Interview with Kjeld Olesen and Nina Gunnarsson from Novo Nordisk

- **By Camilla Krogsgaard and Sidsel Clemmensen**

NB! This interview was done in Danish and the following is freely translated into English.

What is your background and what are you currently working with?

Kjeld Olesen: "I work in Yeast molecular genetics with the development of yeast expression systems. I did my PhD at Carlsberg and stayed there for 20 years, also working with development of production strains."

Nina Gunnarsson: "I am a yeast physiology and genetics scientist, and team leader of a group working with development of yeast expression systems. I have a degree in chemical engineering and PhD from DTU in metabolic engineering. Then I worked in a small startup for 7 years, and now I have been at Novo Nordisk for 5,5 years."

How are Novo Nordisk currently producing insulin? (organism, substrates..?) And from where do you get the substrates?

Kjeld Olesen: "We use S. cerevisiae to produce recombinant human insulin expressed by an episomal high copy plasmid, since we discovered that gives the highest yield. We also use classic yeast molecular biology to knock out genes that encode proteases and other competing pathways.

We cultivate in big tanks in defined synthetic medium containing different refined carbon sources, e.g. glucose, which is provided by several different suppliers, since one of the most important things is to have a stable supply chain of substrates to ensure the production can provide the patients with sufficient amounts of insulin."

When you are developing your cell factories, what characteristics are most important?

Nina Gunnarsson: "Yield is very important, but we also focus on being able to achieve a high degree of purity. Purity in that sense that we want to avoid degradation and certain post-translational modifications, e.g. O-glycosylation. We also consider the downstream

processing and how to improve that. It is also important that the organism does not produce all kinds of other products, which can be hard to get rid of in the purification process."

Kjeld Olesen: "The quality is perhaps a bit fluffy term. But in general, we need to have a very holistic way of thinking."

Nina Gunnarsson: "The process must be predictable, so a well-known organism is also to prefer."

Kjeld Olesen: "Yes, the organism and process must be robust, in case something goes wrong in the process."

If we have our system working and up and running (producing insulin in an alternative organism, that can utilize sustainable substrates), would you change your production method to this? Would you want to be a part of the biobased economy?

Kjeld Olesen: "A lot of our concerns with using e.g. waste from pork production revolves around having to get our products approved by the FDA in order to sell it on the American market. However, other markets might have less strict regulations, so production on less pure substrates could be possible."

Nina Gunnarsson: "The whole production costs a lot of money from development of the therapeutic compound to the purification process, so you would need a really good business case."

How do you see the need for insulin in the future in regards to the increasing demand for insulin and the increasing competition for crops and the refined sugars you are using - how will the current production line be able to follow this development?

Nina Gunnarsson: "We obviously have a big focus on having the most optimal processes with the highest possible yield with the minimum environmental impact. Novo Nordisk operates with something we call the triple bottom line, which covers economical, social and environmental sustainability, so we aim to consider all three aspects as much as possible."

Kjeld Olesen: "We use a very little amount of sugars compared to what is consumed for food, so right now that sort of sustainability is not that a big of a concern for us. We are more focused on our obligation to provide sufficient amounts of insulin to the patients."

So do you think that changing your production organism would be realistic?

Nina Gunnarsson: “Well, it would be a huge project! Because the product quality is so important, it would be a very long and complicated changeover, which will include a new factory and new experts, so it would have to be a very good business case.”

Kjeld Olesen: “The stabile continued supply of substrates is very important. The content of the substrate cannot vary either.”

Nina Gunnarsson: “Maybe this would be more applicable for production of other things than therapeutics - such as bulk chemicals, enzymes or food additives, that do not have the same quality and purity requirements.”