

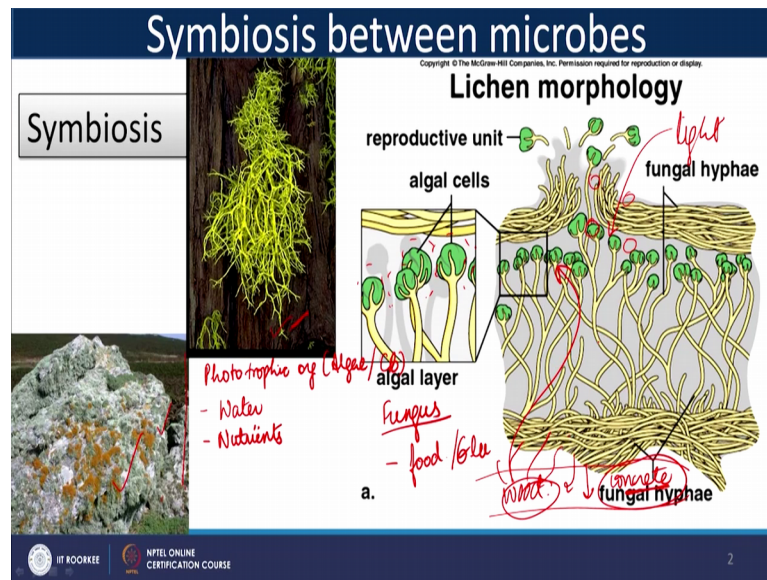
Applied Environmental Microbiology
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Lecture – 26
Microbial Symbiosis 1

Dear students, in today's lecture, we will talk about microbial symbiosis which means that microbes exist with other beings which may be microbial in nature or may be higher order of life forms in perfect; how many with each other and how many in sense that the microbes give something of benefit to the other life living being there; living in close association with and the other living being also benefits the microbes. So, this is a win-win situation and this win-win situation results in some very close associations of microbes with other life forms that at the first glance might actually be even not distinguishable.

So, these indistinguishable close relationships that microbes have with other life forms; can we have different types, we have talked about parasitism where microbes are parasitic they hurt other microbes that is a close association, we have talked about commensalism where they live together happily without affecting each other much and now today, we are going to talk about symbiotic relationships where microbes dwell inside an organism or very close to it and very close association and there is mutual benefit and we will get started with our the first example which is the lichen.

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Now, lichen is a very interesting relationship between our phototrophic living being and a fungus. So, the phototrophic living being could be an algae or it could be cyanobacteria if you remember we talked about cyanobacteria being able to trap the light energy of the sun and convert it into food.

So, this is a very interesting relationship and now let us talk about lichen I am pretty sure that if you have wandered around in the wild or seen at seen the places that still have rock cropping like such as this one forest areas old wood, you must have definitely seen lichen. Lichen comes in different morphologies and here are only two pictures of lichen this is the lichen growing on rock and this is the lichen growing on the bark of tree, it does not always look as pretty as this one or as brown as this one. So, I encourage you at this point to go around and check on the internet the different morphologies and colours of lichen and perhaps go around your own community and find where you can see the lichen outgrowth.

Now, the important thing about lichen is that as I mentioned just a bit ago that this is a association between fungus and a phototrophic bacteria. So, this is your fungal hyphae where the fungus is this is your fungus by the way and then the phototrophic the phototrophic microbes will live very close to it embedded within the fungus. So, neatly embedded with each other that they are very difficult to actually distinguish for example,

if we zoom into this particular portion of the picture you notice that these are your fungal hyphae.

So, this is fungus in yellow and the green cherry sitting on the top is your phototrophic bacteria or algae. So, phototrophic bacteria as I mentioned before could be cyanobacteria or it could be algae now why do algae or cyanobacteria from this intimate relationship with fungus has fungus kidnapped these phototrophic microbe or is it willingly and beneficiary this relationship has been set up.

Now, if you notice that the whether it is on a rock on a wall or on the bark of a tree the lichen is growing on exposed areas; areas exposed to the air the other thing to notice that the media on which the lichen is growing again whether it is bark rock or wall is not very nutrient rich its usually very dry and in dry places, it is not easy for algae or for cyanobacteria to grow. If you remember, I have shown you pictures of cyanobacteria growing in river in lake and have talked about algal blooms in water media what surface waters bodies, but algae will not find it very easy to grow on a bark a rock or on the wall.

So, in that case the fungus what a fungus does it fungus makes this beautiful structure of hyphen, but not necessarily beautiful to humans, but very beneficial for the fungus and the phototrophic microbe they make their structure which drops hydro traps water and prevents dehydration of it does this cyanobacteria have plenty of moisture around them and if they are algae there are plenty of moisture around them and they never feel lack of water also the other thing that other way in which these phototrophic microbes benefit from as.

So, their close association with fungus is that if you remember, how fungus feeds; it releases chemicals acids mostly which degrade whatever complex organics are present, if it is in case of wood or if it is concrete, then it corrodes the concrete we have talked about oxalic acid from fungus concrete corroding the concrete in that case certain nutrients whether micronutrients macronutrients sugars or some other nutrients are released which are eaten by fungus and also are available for the phototrophic microorganism to consume.

Thus if we talk about phototrophic organism which could be algae or cyanobacteria then the benefits are it receives water which it will not receive if it is trying to grow on a rock or on a wall the other thing is it at times depending on where it is growing, it will receive

nutrients, especially if it is growing on a wood or a bark of a tree. So, if you see some kind of algal growth on your wall you can be rest assured and it is not very wet you can be rest assured it is not just fungus, but there perhaps some kind of phototrophic microbe in it.

Now, another thing that another question that should be pretty obvious is these are the things that our phototrophic microorganism is benefiting from what is fungus benefiting from now fungus cannot make its own food it needs to degrade food from its surrounding. So, already trying to degrade wood and is trying to degrade concrete all its trying to degrade drop in case of concrete and rock we know that fungus will not get enough electron donors and will not get enough carbon source to survive and thrive and make this beautiful or let us say just this marvellous lichen structures and forms.

So, if what the phototrophic microbes do is that they are exposed to light and they receive they receive light and they make their own food and when they make their food they share it with the fungus. So, the fungal the fungal member receives food from cyanobacteria or algae that it is associated with thus it is a win-win situation fungus is getting its food cyanobacteria is getting its water and nutrients whether it is micronutrient or macronutrient and this is the first semi-close association that we are talking today in the lecture the lichen.

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Consortium

Epibionts

(a) (b) (c) (d)

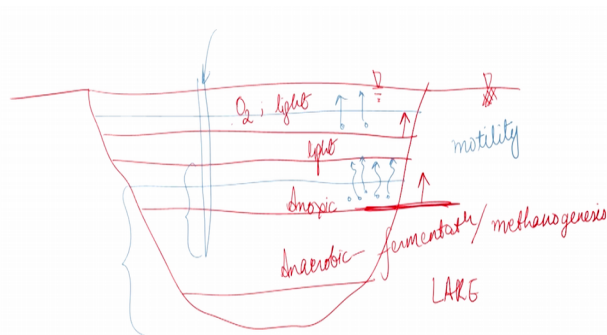
- Of non-motile phototrophic green sulphur bacteria and motile nonphototrophic bacteria
- Green sulphur bacteria are obligate anaerobes = *Chlorobi*
- Stratified lakes world over
- Color depends upon bacteriochlorophyll
- Motility helps in adjusting rapidly to the changing levels of light, oxygen, and sulphide.

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Next we want to talk about is a consortium this often happens in the stratified lake. So, in stratified lakes you remember light can penetrate from some distance towards the bottom of the lake and then it cannot. So, at the top of the surface of the lake you might have phototrophic organisms also oxygen can only penetrate and remain dissolved in water up to some depth.

So, there will have aerobic organisms and then as we go deeper we will have anoxic and anaerobic environment. So, in stratified lakes the mixing is not very common or hardly present now in this kind of lakes we often see a epibiont consortium of microbes now this consortium often includes two kinds of members one is motile and the other is non motile. So, one of them can move the other cannot move.

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Now, in stratified why in stratified lakes consortia is so important because in stratified lakes as time progresses the depth and the area where a nutrient is available changes; so, for example, let us say this is our lake. So, this is our lake and at the top here we have the phototrophic layer which is rich in oxygen and its rich in light. So, most probably we have phototrophy present, we have aerobic microorganisms and life forms present.

Now, maybe the big behave in that cleft is the water is really clear and the light can penetrate here also. So, here we will have anaerobic or anaphoric phototrophic organisms then as we proceed we will have anoxic zone where microbes that digg reduce nitrate sulphate and other electron acceptors will be present and then as you go towards the

bottom will have anaerobic zones where the conditions would be very nice for fermentation and methanogenesis all right. So, as time progresses the oxygen here let us say there is no mixing.

So, the oxygen will start getting depleted and the and the depth at which the oxygen rich aerobic layer ends will move up the depth at which as the nutrients in the anoxic zone such as nitrate and sulphate get reduced the depth at which anoxic zone ends will move up and similarly the depth at which anaerobic zone begins will move up. So, over time, we will notice that our new aerobic zone has perhaps moved up similarly our new anoxic zone has moved up and now there is there are larger portions that are in fermentative and methanogenic stage.

Now, in this case microbes that survive best in presence of oxygen what will they do a microbes that survive best in presence of electron acceptors such as nitrate and sulphate what will they do they need to find a way to move as the layers of the lake move; so, in that; that is the reason why motility is of utmost importance for survival of the cell. So, there are certain cells that are not more non motile and non motile cells well if they cannot move they will die out. So, now, non motile cells the interrelationship with motile cells which stick around it and develop some kind of relationship with it and when these motile cells move by default the non motile cell moves also.

So, let us take a look here now this is your epibionts the green and brown cells that you notice here are your phototrophic bacteria and the tail here that you are noticing is actually a flagellum of nonphototrophic motile bacterias. So, because if the phototrophic bacteria requires light to survive, but these are non motile. So, they cannot move and at the inside at their core they have here a rod shaped motile non phototrophic bacteria now because it is non phototrophic it does not require exposure to light.

So, it will allow as many motile cells as can stick to it to stick around its periphery then when the time comes to move towards light rich area; let us say the turbidity changes with season or the amount of sunlight that is available changes with season in that case this kind of microbe requires to move up or below the depth at which it is present. So, then these microbes will stick to this motile microbe the rod like micro and when the time has come to move it will use its flagella to either move up or to move down.

Now, notice that I mentioned here that both of them are phototrophic whether they are green coloured or whether they are brown coloured both these epibionts are phototrophic now the conventional perception or the typical perception of phototrophy is the green colour, but it can be either green or brown depend on different colours depending on the type and the quantity or percentage of the bacterial chlorophyll that is present in the microbe.

Now, notice here that we have epibionts mentioned here and that is for a good reason because epi means outside biont means alive organisms. So, organisms that stick outside to you are epibionts. Now usually the way it works is that the non motile there this one the ones that are on the periphery, they are phototrophic sulphur bacteria the colour might change depending on the kind of bacterial chlorophyll and then these are your motile non phototrophic bacteria now one thing to notice that we have talked about this in previous lecture that these epibionts which are your sulphur bacteria green sulphur bacteria they all belong to a particular of microbe and that is known as chlorobi, if you remember that they all belong to chlorobi they all are obligately anaerobic.

So, if we come here they will exist in this anaerobic zone where the light can still travel. So, this is where they would like to exist no oxygen present and the light can still penetrate this is where they would like to be present and the other thing is important about them is they are found across the globe they are not limited by geography anywhere there is stratification of water and they because the water is stratified there is a need to move as conditions move.

If water is not stratified it means that there is good level of mixing going on and when there is mixing the nutrition is likely to be available throughout the year throughout the lifespan of the microbe, but when it is not available then the non motile phototrophic bacteria love to combine with the motile nonphotographic bacteria and allow it to carry them to more productive areas nutrient rich areas.

Now, so, this is the benefit that the phototrophic bacteria are getting from attaching to a motile, non phototrophic bacteria what is non phototrophic bacteria gaining from this relationship the obvious answer is food the phototrophic bacteria convert light into food and now they shared this food with the motile non phototrophic bacteria. So, it is almost like a bus ride that they get, but once they have reached to a nutrient rich area they do not

cleave away very quickly because either because their lifespan is over or that the need to move again might arrive very quickly alright. So, these are different examples of consortium.

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Plants as microbial habitats

Mutualism

Soybean root nodules: More root nodules translate into better health and productivity

Phylogenetic tree showing various *Rhizobium* species, including *Rhizobium leguminosarum* and *Rhizobium lotum*.

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Now, the next example we want to look at is plants as microbial habitat we have briefly talked about this how nitrogen fixing bacteria allow the plants such as soybean and other legumes to fix nitrogen and pay protein rich grains protein rich seeds legume this is an example of soybean root nodules.

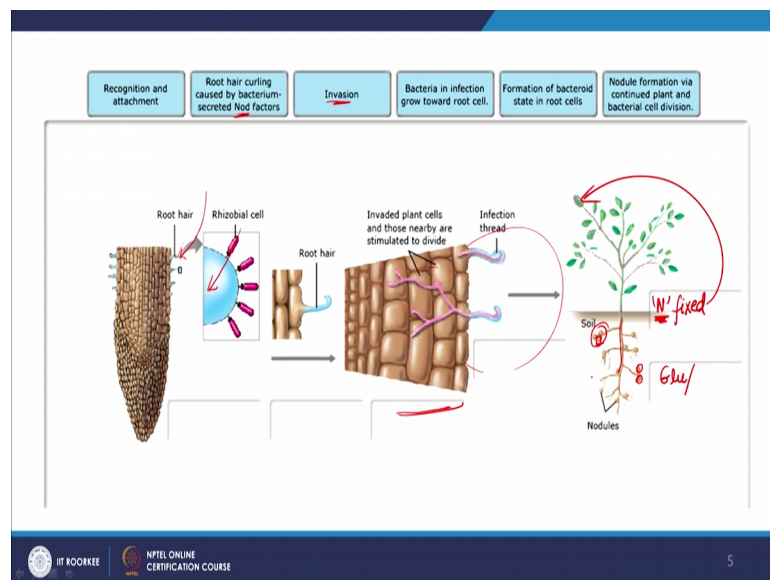
Now if you pull out the root of legumes and soybean and other plants that are fixing nitrogen from air and convert and making new nitrogen rich food for us then you will notice these nodules kind of structure on them and here is a beauty these nodules are a result of a bacterial infection, but the more nodules we see the more bacterial infection, we notice in a plant the healthier that plant appears to be and also the more productive it appears to be.

So, when we do sequencing of these root nodules and we sequence the these components now how will be sequence, we will tear them off, we will weigh them, we will extract DNA, we will attach the right adapters and we will split the DNA into right length and then attached to right adapters and then sequence it using her generation of fourth generation sequencing technique and when we sequence, then we find are not apart from the typical root mike genetic sequences that we will get the (Refer Time: 17:18).

Now this is soybean. So, apart from the typical soybean sequences we find quite a rich diversity of bacterial sequences and many of them at least the one which that I have shown here in this picture are rhizobium leguminosum are of. So, named very appropriately found in rhizobium of the legume and; however, we have we know now that this bacteria that colonize these infected root nodules are very diverse they can be even prettier bacteria and different kinds of microbes actually.

So, we can use our sequencing techniques to find out what kind of microbes are infecting the root now how does it work out how does the infection take place and how does it help plant health and improve its productivity all rightly this is how it usually works.

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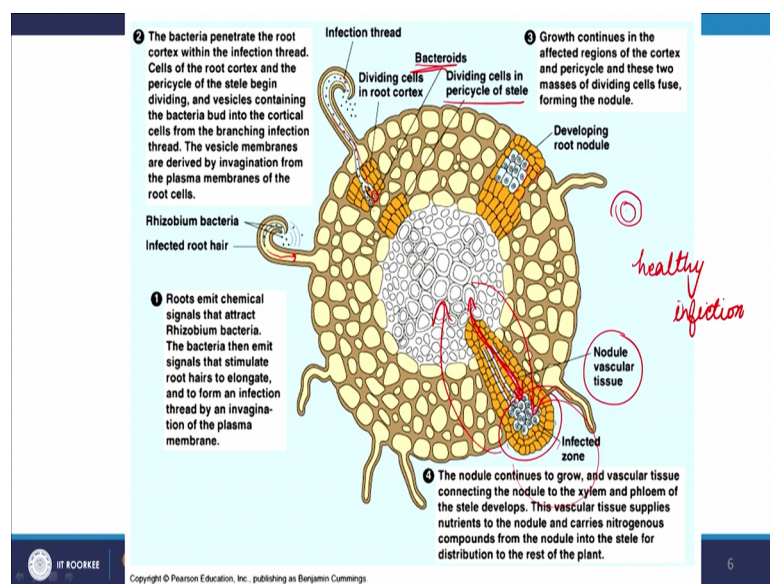
This is your healthy root this is healthy root and says it is prior to infection now when we talk of human health usually if we get infected then this is bad we are unhealthy, but in case of legumes if they get infected by the right microbe its really good for them. So, look here this is your typical root and then there are tiny-tiny root here that represent in any healthy root and the root has rhizobium cells on it now what happens is that these root hair they secrete certain chemicals which attract the rhizobium microbes to it now these rhizobium microbes they are identified recognized by the root hair that yes this is the root microbe that I was looking for now when the microbe had this is the blue microbe enlarged at the tip of this enlarged here.

So, when the microbes have attached to your root hair then they release not factors now these not factors, what they do is that they allow the microbes to invade into the root cells into the root hair. So, now, this is the invasion the invasion step where the bacteria actually invade now when the bacteria invade this root they encourage the cells to grow rapidly. So, now, this is the infection has happened in the root. So, this has been infected once the infected has infection has happened the plant cells are now hijacked by the bacteria and they are stimulated to divide faster and faster now when they divide faster and faster they make a lump or tumour like lump which we call as root nodule.

Now, now this root nodule this; this root nodule is formed and once it is formed the bacteria utilized this to fix to serve as the perfect environment for fixing nitrogen and sending into the letting the plant uptake it now we know that the plant is benefiting from this despite spending more energy in making this tumour like root nodule growth.

It benefits from it because it gets rich amount of nitrogen food fixed nitrogen from atmosphere that it can now make nitrogen rich legumes it can make nitrogen rich fruits and seeds the question is how is bacteria benefiting from it well the bacteria benefits from it because the plants in turn because they are phototrophic and they make food very easily share glucose and other food with the microbes. So, microbes fix the nitrogen for them and plants receive give them food in return.

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So, now let us look at the infection of a root cells a little bit more in detail the first step is that the root as I mentioned earlier the emit a chemical signal that will attract the rhizobium bacteria. So, in this first step the root hair has emitted a signal and the rhizobium bacteria this tiny black dots have been invited and look there is actually an incision here in between the root hair and they are going in deep here and now this root hair has become infected.

So, once the root rhizobium bacteria have been attracted to the root hair the bacteria starts desecrating chemical signals which forced a which hijack the cell that oh go faster grow faster elongate and when they elongate further they actually create an invagination. So, a kind of a tube inside the root hair and then they penetrate it and now once they have penetrated the infection can go this is an infection invagination it can go and it can make bacteroids; so, bacteroids inside the root hair.

Now, these are these root hair are dividing really fast they are in the root cortex. Now since they have reached here the vesicles that contain the bacteria will hijack the root cell that lets divide faster and faster. Now they and they divide in the pericycle of the stele part of the root and then the growth continues you know this is the growth is continuing and these are the cells that have bacteroids; bacteroides in them in the vesicles.

So, it if you remember the virus infection how virus enters the cell it can do two things it can either become part of the chromosome or it can make multiple copies and divide now here the bacteria enters a eukaryotic cell which is a root cell stays there in a vesicle now vesicle is a membrane like structure which allows something to stay isolated from rest of those cells.

So, you root cell does not kill the bacteria and they ask it to divide faster and faster. So, these pericycle of stele cells are dividing faster and faster in the root cortex and more and more of them have bacteria in their vesicles and all of them are hijack and they keep dividing keep dividing are the infected. So, they make a tumour like outgrowth they it will continue to grow and vascular tissue containing through the nodule to xylem and the phloem of the stele would develop. So, now, this is developing the vascular system. So, now, they can have this is nodule vascular tissue. So, they can have a good input of nutrient and they can share whatever nutrients that they have.

So, this is it will allow the transportation of nutrients. So, the nitrogenous compounds are transported from nodule to the rest of the plant and the water and other nutrition is shared with the root nodule. So, this is how root legumes they how this is how the legumes actually are colonized by bacteria and this is a very healthy infection. So, as agriculture in agriculture we want our roots to be infected by the bacteria. Now I have mentioned here our microbiological studies and new tools have give us in and give us an idea of what kind of microbes tend to colonize the roots and form these root nodules and we also know what kind of micro organisms micro..

Microbial communities allow more root nodules to form are more viral and in or more pathogenic and form more root nodules which result in this translate in better health and higher productivity. So, now, we can do a sort of probiotic approach where if we want to encourage root nodules we can actually take the strains that encourage it more inoculate them around the root and the next thing we know is lot of root nodules present in our root system and that will benefit the that will benefit the plant and would benefit the farmer; all right, students, this is all for today.

In the next class, we will be moving on towards public health and how as human beings and as animals; different animals such as cow also you have I have very close relationships with microbes and how we utilize them for our better health.

Thank you.