Yeastilization: From Waste to Value

Overview

The global population is estimated to reach 9 billion in 2050. The increasing world population brings a number of challenges, from food shortage to management of increasing waste generation. One of the solutions is the circular economy with waste. This is where the BioBuilders 2016 picked up. Our aim was to develop a new cell factory able to utilize local waste streams in Denmark to produce valuable products. We aimed to investigate the growth capabilities of the non-conventional yeast Yarrowia lipolytica on several substrates. Furthermore, we aimed to develop a toolbox for the yeast enabling genetic engineering and expression of heterologous proteins.

Team members


Numerous high schools nationwide! The BioBuilders project will be an exciting opportunity for students to work with synthetic biology, and we are soon ready to ship the kits to high schools nationwide. For more information about the project, please visit www.biobuilders.org.

Waste in Numbers

Denmark has been recycling and recovering waste for more than a century. We have used the recycled waste as heat and electricity sources. Further, in recent years, great strides have been made towards recycling still greater amounts of waste.

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<th>Waste in Tons</th>
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Growing Yeast on Danish Waste

Our substrate should be cheap, available and abundant. It should have no competing uses and should be compatible with fermentation.

Yarrowia lipolytica is a dimorphic, non-conventional yeast belonging to the Ascomycota phylum. In the recent years, Y. lipolytica has received increased attention from researchers, as studies have found it to posses great potential for producing industrial enzymes and pharmaceuticals. On top of this, Y. lipolytica has a very broad substrate range, making it perfect for our scop: transforming waste to value.

Biosensor

In Denmark, it is possible for high school students to work with fermentation of plants due to rules issued by the Danish Ministry of Education. As a consequence, very little knowledge is based on the student starch-ethanol and biomass and many will only encounter the field when attending university.

With our biosensor project, we made it possible for high school students to work with biobricks.

A New BioBrick Plasmid

We developed a plasmid that supports the Biobrick stan- and replication in Y. lipolytica. The plasmid (pSB1A8) has a high copy E. coli plasmid for cloning and propagation of DNA. To support the Biobrick standard, only the amplic-

Growing Y. lipolytica on three waste or by-product sources such as glycerol from Demeleek and melasse from Dansukker showed the capability of Y. lipolytica to utilize these carbon sources, demonstrating the very wide substrate range.

CRISPR-Cas9 & Gene Insertion

As a proof of concept, we have shown that we can integrate a gene of interest into Y. lipolytica and further disrupt a native gene of interest. These proof-of-concept experiments validate the use of Y. lipolytica for future cell factory engineering purposes. We have focused on the integration of an auxotrophic marker gene, and disruption of the gene PEX10 necessary in peroxisome biogenesis.

Heterologous Protein Expression

We aimed to create a versatile cell factory, which will in the future be able to produce any product. We wanted to demonstrate this versatility of Y. lipolytica by producing heterologous proteins such as the humanized tandem retin designated green fluorescence protein (hrGFP) and proteins using our own plasmids (pAB1).

Modelling

We used genome-scale modelling techniques to optimize beta-carotene production in Y. lipolytica. We simulated growth and theoretical maximum production yield under different nutrient conditions, creating phenotype phase plans, in order to explore the simulated growth genome-scale modelling techniques to optimize beta-carotene production in Y. lipolytica.

TaqCO

TaqCO is a computational tool developed by DTU Biobuilders 2016 to performamin assembly for any species with sufficient data available. The code preference is based on the species-specific TaqMan Adaptation Index (TAI) and is estimated such that the correlation between protein level and TAI is maximized, mainly inspired by a method described by Gieske et al (2014). The final result is a unique stand-alone application, with fast run-time, available for Linux (by Chris) and a browser with a graphical user interface (GUI). The user is offered the option to optimize sequences such that the sequenced section site are removed during the process.

References