see how microbes impact our health.

Now, it is a very important thing for you to understand that even though we talk about environmental engineering environment is anything that surrounds us. So, we can have at least 3 different kinds of environment broadly speaking we have an external environment and then we have an internal environment and internal environment would include for example, my gut microbes what is going on in my gut I mean know that human gut is heavily colonized by bacteria and other microbes and they are essential most of them are very important for our health if we lose them we fall sick.

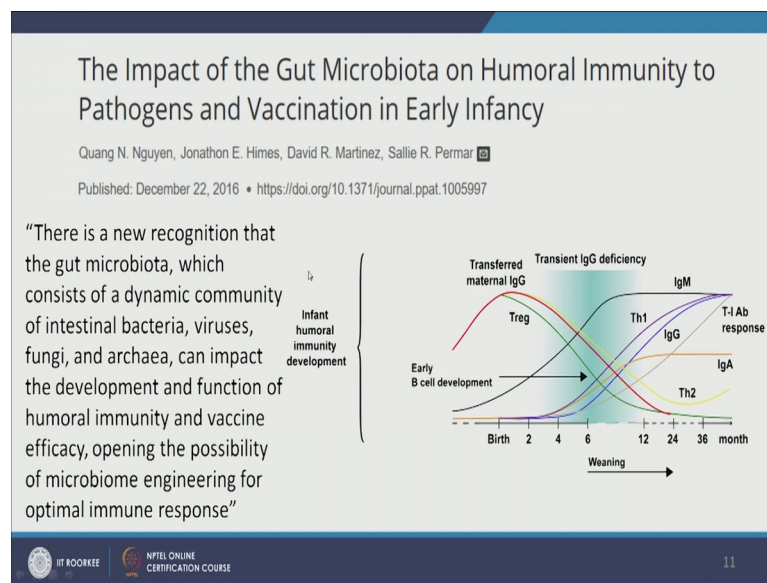
Then we have microbes in the outer environment which impact things like our climate which impact how much oxygen goes out in the atmosphere they influence how our waste degrades how our landfills behave how our wastewater treatment will be successful or not what problems we might face will our sludge float or not and the latest research as represented in the slide is showing that it is not there is no clear division between external and internal environment the external influences the internal and the internal it influences the external let me give you an example.

You are sitting in a room clean room like the one I am standing in right now and a dirty muddy dog walks in then the dog because the dog is bad lets imagine and when the dog shakes itself to get rid of the water he also sprays these micro a bio aerosols, but lot of microbes in the air you happen to inhale some of them not these microbes that are in the air thanks to the dog that walked in will colonize your nose your nose strokes maybe your upper respiratory system your skin definitely and most probably most of them would be benign and you will be fine in fact, healthier now because of the immense diversity in your body.

But maybe one of them is not good for you and you might fall really sick. So, this is external environment impacting internal another example would be I drink water it has pathogens in it and I fall sick. Now let us look at internal impacting the external one of the major environmental challenges in front of our country today are is antibiotic resistance and this is a classic example where the internal exchanges antibiotic resistance with the external and external gifts antibiotic resistance to the internal. So, in very soon and this is also the new motto of a leading research department; department of biotechnology that we have to move towards one health.

So, environmental health public health and veterinary health all have to come under one umbrella because we can no longer distinguish between internal and external; however, in this course we will be focusing mostly on in external environment and this is a beautiful paper that is it is pretty recent.

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And we will talk about this paper later, but for now; I think this is enough to know and this is antibiotic resistance threat.

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A looming threat: on the TB crisis

EDITORIAL

More on Weekend Being

A recent global report says India brings up the rear on most critical fronts to fight the disease

In 2015, India accounted for nearly 2.8 million (27%) of the 10.4 million new tuberculosis (TB) cases in the world, and 0.48 million (29%) of the 1.8 million TB deaths globally. Yet, India brings up the rear on most critical fronts to fight the disease. This has been clearly highlighted by the 'Out of Step' report. Released recently by Médecins Sans Frontières (MSF) and the Stop TB Partnership, the report surveyed 29 countries that account for 82% of the global TB burden.

Lag in practice, policy

Correct and timely diagnosis of TB is the first step in treating the disease and preventing its spread. Unfortunately, both the private and public sector in India heavily rely on smear microscopy as the initial diagnostic test. This is despite smear microscopy's ability to diagnose only about 50% of the positive cases. The over-reliance on the century-old method becomes particularly worrying as the private sector caters to 70% of TB patients.

All children diagnosed with TB must get paediatric fixed-dose combination drugs

About 5,500 of over 76,000 children tested in nine Indian cities have been diagnosed with tuberculosis, 9% of them with multi-drug resistant TB (MDR-TB), highlighting the silent spread of the disease. Though the actual prevalence of MDR-TB among children in India is not known, the results from a limited number of children tested in this sample, under the Revised National TB Control Programme, is worrying. According to a 2015 study, of the over 600 children who had tested positive for TB in four cities, about 10% showed resistance to Rifampicin, a first-line drug. Since the incidence of TB among children is a reflection of the prevalence of the disease in the community at large, the high prevalence of both drug-sensitive TB and drug-resistant TB in children from these nine cities is a grim reminder of the failure of the health-care system to diagnose the disease early enough in adults and start them on treatment. Very often, children who test positive for TB have been

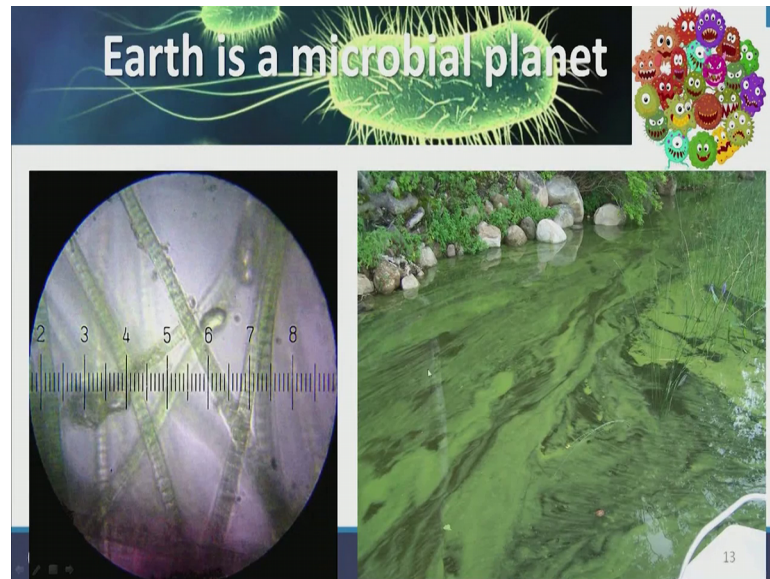
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And I wanted to share this and that how TB antibiotic resistant tuberculosis is a big issue in India and even though it sounds like a very medical problem well antibiotic resistant infection doctors take care of a medical professionals medical scientist bio-technician, but this is very important the latest research suggests that the root cause of a resistance in

to the clauses or any other microbe for pathogens for that matter is not what is happening just inside the hospital just inside the body and what the drugs are doing to bacteria.

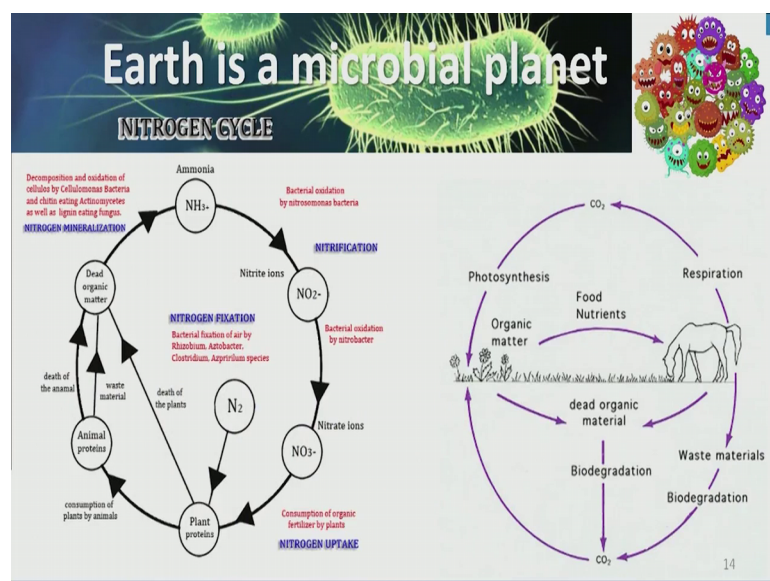
But a large role is played by natural environment and this is where environmental engineering has to jump into action and to help combat antibiotic resistance all right.

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And this is cyanobacteria by the way pictures of cyanobacteria under microscope in lake and remember this is the bacteria that generated oxygen for the first time on earth.

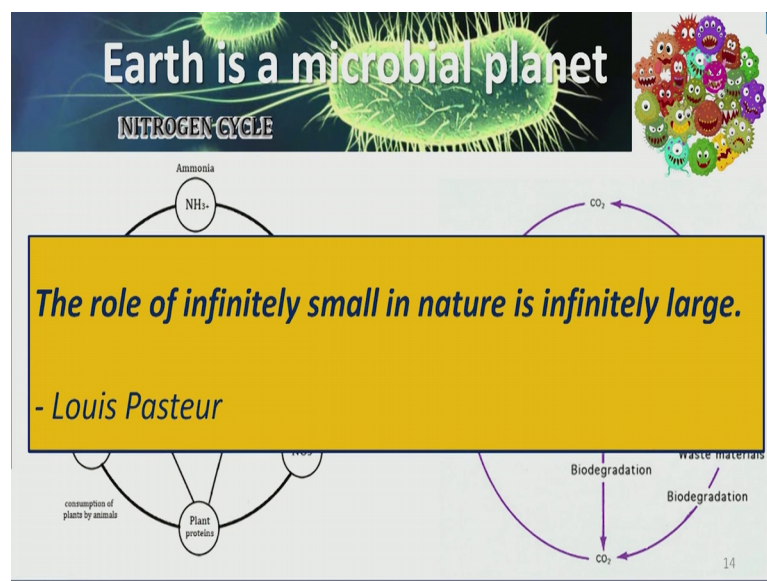
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The other important reason why we should spread awareness of applied environmental microbiology is that microbes not only impact health whether its environmental public or veterinary, but they also impact our climate they impact how our nutrient cycles move in on our earth.

For example here we have nitrogen cycle and carbon cycle both are driven by microbes and this is where it becomes very important to understand that depending on how microbial communities behave our climate will respond to it.

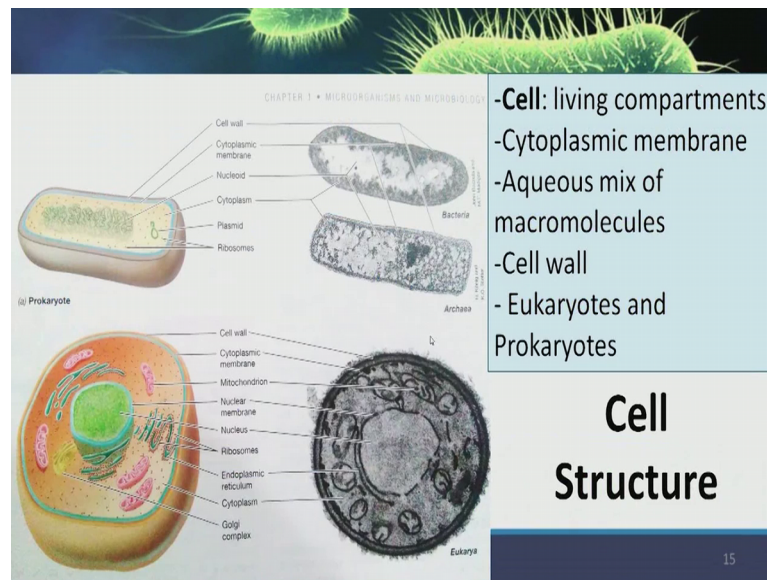
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And I would like to quote one of the early heroes of microbiology Louis Pasteur that the role of infinitely small in nature is infinitely large and this is exactly what we did not; we hope; this is exactly the message that I hope to pass on to you my dear students in this course.

So, because the role of infinitely small in nature is infinitely large lets understand the infinitely small in nature.

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And we start with the fundamental unit of life the cell the cell structure in this slide you will note that there are 2 different kinds of cells on the top we have this very simple container the gray container with a blue inner lining and some liquid inside and this convoluted green mass now let me give it more scientific terms the layer outside is actually cell wall the blue layer is cytoplasmic membrane the orange is liquid here with dots in it is cytoplasm and then this green convoluted mass is actually nucleus. So, it is a genetic code this particular kind of cell is called prokaryote pro meaning primitive karyote means cell.

So, this is a primitive cell not in terms of complexity again, but in terms of time when it first evolved. Now it is very important to note that in this cell it has a very simple geometry and it has only one container. So, it is like a one room house in the bottom we have a eukaryotic cell and I have already mentioned that eu means new karyon means cell. So, in this we have a container an orange container with a blue inner lining some orange just a liquid here with dots and look there are multiple organelles within this compartment there is a sink mitochondria there are these blue endoplasmic reticulum and then there is this green nucleus for the blue lining around it and the Golgi complex is yellow.

Now, each of these organelles that I mentioned nucleus mitochondrion Golgi body and endoplasmic reticulum they have their own membrane. So, this cell in in some way

behaves like a building with multiple rooms. So, if this is a building it has multiple rooms inside it under the hand prokaryote has only one room. It is a one room house now cell is the smallest living independent living being and to announce more about the cellular components. It is very important to understand both of them will have cellular membrane and more often than a not a cell wall inside they were cytoplasm; cytoplasm is a water based mixture of macromolecules biomolecules which are very important and this is where life happens the biochemistry of life happens.

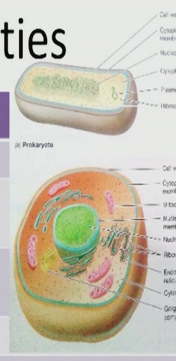
Now, this green mass both in prokaryote and eukaryote is new nuclear material which is genetic code this is the code of life and there are four alphabets in code of life ATGC and in next lecture we will be talking about ATGC more, but for now it is important to understand that the information stored in this green mass is what tells cell how to deal with the day to day activities the minute to minute challenges and issues that come up these in code how life will work for this cell in terms of a eukaryote; let us take our example our green mass the DNA will decide how what color our skin would be whether we are going to be humans or not in the first place.

How our hair will turn out and what diseases we are susceptible to I want to mention that these 2 pictures on the left for prokaryotic and eukaryotic cell or artistic impressions these bodies do not really have these colors, but they are helpful to visualize in a class and the right side we have more real microscopic images of 2 prokaryotes and the eukaryotes and the good question that I want you to think about is why do we have 2 prokaryote take examples and one eukaryotic example.

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Cell Structure and activities

	Eukaryotes	Prokaryotes
Size	5 – 100 μm	0.1 – 5.0 μm
Organelles	Mitochondria, Chloroplasts, ER, Nucleus, etc	None
Genome organization	Linear	Circular
Genome size		Yes
Cell wall		If present, has mucopeptide or peptidoglycan
Other peculiar features	Introns and Histones are present; Plasmids are rare	



The diagram illustrates the structural differences between prokaryotic and eukaryotic cells. The prokaryotic cell is shown as a small, oval-shaped cell with a thick cell wall and a single circular chromosome. The eukaryotic cell is shown as a larger, more complex cell with a cell wall, a large nucleus containing a nucleolus, and various organelles including mitochondria, chloroplasts, and the endoplasmic reticulum.

Labels for Prokaryotic Cell: Cell wall, Cytoplasm, Nucleoid, Plasmid, Ribosomes.

Labels for Eukaryotic Cell: Cell wall, Cytoplasm, Nucleus, Nucleolus, Mitochondrion, Chloroplast, Endoplasmic reticulum, Golgi complex.

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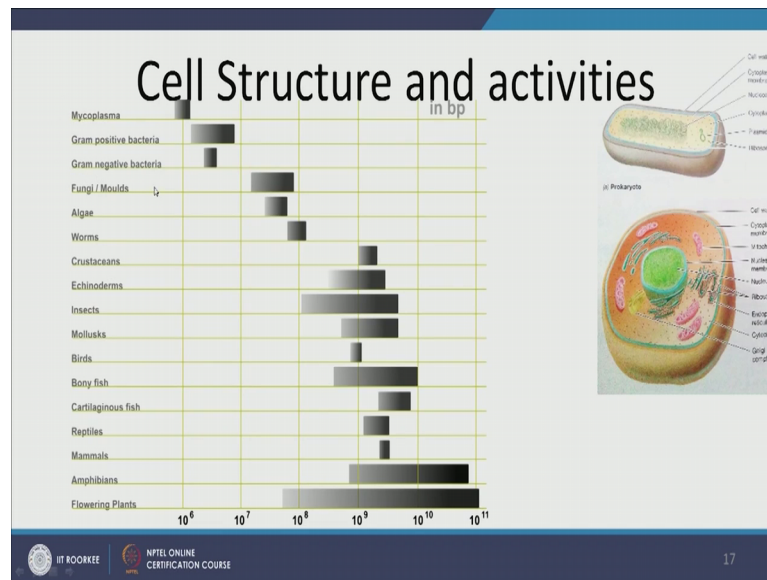
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We will talk about it in next lecture, but for now I think it is important to note that eukaryotic and prokaryotic cells are different from each other and I go over these details this in detail later.

So, for now it is just important to know that the pro eukaryotic cell is much bigger than prokaryotic cell eukaryotic cell has multiple organelles with separate individual membranes whereas, prokaryote does not; the nuclear material in eukaryote or genetic material in eukaryote is much longer and much more complexed and one in prokaryote eukaryote also eukaryotic nuclear material also have very interesting things like histones and do not have plasmids that are very often found in prokaryotic cells.

Another important difference in between prokaryotic and eukaryotic cell is the off size.

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Most prokaryotic cells here the examples are mycoplasma gram positive and gram negative bacteria and less than ten to power seven base pair per cell most eukaryotes are much larger and that is the reason why it is; it was so, easy and so convenient to a sequence the bacteria much before we could manage human genome.

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Cell Structure and activities

	Eukaryotes	Prokaryotes
Size	5 – 100 μm	0.1 – 5.0 μm
Organelles	Mitochondria, Chloroplasts, ER, Nucleus, etc	None
Genome organization	Linear	Circular
Genome size	Large	<10 ⁷ base pairs
Cell wall	Present	If present, has mucopeptide or peptidoglycan
Other peculiar features	Introns and Histones are present; Plasmids are rare	-

Labels on the right side of the slide (for diagrams): Cell wall, Cytoplasmic membrane, Nucleoid, Capsule, Flagellum, Ribosomes.

Labels on the right side of the slide (for diagrams): Cell wall, Cytoplasmic membrane, Mitochondrion, Vacuole, Nucleus, Nucleolus, Ribosome, Endoplasmic reticulum, Cytoplasm, Golgi complex.

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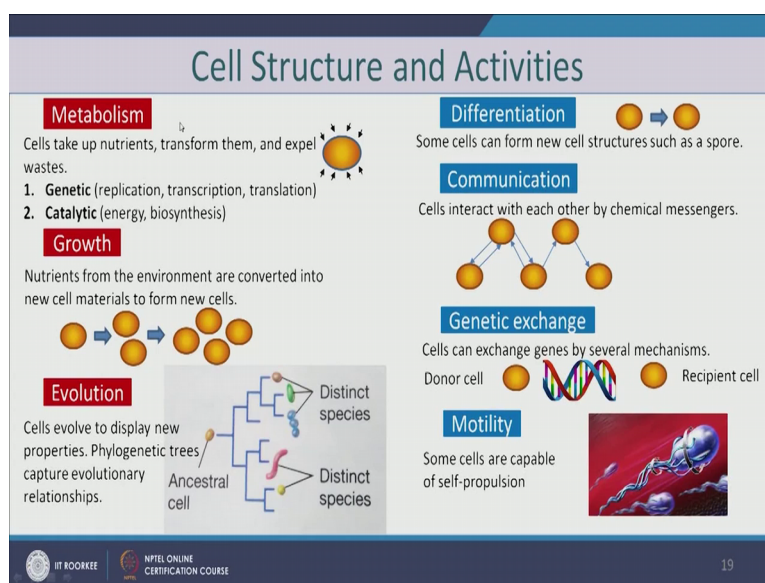
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In fact, it is interesting to note that in case of prokaryotes which are bacteria is an example of prokaryote by the way in case of bacteria an individual life could sequence the entire genome, but for human for many years we had to rely on a massive human

genome project and then there are other difference between eukaryote and prokaryote would be the eukaryotic genetic material is linear.

Whereas for prokaryote it is circular, if we could unwind if we could unwind this green convoluted mess here for a prokaryotic cell it will come out to be a perfect circle and for eukaryotic will come out to be a straight line, all right; now what does a cell do.

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And what is structure is now on left side we have important activities of the cells such as metabolism growth and evolution that it does on its own individual level on right side we had differentiation communication genetic exchange and motility this is how a cell behaves interacts with its environment and its colleagues.

So, let us look at metabolism cells take up nutrients they transform them and expel the waste much like what higher orders of life do and in terms of metabolism it has 2 components genetic and catalytic now genetic activities would include replication transcription translation we will go with them in next class in much detail. So, for now it is just important to know that when it comes to cell activities it has 2 components genetic and catalytic which is basically genetic and chemical the other important activity for cell is to grow right a cell needs to multiply it needs to grow.

So, from one cell it needs to gather enough material enough energy and enough nutrients that it can divide into 2 daughter cells and so on and so forth; the other activity that cells

participate in is evolution cells evolved at every step of reproduction. So, here we have one cell and it splits into 2 daughter cells right here in between this the replication process there is a possibility that the cell the daughter cells will be slightly different from the parent cell and the reason for that is evolution the genetics within the microbes their genetic material evolved change and as such here we might have one ancestral cell and it will create many progenies of which the progenies might go and cluster into unique species and from one ancestral cell we might have very distinct species that look different behave different and are genetically very diverse.

And in fact, there is a belief in biological community that we all have one common ancestor Luca and we will talk about it in the next lecture then we have a on the right we have differentiation communications genetic extinct and motility and I like to just quickly summarize here that differentiation is when a cell can transform into something else. So, we have a living vibrant cell, but conditions around it go really bad and it decides to become a spore. So, this is different from growth please keep this in mind then we have communication much like human beings cells also communicate with each other they produce these beautiful chemicals which act like messengers and anyone who catches the chemical and knows how to decode it will know what the message is.

And our science suggests that antibiotics are produced by bacteria to talk to each other and to ward off unfamiliar unfriendly microbes the other thing cells do is genetic exchange this is really cool a cell can share its genetic material with another cell just by touching this is almost like saying if I have curly hair and I touch my friend who has straight hair I can give her genes to have straight hair cool right and this is also a reason why we have a lot of environmental challenges. So, this is very relevant.

And next important thing is motility cells have capacity some cells not all have capacity to propel them and in this picture you have this beautiful microbe with lot of appendages here what they do is they twist in a particular direction and they act like a super coiled spring. So, the moment the bacteria decides that I am ready to move forward it will let us spring on coil and its propelled forward when it wants to change direction it just asked its flagellum to no, it does not ask the; it just whips its flagellum in opposite direction and it comes to a standstill and then it can call in a different direction to oppose it to which it wants to go.

So, here we have cell structure and activities a good summary of them and in the next lecture I will cover more details about cell structure and activities we will also talk about other applications of applied environmental microbiology what you are going to learn in this course and how, it will help you become better environmental engineer environmental scientist and just improve and if nothing else it will definitely improve your awareness and understanding of microbiology around you help you become better and more productive engineer.

So, I will stop this lecture here and continue in the next lecture.

Thank you.