**Computational Systems Biology**
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**Lecture - 76**
**Constraint Based Modeling of Metabolic Network: Applications**

In today's lecture, we will continue with applications of constraint based modeling and we will look at the example of metabolic engineering going away from drug identification and we will look at how Lycopene biosynthesis was engineered in E. Coli using information from computational approaches and flux balance analysis. So next example is let us switch to metabolic engineering. Lycopene is an important Nutraceutical.

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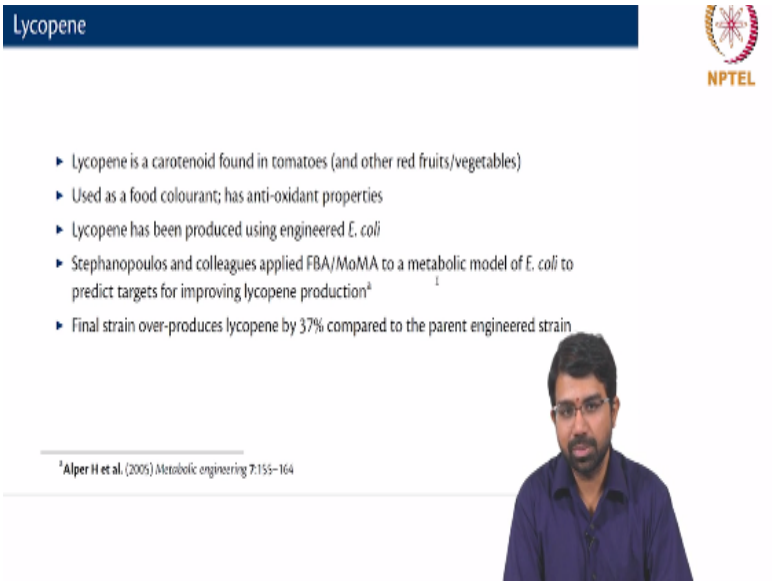


It is a carotenoid it is found in tomatos and so on and other red fruits. It is used as a food colorant and has anti-oxidant properties and it has already been produced using engineered E. Coli and Stephanopoulos and colleagues applied FBA and MoMA metabolic model of E. Coli to predict targets for improving lycopene production. And they finally came up with a strain that over produced lycopene by 37% compared to the parent engineered strain.

So the parent strain was already an over producer and they improve upon that by 37% solely using an FBA MoMA approach. So what did they do?

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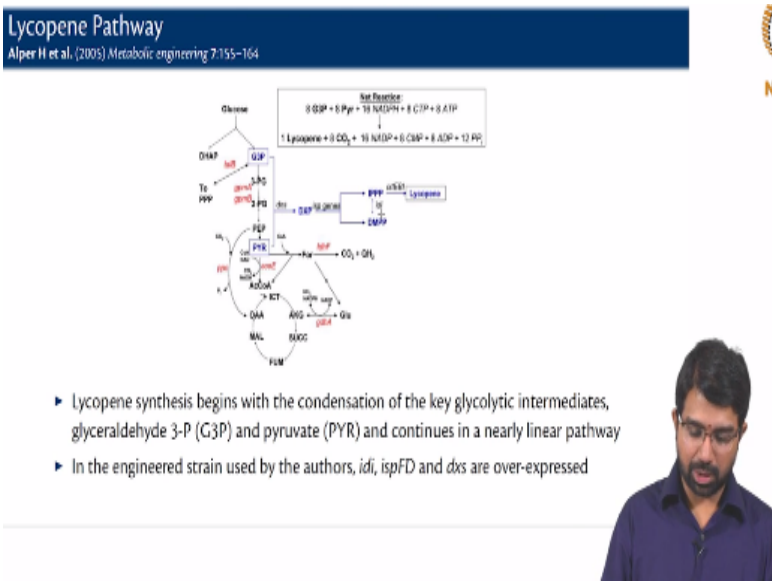


This is how the pathway looks so that glucose that goes to DHAP G3P and then to DXP finally here to lycopene. And the net reaction is something 8 G3P + 8 Pyr + 16 NADPH + 8 ATP producing one lycopene. So this is the reaction with engineered strain used by the authors had 3 genes over expressed so that isp, dxs and idi. So these are the 3 genes that were over expressed so that is the most common kind of over expression that one would express wherein you over expressed the pathway genes themselves all the pathway genes.

So this is like the important sub branch that goes towards lycopene over expressed all of these.

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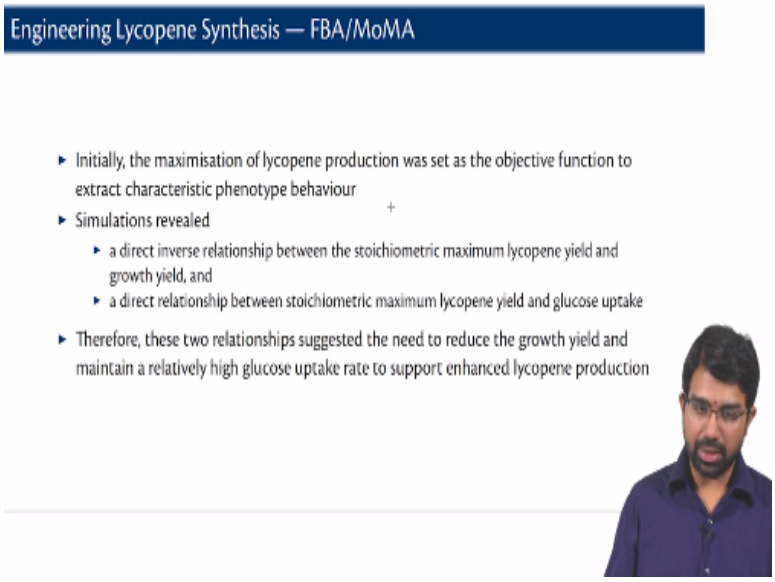


So initially what they did was they try to maximize lycopene behavior as the objective function so they set up lycopene production as objective function and to figure out the

phenotype. They found that there is direct inverse relationship between stoichiometric maximum lycopene yield and growth. So this is not a growth associated metabolic higher the growth lower the lycopene, lower the growth higher the lycopene.

And they also found a direct relationship between stoichiometric maximum lycopene yield and glucose uptake. So you need to have a tradeoff so you want to reduce the growth yield somewhat and also maintain a high glucose uptake rate for enhanced lycopene production.

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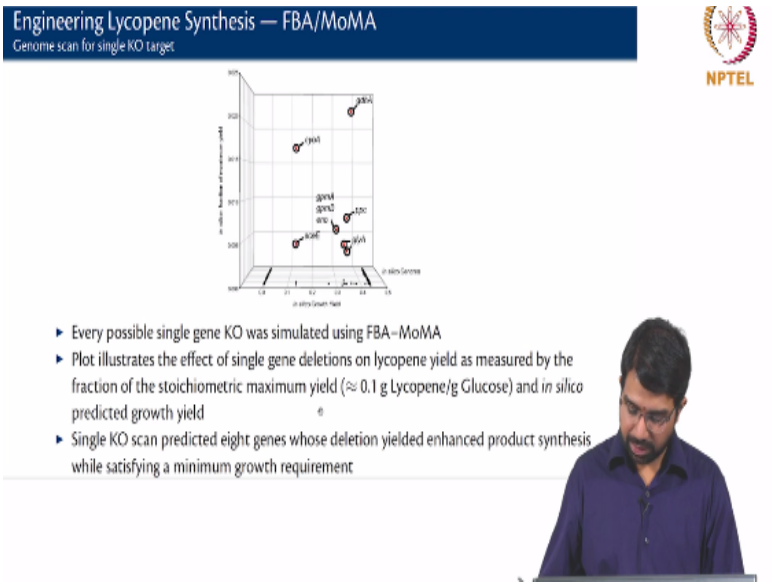


So the method they did was they did to knock out every genes to see if it could positively impact lycopene production so they knocked it out and simulated using FBA and MoMA. So the plot illustrates the effect of single gene deletions. So the y axis is the in silico fractions of maximum yield and the x axis in silico growth rate. So you see that sometimes growth is very high. So here the growth and lycopene are low.

So here both growth and lycopene are reasonably high. So gdha is a good knock out or just talks about some in silico geno what are all the reactions or something like that. So they had a single knock out scan they predicted 8 genes whose deletions yielded enhanced product synthesis.

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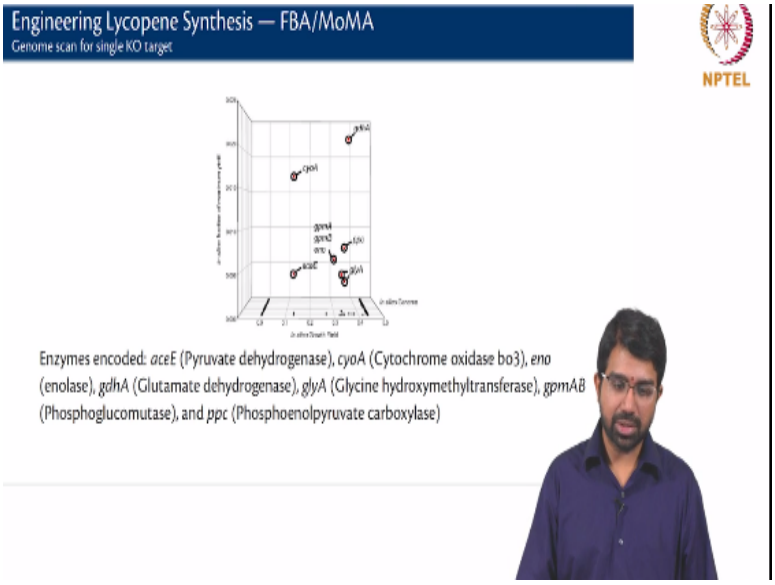


And these enzymes included aceE cytochrome oxidase, glutamate dehydrogenase, glycine hydroxymethyltransferase and so on so these are the genes that they found quite interesting. As you see Pyruvate dehydrogenase is probably involved in pyruvate production which is an important input to this and some of these might just be manipulating the (()) (04:00) balance because if you see that there were lots of NADPH involved and so on.

So this is importance of doing a systems-level metabolic engineering because you will be tapping into the whole metabolic network not just the product forming reactions and so on.

So we look at traditional metabolic engineering, a lot of wisdom is about let us go and maximum the pathway enzyme and so on, but it turns out that you can identify many sort of non intuitive reactions by doing a system-levels study.

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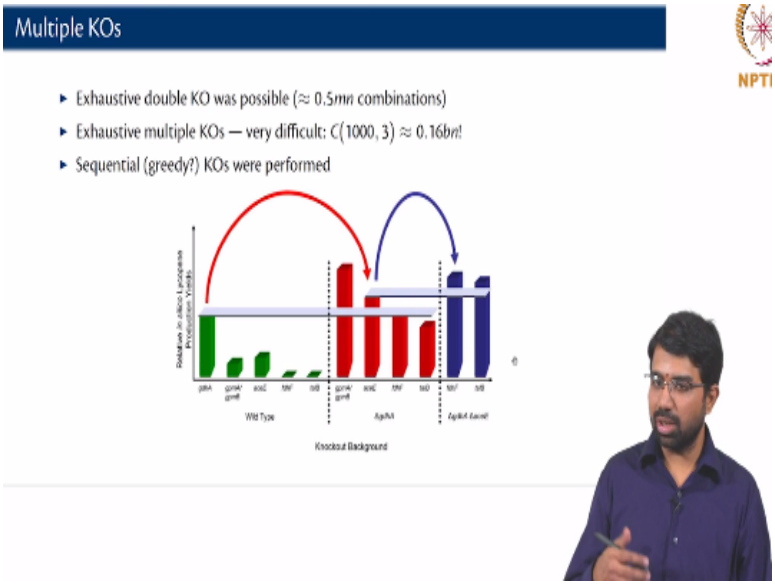

They also try to do exhaustive double knock outs. You knock out 2 genes at a time but that is reasonable because we have around thousands genes and you are talking about half a million combinations. As we discussed in the previous class about synthetic lethals, but exhaustive multiple knock out is much harder if you are talking about thousand choose 3 that is like 1.16 billion. So what they did was sort of greedy knock out.

So they took the best double knock out and knocked out one more gene from that. So they took the best double knock out strains and did a single gene deletion on that strain that is only about doing like 1000. So if you take the best 8 or something and do a single gene deletion you are talking about 8000 LPs to solve that is not a big deal.

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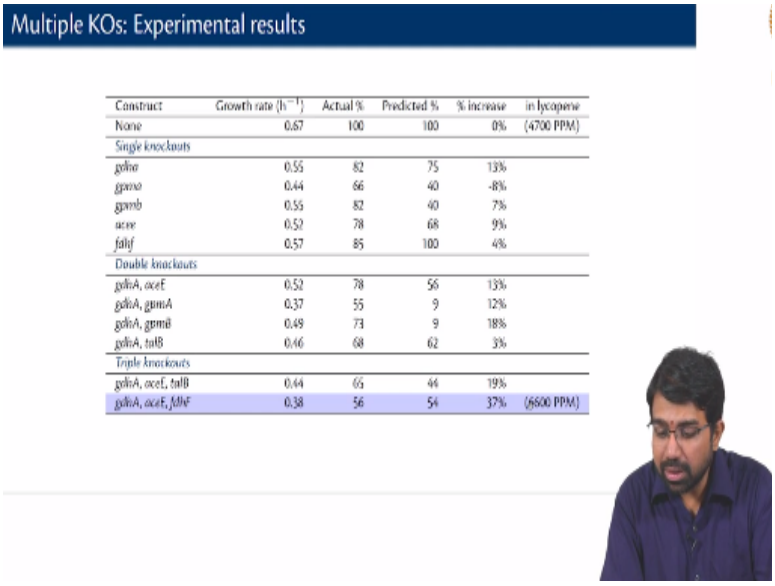


And this table is very interesting. The FastExcel works well for getting a lethal phenotype. Here you want a non lethal phenotype so the same assumption would not actually hold that easily. So you will have to it is kind of tricky so we have not got in around to that yet. So this table is very interesting and this kind of summarizes the entire approach. So it tells you that predicted increase and actual increase will not match, may even vary in direction but a very likely to hit a good strain by doing this modeling.

So remember the model is wrong, but it is quite useful. So here we have is like five single knock out here and four double knock out and a bunch of triple knock outs. So the growth rate is listed here the actual growth rate, the predicted growth rate in percent and the increase in lycopene production. So the predicted increase in lycopene was 54%, but in reality you got about 37% not bad.

Whereas here you predicted a 100% increase and you only a 4% so there are going to be these kinds of mistakes, but the bottom line is if you wanted to try about 10 strategies you are very likely to get successful in at least one of them. So here we have about 10, 15 strategies and one of them was a big winner. So this is the bottom line and basically you had about 6600 ppm of lycopene that was produced by this process.

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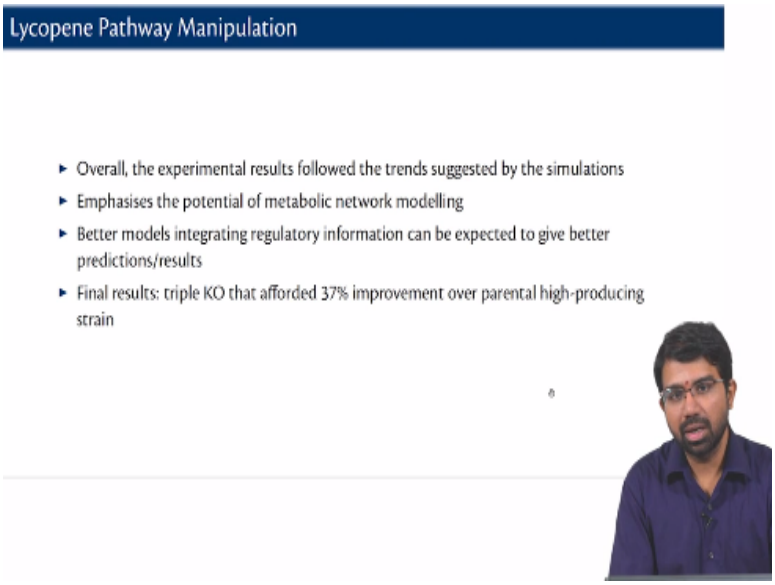


So to summarize the experimental results sort of followed the trends suggested by the simulation not exact quantitative values although the study was done in 2005 and since then the accuracies have really improved for flux analysis and you can actually try and fit some flux. If you fit some fluxes, you will be in a much better position if you measure a few fluxes and fit them better.

You can already get along with FBA by just measuring the uptake rate and feeding them into the model it is reasonable enough, but you can do better than that and this is of course emphasizes the potential of metabolic modeling and you can anticipate better results if you integrate more regulatory information and so on without using any regulatory logic we have been able to get such a reasonable prediction.

So the final result was a knock out a triple knock out that afforded 37% improvement over the parental over producing engineered strain. I hope today's lecture convinced you that computational approaches or just flux balance analysis are very useful for metabolic engineering for predicting strategies for metabolic engineering and in the next video we will

look at an integrated approach that combined various system levels aspects to predict what are the all the high confidence drug targets in tuberculosis.