**Course on Industrial Biotechnology**
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**Mod01 Lecture02**
**Development of Industrial Strains**

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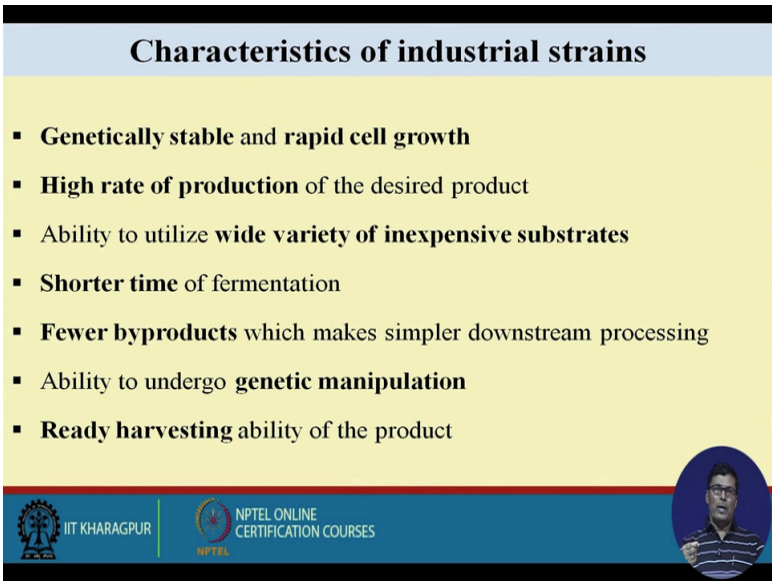


So development of industrial strain, the industrial strain appears to be the one of the important factor that we have with the particularly in the bio chemical industries and this industrial strain should have different characteristics and first characteristic is to be the genetically stable and genetically stable means the bio chemical charactostics of the organism should be same, because you know that organism microorganism has stable generation, as the they have change the generation, the characteristics of the organism should not be changed, genetically is stable organism is very important.

Now if you compare these with the wild strain, wild strain this is most unstable genetically unstable. So suppose we work with the wild strain and get some good product formation, but next time we can have the repeatability of these reactions. Now most of the industry, they when they involved they never they think about that you know that they cannot compromised with the productivity. Productivity of the industry should remain same, because the at the end of day the (())(1:39) management will see that how much product is produced in the industry if the product formation is less naturally that is not desirable for any industry.

So they are come up with lot of (())(1:51) question to the to the operation people. So genetical stability of the organism is very important and it should have rapid growth, when (())(2:00)

would have rapid growth characteristics and the high production rate, because the here I **to** want to (())(2:08) tell you that the product concentration plays very important role in any kind of chemical and bio chemical industries. Now (if we) if the product concentration is less than our recovery cost, because I told you that whenever we market any kind of product, it should in a purified form if the concentration is very less your purification cost will be very high which is undesirable. So we have the so this industrial strain has high productivity than the high rate production high concentration of product that formation takes place.

The another very important factor is that you know, to utilize the wide variety of the inexpensive substances, because you know that organism should have the capability of wide type of different type of substrate. If they use one type suppose, they are using one type of substrate for the timing it may be cheaper, but as the time passes or (())(3:08) then since that showed with the peoples (())(3:11) the source to form where he (())(3:13) is collecting the raw materials thereby finding that this has lot of demand they keep on rising increasing the price of that particular material.

So if you have multiple choice of the raw materials naturally that you know you can you can control the cost of your production to a great extent. Their short time of the fermentation, because it is because we know in the industry every time is very costly, because they count money for that. So the time of fermentation plays very important role in the fermentation industry that should be as lowest possible, because by using the industrial strain that time of fermentation can be reduced, the fewered than in a byproducts formation that is also very important aspect that byproduct formation should be as low as possible, more byproduct formation that means your substrate will go for some not to produce your desire product, but the other than the desire product which is not to acceptable.

So industrial strain give you fewer byproduct that is most desirable thing, ability to undergo genetic manipulation, because the why it is required, because to get desired change in the organism so that we can increase our productivity increase our product concentration we can get our desire product and ready harvesting that is also very important, because cell particularly we have if I told you that different type of cells we have, we have bacteria, we have algae, we have fungi, so you know in case of bigger cells like fungi, we do not have any separation problem, but when you go for the when you go for bacteria, the bacteria is very tiny particle.

So bacterial size varies from 0.5 to 2 micron, so separation problem is there, so harvesting is a very important aspects of that, so how quickly you separate that organism that is also very important that plays very important role. So what we have covering in this course that not only on the development of this how the industrial strain and how to preserve this strain that also we are going to discuss.

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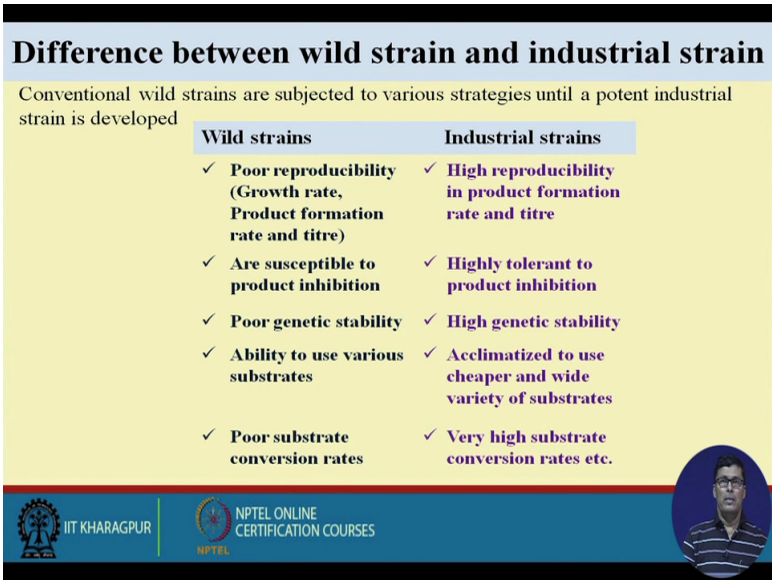


Now let initially let us discuss about the what is the difference between the wild strain and the industrial strain, as we know wild strain, basically by the in the environment, because you look at this soil we have different type of bacteria that is available different type of organism whatever organisms we have all organism most of the organism is available in the soil. So that that if we bring that an isolate in the lab and this organism we consider is a wild strain.

So wild strain is the reproducibility that is growth rate and product formation rate and titer is very poor as compared to the industrial strain. Industrial strain that the high predictability in product formation rate and titer and I told you that concentration of the products has great influence on the cost of production of because recovery cost will be reduce to a great extent if the concentration product is very high. Then another important that drawbacks is that that wild strain, they are susceptible to the product inhibition, because as the product concentration increases it inhibit the growth of the organism, so in case of industrial strain high tolerance on the product inhibition, I can give the example of the yeast that you know approximately one gram one gram of glucose produces 0.51 gram of the ethanol.

Now if I want to increase the alcohol concentration in the fermentation, but now if we look at in the early days that most of the fermentation industry, they produce the ethanol about 7 to 8 percent and now it is the industry they are producing about for 14 to 15 percent. Now for producing the 14 to 15 percent there so the glucose concentration should be as high as 30 percent which is very high and as we increase the glucose concentration than the osmotic pressure on the fermentation block will be high. So in that cusses the shrinkage of the organism.

So organism cannot grow properly, so that is the that means the organism should have the osmo tolerant. So industrial strain they have that capability they can withstand that high osmotic shock this is another advantage we have, then poor genetical stability I told you before also this is wild strain, they have poor genetical stability whereas the industrial stain, they have high genetical stability. Ability to ability to use the various substrate here also we ability use the various substrate, but we use the cheaper substrate and wide variety of substrate can be used by the industrial strain and in case of wild strain we have the poor substrate conversion rate and here very high substrate conversion.

So this is the different advantages we have when we use the industrial stain, so that is why the industrial strain is preferred by the industry and naturally the cost of the industrial strain is very high, because this is the specially prepared by the different lab for the industrial purpose.

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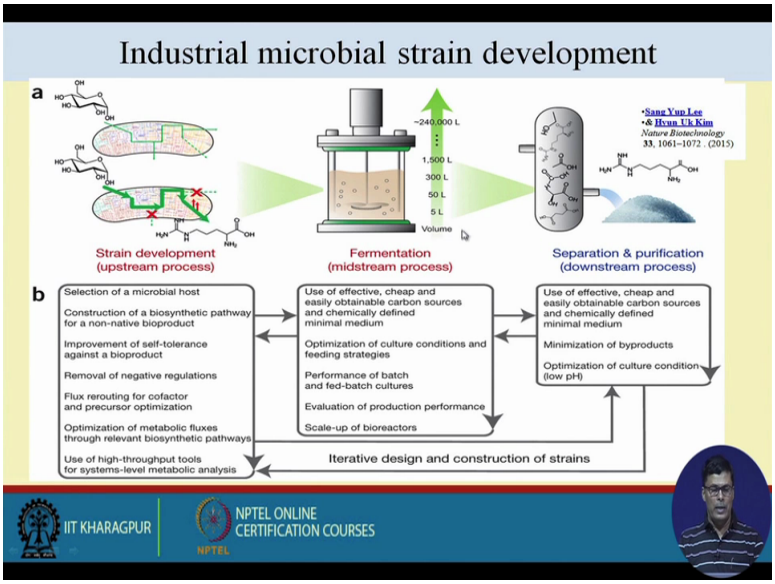


Now if you look at the how the industrial strain development is taking place that we have 3 things we have we can see, the strain development that is in the upstream processing then

midstream process and downstream process that that is like this, in the strain development, first we select the microbial host so that where we want to have the changes that you know, then construction of the biosynthetic pathway for the non-native bio products. Improvement of self-tolerance against the bio products, removal of the negative regulation, flux (())(9:15) for cofactor and precursor the optimization, optimization of metabolic fluxes through the relevant biosynthetic pathway, use of high throughput tools for system level metabolic analysis .

So we know that this is the this is then that we want to work with the for the strain development, because I shall discuss that how we developed it, but this is the main purpose of the in this way we want to guide the organism, in such a way we can get the higher amount of product formations, then if you come to the fermentation process, use of effective and cheap and easily obtainable carbon source and chemically define media, because this the this should influence that one then optimization of culture condition and feeding strategy, performance of batch and fed-batch culture, evolution of product performance and scale up of bio reactors that is very important.

And in the separation use of effective and cheap keep and easily obtainable carbon source and chemically defined minimal media and minimization of byproducts I told you before and optimization of culture condition that is are at a different pH we to want to carry out. So this are our interactive things that we have that is how it influencing the fermentation process.

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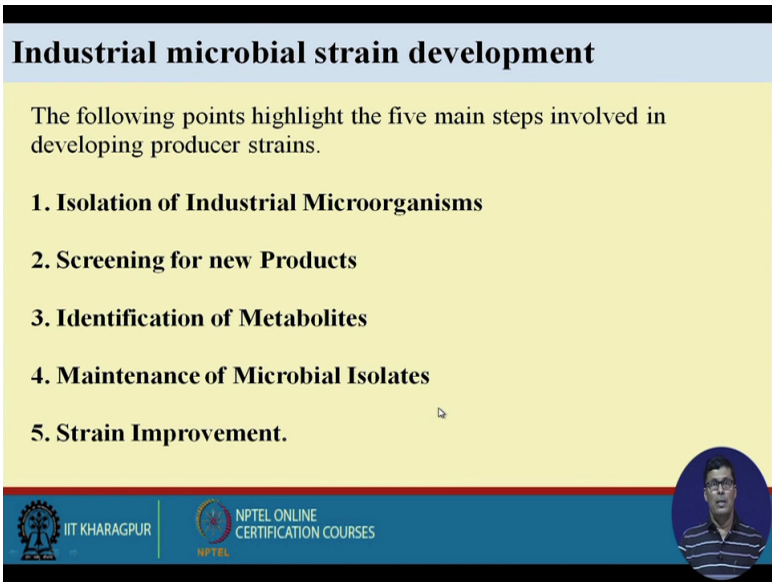

Now industrial that that is strain that is develop in that is five main steps involved, one is the isolation of the industrial microorganism, which organism we are looking for which organism we as for example saccharomyces cerevisiae that is used for alcohol production, now the suppose the saccharomyces cerevisiae is mainly use the hexo sugar.

Now we want to use the also the pentose sugar, because the suppose we want to use some kind of pentose sugar for the formation of ethanol, naturally that we shall have to improve upon this organism we shall have to do the genetic modification of the organism to have the capability of the organism so that you know we can covert the pentose into the ethanol. The screening (of) for new products that we that we that is very important then identification of metabolites that is also important, maintenance of microbial isolates and strain improvement. These are the several things that is involve with the industrial strain development.

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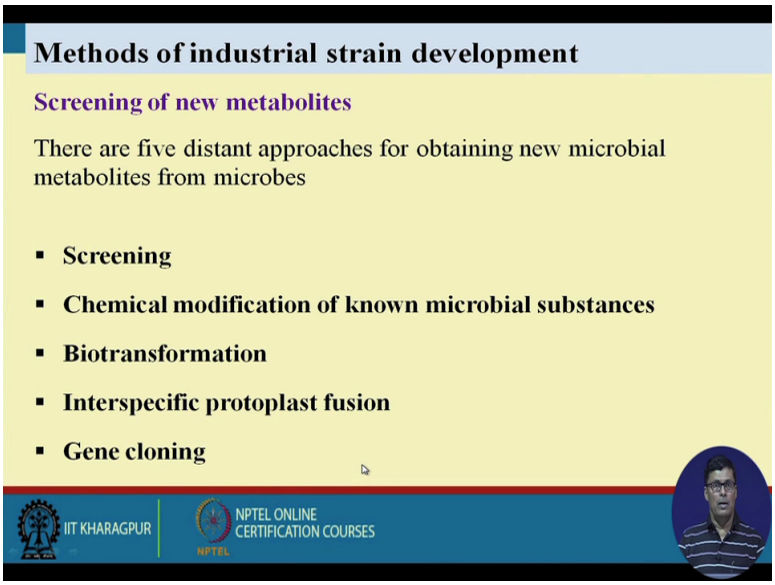


Now another that the other five distant approaches for obtaining the new microbial metabolites from the microbes that is screening we do the screening we do the microbial screening and try to find out which microbes is good for the product formation, then chemical modification of known microbes have microbial substances, bio transformation that also by we can easily carried out with the help of microbes and interspecific protoplast fusion, because we know the different organism has different characteristics.

So if you do the protoplast fusion, it is possible that characteristics the another organism can switch over to other organism and you get the properties of that organism in the system and genetic cloning I told you that insulin, it kind of disease this is used for the diabetic patient

and insulin is kind of protein that is available in the human system. So we can we can produce microbially how we can produce we isolate the gene, which is produce that insulin and we with that gene we over express in the in out host that is E. Colli (())(13:05), so that we can pipe through the fermentation process we can produce the insulin and we can use for our day to day life.

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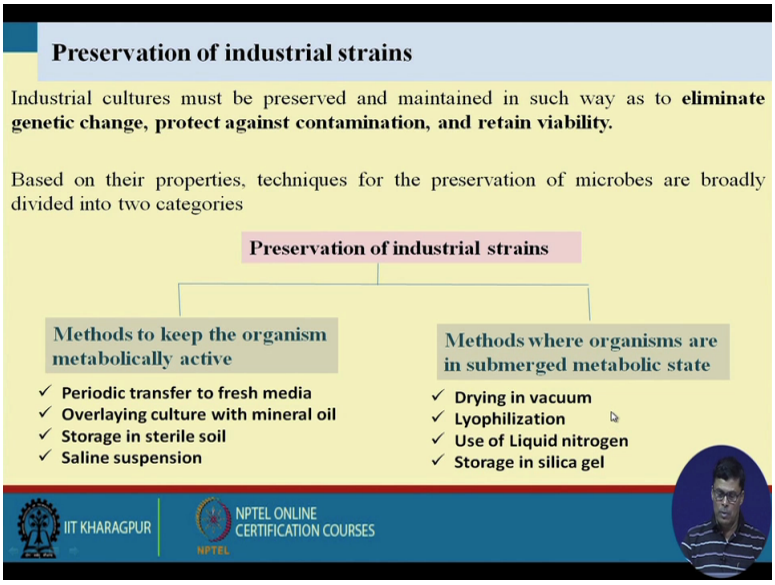


Then question comes how to do the preservation of the industrial strain industrial culture must be preserved by maintaining such a way to eliminate the genetic changes protect against the contamination and retain viability. This is very important. All these 3 factors is to be considered that one is we should keep in a manner so that you know genetic change should not be there. So you should not keep exposure to the UV rays and other chemicals other the adverse conditions so that some kind of changes can take place and then protect against the contamination, because our air atmosphere contains lot of microorganism, because particularly contamination problem is very problematic in the industry I work with citric acid industry I can we use the cane molasses (())(14:10) for the production of citric acid and we observe that our main problem this fermentation process with the contamination by the yeast cells, because cane molasses can be easily used by yeast cells for the production of ethanol.

So and if you look at the doubling time of yeast is much less as compared to fungi. So yeast can grow very rapidly as compared to fungi. So that is why that our the citric industry (()) (14:40) well scaled of these yeast cells and we try to remove this organism so that we do not have any (())(14:47) problem with the citric acid productivity and retain the viability, because viability as you know that the organism should be viable, so that we can it can grow properly

and give the desired product and mostly that industrial fermentation process the when we when you do the inoculation of the organism with the low, it should be either mid lock phase to lead lock so that that organism should grow in a proper manner.

Now about the preservation of the industrial strain is concerned, the methods to keep the organism metabolically active, the periodically transfer to the fresh media, this is one important aspects that we have, overlaying the culture with the mineral oil that is another thing that we have, storage of sterile soil then saline suspension. These are the different methods through which we can keep this when the organism metabolically active, the methods where the organisms are submerged in metabolic state, like drying in vacuum then lyophilization.

Lyophilization consider as a phis drying, phis (())(16:00) drying phis drying means that organism that with the frozen then they do the then that water molecule that presents in the solids phase (())(16:11) that will go to the vapour phase. So the organism can be preserve for a longer period of time around 30 years we can preserve that organism in the viable conditions, then use of liquid nitrogen a that is another way that we can do that, then storage of silica gel, storage in silica gel that is a another way we can preserve this microorganisms.

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**Preservation of industrial strains – Comparison of various methods**

| Method | Merits and de-merits |
|---|---|
| **Periodic transfer** | Variables of periodic transfer to new media include frequency, medium used and holding temperature. This can lead to increased mutation rates and production of variants. |
| **Mineral oil slant** | A stock culture is grown on a slant and covered with sterilized mineral oil; the slant can be stored at refrigerator temperature. |
| **Minimal medium, distilled water, or water agar** | Washed cultures are stored under refrigeration; these cultures can be viable for 3-5 months or longer. |
| **Freezing in growth medium** | Not reliable, can result in damage to microbial structures, with some microorganisms, however this can be a useful means of culture maintenance. |
| **Drying** | Cultures are dried on sterile soil (solid stocks), on sterile filter paper disks, or in gelatine drops; these can be stored in a dessicator at refrigeration temperature or frozen to improve viability. |
| **Freeze-drying** | Water is removed by sublimation, in the presence of a cryoprotective agent; sealing in an ampoule can lead to long term viability, with 30 years having been reported. |
| **Ultrafreezing** | Liquid nitrogen at -196°C is used, and cultures of fastidious microorganisms have been preserved for more than 15 years. |

Now the preservation of the industrial strain and comparison with the different methods is given here, one is periodical transfer the variable of periodical transfer to new media includes the frequency, media used and holding temperature. This can lead to increase the mutation rates and production of variant. Then mineral oil slant, this is another important aspect that

we have, a stock culture is grown on in a on a slant and covered with sterilized mineral oil and slant can be stored at refrigerator temperature.

The then minimal medium and distilled water or water agar that is the use the washed culture was stored under refrigeration and this culture can be viable only for 3 to 5 month or longer not for very long period of time. The freezing of the growth media, a not real reliable, because can result in damage of microbial structure, with some microbes and however this can be useful mean of the culture maintenance. Drying this is another important thing that is the culture are dried on sterile sterilized oil soil and on sterile the filter paper disk in gelatin drops and these can be stored in a dessicator at refrigerator temperature or frozen to improve the viability.

The most the technique that I was telling you that which is used by the industry that is the freeze drying technique. Freeze drying technique, water is removed by sublimation process, in presence of cryoprotective agent and sealing it in a ampoule and can lead to the long term viability with 30 years having been reported, because 30 years we can preserve this organism in the viable condition. Then ultra-freezing, this is another aspect that we have liquid nitrogen at minus 196 degree centigrade is used, culture or fastidious microorganism have been preserve for more than 15 years. So these are the different way we can preserve the microorganism.

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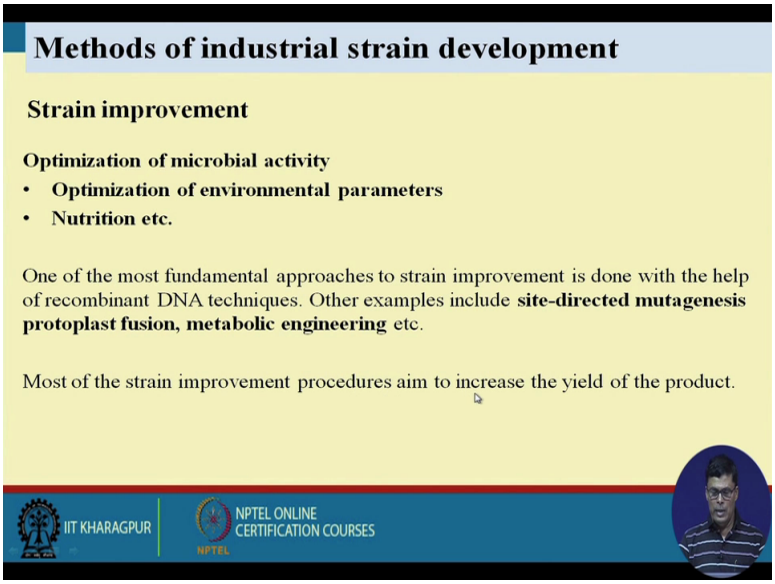


Now let me discuss about the method of industrial strain development. The strain improvement here, optimization of microbial activity we can do by optimizing the

environmental parameters, like the in a temperature, pH, substrate concentration I told you that osmo tolerant culture that can we increase the microbial activity. Then nutrition this is very important ligand, recently we work on that and we find that that you know particularly if that, in case of when we use some kind of waste material for the production of some useful product, waste material usually contain does not contain much of nutrient has less (())(19:45) nutrients I am also if with the micro (())(19:48) we add to the waste material then your microorganism can grow properly and give the desired product that that is there.

One of the most fundamental approach is of the strain development is done with the help of recombinant DNA techniques, because I told you the example is the insulin production and that the other examples as site-directed mutagenesis, protoplast fusion and metabolic engineering, metabolic engineering plays very important role, because you cannot (())(20:18) the metabolic pathway to get the desire amount of product, because this is very important. Most of the strain improvement procedure aim to increase the yield of the product. This is the main purpose that.

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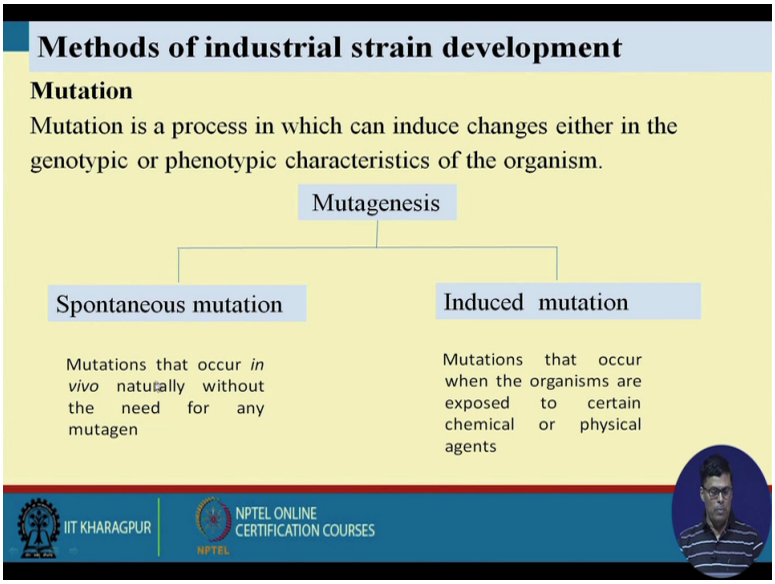


So mutation is very known technique that is used in the industry for the improvement of the productivity of the strain. Mutation is a process, which can induce changes either in the genotype or phenotype characteristics of the organism. The two type of changes that occurred in the organism, one is genetically another phenotype change that can be done with the help of mutation and mutation may be of two type, one is call spontaneous mutation another is induced mutation.

The spontaneous mutation, which is occurred in vivo, (())(21:05) in vivo means they are itself organism itself the mutation that occurs naturally without the need of any mutagens. The induce mutation the mutation that occurs when the organisms are exposed to certain chemical or physical agents.

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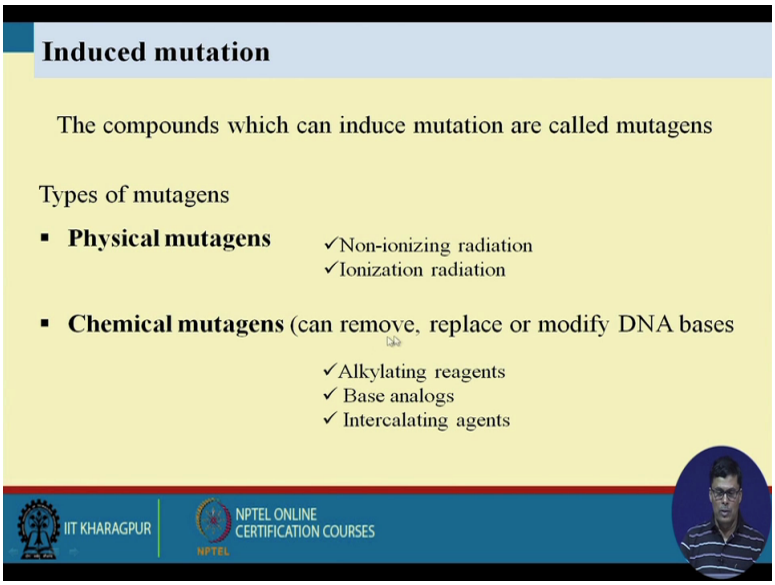


So now we have different type of mutation we have **we have** physical mutation, we have chemical mutations. Physical mutation we have non-ionizing the radiation, ionization radiation then chemical mutagens we have alkylating agent, base analogs and intercalating agents.

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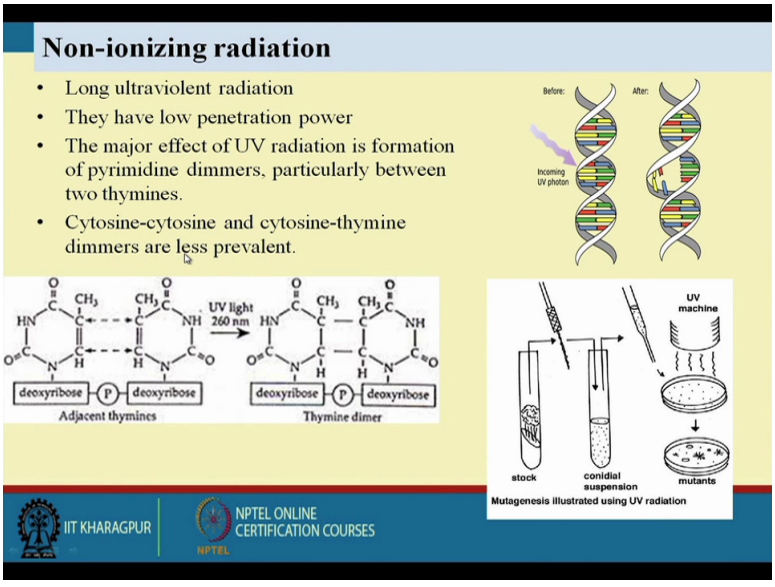

So different type of mutagens we use in the industry for the development of the strain. Let me take the example of the non-ionizing the radiation examples is the ultraviolent radiation and they have long penetration power you know that ultraviolent radiation as very shorter wavelength and major effect of ultraviolet radiation is the formation of pyramid in dimmer, because you can see that here, 2 nitrogen bases is there, this is this is thymines and there how the dimmer formation is there that you can see that here dimmer formation takes place, but cytosine-cytosine and cytosine-thymine dimmers are less prevalent.

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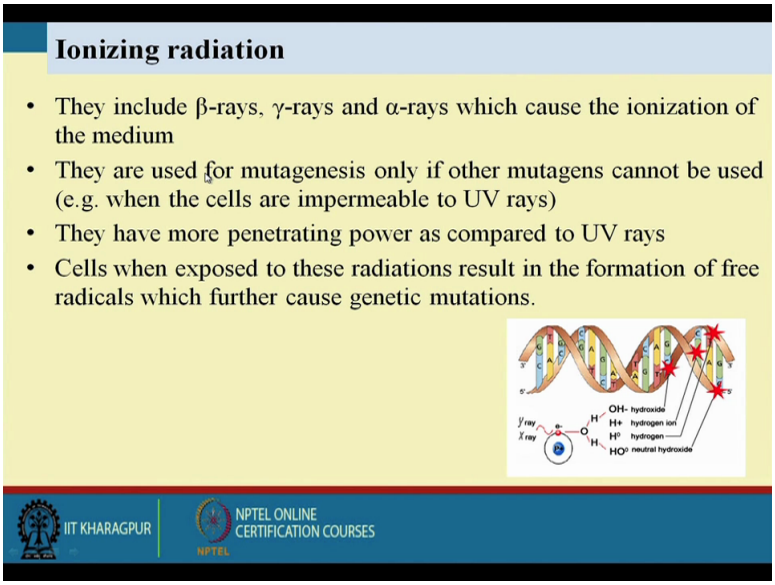


Now ionization radiation this is actually apply where that non-ionization that radiation is **is** not effective, they include the gamma the beta rays, gamma rays, alpha rays which causes the ionization in the media. They are used for mutagenesis only if other mutagens cannot be used when the cells are impermeable to the UV rays. They have more penetrating power as compared to UV rays. Cells when exposed to these radiations results in the formation of free radicals which further cause the genetic mutation. So this is the (())(23:10) effective more effective as compared to non-ionizing radiation.

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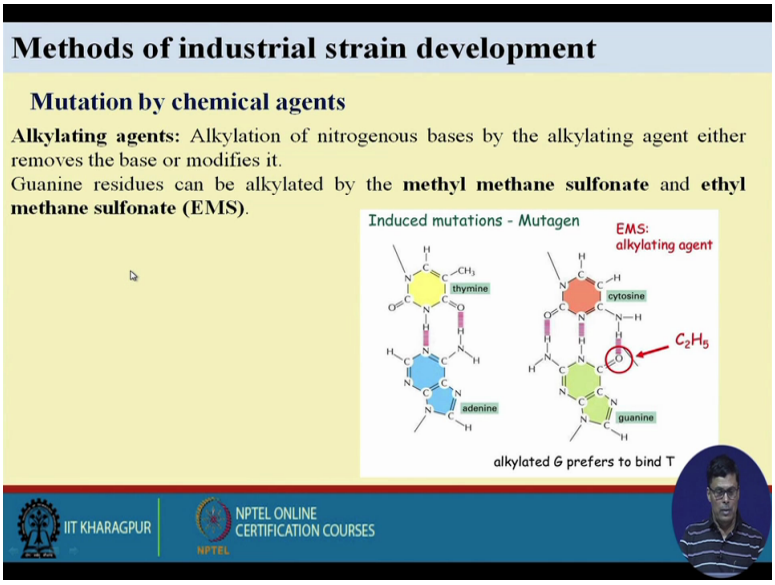


Now we have chemical mutagens that we use mainly, this chemical mutagens we use to change the nitrogenous bases that you know alkylation of nitrogen bases by alkylating agent either remove or the base is or base or modifies it you can see this is the thymine, inenine cytosine and guanine that here how by using EMS, EMS means the ethyl methane sulphonate, methyl methane sulphonate, there are two alkylating agent that can be used for mutation purpose. This is how we use to change the characteristics of this nitrogen base that is call there is part of the gene.

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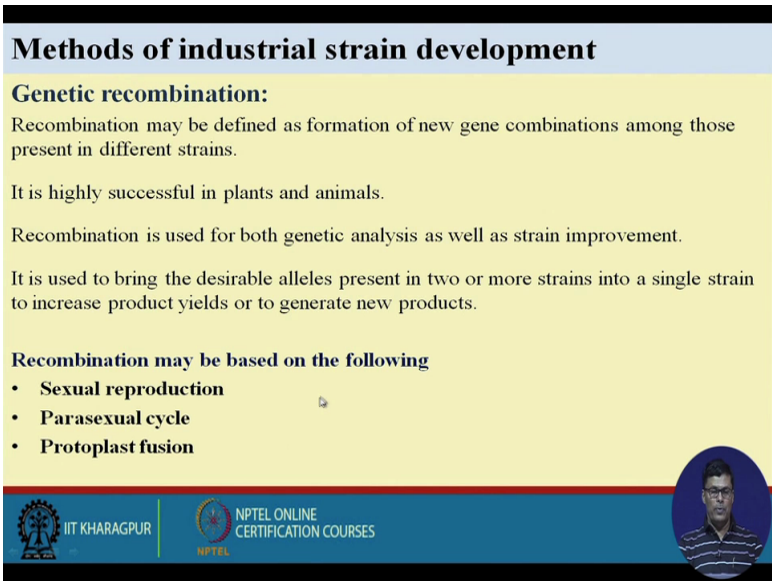


Then methods of industrial strain improvement that is also very important. The one is the genetic recombination the recombination may be defined as the formation of new gene

combination among those present in the different stain as I told you suppose one organism does not have a particular gene we want that particular gene in that particular organism. So we try to take the gene from other organism and we clone in a kind of plasmid and then (()) (24:38) when these organism to get the desired property on the in that particular organism atht is call recombination techniques and it is highly successful in plants and animals.

Recombination is used for both genetic analysis as well as strain improvement. it is used to bring the desirable the present 2 or more strain into single strain to increase the product yield and to generate the new products. The recombination may be based on the following sexual reproduction, Para sexual cycle and protoplast fusion. The different base that we can do the recombination.

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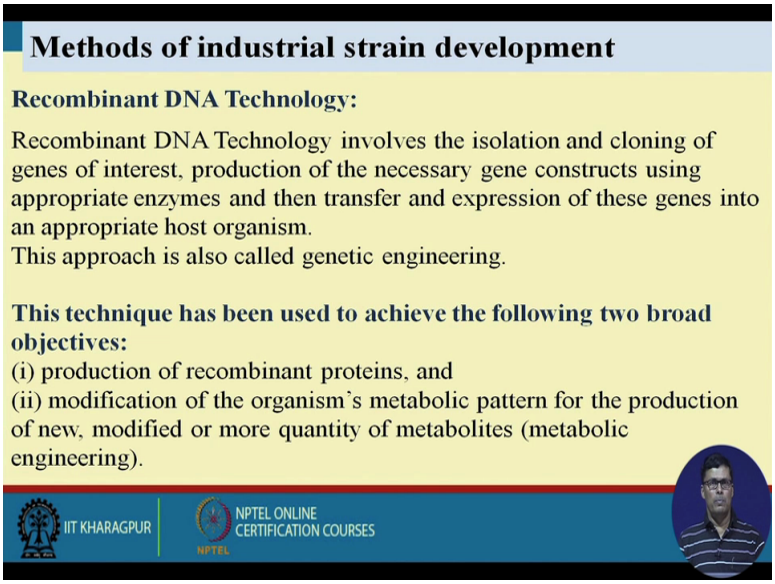


Now finally I want to tell you that recombinant what is the recombinant DNA technology you might be knowing that see, let me tell you the recombinant DNA technology involve the isolation and cloning of the gene of interest, because gene of interest me the what particular gene you know that the why if the gene is coding a particular protein and the production necessary gene construct using the appropriate enzymes and then transfer and express this is into the appropriate host organism.

So this is the technique this is the kind of innovative technology that I recently has develop to get the desire characteristics of the microorganism that we call it genetically the improvement of the microbial strain and this technique has been used to achieve the following two broad objectives: one is the production of recombinant proteins as I mention that this is very

important and modification of the organisms, metabolic pattern for the production of new modified and more quantity of metabolites that is a metabolic engineering when we when we study the different bio chemical pathway so we easily find out that you know which pathway can give us the maximum amount of product formation.

So through this metabolic pathway is we can through this recombinant DNA technology we can develop that particular desire enzyme. So that the activity of that enzyme can be increased so the we can be increase the productivity of the particular product metabolize to a great extent. So this is the whole things that I want to emphasize here that is there how the industrial strain development are how preservation takes place and this is largely used by the industry for getting desired products, thank you very much.