Plant Cell Bioprocessing
Dr. Smita Srivastava
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture – 20
Biotransformation in plant cultures

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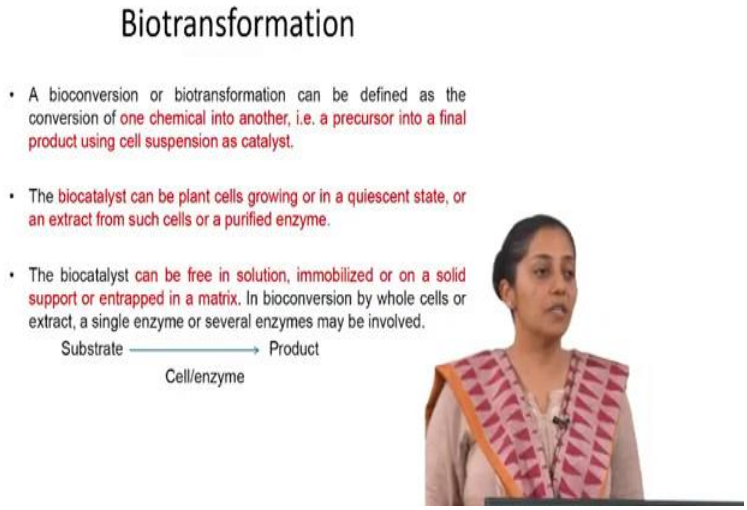

So, today we are going to discuss about Biotransformation and plant cell immobilization and its applications. So, starting with biotransformation; what is biotransformation to you? Biotransformation is done using any living organism, you must have already read about it. So, what is biotransformation? How is it different from de novo production using bioprocesses? So, majorly what kind of conversions come under biotransformation? The question was how it is different from de novo synthesis? What is de novo synthesis?

From the beginning. So, a will get converted to b, b will get converted to c and this may not be structurally related. They can be n number of conversions with other intermediates more than one which will combine together to give you the product moiety. Now, how is bioconversion where is it placed in this synthesis? So, generally changes in the functional group, addition of certain moieties in the parent molecule. So, these class of reactions come under biotransformation.

Sometimes a low cost substrate can get transformed to give you a high cost product; now for example, glycosylation in the molecules or demethylation or methylation. So, when we say low cost to a higher cost which means sometimes the lead molecule may be suffering from problems like not soluble in water or toxic. But by biotransformation, the chemical structure is changed such that the functional moieties are changed such that either by replacement or displacement or additions you get rid of these limitations.

So, now when in plant cell technology, the whole cell the plant cell has a rich repository of enzymes. So, generally in biotransformations these reactions are carried out. So, what will be involved in all these reactions?

Student: Enzyme.

Enzymes. So, either you have a purified enzyme and then carry out these reactions or you use a rich repository of enzymes. If there are more than one steps involved in this complete biotransformation then rather than using a package of purified enzymes or a single enzyme you can use the whole cell which is nothing, but a rich repository of a number of enzymes dehydrogenases, hydrolysis then what else, redox reactions. So, you have so many different kinds of enzymes which can bring about these changes.

So, what is it, the one chemical is changed into another where the precursor is converted into a product using cell suspension or your biocatalyst. Now, the biocatalyst can be plant cells growing or in quiescent state which means resting cells, they either can be in the active phase or can be the resting cells. So, why it is linked to plant cell immobilization you will be able to understand when I say if it is in the quiescent state, how we can exploit this at large scale.

So, the biocatalyst can be free in solution if it is possible, or immobilized or on a solid support as a film or entrapped which is immobilization in a matrix. Or even you will say there are different kinds of immobilization we will be talking about it, either surface immobilization or embedding it or encapsulating it; so, there are different ways.

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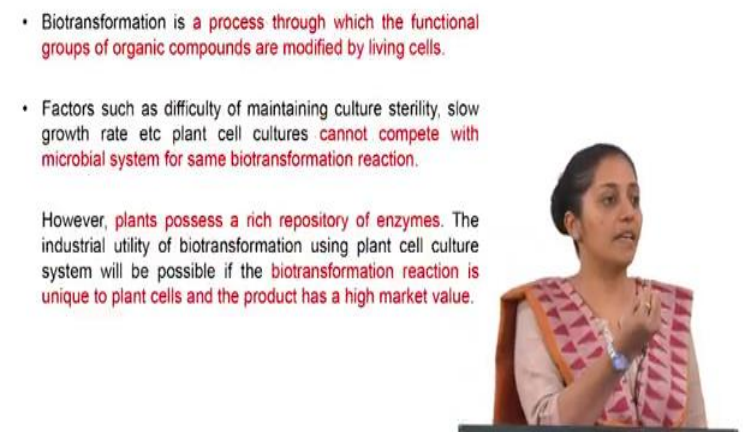

So, again reiterating; what is biotransformation? Biotransformation is a process through which the functional groups of organic compounds are modified by the living organisms. Now, why do living organisms do that, they need to do it not everything is de novo synthesized by the cells. So, what can be the different reasons? Why the cells are doing this? Defense, what else. So, which means to overcome either toxicity or to convert.

So, stress is one form. Other reason can be in order to incorporate and utilize somewhere else; that is, in other metabolism. So, that can also be a reason for biotransformation. So, factors such as difficulty of maintaining culture sterility, slow growth rate etc in plant cell cultures cannot compete with microbial systems for same biotransformation process.

So, we know the limitations of plant cell cultures, they are slow growing. At large scale imagine heterogeneity and scale up can be an issue. Generally, at high cell densities they will have non-Newtonian behaviour. So, the scale up becomes limited.

So, then how high cell density can be obtained? Cell immobilization. You can still have higher cell density, but you can avoid shear sensitivity in comparison to microbes, which can be a problem, when you are using free cells. Mass transfer limitations because they tend to aggregate together, the cell to cell contact is high that can be a problem.

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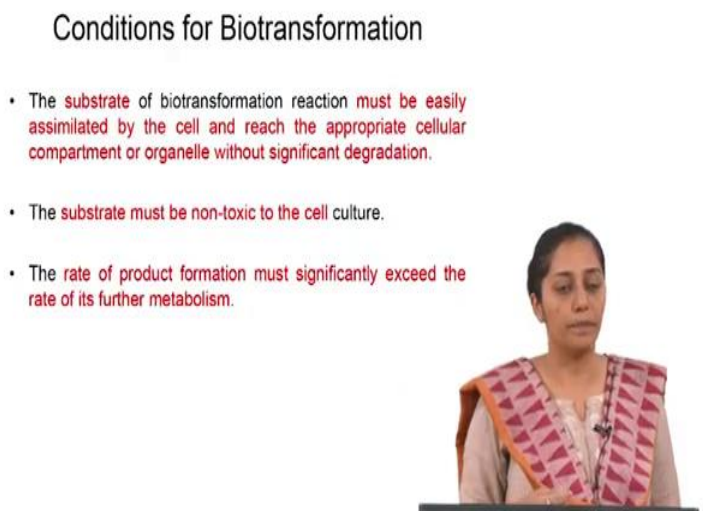

Now, what is a key here, whatever process you use for biotransformation? The substrate has to be made available to the cell. So, which means that the substrate has to enter into the cell, gets biotransformed into the product and the product should be able to come out in the solution. So, that is a prerequisite. Now the substrate must be therefore taken in and converted. It is not necessary that all time the substrate will be taken in.

In detoxification reactions maybe there can be enzymes which can be exuded which are used in biotransforming the toxic metabolite into a non toxic form. So, but generally in case of multiple step biotransformation reactions, the precursor moiety is to be taken in by the cell and then biotransformed into the final product. Now, what is key there? It is that the precursor itself should not be toxic to the cells, when it been taken in by the cells.

Sometimes what happens you will see that some of the precursors when they are produced intracellularly they are not toxic or the same precursors when given exogenously are toxic. So, how is that possible? The cells do not die because we know biosynthetic pathway that precursor is getting formed, but if you will add that precursor exogenously they become toxic to the cells. So, how is the cell then synthesizing and managing? Very nice so, there are enzymes; so, this is also class of biotransformation to prevent itself from the toxic effect of that metabolite.

So, it is formed and either it is biotransformed to make it to detoxify it or it is and then it is stored at a suitable position with the enzyme nearby. So, that when the need arises the enzyme can convert it into the required metabolite. What other prerequisite? The rate of product formation must significantly exceed the rate of further metabolism then only it is advantageous.

If you want a desired biotransformation reaction to happen then the rate of formation has to be more than the rate of its subsequent metabolism, if the cell does not need it has to be metabolized or it has to be leached out. So, the rate of transfer of the product outside then becomes more limiting than the rate of biotransformation for the success of such process or its further metabolism rate.

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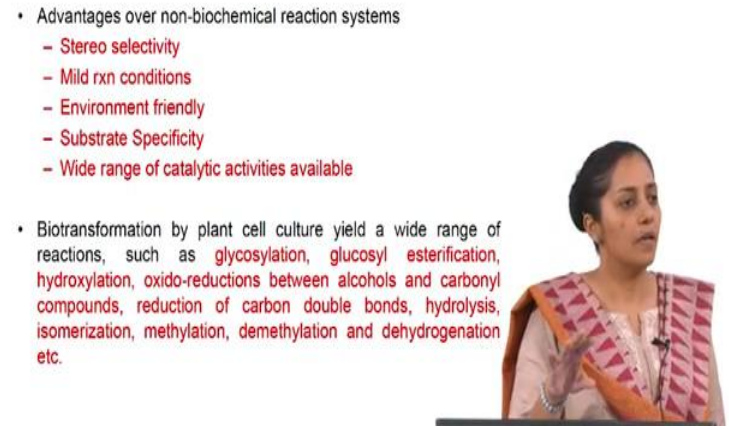

Now, what are the advantages of biotransformations over chemical transformations? What are the advantages? This is; this you must have already read when we use enzymes, how are enzymatic reactions better than chemical reactions. So, high specificity means what? There is stereo-specificity and regio-specificity, I hope you understand.

So, enzymes give you the leverage, enzymes provide you stereo-specificity and regio-specificity and at the same time milder conditions, lesser toxic byproducts. So, they are more environmental friendly processes. Wide range of biotransformation reactions can

be carried out using plant cells as repositories or plant cell enzymes as bio-transformants, but here we are talking about plant cell as it is as repository of enzymes.

So, what kind of reactions for example, glycosylation, glucosyl esterification, hydroxylation, oxido-reduction between alcohols and carbonyl compounds, then reduction of carbon double bonds saturation-unsaturation then hydrolysis isomerizations sometimes for example, I must have also talked about vitamin E alpha tocopherol during my earlier discussions.

So, tocopherols in nature they exist in four different isomeric forms; so, these are alpha, beta, delta, gamma. So, there are four different forms, but the most bioactive form which is preferentially absorbed by humans is used in your vitamin E capsules and are in cosmetics where the price rises the minute it is added. So, that is alpha tocopherol.

Now, therefore, when you use plant cells versus chemical synthesis then plant cells can produce the natural form of alpha tocopherol, where the chemical synthesis leads into a racemic mixture; even methylation, demethyation, dehydrogenation all these reactions also come under biotransformations.

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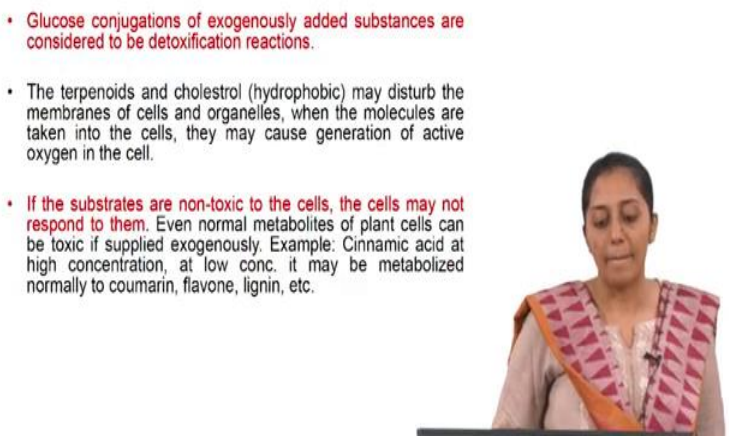

Now, glucose conjugations of exogenously added substances are considered to be detoxification reactions generally. So, if glycosylation is needed. So, then glucose, moiety, addition is needed then plant cells can be used as biotransformants. The

terpenoids and cholesterols which are hydrophobic may disturb the membranes of the cells and organelles when the molecules are taken in the cell directly. So, they may cause generation of reactive oxygen species in the cell.

So, therefore, when these kind of products or substrates are given then the cell would glycosylate it or add the glucose moiety to detoxify. If the substrates are non-toxic to the cells, the cells may not respond to them.

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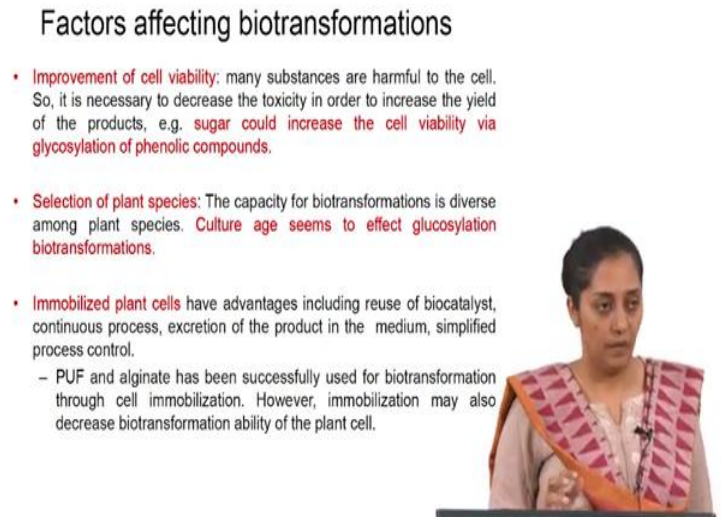

So, what factors will affect your biotransformation efficiency; improvement of cell viability. So, you need to see at what stage the cell is whether the resting cell is needed or whether you need the cells in the active phase. So, many substances may be harmful to the cell. So, it is necessary to decrease the toxicity in order to increase the yield of the product.

For example, sugar could increase the cell viability via glycosylation of phenolic compounds. Now, selection of plant species: the capacity for biotransformation is very diverse in plant species where now culture age is also an important factor; what age of cells, what phase of cells; which means whether active cells or resting cells. Now, immobilization of plant cells- it has advantages; what all advantages, reuse; It makes the process continuous, reuse of the biological catalyst is possible, but what is the prerequisite here, the product must be excreted out what kind of immobilization materials are known PUF; PUF stands for any guess?

Student: Polyurethane Foam.

Very nice Polyurethane Foam, it is an immobilization material, inert material which has been successfully used to immobilize plant cells.

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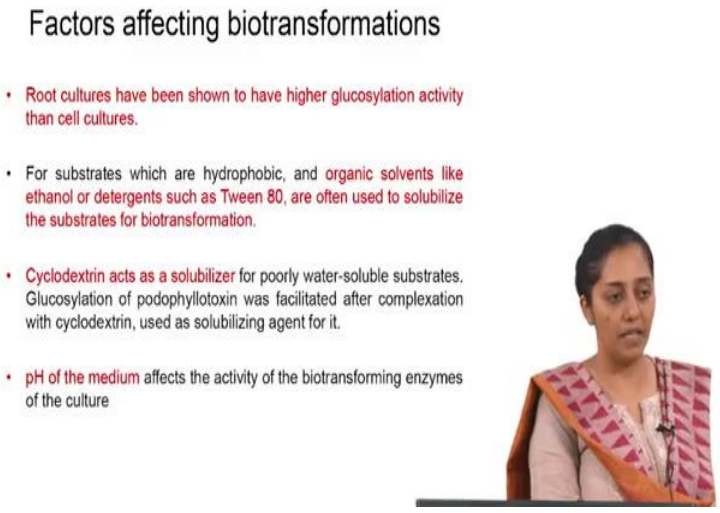

But we need to take care while selecting the material and depending on the species on which we are working. So, we will see in details when we go on to plant cell immobilization. Now, what are the factors affecting biotransformation? For example, root cultures they have been shown to have higher glycosylation activity than cell cultures. So, sometimes it also depends what kind of cultures you are using. So, organ cultures like root cultures may have better biotransformation ability than the free cells in suspension.

Now, for substrates which are hydrophobic and organic solvents like ethanol detergents like tween 80 are often used to solubilize the substrates for biotransformation. I said it is very important for the substrate to be readily available to the cell for uptake. Now, some of these substrates cannot be taken as it is by the cells maybe because of the toxicity. So, in order to facilitate its transport inside the cell, one of the molecules which is called as cyclodextrin is used. It is very commonly used to encapsulate the substrate and such that it can form a vesicle like structure which can be easily then taken in by the cell.

So, cyclodextrin acts like a solubilizer for poorly water-soluble substrates. Glucosylation of podophyllotoxin was facilitated after complexation with cyclodextrin as it is it cannot be taken in, it may be toxic. But when you complex it with cyclodextrin then it was able to get glycosylated. Now, pH of the medium can also play a role affecting the activity of the biotransforming enzymes.

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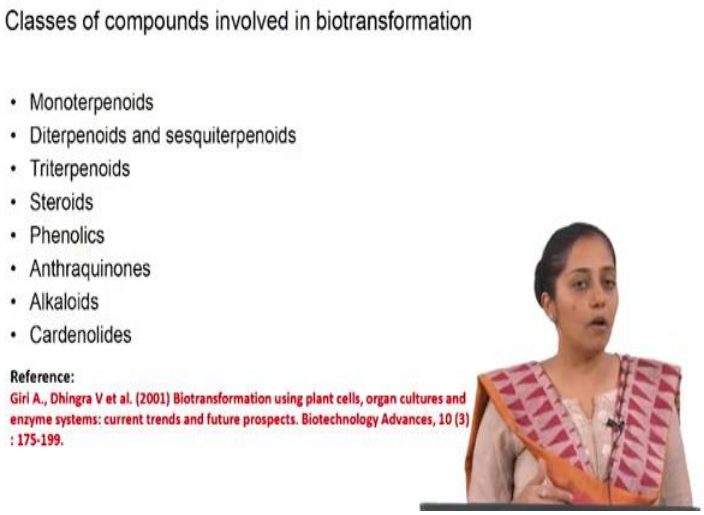

So, what are the class of compounds, which can be involved in biotransformation: Monoterpenoids, diterpenoids these are some example class of compounds secondary metabolites which go under biotransformation reactions in plant cells.