

**Cell and Molecular Biology**  
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**Week 03**  
**Cellular Homeostasis**  
**Lecture - 10**  
**Cell Growth Medium**

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And in the course, Cell and Molecular Biology, we are discussing the different aspects of the molecules, how they interact with each other, and how these interactions result in the different types of processes. In this context, so far, we have discussed the origin of life, the evolution, and the cellular structures, whether they are prokaryotic or eukaryotic. And then in the previous lecture, we discussed why growth is very important for the different types of organisms.

So, we have discussed that growth and reproduction are the fundamental aspects of any living organism. So, there are distinct properties of a living organism. It should be responsive to the different types of stimuli. It should be, you know, very complex having the organizations and at the most important.

The property of a living organism is that it should be able to have growth potentials, and these growth potentials should be endogenous rather than exogenous; it should also be able to reproduce and give rise to offspring. Now, in the previous lecture, we discussed the growth, how the different phases of growth are present in the prokaryotic system as well as in the eukaryotic system. What are the different places where it can be controlled so that you can have growth in very, very tightly controlled processes? We have discussed how the growth is being controlled at different stages in the prokaryotic system and what different cellular machinery is required for controlling cell division within the prokaryotic system. Then we also discussed the eukaryotic system and the different stages where growth can be controlled. Now, when you talk about growth, it is very, very important.

And if you recall from the previous lecture, we discussed that for growth, energy is very, very important. And because the energy is required in terms of, you know, driving so many interactions, whether it is being done by the ATP and the ATG complexes and all that. So when you talk about growth, the growth requirements are very different. We have discussed prokaryotic cells, eukaryotic cells, and within the eukaryotic cells, we have also discussed animal cells, plant cells, fungi, yeast, and so on. So when you talk about growth, every organism's requirement for growth is very, very different.

And this is the topic that we are going to discuss in today's class: how different organisms acquire nutrition for their growth and what their requirements are. So when we talk about

the growth media, it is actually a complex material or solution. The main purpose of growth media is to provide the essential biomolecules or nutrients so that the organism can build the basic biomolecules. When you talk about the basic molecules, you are going to talk about proteins. You are going to talk about carbohydrates.

We're going to talk about lipids and nucleic acids in the form of DNA and RNA. So when we talk about the growth media, the growth and multiplication of organisms used as the expression system require suitable biochemical and biophysical conditions. The biochemical that is the nutritional condition can be provided by the use of various nutrient media. Depending on the special need, different types of media have been developed for expression to achieve growth, multiplication, and overexpression. So when we talk about the growth media, it is required for the organism to build its building blocks.

And the building blocks are proteins, carbohydrates, lipids, and nucleic acids. When we talk about carbohydrates or when we talk about proteins, proteins are made up of amino acids. An amino acid is a building block, and it actually works as a building block, which means that proteins are mainly used for making different types of structures. For example, the plasma membrane's protein is being used to make the multimeric protein complexes so that they can participate in two different types of reactions and so on. So their main job is to serve as a building block.

And the proteins are made up of the different types of atoms such as carbon, hydrogen, oxygen, nitrogen, and sulfur. Similarly, for carbohydrates, the monomeric unit is sugar. Right. It could be a monomeric sugar such as glucose, fructose, mannose, and galactose. And then these sugars are burned in the different types of carbohydrate metabolic pathways such as glycolysis, the Krebs cycle, and so on.

By using those metabolic pathways, the sugar molecules are used to produce energy. So the main purpose you require carbohydrates is that the organism requires carbohydrates because it wants to produce energy. And the carbohydrates are mainly made by three atoms, such as carbon, hydrogen, and oxygen. There are carbohydrates where you actually have the other atoms as well, but that proportion is very, very, very minor, such as with chondroitin sulfate and some of those kinds of carbohydrates. But they are specialized carbohydrates.

They are not being used. They are not being used for energy production. Similarly, we have lipids. Lipids are made up of fatty acids. And these fatty acids are also being used to produce energy, and the lipids are made up of different types of atoms such as carbon, hydrogen, oxygen, phosphorus, and sulfur.

Then we have nucleic acids, which are made up of nucleotides, and nucleotides are used to carry information from one generation to the next. This, anyway, we are going to discuss in detail when we talk about the genomic DNA or the genomic content of the organism in the

following modules. The DNA and RNA are made up of carbon, hydrogen, oxygen, nitrogen, and phosphorus. So, what you see here is that even if you have to synthesize the proteins, carbohydrates, lipids, or nucleic acids. You basically required a nutrient source that can provide you with carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and nitrogen.

So basically, you require a source of nutrition so that you can provide these nutrients or these atoms, actually. This can be provided in a complex form, or it could be provided in a simpler form. Apart from these requirements, you also require a very small quantity of minerals and different types of vitamins that are actually going to function as coenzymes. So, considering these requirements for the organisms, people have developed different types of microbiology media or different types of media for the different types of organisms. So let's first talk about the microbiological media, and then we will talk about the eukaryotic media.

So when we talk about microbiology media, remember that when you are going to provide any media, your primary objective should be that the media is able to provide these atoms. so that you can use these atoms to synthesize first the monomers, and then these monomers will come together to give you the polymer or the complex molecules. So, for example, if you use these atoms, they are actually going to be used for the synthesis of the amino acids, and then these amino acids can be used for the synthesis of proteins through a very complicated process of translation. And the same is true for the nucleic acid as well. So when we talk about microbiological media, right, you are actually going to use the different types of constituents.

So you are going to use the amino nitrogens and growth factors. You're going to use the energy source. You're going to buffer. You're going to explore different types of minerals and metals, selective agents, indicator dyes, and gelling agents. So when you require the amino nitrogen, which means this is actually going to provide the nitrogen for the organisms, you can actually use the peptones, protein hydrolysates, infusions, and extracts.

Similarly, the growth factors are also required. So you can actually use the blood serums, yeast extracts, vitamins, or NAD. Then you require the energy sources that are actually going to provide the carbon. And for that, you can actually use the sugar, alcohol, and carbohydrates. Then a buffer is required because we are using the in vitro system.

So the pH should not change. So for that, you can actually use phosphate, acetate, and citrate. Remember, the phosphate you use for the buffer is also going to be a source of phosphate, right? Then you are also going to use the mineral salt and metal. So you can actually use the phosphate, sulfate, magnesium, calcium, and iron. Then you are going to use the selective agents.

Selective agents will be used for the selection of the organisms. So that may involve the application of recombinant DNA technology, where you are going to use a selective agent to

select the transformed cells from the untransformed cells. So, for that, you are going to use antibiotics, dyes, and chemicals. Similarly, you are actually going to use the indicator dyes so that you can see whether the media is good or bad. For that, you are going to use phenol red or neutral red.

And ultimately, you are going to use the gelling agents. Gelling agents are going to be used for converting a liquid media into a solid media. So you're going to use agar, gelatin, alginate, and silica gel. Now, considering these nutrients or constituents that you are going to get from these complex substances, people have developed different types of microbiological media. So they have used different types of media.

So one is called M9 minimal media, M63 media, LB media, SOB media, and so on. And when you see the composition, right, for the minimal media, it is going to be sodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, and ammonium chloride. And this is going to be used for the cultivation of all sorts of, you know, strains of E. coli. Similarly, you have the M63 minimal media where you are going to use the ammonium sulfate, potassium hydrogen phosphate base 6 and the ferrous sulfate and that you are going to use for the cultivation and maintenance of the E.

coli. Similarly, we have the LB media where you are going to use 1% peptone, yeast extract, and 1% NaCl for E. coli growth so that you can prepare the plasmids and use them for protein production. Similarly, we have the slightly modified version of LB media, and there you are also going to use that for the propagation of E. coli. Similarly, we have SOB media, which contains 2% peptone, 0.

5% extract, NaCl, KCl, and  $MgCl_2$ , and it is used for making high-efficiency competent cells. Then we have the SOC media, and that is going to be used for making the competent cells. Then we have the YT media that is for peptones, X extract, and NaCl, and that is going to be used for fast DNA production. Similarly, we have the super broth where you are going to use 3.

2% peptone, 2% yeast extract, and 0.5% NaCl, which is used for the high yield of plasmid DNA and protein production. So all these microbiology media, some are minimal media, some are defined media, and some are very, very complex media. For example, the media in which you are actually adding the salts and all those kinds of things are actually being called defined media. Right. So where you are actually having the media component that is defined.

So these are called defined media. Where you have the defined amount of disodium hydrogen phosphate or potassium hydrogen phosphate, sodium chloride, and ammonium chloride compared to, for example, the LB media, it is not a defined media because you have the peptone and yeast extract, and peptone is actually a very, very complex biological material. Peptone has a lot of cellular material, so the composition of the constituents

present within the peptone is not very much under the control of the user. So that is the same thing as true, like, for example, you have the M63 minimal. So all sorts of minimal media that you use are actually the defined media, and defined media is used in those cases where you are trying to optimize the different types of parameters. And you also want no contamination or any kind of interference with the complex biological substances from the microbiology media.

So in those cases only you are actually going to use the defined media. Now how are you going to prepare the media? So to prepare a media, we have taken an example of how you can prepare the LB medium. To prepare a bacteriological media, dissolve the components according to this particular table. For example, in LB media, you are going to take 1% peptone,

5% yeast extract, and 1% NaCl. And then you dilute that into one liter of distilled water, right? Cover the top of the flask with cotton plugs and autoclave the solution at 121°C for 20 minutes. So when you do so, you are actually going to make the microbiological media. Remember that you are using autoclaving for sterilization only in the case of microbiology media. So, while you are doing the autoclaving, you are supposed to take a lot of precautions, right? What precautions do you have to take? Number one is that the ratio of the media volume to the cultural flask should be defined, right? For example, when you are making a 250 ml media, right. Then it has to be prepared in one liter flask so that you are actually going to have 750 ml of oxygen.

Right. So you are actually going to have the three-fourths oxygen, and that three-fourths oxygen is good enough to provide the radiation. Because remember that once you are going to prepare the media, for example, if you have taken a flask, you are going to put a cotton plug on top. Once you put the cotton plug in, it is going to be disconnected from the outside, right? So there will be no oxygen that it can actually take up from outside. That means the bacteria have to survive on whatever oxygen is present within this flask. That means you should have a sufficient ratio of media volume to culture flask so that you can provide enough aeration for the bacteria to use in their various metabolic pathways.

The media components are hygroscopic, and while weighing, you should avoid moisture; they should be stored in a cool and dry place, which means all the media components should be very dry so that you can add these media components in defined conditions or defined amounts. So you should be very careful when weighing these media components to avoid putting moisture into the media component. And that's why you should store the media in a cool and dry place. While autoclaving, open the autoclave only when it is cool. Remember that autoclaving media means you are actually going to autoclave something at 121 °C.

And at this temperature, the water is actually going to be boiling even when the autoclaving is done, so if you open the autoclave, this boiled water will actually spill and could cause

injury. That's why you should be very careful; you should only open the autoclave when it has cooled down. Then you avoid charring the media component. So you should have a sufficient quantity of water within the autoclave when you are doing the autoclaving so that there is no charring of the media component. It means there should be no burning of media components.

Then the solid media should be poured into a plate once it has cooled down. So when you are preparing the solid media, you can actually prepare the media in two ways. One is the liquid media; the other one is the solid media. Solid media where you are actually going to put the gelling material right so in that case you should let the media to be get cooled down sufficient enough so that when you add the antibiotic the antibiotic should remain within the solution right and should be active right and that is only possible when you cool down to around 50 to 55 degrees celsius and then only you add the antibiotics The various antibiotics or nutrient supplements should be added to the media when the temperature is below 50 degrees Celsius. So this is all about how you can prepare the microbiology media, how you can autoclave it, and what different types of precautions you should take.

So to explain these phenomena, I will take you to my lab where my students will show you how to prepare the microbiology media, how to prepare the cotton plugs, and how to perform the autoclaving. In this video, we demonstrate how to prepare bacterial culture. For preparing cultured broth, we need three components. One is pepton, yeast extract, and sodium chloride. For 100 ml of cultured broth, we need 1 gram of peptone, 0.

5 grams of yeast extract, and 1 gram of sodium chloride. I am going to weigh individual components and dissolve them in double distilled water. Then we have to autoclave the medium. Before weighing, care should be taken to ensure the spatula is clean and the balance is in place. After weighing, we have to clean the spatula and return it to its original position, and during weighing, care should be taken to avoid contact with any of these media components. After weighing the media components, we have to dissolve them in double distilled water, initially dissolving in 80 ml of distilled water; once the components are completely dissolved, we have to make up the volume to 100 ml.

While it is steaming, we have to prepare cotton plugs for the flask. To prepare cotton plugs, you have to take one thick layer of cotton and fold it like this with both hands. Once the media resolution is complete, we have to pour it into the flask, up to 100 ml, so we use only one-third of the remaining space; the empty space is used for aeration purposes and also ensures proper autoclaving. In order to check whether the components are autoclaved or not, we use a paper-based sterility indicator that we have to paste onto the flask before autoclaving. If the autoclave process is completed properly, we will see the white strips turning into black ones.

So this is the indication that the autoclave process is complete. Now the media components are completely dissolved. Now we have to pour it into the flask. Tap the mouth with a cotton

cloth and rub a little oil directly around the mouth.

Now this is ready for the autoclave. Once the media preparation is complete, we have to sterilize the media in order to use it for further applications. This is a typical autoclave where you can see the temperature and pressure indicators, and these are the pressure knobs. This one is the quick pressure release knob that you can use when you are in a hurry. You have to use this one, but I would prefer not to use it; let it go on its way. We have to turn on the autoclave, so you can see here the bulb is glowing.

Before keeping the media components in the autoclave, make sure that the heater inside the autoclave is submerged in water. Now I am going to keep the media components in the basket that we use for autoclaving. Then keep this one inside the autoclave. While closing the autoclave, make sure that you are closing it in the opposite direction. Once the pressure and temperature reach 121°C and 15 psi, you have to hold that point for 20 minutes.

Then you have to turn off the machine, let it cool down, and remove the components. The same procedure applies while opening; you have to open it in the opposite direction. To conclude the video demonstration, we have discussed how to prepare bacterial culture media and how to prepare cotton blocks and autoclave them. During the culture weighing of the media, we have to make sure that the media components should not be exposed to air because those substances absorb moisture and become liquid. Another thing is that for cotton plug preparation, we have to take a single layer of cotton and then fold it.

After autoclaving, we should not release the pressure in a single shot; let it go and come to normal pressure, then we have to open the autoclave. Now, I'm sure you understand how you can prepare the microbiology media. The procedure for preparing microbiology media will be applicable to all the media where you are actually going to do the autoclaving. So when we talk about eukaryotic media, the simplest eukaryote is yeast. So these are some of the media that you can actually use for the yeast propagations.

So where you have the CSM media, you have a YPD, broth; we have the YPGal, and you also have the standard minimal media where you are actually going to have that. And as far as the composition is concerned for the CSM media, you are actually going to have the CSM at 0.

74 grams. Then you have the yeast nitrogen base, which is 6.6 grams. And you are going to have the ammonium sulfate, which is going to be 5 grams. You need to have glucose as a carbon source for 20 grams. And then you are also going to have agar, which is going to be a gelling agent. And this is what you are going to use to make the agar plate, which will be used for the growth of *Saccharomyces cerevisiae* and for preparing the competent salts. Similarly, we have the YPD broth, where you're going to have 10 grams of yeast extract, 20 grams of bacto-peptone, and 20 grams of dextrose.

And this is commonly used as a medium for the maintenance and propagation of *Pichia pastoris* and *S. cerevisiae*. Similarly, we have the YP-Gal. YP-Gal is actually a standing media for the *S.*

*cerevisiae* omitting the glucose repression. And it is going to have 10 grams of Bacto-East extract, 20 grams of Bacto-Peptones, 100 ml of 20% Galactose, and 50 grams of Agar. Then we have the standard minimal media or SD media, and there you are going to have 6.57 grams of yeast nitrogen bases with ammonium sulfate and without amino acids and sugar. This is required, so you're actually going to add these amino acids and nucleotides at a concentration of 50 micrograms per ml. And this is the base medium for the preparation of minimal and synthetically defined yeast media.

And how you're going to prepare is just going to be the same procedure that we have discussed for the microbiology media. You are actually going to take the media components, dissolve them into the 950 ml of water, and then autoclave. Autoclave allows the media to cool down to 50-60<sup>o</sup> C, and then you add 50 ml of filter-sterile 40% glucose so that the final concentration becomes 2% and adjust the volume to 1 liter if necessary. Remember that the glucose is very susceptible to degradation or charring, actually.

So we don't add the glucose, and then we do the autoclaving. We have to use the sterile glucose filter separately, and we have to prepare all other components from the yeast media, and then we have to add them according to our requirements. This is true not only for glucose, but for all other small nutrients like nucleotides, amino acids, and all that. Right. So all these, you know, the temperature-sensitive chemicals or the constituents we should add separately by doing the filter sterilization. Now let's move on to the slightly complex media, which is the mammalian cell culture media.

I have taken an example of the DMEM media, and there are so many media available for mammalian cell culture. You have RPMI media, DMEM media, MEM media, and so on. When you want to make the DMEM media, you are actually going to use the DMEM at 13.4 grams per liter.

Then you are going to add the sodium bicarbonate, which is from the 3.7 grams per liter solution. Then you are going to add the FBS, which is 10%, and you are going to add the antibiotic, which is 1%. You are not going to use or put the BS and the antibiotic at the beginning. So, how are you going to prepare the cell culture media? So add 13.

4 grams of dry powder to the water and mix it to dissolve completely. Then you add the 3.7 grams of sodium bicarbonate, mix completely, and adjust the pH to 6.9 to 7.1 using the NaOH or NaCl.

Finally, add cell culture-grade water to the media to bring it to the final volume. Sterilize the solution by using a sterilized membrane filter with a pore size of 0.22 microns.

Remember to use filter sterilization for all the cell culture media with the help of the 0.22 micron filter. Then you add the supplements, such as antibiotics and a serum, and then you can sterilize using aseptic conditions.

Since this is a very complicated process, I thought, why not take you to my lab where the students are actually going to explain to you how you are going to prepare the media, how you are going to assemble the filtration unit, and how you are going to prepare the filter-style media. In this video, we will demonstrate how to prepare mammalian cell culture media. For that purpose, we need Hi-Cell media, which is DMEM, Dulbecco's Modified Eagle's Medium, high glucose. And we need FBS (Fetal Bovine Serum).

And we need an antibiotic cocktail, comprised of streptomycin and ampicillin. The basal media provide inorganic materials and amino acids, which are required for the basic development of cells. And the FBS is used to provide both factors to the cell. We cannot autoclave this media because it might degrade its components.

For that purpose we use 0.22 micron filters. This is a 250 mL bottle top filter. Now I will demonstrate how to prepare filters for media. We have to pack it closely so that it doesn't allow any leakage, and after this, we have to put it in for autoclaving. This is an autoclavable bottle for filters. After we pack the filter, we have to keep it for autoclaving.

For that purpose, we use an indicator to check whether our filter has been autoclaved or not. When the lines on this clip turn black, it means that the filter has been autoclaved. In order to prepare media, we will now be adding the basal media to the already autoclaved double distilled water. We can use double-distilled water or Milli-Q water for that purpose. After adding media, we need to stick it on a magnetic stirrer for the components to dissolve completely. We can either use double-distilled water or millicube water, but double-distilled water is more preferable, as it contains more ions than millicube water.

After the media components have dissolved completely, we need to set the pH of the media. For that purpose, we can either use a pH meter or pH strips. In this case, we cannot use a pH meter as the bulk of the pH is sensitive to the media components and may get corroded. After the media components have been dissolved completely, we will be able to set the pH of the media. After the components of the media have been completely dissolved, we now need to adjust the pH of the media.

The bright red color indicates that the pH of the medium is in the range of 7.2 to 7.4. If the color of the media turns purple, then it indicates that the media is basic. If the color of the media turns yellow, it indicates that the media has become acidic.

Now we will check whether our media falls within the range of 7.2 to 7.4. After the media has been set, we now need to filter the media inside the biosafety cabinet, as we have added the constituents in their non-aseptic condition. After the media components have been

completely dissolved and the pH has been set, we now need to sterilize the media using a membrane filter. For that purpose, we use class II biosafety cabinets that are used for handling mammalian cell cultures. So this is a typical biosafety cabinet in which we perform the filtration of media.

This is the control panel that is used to operate this machine. This is the on-and-off switch. This is the switch for normal light. This is the switch for the UV light. Now I will demonstrate how to filter the media. Now we are going to filter the media.

For that purpose, we need a suction pump that can be connected to the bottle top filter. The suction pump is for extracting the air from the bottle top filter so that we can filter the media. Initially, we need to check the bottle top filter with less media to see if there is any leak or not. For that purpose, we are going to add around 50 to 100 ml of media. As we can see that there is no leakage in the filter, we can proceed with the filtration. After the media has been filtered, we now need to add FBS and antibiotics in order to make it complete media.

The complete media comprise serum, whereas the incomplete media do not contain serum. We are adding 100 ml of Fetal Bovine Serum in order to make it a 10% FBS-containing serum. With this, we have prepared 1 L of complete DMEM media comprising 10% FBS and 1% antibiotic solution. So far, we have seen how to prepare cell culture media for mammalian cells, although there are some precautions to be followed. Like when we set the pH of the media, we need to use pH strips instead of using the pH meter.

There are some components in the media that can clog the bulk of the pH meter and reduce its efficiency. Certainly, when we use the media, we need to thaw the media from 4 degrees to 27 degrees. But we need to thaw it first to room temperature and then to 27 degrees to avoid changes in the pH of the media. And also, if we are producing the media in larger quantities, we have to aliquot to transfer our requirements and then use it in order to avoid contamination and changes in the pH of the media. Thanks for watching the video. Once you prepare the filtered sterile media, you are actually going to check or take different types of precautions so that you do not prepare a medium that is good for the propagation of cell culture or propagation of mammalian cells.

So what you are going to do is take care of the pH. So pH should be between 6.9 and 7.4, and slightly acidic pH or slightly basic pH is going to destroy the cell. So if it is going to go beyond this, it is going to do. Then you also have to do the filtration at a very low speed. So you should not do a very fast filtration because if you do fast filtration, it is actually going to compromise the pore size of the membrane that you are using for the filtration.

And that may actually be responsible for bringing the bacteria present in the air. Right. And that's how it is actually going to be contaminated. Then, your serum, which you are going to use, has to be heat-inactivated so that it actually destroys the complement and other kinds

of factors, so that you do not kill yourself. Right. Remember that the serum actually has a lot of immunologically active molecules.

So you should perform the serum heat inactivation so that you can remove these immunologically active molecules. Then you have to add the antibiotics after you are done with all these filtrations, and they are going to add the sterile antibiotics. Once you are prepared with the media, you are going to use decontamination checking, right? Because it has to be done before you add this media to the mammalian cells. How are you going to do that? You are actually going to take a 10 mm dish, aren't you? And you are just going to add 10 ml of your media. Right? You're just going to take the 10 ml of media and put it in here.

Right? And then you have to cover this with a lid. Right? And then put it in the incubator for 24 hours. So then you put it in the incubator and let it remain there for 24 hours at 37°C. And if you do so, if there is a problem in your filtration, if there is a problem in your serum and all those kinds of things, you will actually see that the bacteria is propagating in your media. If the bacteria are not propagating for 24 hours or 48 hours, then that means that the media you have prepared is sterile and the media is good for your mammalian cell culture propagation. So this is the DMEM media that you are going to prepare. Remember that the media has to be prepared in a small aliquot so that if there is a problem, if there is contamination, it should not spoil your media, actually the complete media.

So you should not have prepared one liter of media. Instead, you can actually have four aliquots of 250 ml of media so that even if one aliquot becomes contaminated, you can discard it and use a fresh one. Now let's move on to the insect cell lines. So insect cell lines also require special media. So you actually require the different types of Grace's insect media.

You require Hink's TNM-FH insect media and insect IPL 41 insect media. Then you require the TC-100 and all of that, right? And the insect media are being used for propagating the insect cell line. And the insect cell lines are being used to propagate the Baculovirus. And that's how you are actually going to use it for protein production. So these are the compositions of these media, and these are the applications.

So, for example, Grace's insect media are being used for the growth of the Spodoptera cell line or SF9 and SF21 cell lines. Then you are also going to have Hink's insect media, which is going to be used for the culture of the Cabbage looper or the Trichoplusia ni cells. Then you also require this media, which is going to be used for the SF9 and SF21 cells. And then you are going to use the TC100 media which is for the propagation production of Baculovirus. And then you are also going to use these media for the mosquito cell lines, especially the Aedes. And then you also require Schneider's Drosophila media, which is going to be used for the in vitro culture of Drosophila melanogaster clusters.

So these are some of the cell culture media that you are going to use for the propagation of

microbiology media, like you are going to use for the growth of prokaryotic cells. And then you can actually use some of the eukaryotic media for propagating the yeast culture media, mammalian cell culture media, or insect cell lines. So, with this, I would like to conclude my lecture. Thank you.