Genetic Engineering- Theory and Applications
Professor Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati
Module 1
Introduction and Basics of Biological System
Lecture 1
Cellular Structure (Part 1)

Hello everybody. This is Doctor Vishal Trivedi from the department of bioscience, and bioengineering, IIT Guwahati. And in this course, we are going to discuss about the different processes as well as the underlining principle related to biotechnology.

So, the first question comes, what is the biotechnology? So biotechnology is the technological aspect, developed by the humans to exploit the living organisms for their own benefit, which includes the plants, the microorganisms, and the other small organisms. And the purpose of different aspects or purpose of different processes which are involved in biotechnology is, to exploit the different organisms or to increase the potential or different organisms by employing the biotechnology regulated principles.

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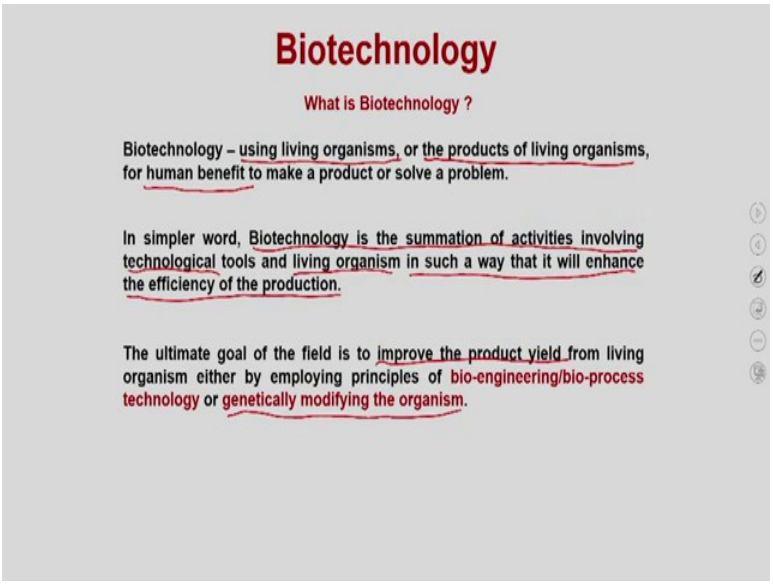

So let us see, in definition, what is mean by biotechnology is. Using the techniques you exploit the living organism or you enhance the product of the living organisms for the human benefit. So, it is a very important aspect of biotechnology that whatever the principle or the processes you use, we are going to use them to exploit or enhance the production of the different products, from the different organisms.

In simpler word what is mean by the biotechnology is, that biotechnology is the submission of the different activities which involving technological tools and the living organisms. So that is very important. Bio technology means that technological tools which are being used or which are being developed by the human being and they will be used on the, living organisms in such a way that it will enhance the efficiency of the production.

And as the purpose suggest, the ultimate goal of the biotechnology is to improve the product yield of the living organisms, either by employing the principle of bioengineering or bioprocess technology. Or in some cases when the bioprocess or bioengineering technology or processes are not capable enough to enhance the production, we also would like to genetically modifying the organisms. Now the question comes, when we say about the biotechnology, how many biotechnology products you are using in your daily life and whether you know any of the biotechnologists so far.

One of the classical examples of the biotechnology product is the curd, which we are preparing in our home, and that work is being done by one of the biotechnologists in your home that is your mother or the grandmother. And these people are doing this curd making from many generations and what they have been trained that, you take the small amount of

inoculum or the preformed curd and add it to the milk and then you wait for some hours and then the whatever inoculum you have added that will convert the milk into the card.

But what happened in many times that the curd what you are going to make is sometime is sour in taste or sometime it is in sweet in taste. So this difference in the curd making is mainly been because sometime you are adding more inoculum or sometime you are adding less inoculum.

In some cases you are actually forget to discontinue the fermentation process. And that is how the bacteria which is present in the curd is utilizing the sugar in the milk and it is producing the acid and that is making the curd very very sour. So the way the curd is being produced in our home, mostly been done simply by the experience instead of understanding the basic principle or the basic or the many factors which are governing the curd formation.

You can imagine that this kind of random behaviour or the random way in which you are making occurred is not going to work when you are actually making the curd in a dairy industry.

For example, if you are working in a company and you would like to develop the curd, you have to ensure that when the curd will reach to the customer, it will be always be of same taste, same texture, and the same quality and that would always be achieved simply by understanding the role of different factors which are involved in making the curd. And also you have to know how to modulate these factor in such a way that you will maintain the quality of that particular product.

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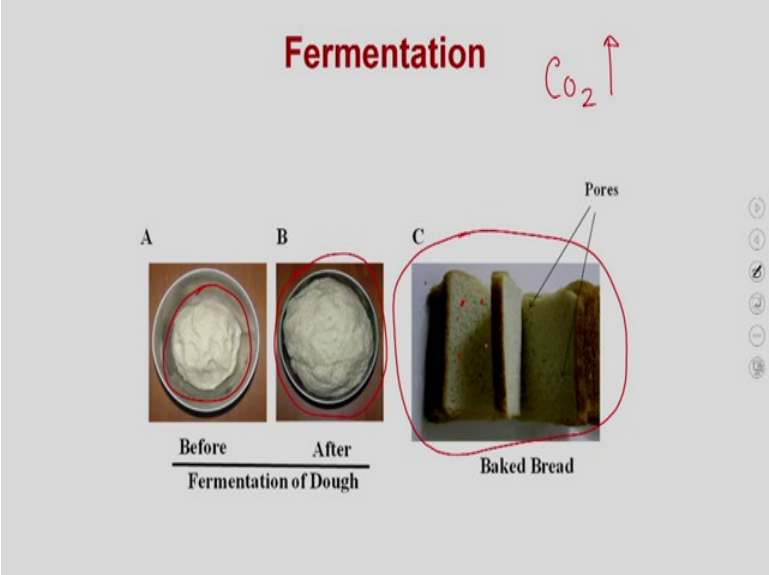

Now let us see the another product which you are using in your daily life. So this is actually a bread which you are using in your home. So how the bread is formed, the bread is formed simply by having a dough, which you are and to this dough you are adding the yeast and then you are letting this dough to incubate or remain as such for some time.

What happens is, when you add the yeast to this dough, the yeast is utilizing the glucose which is present inside the dough, and by utilizing the glucose, it is producing the carbon dioxide. And that carbon dioxide is a gas. So that carbon dioxide comes out from the dough, and that is how when it comes out, it actually makes the, bubbles inside the dough. And that is how the dough get swollen up, and as well as it becomes very fluffy and that is all you will see that there are small small holes inside the bread and that makes the bread very spongy as well as good in taste.

Apart from, making the different types of products, the main aspect of biotechnology is to improve the yield or the productivity of food grains from the plants as well as the food items from the different microorganism. Why the increase in productivity is important, because the human population is increasing.

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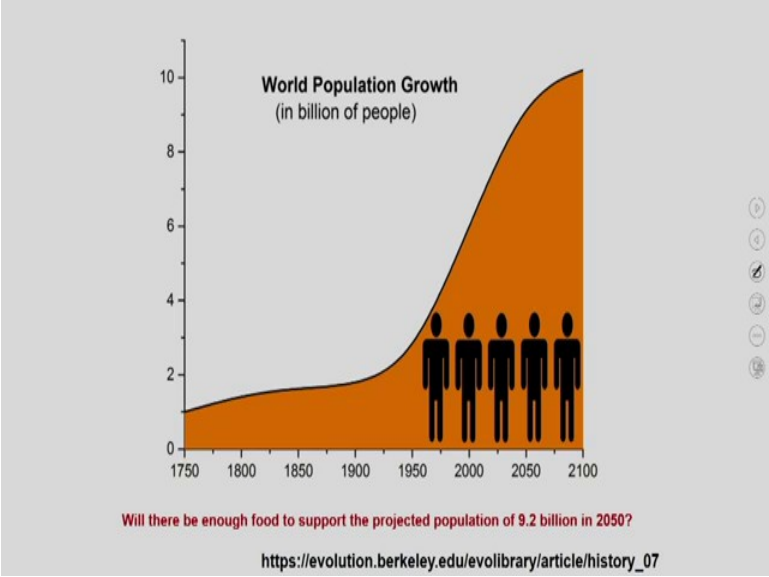

As you will see in this graph but we have shown that the human population is increasing from several hundred years, and what you see is the estimated human population is going to be 9.2 billion. This means, you know that the land is limited. So, you cannot increase the productivity simply by having the more and more land and more and more, farmlands.

So that is why you have to increase the productivity. And that is what the place where the biotechnology is going to play a crucial role. what it is going to do is you have to improve the crop yield, simply by either increasing the productivity or decreasing the losses. So, apart from increasing the product yield from the crop plants, the biotechnology is also having a significant role in many other aspects related to human being.

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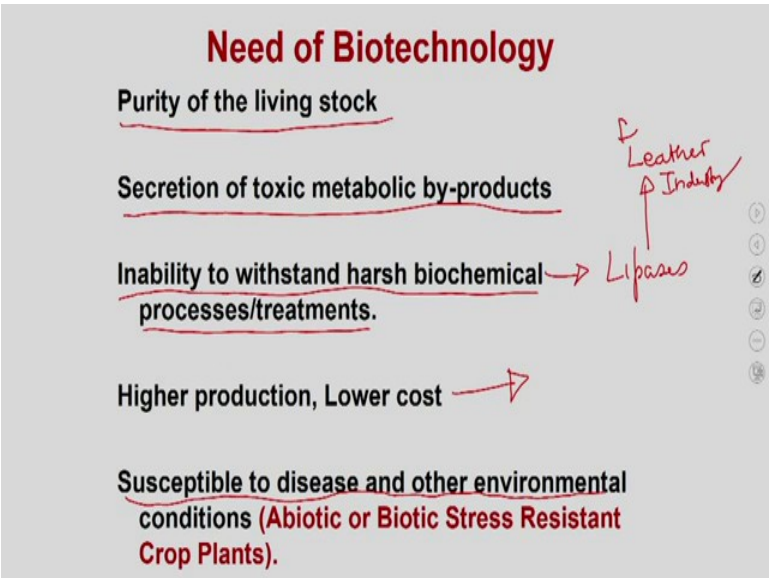

One of the major aspects in which the biotechnology is being used is to derive the pure living stocks. What happened is that when the different living organisms are growing or different living organisms are living together in a society, they are mating with each other and that is how they are making the strains impure or they are making the species impure.

Impure species are sometime acquiring a bad trade or sometime they are losing the good traits. And because of that it is important for us to develop the pure strains, and the advantage of pure strain is that you will know the quality as well as the features of these strains. And that is how you can actually mix the particular pure strain with the another particular pure strain to develop a unique, property into an off springs.

The other aspect is the secretion of toxic metabolic by-product. So, many of the plant as well as the micro organisms are the source of the, the different metabolites or they are being used as a source of developing the drugs. But what happened is that when they are being used as a source of different biotechnology related product, they are also having the metabolic reactions for developing the by-products. And sometime these by-products are toxic in nature.

So, with the help of biotechnology or the employing the biotechnology tools, what you can do is, you can increase the productivity of the desirable product and you can decrease the productivity of the toxic metabolic by-products. The third aspect is that, inability to withstand harsh biochemical processes or treatment.

What happened is in the biotechnology industry, or in some, industrial processes, you are using the different types of enzyme and these different types of enzyme are, has to withstand the different types of, biochemical processes or the harsh treatments. These harsh treatments are not good for these enzyme. That is why you have to develop the enzyme, which can withstand the, these harsh treatments.

One of the classical example in this category is the lipases. Lipases are the enzymes which are having the role in the, leather industry. So, if you develop a lipase, which is actually going to work in the leather industry, but the lipase are sensitive for the harsh treatment or the temperature which are being used in this particular industry. So because of this you can actually… if you genetically modify or if you develop a lipase which can work under these harsh conditions, you can actually increase or you can increase the productivity of that particular product from the industry. Then this aspect we have discussed in detail that you

can, the ultimate goal of the biotechnology is to increase the yield and once you increase the yield of the crop or once you increase the yield of the productivity from the crop as well as the microorganism, that will eventually going to lower down the cost of that particular product.

And then at the end you are also going to use the biotechnology to develop the crop plants which are going to be resistance for the abiotic or the biotic stress. How would that going to a help to the consumer is that, once you develop a crop, which is the resistance for the abiotic or the biotic stresses, it is going to withstand those climate conditions and that actually eventually going to increase the productivity.

One of the examples is that in the tropical country you are in African countries you have a very, very diversified weather. So for example, if you are going too much rain then that too much rain is going to clog the farmlands and that actually will reduce the availability of oxygen, which is available for the three or which is available for the crop to function. And because of that, many crops are going to die.

If you develop a crop which can withstand the water clogging or which can withstand these kinds of environmental conditions, then you can actually save these crop plants and you can actually eventually reduce the losses. On the other hand, you can also develop the crops which are resistant for the insects. So once the insect will bite, they will not be get affected by the insect biting. And that is how you can actually avoid the losses also.

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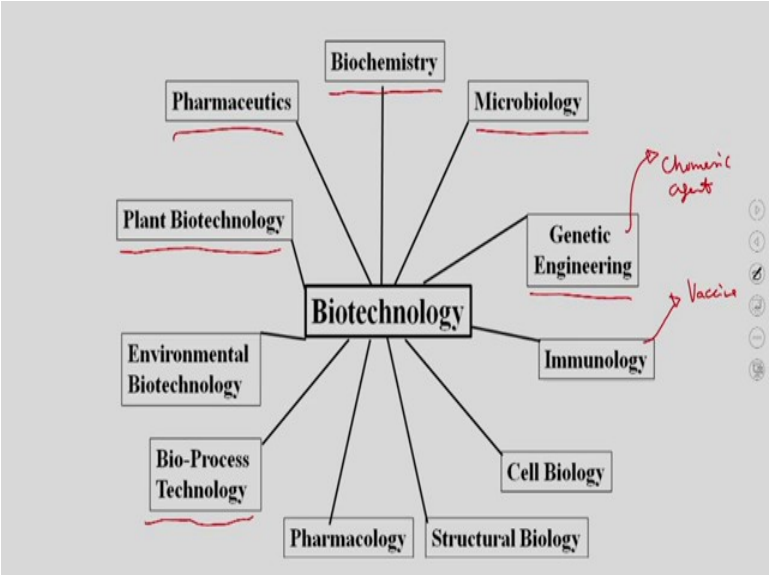

Since the biotechnology is having a complicated… is a science. It actually requires the inputs as well as the knowledge of different fields which are being taking part in developing the product. What are these fields?

You need to have a knowledge of biochemistry, microbiology, genetic engineering that genetic engineering knowledge can be used to develop the chimeric molecule or the immunology, which can be used to develop the vaccine or the cell biology structure, biology and pharmacology that will help you to develop the drugs which are important for the human being.

On the other hand, you also have to take the inputs from the environmental biotechnology. These environmental biotechnology inputs are important. While you are doing all these biotechnology related processes that should not affect the local environment or the global environment in such a way that it should not be a situation that these environment is going to be unsuitable for living, for human being as well as for the other animals.

Then you also need the inputs from the biotechnology as well as plant biotechnology as well as the pharmaceutics to develop the new or new drugs and you also need the inputs from the bioprocess technology. The bioprocess technology is going to help you to up regulate or down regulate a process in such a way that you do not have to compromise with the quality of the product as well as you will increase the yield or you will increase the productivity.

Since the biotechnology is a very very vast field, it did not develop in a single day. It requires continuous learning processes through which the humans have developed this particular field.

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Table 1: Important milestones of Biotechnology

S.No.	Time Period	Major break-through
1.	7000 BC-100CE	• Discovery of fermentation • Crop rotation as a mechanism to improve soil fertility. • Animal and plant products as a source of fertilizer and insecticide respectively.
2.	Pre-20th Century	• Identification of living cell and bacteria • Discovery of small pox vaccine, rabies vaccine. • Process development to separate cream from milk. • Discovery of artificial sweetners, "invertase". • Discovery of DNA and chromosome responsible for genetic traits.
3	20th Century	• Discovery of Pencillin. • 3-D Struture of DNA. • Fabrication of artificial limb and arms. • Production of human insulin in bacteria "Humulin". • Discovery of PCR. • Gene therapy. • Procedure for artificial insemination and test-tube baby. • Cloning of first mammal "Dolly".
4	21st Century	• Vertebrate, invertebrate and bacterial genome sequences. • Completion of Human Genome sequence. • Sequencing of Rice genome. • Discovery of Nano radio. • Invention of Bionic leg.

And because of that, the biotechnology has many applications and the humans have learned the different processes as well as the principles related to biotechnology to achieve as well as to improve the, product from the living organisms.

What are these milestones? One of the initial discovery by the humans is that they have discovered the process of fermentation. The classical example indicates of fermentation is they have developed the alcohol and they have developed how to make curd, and that actually has been the first documented discovery of the related to biotechnology.

After that they started absorbing very minutely the development of different crops as well as their productivity and what they have noticed that once they rotate a particular crop in a particular combination that actually give them the more food grains. One such example is that if you grow a leguminous plant versus any other crop. So, what they have found if they rotate a crop with the leguminous crop, the productivity of the land remains intact.

At present, we know the reason behind this particular kind of observation, but that time the people were not being able to understand why it is happening. But as a practice they were making a crop rotation and they were growing the leguminous plant followed by the non-leguminous plant.

Then to improve further the, the productivity from the land they started using the animal as well as the plant product as a source of fertilizers, and this information or the knowledge we are still using before increasing the productivity from these plants.

Then in the pre 20th century, they have started developing the living cells as well as they started identifying the bacteria. This actually helped them to utilize these living cells or bacteria for different types of applications. For example, they started developing the bacteria so they can use that for making the curd or other kind of fermentation products.

Then they discovered the different types of vaccines, like polio vaccine or the small pox vaccines or the rabies vaccines. These vaccines have given the protection to the human being against the diseases and that is how they were actually reducing the loss of life by death and they were also giving the immunity to the living beings and that is how it helps to the human being in many ways.

Then they have developed a process to separate the cream from the milk. This is actually important discovery because by this time they were started developing the cream from the milk and as a result they have started making the ghee as a biotechnology product as well as the other vegetable oils.

Then they discovered the artificial sweetener invertase. By the end of the pre 20th century they also could have understood that the DNA is the genetic material and that how they were started, the mutual interaction between the two different species and how that helped in developing the off springs and how the genetic information from one generation goes into another generation.

But with the beginning of 20th century, the first thing what they had developed is they are asked to probably not Pencillin. Pencillin you all know that pencillin is a molecule which is been used to kill the bacteria, and pencillin discovery or we all know what is the story behind the, how the pencillin has been discovered and all that. And then they also saw that 3-D structure of DNA that could have helped and to understand the molecular basis of different processes.

For example, transcription as well as that translation and the underlining mechanism, how these processes are working, how one DNA is different from the other form of DNA and so on. Then they developed the artificial limbs and arms. So once they develop the artificial limbs and arms, those... It was becoming a very very important aspect because those who were losing the limbs or the arm, these artificial limbs and arms, were giving them the hope that they could be able to walk or they could be able to work independently.

Then comes insulin, so with this they have developed an insulin in bacteria. Before this people were using the insulin but that was directly isolated from the other animals such as pig and with this they have started making insulin in bacteria and that actually helped them to take care of the disease which is known as diabetes.

By 20th century in the middle of 20th century they started, they also have developed the PCR. With the discovery of PCR they have developed a diagnostic tools and the PCR is allowed them to identify different infectious organism such as HIV, hepatitis and all other kinds of infectious organisms, and PCR has also enabled them to use this particular technique to identify the contamination of microorganism as well as to see whether a particular biological sample is a pure or it is contaminated with something.

And then they also did the gene therapy or they have developed the principles, how to use the, the gene therapy aspects. And then they have developed the artificial insemination as well as the test tube baby.

The artificial insemination as well as the test tube baby has revolutionized the field of the OB gyn and that actually helped to the couples who are not being able to have their own kids. And because of these particular two techniques, they could be able to do the artificial fertilization as well as they would be able to prepare the baby in a test tube and then they can implant that baby into the womb of a woman. And that is how the people were getting their own babies. And then by the end of 20th century, the humans have developed the first animal and that is called as Dolly.

So, that Dolly is being developed completely under the in vitro as well as the artificial conditions. By 21st century, the focus has been gone towards the genomic era, and then they have started making the discovery or the progress in terms of making the, different, genomic sequences and they started sequencing the whole genome from vertebrates, invertebrates, bacteria, humans, and what is the advantage of these genomic sequences is that this will allow you to identify the different doc targets.

This will allow you to understand the complex metabolic reactions. This will allow you to interplay of different proteins or different pathways in operating within the within the particular organism. It will also help you to understand the particular metabolic pathway and how this particular metabolic pathway is affecting the production as well as the downstream effects in a particular organism.

So with the discovery of genomic sequences, the humans have acquired the ability to modify as well as to change the, organisms in such a way that it will increase its productivity and it will be more useful for the human beings.

Ultimately, they also did the sequencing of rise genome. So with the sequencing of rise genome, they have done the same thing. What they have understood is that, what are the genes are important for the different types of abiotic as well as the biotech stresses in the rice and how they can manipulate to improve the crop as well as its quality as well as its productivity.

Then they have this also discovered the nano radios or other nano devices and then they have discovered bionic legs and they are also… this development is or the biotechnology development is a continuous process and it is still continuing and people are developing the stem cell therapy and downstream many things actually.

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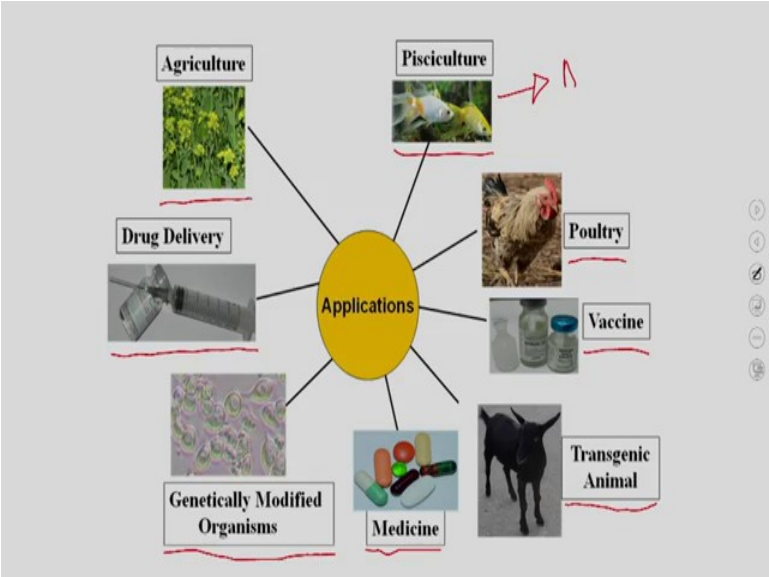

So, actually the biotechnology has application in diversified fields. It has an application in the pisciculture where you can actually increase the productivity of fishers in a pond. Moreover with the genetic injury as well as the other kind of biotechnology, principles. You can also develop the ornamental fishes and that actually will increase the demand of these fishes in the market.

Then you can also contribute in terms of poultry with the help of vaccines and with the help of different types of diagnostic tools, you can actually control the diseases in the poultry farms, and that is how you can increase the productivity.

Earlier what happens is if you have any disease or if, if you have any infection in the poultry, it spreads very fast into the whole colony and that is how you are going to have the losses. then they have developed the different types of vaccine, which we have already discovered.

Then they have developed the transgenic animals, which have a role in providing the new traits to the existing animals, medicines anywhere they have discovered because with the knowledge of the different types of drug targets, as well as the bioactive molecule present in plants, they have been able to develop different types of medicines for, for taking care of the, diseases.

The genetically modified organisms, so with the help of recombinant DNA technology as well as the other biotechnology aspects, they are being able to genetically modify a particular organisms and lately they have also having the applications in the field of drug delivery because it is not important that you have a drug. It is also important that you deliver the drug to a particular site of action so that it would work more optimally. And then at the end, the biotechnology also has an application in the field of agriculture so that you can increase the productivity from the crops.

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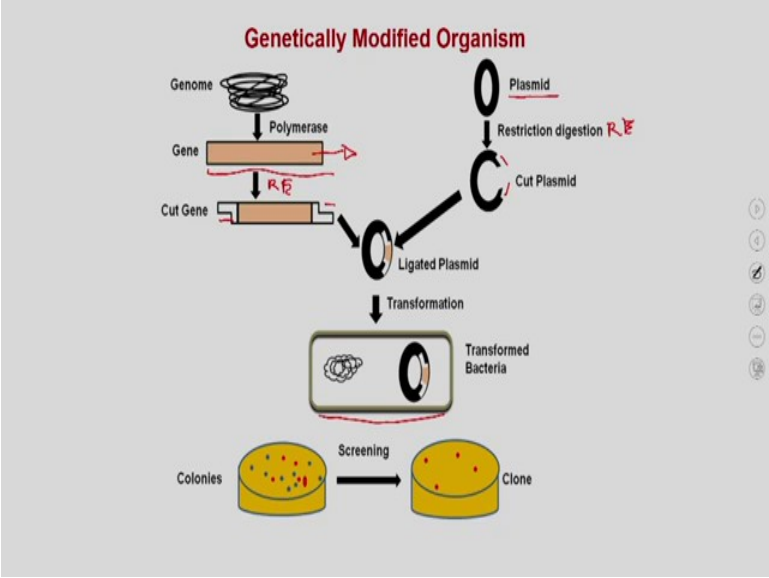

In a typical, biotechnology protocol, what you have is, you are actually developing a genetically modified organisms or you are actually going to improve the existing organisms. How that happens is that, I will take you to this processes by one of the example. How you do that is that if particular organism has a genome, then what you do is you do a PCR reactions and that PCR reaction is going to give you the DNA which is corresponding to that particular

gene which you would like to introduce into the new organism and this particular gene is important for giving a specific trait.

Then what you do is, you take this gene which is actually a PCR product and then you cut this with a set of specific restriction enzymes. So once you cut this with a specific restriction enzyme, it is going to develop the sticky ends and these are the sticky ends on both the sides are going to be used for the downstream applications.

Similarly, you are going to take the plasmid, this plasmid you are going to use and then you also going to perform the restriction digestion of this plasmid as well as with the same set of restriction enzymes, and as a result, the plasmid is also going to have the sticky ends. And then what you do is you take this plasmid, the cut plasmid as well as the gene, put them together and put it for the ligation reaction. As a result of ligation, what you are going to develop is chimeric plasmid, these chimeric plasmids.

Then you are going to transform into the bacteria or the other organisms which you are interested to modify. And as a result, what you are going to get at the end or the result of this transformation is that you are going to get new or the new organisms with the additional trade which is being provided by this particular gene.

So, by following this kind of schematic pathway, or by following this kind of reactions you could be able to modify this particular organism and you could be able to develop a new genetically modified organisms.

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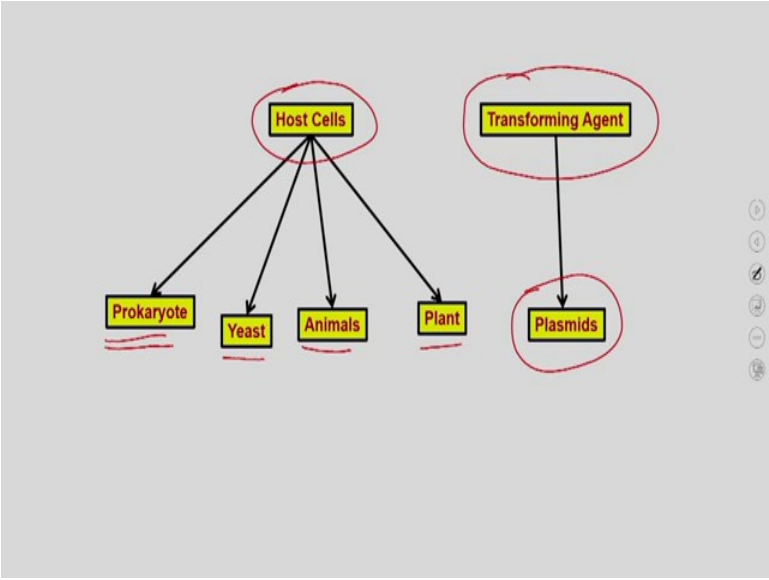

For this particular kind of protocol, or for this kind of schemes what you need is, you need two different objects. What you need is, you need the host cell, the host cell, which you are going to use for modifications. These host cell could be prokaryotes, it could be yeast, it could be animals, or it could be plant species, the plant which you are interested to modify, and then what you need is the transforming agents.

These transforming agents could be the plasmid, the plasmid, which are corresponding to that particular host cells. So there are plasmids which are propagating in prokaryotes. There are plasmids which are specifically for animal or the plant.

So, what you have to use is you have to take the plasmid of the corresponding, host. So to understand this scheme as well as to the plan, we have to understand this host as well as these transforming agents separately.

So, let us start with the host cell, and first we will understand how you will use prokaryotes cell or under what condition you will use the eukaryotic cells, which includes yeast animal as well as the plant.

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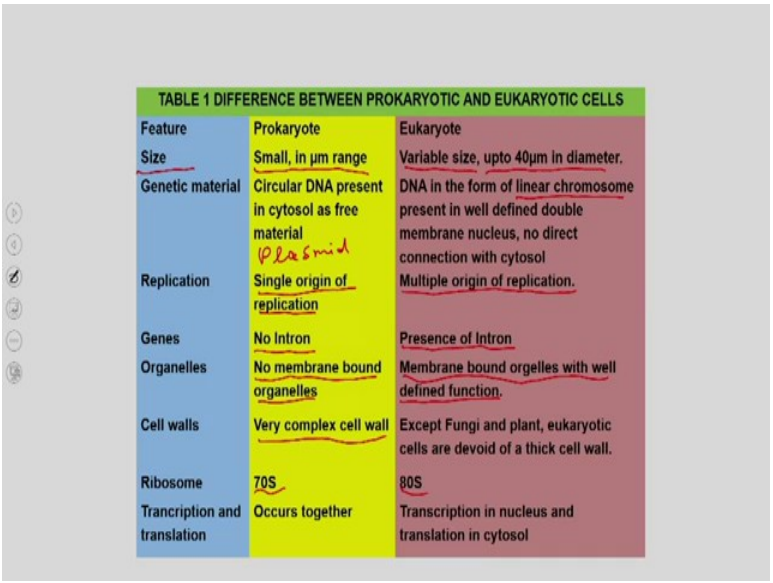

So the prokaryotic as well as the eukaryotic cells have the distinguish and the significant differences and these differences are important to understand so that you could be able to exploit these differences and you could be able to choose the suitable host for your downstream applications.

What are these differences? These differences are that first of all, one of the initial differences is that prokaryotes are small in size. They are in the micrometre range, whereas the eukaryotes are of variable size , some eukaryotes are, have 10 micron meters, some eukaryote cells are 30 micro meter and so on.

So, they could be in the larger in size. Then for the genetic material the prokaryotes are having the single chromosome of, single chromosomes, circular chromosome and whereas, the eukaryotes are having the DNA in the form of linear chromosome and these chromosomes are arranged and inside a double membrane organelles that if called as nucleus. Apart from the circular DNA, the genetic material as a genetic material, the prokaryotes will also have the extra chromosomal DNA and that is called as the plasmid.

As far as the application is concerned, the prokaryotes are replicating with the single origin of replication, which means that the Chromosomal DNA have the single origin of replication and using that origin of the application, it is actually synthesizing the whole genome. Whereas in the case of eukaryotes you have the multiple origin of replications and that is important because once you have the multiple origin of replication, you could be able to complete the replication in a very fast processes

Prokaryotes gene do not contain the introns. We will discuss about the introns in subsequent lectures. So introns are actually the non-coding region presenting the genome. So these regions are not present in the case of prokaryotes, but these genes or these regions are present in the genes of the eukaryotic system.

Then Organelles, there is no organelle membrane bound organelles present in the in the case of prokaryotes, whereas the membrane bound organelles are present in the case of eukaryotes. This also has a significant, advantage to the eukaryotic system that they have the membrane bound organelles, and that also we will discuss in a subsequent lectures, the relevance of having a membrane bound organelles. Then cell wall is very very complex cell wall, which is present in the prokaryotic system, whereas in the eukaryotic system, except the plant and the fungi, the cell wall is not present in any of the eukaryotic system.

The Ribosome which is actually the protein production machinery is present in the case of… is presenting the prokaryotes, which is 70S, the ribosome is of 80S in the case of eukaryotes and the transcription as well as the translation occurs together in the case of prokaryotes,

whereas it occurs separately in the case of eukaryotes because the transcription occurs in nucleus, whereas the translation occurs in Cytosol.

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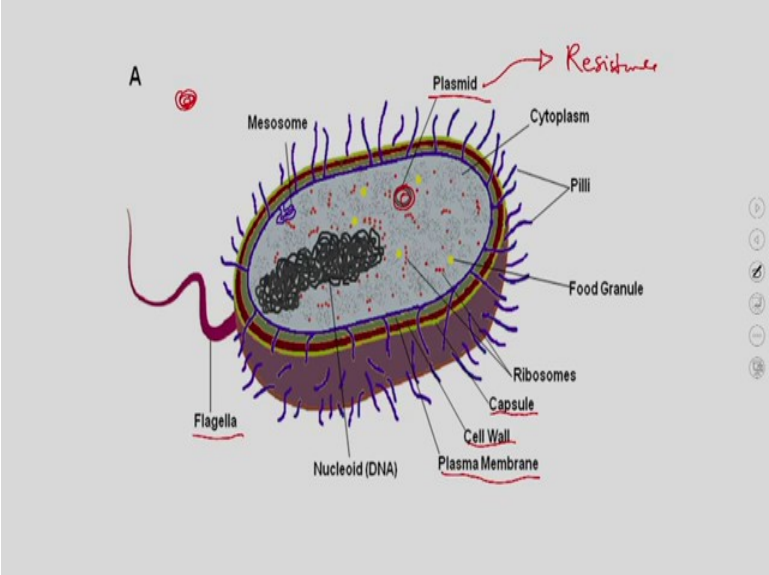

So, let us understand the structure of a bacteria, which actually contains the three different layers. What you have is the outside capsule, then a capsule followed by the cell wall and then below the cell wall you have the plasma membrane. All these, three layers are important for the bacteria to be protected from the, changes, in the micro environment.

For example, if you keep a bacteria in water, which is actually going to be the hypertonic, these layers are going to protect the bacteria and they will not allow the loss of water from the bacteria. Capsule is made up of polysaccharides and it is actually impermeable to the water, whereas the cell wall is made up of different types of lipids as well as different types of material that we are anyway going to discuss in a subsequent slide.

The plasma membrane is a typical plasma membrane. It is the semipermeable plasma membrane, which is presented inside and the plasma membrane is made up of lipids as well as the proteins. Inside the cell what you have is the cytosol as well as the genomic DNA. The genomic DNA is present in the form of a single chromosome. Apart from the Genomic DNA, you also have the extra chromosomal material, which is called as the plasmid.

These plasmids are being exchanged between the different bacterial species for many purposes. For example, these plasmids are actually being used to provide the resistance, against the antibiotics. So what happen is suppose some bacteria has the ampicillin resistance by using these plasmids, they can actually exchange these plasmids between the two

organisms and that is how you are actually increasing the number of bacteria which are actually going to harbor this particular plasmid and will have the antibiotic resistance in due course.

Similarly, you will also can think about that some of the plasmids are also having the other kinds of features and so bacteria are normally uses these plasmids to exchange and to spread their particular property.

Apart from this, you also have the flagella, which is actually attached to the cell body through, to the bacteria. These flagellas are made up of the flagellin protein and the purpose of these flagella is to provide the swimming effect or with the with the help of the Flagella, the bacteria can move from one place to another place.

You can imagine a situation that if you add a food particle, for example, if you add the glucose, the glucose molecule will attract the bacteria and bacteria has to take that glucose molecule. So in that process, what will happen is the flagella will move and the bacteria will go to that process to that particular glucose molecule with the process known as the Chemotaxes.

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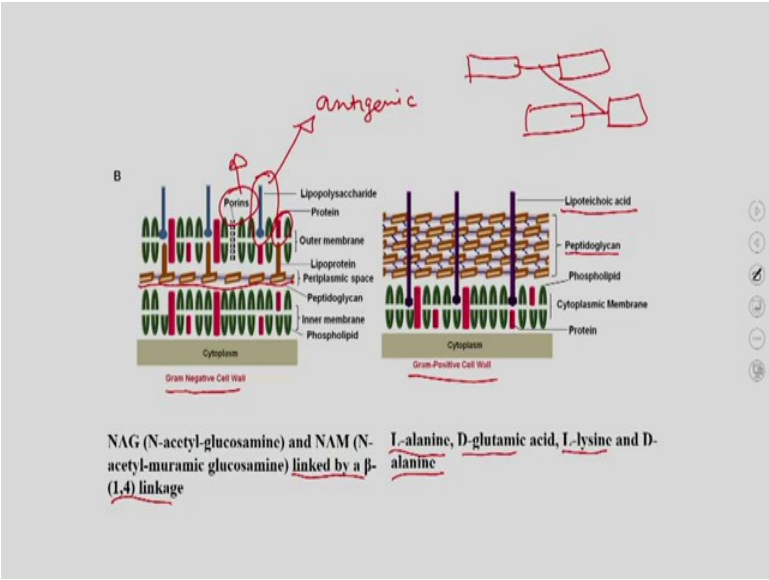

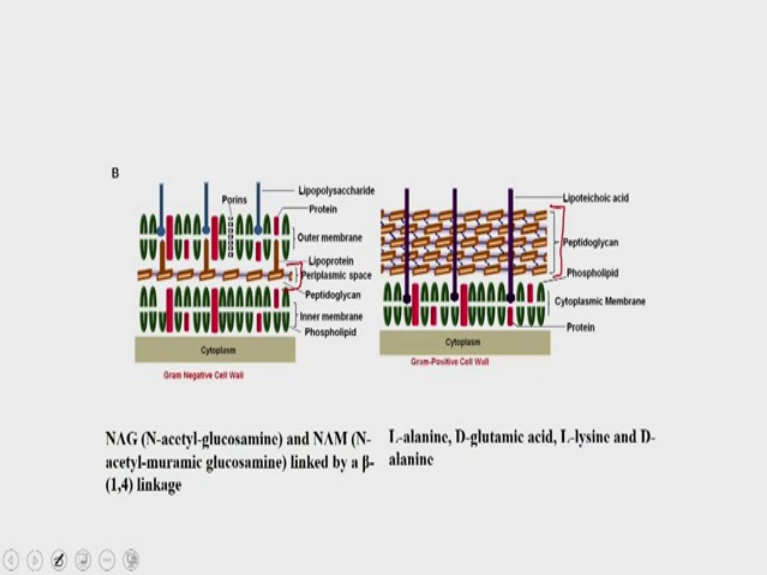

So apart from this they also have very, very complex cell wall. These cell walls are made up of different bio molecules and the cells based on these cell walls, the bacteria are divided into two groups. One is called gram negative bacteria, and the other one is called as gram positive bacteria.

So, in the gram positive bacteria, what you have is a thick layer of peptidoglycan. This peptidoglycan is actually a complex biomolecule where you have the alternate arrangement of the sugar molecule that is called an N Acetyl glucosamine as well as the N Acetyl Muramic Acid and these N Acetyl glucosamine it or N Acetyl Muramic Acid are linked by a chain of Beta one four linkage.

So, you can imagine that the NAM as well as the NAG are connected to each other and these polymers are running towards the peptidoglycan chain, and then these NAM or the NAG polymers are being linked by each other. So one layer is this one, another layer is this, like this and all these are being connected by a peptide chain.

These peptide chain is made up of four amino acid, that is L- alanine, D Glutamic acid, L lysine and D Alanine. So, because of this interchange peptide bond, the peptidoglycan is giving a very, very strong a hold to the cell wall, and because of this thick layer of peptidoglycan, the gram positive bacteria are more resistant for the osmotic changes in the environment.

Next to the peptidoglycan layer, you have phospholipid, and then you also have the lipoteichoic acid in the case of gram positive bacteria. Whereas in the case of gram negative bacteria, as you can see, it has a very very thin layer of the peptidoglycan and below to this

you have the plasma membrane and you also have the LPS, which is present in the ground negative bacteria. These LPS is a source of the antigenic reaction, to the host.

So, with the help of these LPS, which is present on the surface of the cell wall, the bacteria are inducing the immune cells in the host and causing the downstream immune reactions. You also have the different types of protein which are present in the cell wall. You also have the porin, the purpose of the porin is to take up the nutritional molecule from the microenvironment or the place where the bacteria is present.

Porin is also important for taking up the water molecules and below to the peptidoglycan layer you also have the plasma membrane and then you also have in this plasma membrane, you have the lipid as well as the protein molecules.

What we were discussing, we were discussing about the different types of cell wall, which is present in the gram positive as well as the gram negative bacteria and in this discussion, what we have discussed, we have discussed about the different or the contrasting feature between the cell wall of a gram-positive as well as the gram negative bacteria.

So these contrasting features can be exploited because in the case of gram positive bacteria, you have a thick layer of peptidoglycan, whereas in the case of the gram negative bacteria, you have a very very small peptidoglycan layer. And these differences could be exploited by the stains which can work on the presence of the peptidoglycan layer and these strains could be used to distinguish between the gram positive bacteria versus the gram negative bacteria.

In this context, one of the popular stain which has been developed by a Danish scientists known as the gram stain and this sustaining procedure is called as the Gram staining.

So let us discuss about the gram staining and how the gram staining procedure can be used to distinguish between that gram positive bacteria versus the gram negative bacteria.

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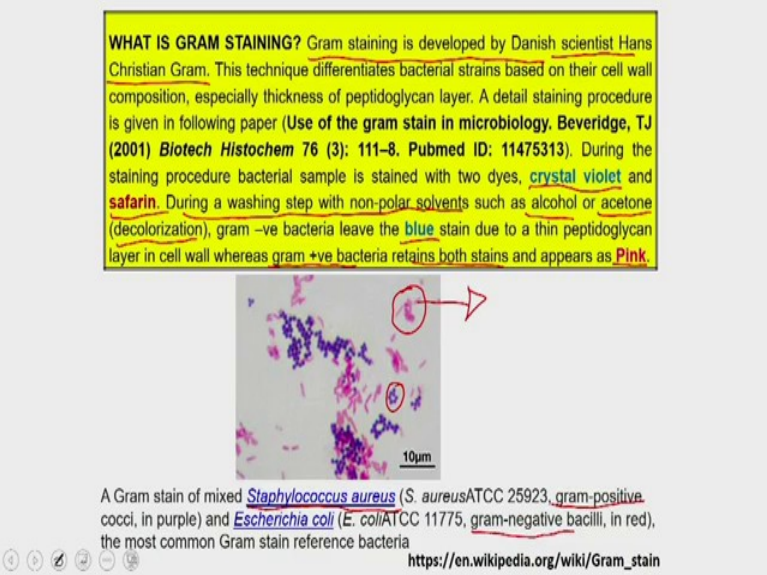

So the gram staining, gram staining is a technique which is developed by a Danish scientists known as the Hans Christian gram, and in this technique, the bacterial strains are going to be stained by the two different dyes. One is called as the crystal violet, which is a blue or violet in colour, whereas the other one is called as the Safarin, which is actually the red or the pink in colour.

So, in this procedure, what you do is you make a thin layer of or thin smear of the bacterial cell and then you stained with the gram stain, which is actually a mixture of crystal violet and the safarin and then during a washing step, what you do is once staining is over, then you wash the a stain with a non-polar solvent.

These non-polar solvent such as the alcohol or the acetone can be used and that actually will remove the stain from the bacteria. And what happened is in the case of gram negative bacteria, which actually containing the very thin layer of a peptidoglycan layer, it actually going to leave the blue stain, which means the gram negative bacteria is going to appear as red. Whereas the gram positive bacteria is having a thick layer of peptidoglycan layer, and because of that, it actually stains, retains the both stain and the, it appears as the, a dark pink or the purple in colour.

So as you can see, this is a, is this is a mixture of Staphylococcus Aureus, and the E Coli. So Staphylococcus Aureus is a gram positive bacteria, whereas E Coli is a gram negative bacteria, and both are these bacterias or bacilli are mixed in this particular sample, and then it will be stained by the gram stain.

And after the discoloration step, what you can see is that the gram positive bacteria, which is the Staphylococcus Aureus, is taking up both the stain and appearing as the purple, whereas the E Coli which is actually leaving the blue colour, is appearing as the red in colour. So these are actually the, E Coli cells which are appearing in red, whereas these are purple colour.

The Coli or Bacilli are appearing from the gram positive bacteria that is Staphylococcus Aureus. So the gram staining is a very, very popular technique to distinguish between the gram positive as well as the gram negative bacteria and using this particular stain the people have classified different bacterial strain, either it is a gram positive bacteria or the gram negative bacteria.

So, with this we would like to conclude our lecture here. And, what we have discussed in this lecture is we have discussed about the basic contribution of the biotechnology in the welfare of the human being. We have also discussed about the different types of products, what has been developed and how the biotechnology as a field, is been evolved from last several hundred years.

And we have an in continuation to that we were now discussing about how to generate the genetically modified organisms. And in that context, we were studying the host strain and we have to study the transforming agents. So, within the host strain we have so far discussed about the prokaryotic cells.

We have discussed about the structure of a typical bacteria, and then we have also start this study about the cell wall, which is either from the gram positive cell wall or the gram negative bacteria cell wall.

Then we have at the end we have also discussed about the gram staining which actually been used to distinguish between the two different types of bacteria and Gram staining is utilizing the fact that it actually works on the level, the thickness of the peptidoglycan layer and subsequent to this lecture we are also going to discuss about the, the different hosts cell that is the eukaryotic cells.

So in that discussion we are going to discuss about the different types of organelles which are present in the eukaryotic cell and if you remember while I was discussing about the prokaryotic cell, I have mentioned that the eukaryotic cells are containing the membrane bound organelles.

So, we are also going to discuss what is the advantage of having a membrane bound organelles present in the eukaryotic cells? And the eukaryotic cell could be unicellular such as the yeast or eukaryotic cell could be multicellular such as the plant or the animal. And at the end we are also going to discuss about the differences between a plant or the animal cell.

And subsequent to this lecture, we are also going to discuss how to separate the different organelles which are present in the eukaryotic or eukaryotic organelles. And we are also going to discuss the different fractions, which you can isolate from the bacterial cells for the protein production. So, with this I would like to conclude our lecture here and in the subsequent lecture we will discuss about d, eukaryotic host cells. Thank you.