Aspects of Biochemical Engineering
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Lecture – 02
Microbiology - II

Welcome back to my course aspects of biochemical engineering. In the last lecture I try to discuss the lot classification of the microorganism. How the microorganism can be classified, and what are the different microorganism is available. Now in this lecture we I am going to talk about the how we do the nomenclature of the microorganism, how will name the microorganism. Then also we will discuss the how this organism that grow in the different media, and what are the different components that are required in the media for the growth of the organism.

How different that you know different chemicals that influence the growth of the particular organism. And then also I am going to discuss that how this organism can produce the different type of products.

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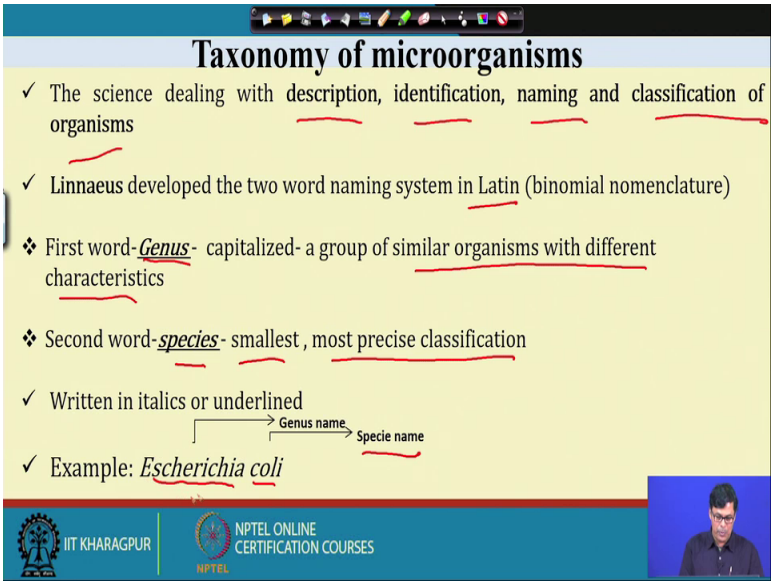

So, if you look at the first let me talk about the taxonomy of the microorganism. This is very important. Because how we how we name the microorganisms. This is the science dealing with because science dealing with description identification naming and classification of the organism. The taxonomy deals with like this, that you know that

when you put the name then these are we take into account the description identification naming and classification of the organism. The linus in linnaeus development of 2 words, that is in Latin. One is called genus, another we are called spaces.

Genus capitalized a group of similar organism with different characteristics. Here I want to point out when you write a genus always we start with the capital letter. But when we write the species, we write with the we start with the small letter. This is the basic difference we have between the genus and the; species with the smallest and most precise classification. So, this is the basic difference between genus and the species. Now I can give the example of esherichia coli, largely used in the biochemical process. Now escherichia is the genus name, and the coli is the species name. So, you can you can see this is escherichia, it is we have this is genus and e coli is the s the species.

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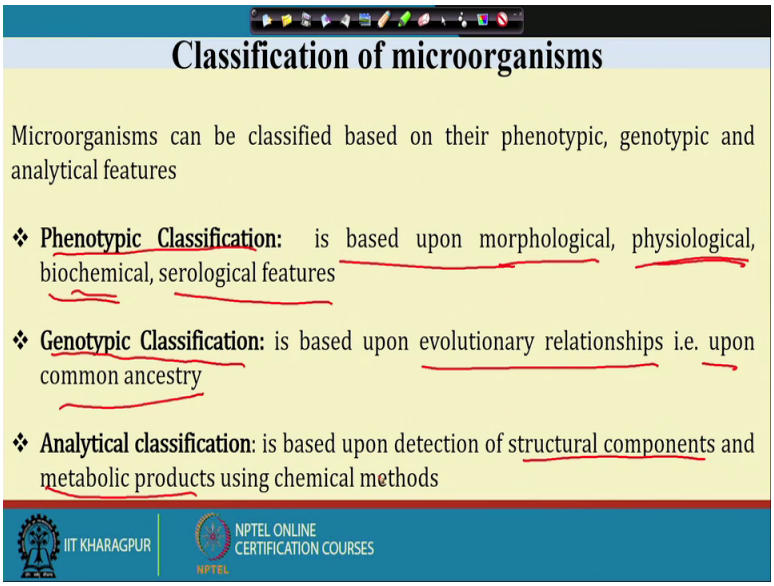

Now, if you look at that you know that classification of the microorganism in the last lecture also I try to talk about the classification. That is different type of classification. We on the basis of prokaryote then a eukaryotes. Now here the classification is on the basis of other types, that one is called phenotype another is the genotype and analytical classification. So, you know these are the now we are slowly go little bit deeper into this microorganisms. Now if you look at the this phenotype, what do you mean by phenotypes? This is I phenotypic classification is based on morphology, physiology, physiological, biochemical and serological feature.

So, you know that we see the organism under the microscope how it looks. Then physiological the physiological characteristics we take into account. Then biochemical we can do some kind of biochemical analysis through which we can find out what is the characteristics, and serological means we take out the liquid and ionized in the and find out the what are the different speciality we have.

So, on the basis of phenotype characteristics can be done. Now previously here I want to point out that immediate that early days that the identification of the microorganism mostly done on the time on the basis of phenotypic fixing. Now pleasant now a days we usually do the classification on the genotype classification. We want to have more definite that what organism it is, may definitely we want to tell that of what is the you know, what is the particular organism is present there. So, it is based on the relationship it is upon the common ancestry.

Because what is the heredity? What is the heredity of this on the basis of energy? We try to find out this this I do the classification of the organism. Then analytical classification is based upon the detection of structural component and metabolic product using the chemical methods.

So, you know that now the microorganism can be can be classified in 3 different ways. One is phenotype also genotype and also analytical, the on the basis of that we can do the classification of the microorganism. Now here I want to elaborate a little bit.

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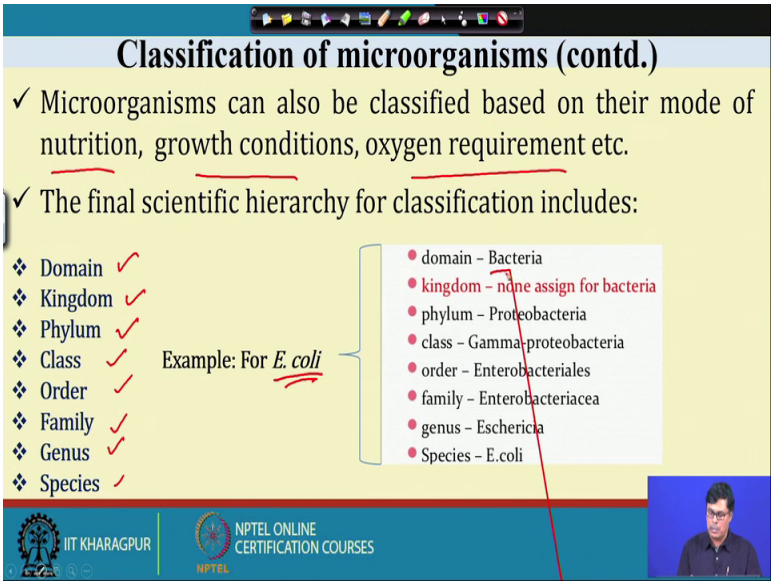

Now, the microorganism can be classified based on the mode of nutrition, growth condition and oxygen requirement etcetera.

Now, first scientific energy for classification includes, this domain, then kingdom phylum, class, order, family, genus and species. As for example, we have given the example of (Refer Time: 05:47) e coli. Now if you look at the (Refer Time: 05:50) coli then domain is the bacteria. The bacteria is the domain bacteria is the domain. Then kingdom known assigned to bacteria.

And then phylum is the proteobacteria. The class c is the gamma proteobacteria. The order is the enterobacteriales. Then family is the enterobacteriacea, then genus is this eschericia and species is e coli. So, this is the how the complete classification of the microorganism can be given.

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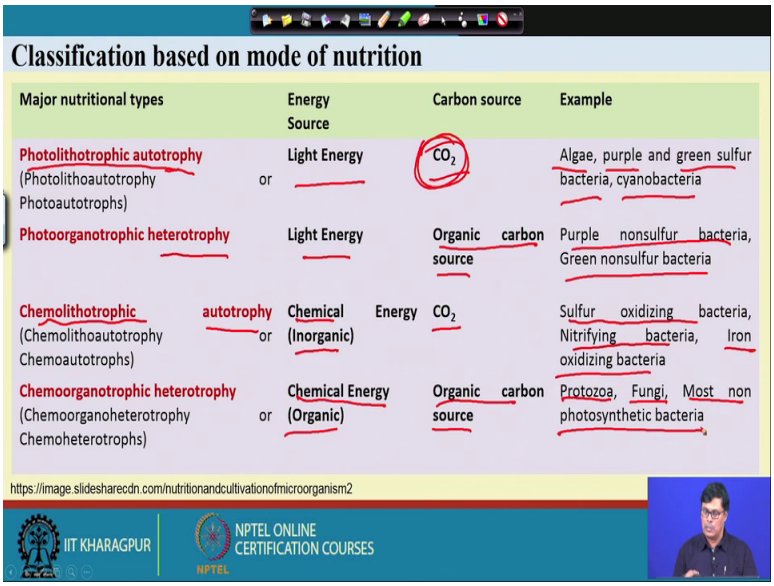

Now, this is now as I pointed out the classification also can be made on the basis of the nutritional requirement, or in the process conditions. As for example, nutrition then temperature then pH, and then some other factors also we can we can consider. First let us consider the nutrition. Nutrition is the as for example, for the photolithography organism this is autotrophs. Autotrophic organism means they use the carbon dioxide as a they has a carbon source. This is a carbon source is the carbon dioxide.

So, they fix the carbon dioxide. Now here I want to stress one point, that I told you in the last class that we have similar environmental concern nowadays due to the due to the availability of more carbon dioxide in the atmosphere. Now due to the presence of carbon dioxide it causes some kind of greenhouse effect that increases the temperature of the atmosphere is the great concern throughout the world. So, this this. So, this organism has the capability to you know fixing this carbon dioxide and from the biomass. So, they can remove the carbon dioxide content from them from the atmosphere. So, they use the light energy and the examples are algae purple, green, sulfur bacteria and cyanobacteria. Then photo organic trapping trophic hetero trophic this is light energy, they use the organic carbon source partner non-sulfur bacteria and green non-sulfur bacteria.

We have green chemolithotrophic organism. We have auto tropic chemicals that is used as the inorganic chemicals used mostly as the energy source, and then carbon they fix the carbon dioxide examples of sulfur oxidizing bacteria, nitrifying bacteria and iron oxidizing bacteria. Now if you look at the chemo oregano tropic the hetero troops this is they use the chemical energy, this organic compound as a source of energy. Then organic carbon they use as a carbon source examples is the protego fungi and most non-photosynthetic bacteria. The on the basis of nutrition also, you see we can we can do the classification of the different organism, between the algae, then you have bacteria then fungi that all that can be classified.

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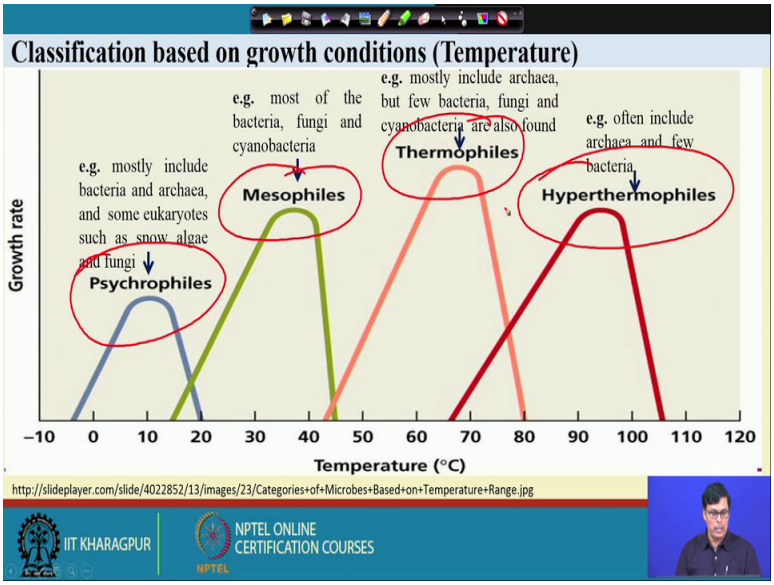

Now, let us come to the temperature. How on the basis of temperature we can do the classification?

We have different type of bacteria, we have psyrophilic, psychrophiles we have mesophiles, we have thermophiles, and we have hyperthermophiles. Now very interesting psychophiles is usually they grow at very low temperature; that here particularly in the cooler region. And I here I want to buy, I want to tell you that particularly in the Himalayan region, our ambient temperature is quite low. So now, there is a there is a great concern that how we can be degrade we can we can dis implicate the waste material. Because most of the organism that degrade the organic material, they are mostly mesophilic or thermophilic, but you know not psychopathic. So, a lot of attention has been paid on the psychopathic organism, how this organism can grow at low temperature.

So, they can they can save good our environment. The mesophiles we have lot of examples we have bacteria fungi and cyanobacteria. And thermophiles we have bacteria fungi cyanobacteria also found in the in the thermophiles. They work at the very high temperature 45 to 6 degree centigrade. And then (Refer Time: 10:22) you see this is 40 to 80 degree centigrade that we have and hyperthermophiles is 70 to 105 degree centigrade. This obtained includes the archaea and few bacteria. This is extreme for particularly in the volcano, that we can we can identify the organism which can be of hyperthermophiles because in the volcano we have very high temperature. And organism that survivor there that we withstand very high temperature.

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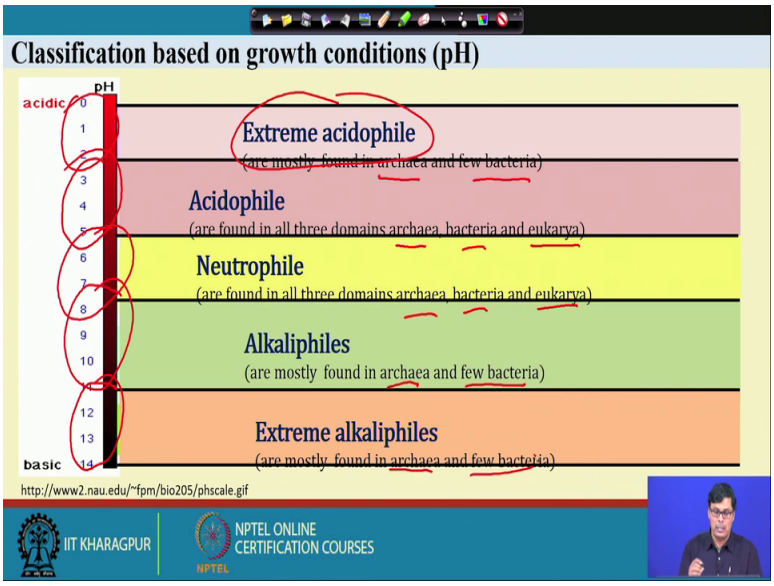

Now, on the basis of pH also we can classify the organisms. We have the examples of this extreme acidophiles, extreme acidophies they grow in between 0 to 2, you can see this is the 0 to 2 that we have. The acidophiles usually grow in between 3 to 5, this is the acidophiles that it had neutrophils usually 6 to 8, this is 6 to 7 or 7.5 alkaliphiles this is usually 8 to 11. That we have extreme alkaliphiles is usually 11 to 14. This is so, on the basis of that you know that pH also we can classify it now extremophiles. We have the examples is the archaea and the bacteria. Then acidophiles we have archaea bacteria and you eukaryote, this is I can give the example, that wastewater treatment processes particularly anaerobic digestion process we use the archaea for the product for conversion of organic wastes to methane and carbon dioxide.

That is used the neutrophils is the archaea bacteria and eukarya that is have alkaliphiles this is the archaea, and if you have bacteria and also extreme alkaliphiles is they are archaea and few bacteria. This is available.

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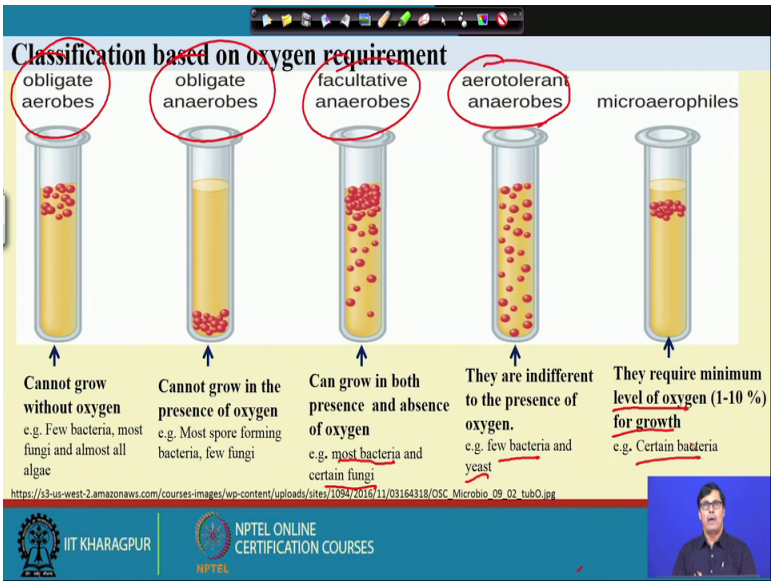

Now, on the basis of oxygen requirement also we can do the classification. We have we have different type of organisms, we have obligated aerobes obligate a anaerobes. But what is the difference between the of what is obligate? Obligate means they required molecular oxygen for the growth and metabolism of the organism. And because if you say obligate adopts means they required molecular oxygen, not the oxygen in other form. But if you look at the obligate anaerobe, they cannot withstand the molecular oxygen. Because they if little molecular oxygen.

Present in a in their system it will rupture the cell. The other cell will be killed. Now question come how it happens. Because we know that it does not have the enzymes, like catalyst or you know superoxide metis. Because you know catalysts we know that when oxygen take by the living cell it converted to hydrogen peroxide. Hydrogen products is the strong oxidizing isn't, and this is usually degraded by catalyst engine. So, but this is lacking in the in the obligate anaerobe. So, and not only they do not have the enzyme life superoxide mutates. Because some our super oxide formation is there therefore, the degradation of the superoxide that the enzymes are missing. So, if any the highly oxidized little metal present in the in the system may be every possibility that bio molecules present in the or living organism, their characteristics will be change and they will be killed.

So, and there is the another organism what do you call faculty dip analogue faculties. Dip is a is a typical organism which can grow both in presence of in presence of oxygen or in absence of oxygen. Because here you can see most of the bacteria and certain fungi they have this unique character. I have I can give you one example that if you know I was talking about the saccharomyces cerevisiae. Saccharomyces cerevisiae under aerobic condition, it produces the bickersons under anaerobic condition it produces the ethanol. So, it has the dual characteristics, now we are walking with the hydrogen production processed last 2 decades. And we find that (Refer Time: 14:45) is a very potential strain for hydrogen production. And this organism has similar type of characteristics and the little bit condition, it produces the same mass, but under anaerobic condition it produces the hydrogen.

Now, then we have aero tolerant anaerobes. So, this is a aero tolerant anaerobes we have. And they are they are indifferent to the presence of the oxygen. That is examples of you bacteria and the e cells. And micro aero files they required minimum level of oxygen for their group. They do not required huge amount of oxygen micro aerophilics they are require very small amount of oxygen for the growth and metabolism.

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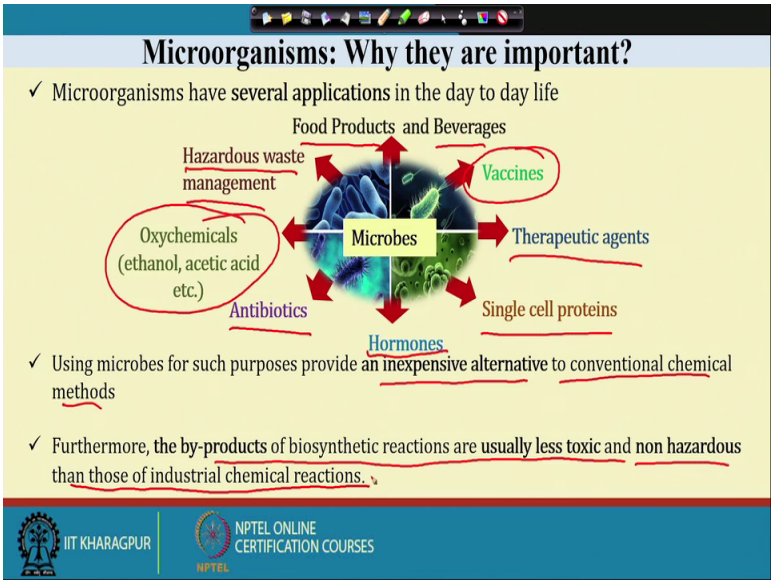

Now, that another interesting thing that how this organism plays the important role. Because this organism can lead to a different application in the our day to day life. As for example, some organism can be used for the production of food products and the

beverage. As for example, I can give the example of for this I know that we I last year yes in the last lecture I try to point out that cheese making industry. The cheese is a kind of product through which you can preserve the milk protein and fat for a longer period of time. So, when beverage industry I talked about the alcoholic beverage, like a beard and wine that is produced through the alcoholic fermentation process. This is considered as a non-mystery liquor.

Then hazardous waste management that is largely used I told you, that whatever after any kind of most of the biochemical and chemical industry, they pose the environmental pollution problem, because they are if you throw the this waste water, through the water sources then what will happen, the whatever bacteria whatever microorganism present in that atmosphere they will grow in the water and they pollute our water shrimp. So, to safeguard that we shall have to treat the water, and so this microorganism can be; I mentioned in the last class that more than 70 percent wastewater treatment processes are controlled by the biological means.

Now this can be used for the production of vaccine, we have this a lot of application of our day to day life, because particularly for our children we have we know that polio vaccine, because the lot of children was greatly affected due to folio, and now this has been recovered with the development polio vaccine. Not only that we have other several vaccines we produce, they have got different safe guard diseases. And then therapeutic agents we produce as per exam I told the examples of penicillin; that is, the produce streptomycin, different type of antibiotics we can produce from this microorganism. Single cell protein this is another area because this microorganism grow very fast as compared to any other living cells. So, they can be used as a very cold good source of protein.

Now, question comes this is not used as a human food, the reason is that most of the this microbial protein content the nucleic acid. And this nucleic acids called is some kind of gout and kidney stones. So, if you want to use this microbial protein for a human food. So, you have to remove the nucleic acid. If we remove nucleic acid, then we can use as a source of protein. And that is the hormone that is largely used for the growth of the plant cell also other different purpose. Then antibiotics I told you oxy chemicals are largely used we have beaten all acetic acid citric acid different type of chemicals we can produce with the from this microorganism. So, it has several uses. Then this microorganism

provides the inexpensive alternative to the conventional chemical methods. This is this is very, very important thing that we have. And furthers the byproducts of biosynthesis.

Reaction usually less toxic non hazardous than other industrial chemical reactions. Because if you look at the industrial chemical processes when we get a certain product with the main product we also produce some kind of by product with some kind of hazardous the effect on our on the environment. So, that kind of problem we have very less in case of biochemical industry, and that is why that this this process are more or less what you call environmentally friendly.

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Now, question come how you do the culture of this microorganism, how this is the different type of methods we have, I can show you this is broad culture this is agar plate, this is slant culture, stack culture collection. So, this is the this is the broth culture broth is the kind of media. At the media where the organism grow you can see when the organism grow it will be little bit hazy. And then the agar plate agar plate where we put the auger or the agar is kind of solidified media, where we put media there. And when the organisms grow you can refine the different colony formation is there one dot is one colony.

And one dot signifies the presence of one living organism. Then with a slant culture we can this slant culture we can do that in this slant in the taste tube, we prepared that stab culture we can do because stab culture usually we do for the growth of anaerobic

organism because inside, that you know we do not have any presence of oxygen, the molecular oxygen. So, most of the analog can grow it here and culture collection this is usually collected in the form of lypol. Lyphilization is kind of freeze drying technique through which we can preserve the organism part for over several months or so, to 3 years we can preserve the organism. So, this is how the microbial culture can be done.

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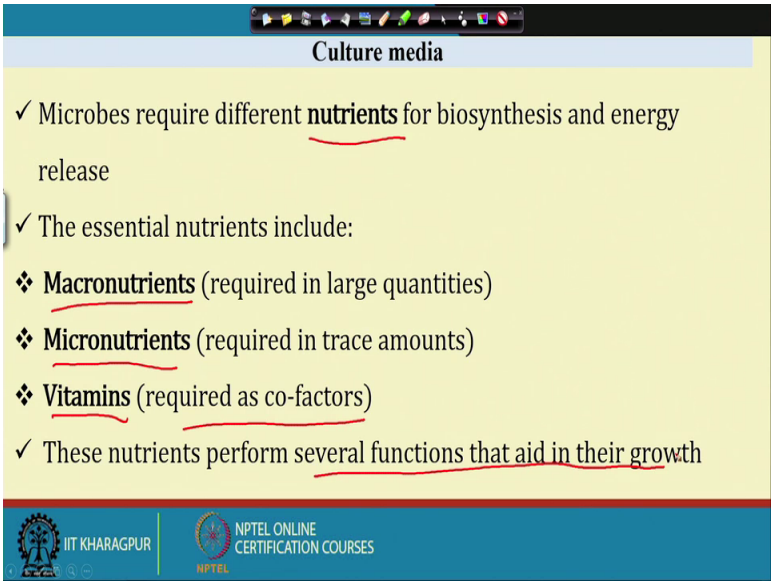

Now, question come culture media. If you look the culture media, microbes require nutrient iii mentioned before, that it causes require nutrients and what the nutrients can be classified in 3 different way. One is called macro nutrients, and the micro nutrients and the vitamins. Now micro nutrients means which is required in the large amount. I can give the example that in bakers in the industry when we produce the bakers; is usually we use the carbohydrate. And we use sugar as a source of carbon which produces the bakers yeast. Now if you look at the stoichiometry, one gram of sugar produces 0.5 gamma bakers yeast. So, you know that that largely contribute for the bakers yeast formation process. So, this is called macro nutrients.

But micronutrients means which is required very small amount. What are those? These are mostly the metal ion. Now that you know that if you look at if we look our metabolic pathways we will find different enzymes. They participate in the in the different metabolic reactions. And this most of the enzymes they required the metal ion as a cofactor. So, that is that is called they required any very stress amount. That is why we

call it the micronutrient. Vitamins also required as the cofactor. This is then these nutrients perform the several function that aids about the growth.

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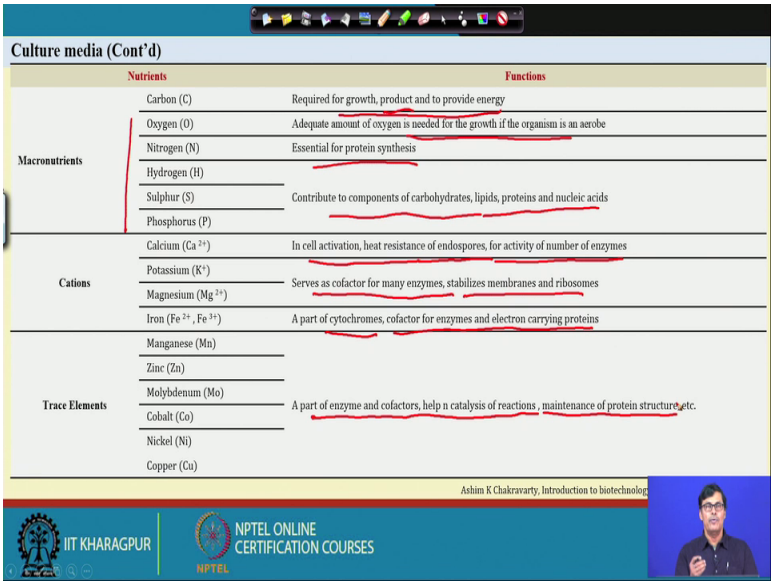

Now, I can give you the typical example the micronutrients; we have carbon, oxygen, you see that we have here carbon, oxygen, nitrogen, hydrogen, sulfur and phosphorus. The carbon required for growth product and it provides energy. Because carbon the carbon plays 3 different role. One is for the growth of the organism, I told you then it also causes the produces the energy. I told you that that when we take glucose it a pass for first pass through the end of my rope pathway then to TCA cycle. Then it produces ATP NADH and this is used as a source of energy.

And then it also produces different products. I asked for example, citric acid one mole of glucose produce one mole of citric acid as here. So, you know there, but one mole of glucose produced 2 moles of ethanol. So, you know if you look at that stoichiometry is very vital. So, it produce from the carbon source we gave the different products the oxygen is required for the growth of the organism. Because this is the major limiting factors of the aerobic organism, because microorganism can take the oxygen which is dissolved in the water they cannot take the oxygen which is present in the air like our system.

The nitrogen is the essential for the protein synthesis, because that is also very, very much important for the growth of the cell. Hydrogen, sulfur and phosphorus contribute

components of carbohydrate lipids and proteins and nucleic acid. Now these are the metal this is calcium, potassium, magnesium and iron; the calcium thus this is used in cell activation, heat resistance endospores and for activity of the number of enzymes. I can give the example of alpha amylase enzyme, in presence of calcium ion the thermo stability of the enzyme that increases. The potassium and magnesium has solved the cofactor of many enzymes and stabilize the membrane and ribosomes. Iron a part of cytochromes cofactors for enzymes and electron carrying protein. We have manganese, zinc, molybdenum, cobalt, nickel and copper and copper.

In a part of the enzyme cofactor to help in catalyzing reaction and maintenance of the protein structure. So, we can here I can we can we can we can find out what is the utility of different component present in the media.

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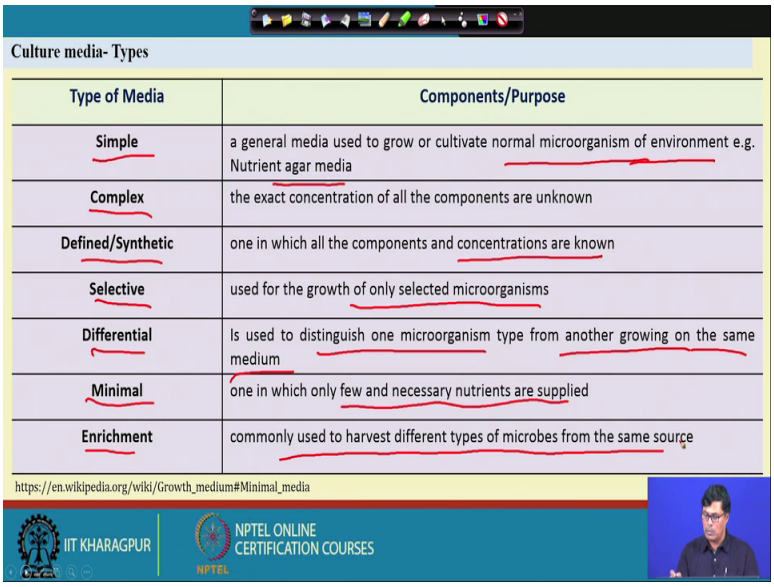

Now, now if you look at the media, and that work of composition it can be classified in different ways. One is called simple media, then one is called simple media, then complex media, different synthetic media, selective, differential, minimal and enrichment media. So, you know it is simple media means that general a general media used to grow or cultivate the normal microorganism of environment and nutrient agar media. Now complex media where the composition of the media is unknown. That is called complex media and the synthetic media means where the composition of the concentration is know.

You know, the exact composition of the media that is called synthetic. Selective means, the growth only selectively microorganism which is because some organism required the selective nutrient for the growth and metabolism the in presence of some specific chemicals it can grow; that is the selective media, differential media it is either used to distinguish one type of organism for another type of organism growing in the same media. And minimal media is basically contains some kind of minerals of one invasive few are necessary nutrients are supplied. And enrichment media commonly used for harvesting different type of microbes from the same sources.

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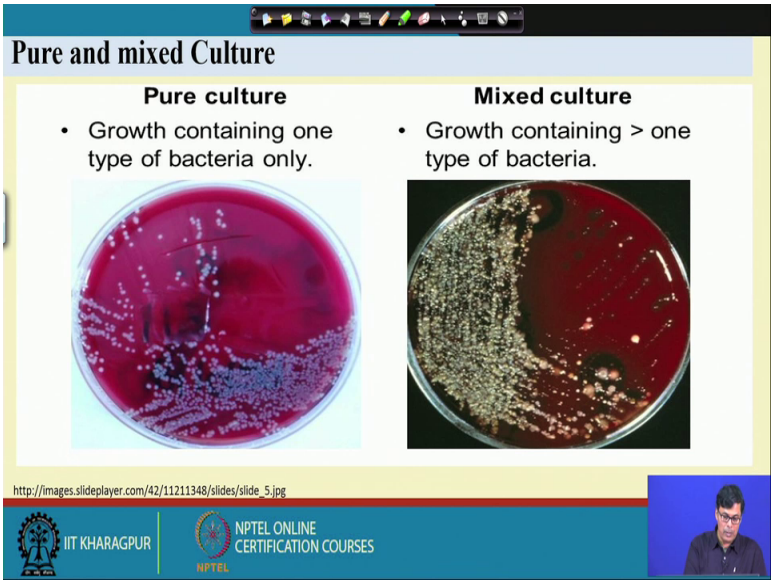

Now, this is how it looks at the pure culture it looks. So, you can see the pure culture it is like this that you know.

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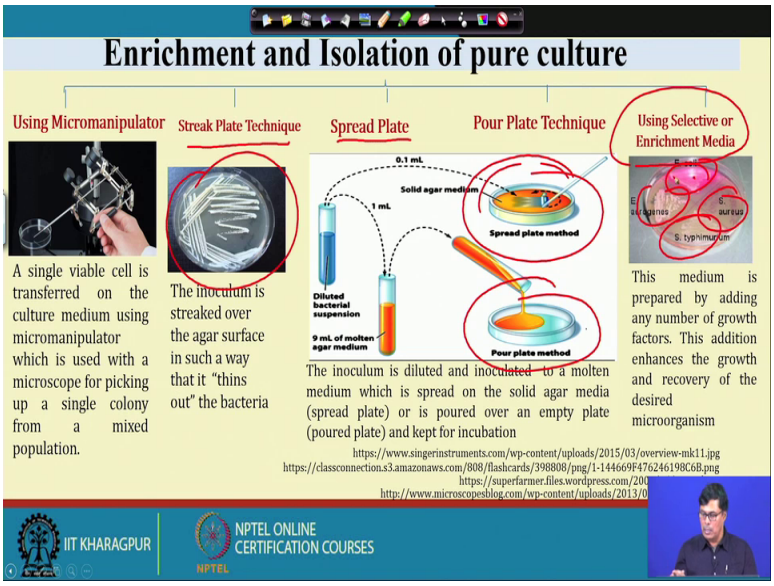

The pure culture is a similar type of you know that that you know that colony you can find out.

But when you have meat culture that is presence of different type of over that we have different type of colonies we have, you can see that you know that that indicates the different type of organisms are present in the culture. So, this is how we can differentiate from and not only that if you see under the microscope from the morphological study also we can find out that whether your organisms are pure. Or I can give a typical example of the this that pure culture that we have.

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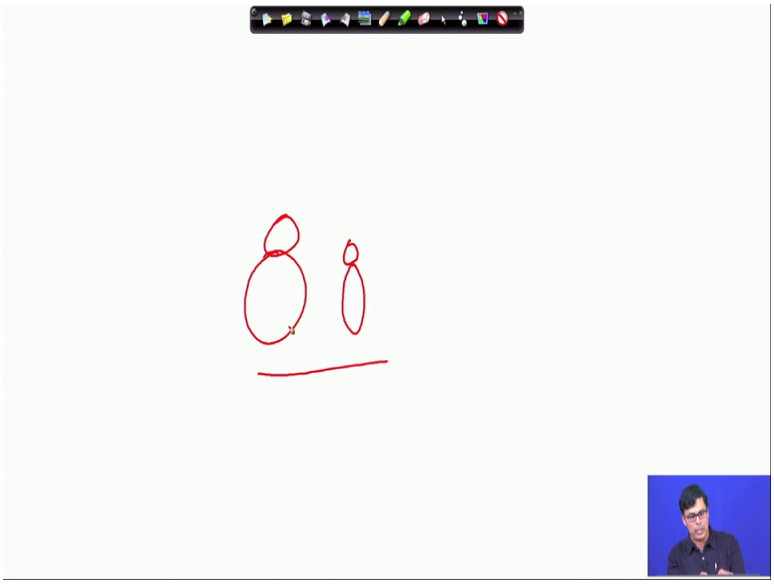

That you know that in case of yeast suppose we have our desire yeast morphologies like this. And whilst strength or organism is like this. So, if you would see under the microscope, even if the same yeast is there, but looking at the morphology we can easily find out. Whether it is contaminated with the other yeast cells or not form the micro microorganism we can easily find it out. So, this is now the how we do the isolation is very interesting that by using micro manipulator we can we can take, we suppose we when you do the plating, from the one I told you of one particular colony is for the growth of one particular cell. You can take it and then you can grow in the in the media. That you know that is how you can do that. Then we can stick plate you can do it this is the streak plate we do you can we can we can in the in the agar media, solid media we consider we can put the streaking you can seed. And then spread plate you can spread it and pouring it and this is spread and this is pouring it. And from that we can we can we can we can grow the culture in this media.

And this is the selectively enrichment media. You can see the different color by using different ingredient spirits you can have different we can put the different antibiotics on the on the basis of that we can find out that the growth of the different organisms in the in the culture media.

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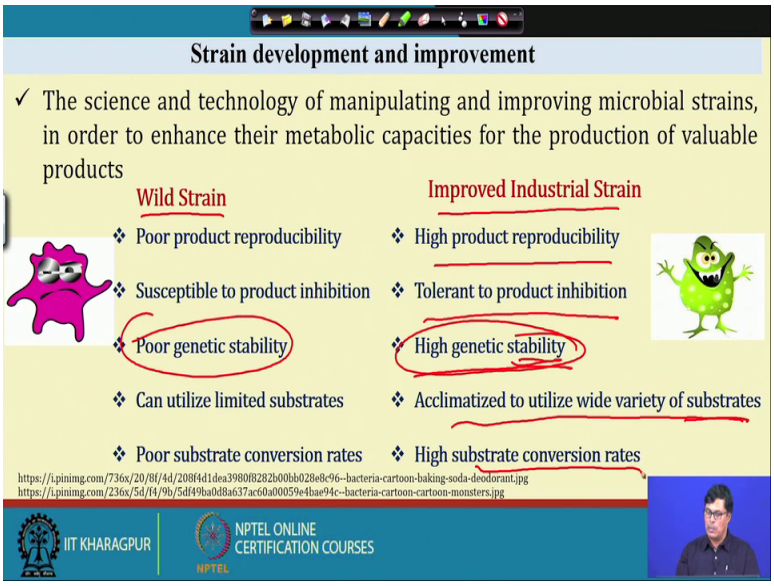

Now, industry as well biochemical industry is concerned that we have 2 type of strain we use. While strain and they improve micro industrial strength. While strength that is the organism that is the available in the nature in the soil. We know then the soil all kinds of organisms are available. An industrial strain we specially prepared for the industrial purpose this can withstand the adverse circumstances. As for example, if you look at that it is a higher productivity tolerance of the product inhibition, high genetic stability. This is very important factor because if you look at that here this while strain.

If you use the while strain for industrial purpose one particular batch will give you good results another batch will not give that the good results. So, this is not acceptable by the industry. It should be the hygienist ability. The genetic stability is a very important factor. The acclimatize say to utilize the wide variety of substrate, that reduce the cost of the product and also high substrate conversion efficiency. This is help in the waste water treatment process, because load of the wastewater treatment processes will be less.

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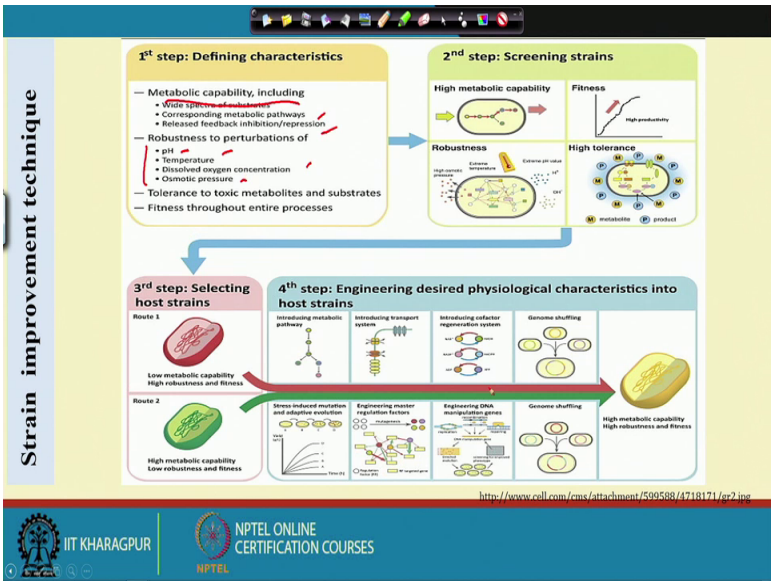

Now, this is the process through which we can we can produce the genetically modified strength order. We can we can produce the industrial strength not genetically industrial strength. First it the defining characteristics that we have as for example, we can have the metabolic capability including the white cell species the spectrum of substrate corresponding metabolic pathway release of feedback inhibition and depression. The robustness of the culture that whether it can grow a highway the change of pH temperature dissolve oxygen concentration osmotic load. I can give the different specific example. Suppose when we talk about the alcohol making industry if the high alcohol concentration you want to produce. That organism might be having some kind of inhibition characteristic or some kind of osmotic shock. Because as we increase the glucose concentration osmotic pressure increases that may cause us some kind of effect on the organism. So, those are the things we take into account when we try to develop some kind of this this microbial strain.

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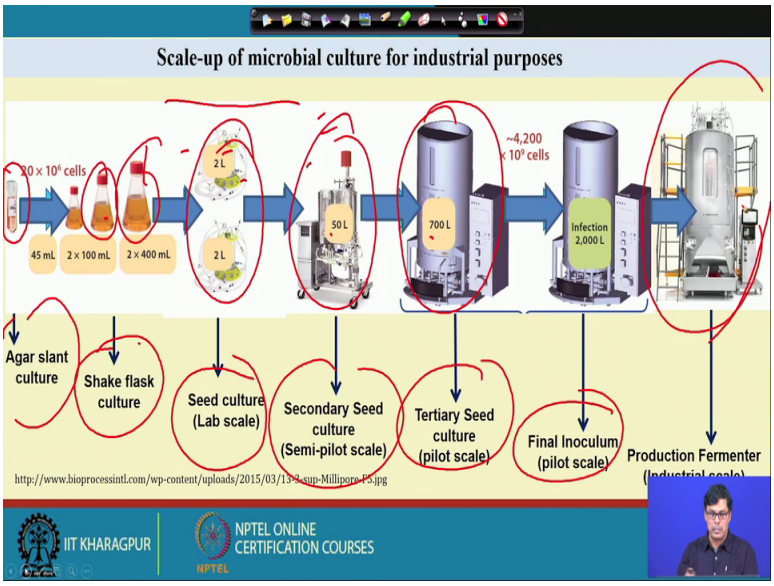

Now, later let me and finally, let me tell you how we develop the organism, that we say we start with this agar slant culture. Then we this is the agar slant we produce that then slant, this is safe flock cultured. This is the flocks we do they put it in the sacred. Then we put in the in the in the fermenter this is a small fermenter we have. Then we a secondary seed culture we produce little bit bigger current. Then tertiary seed culture it will again 50 liter to 700 liters, and 700 liters to that 2,000 liters, then this is we go for the production parameters see. This is how slowly we develop the culture for the industrial processes.

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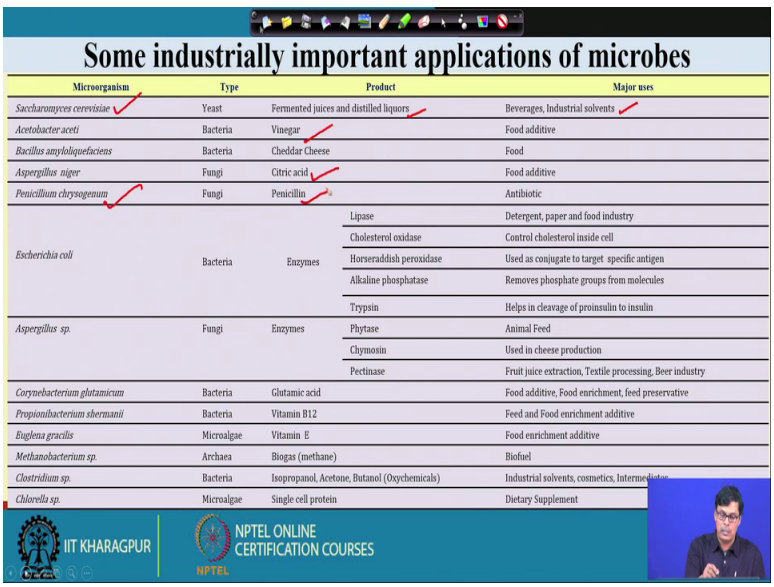

Microorganism	Type	Product		Major uses
Saccharomyces cerevisiae	Yeast	Fermented juices and distilled liquors		Beverages, Industrial solvents
Acetobacter aceti	Bacteria	Vinegar		Food additive
Bacillus amyloliquefaciens	Bacteria	Cheddar Cheese		Food
Aspergillus niger	Fungi	Citric acid		Food additive
Penicillium chrysogenum	Fungi	Penicillin		Antibiotic
Escherichia coli	Bacteria	Enzymes	Lipase	Detergent, paper and food industry
			Cholesterol oxidase	Control cholesterol inside cell
			Horseraddish peroxidase	Used as conjugate to target specific antigen
			Alkaline phosphatase	Removes phosphate groups from molecules
			Trypsin	Helps in cleavage of proinsulin to insulin
Aspergillus sp.	Fungi	Enzymes	Phytase	Animal Feed
			Chymosin	Used in cheese production
			Pectinase	Fruit juice extraction, Textile processing, Beer industry
Corynebacterium glutamicum	Bacteria	Glutamic acid		Food additive, Food enrichment, feed preservative
Propionibacterium shermanii	Bacteria	Vitamin B12		Feed and Food enrichment additive
Euglena gracilis	Microalgae	Vitamin E		Food enrichment additive
Methanobacterium sp.	Archaea	Biogas (methane)		Biofuel
Clostridium sp.	Bacteria	Isopropanol, Acetone, Butanol (Oxychemicals)		Industrial solvents, cosmetics, Intermediates
Chlorella sp.	Microalgae	Single cell protein		Dietary Supplement

Now, this is some industrial uses of some kind of microbes that has been shown here, that I do not like to discuss in details. Because I will discussing this different byproducts that there, I shall discuss some of this. Only I want to highlight that that saccharomyces cerevisiae again produce some fermented juice or destroy liquor, as using the beverage and industrial solvent. Acetobacter aceti can be used for the production of vinegar. Then aspergillius niger for the producing citric acid, penicillium chrysogenum can be used for the production of penicillin.

So, this is this particular lecture we gave you the some broad idea of that how the microbes that you know that behave in a different way, how it differs from each other with respect to their nutrient. Because they use some organism required carbon dioxide as the carbon source some organism required inorganic carbon as inorganic material. Somewhere organism required as the organic carbon as the carbon source. So, you know that how this is the some organism can grow at low pH, some organic cause we can grow a neutral pH, some organs can grow in alkaline pH. The temperature also we have psychrophilic mesophilic and thermophilic.

The different and nutrient also that the organism that that nutrient that differ from each other on the basis of that we can do the classification. So, this give you the broad idea on the microorganism how that different microorganism look round a microscope. How they grow in the microscopic, how we do in the culture we start with slant, then sake plugs then we form interest slowly. Usually the size of the inoculum should be 5 to 10 percent of the production media that usually keep in the mind, when you go for the development of the of the inoculum.

Thank you very much.