Yeastilization

From Waste to Value

DTU BioBuilders

DTU

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Overview

The global population is estimated to reach 8 billion in 2026. The increasing population of the world brings a number of challenges from food shortage to management of increasing waste generation. One of the solutions is a circular economy with zero 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. Further, we developed a toolbox for the yeast **enabling genetic engineering** and **expression of heterologous proteins**.

Attributions

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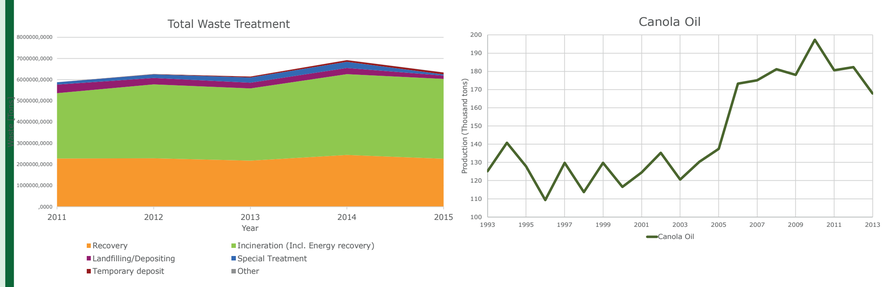
Waste in Numbers



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.

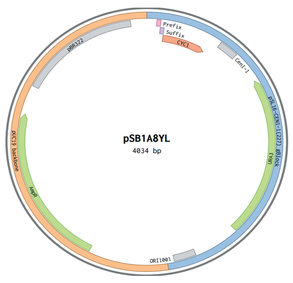
Denmark produces 167,800 tonnes of canola oil annually. Canola oil sediment is a by-product from this production amounting to ~ 3% per weight of the canola oil production. With a stable production this by-product amounts to more than **5,000 tonnes each year.**

Yarrowia lipolytica

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

A New BioBrick Plasmid

We developed a plasmid that **supports the BioBrick standard and replicates in *Y. lipolytica*.** The plasmid (pSB1A8YL) has a high copy *E. coli* part for cloning and propagation of DNA. To support the BioBrick standard, only the ampicillin resistance and origin elements of the plasmid are used. The *Y. lipolytica* part of the plasmid is based on pSL-16-CEN1(227), as it has been found to exhibit high transformation efficiency compared to similar plasmids. The plasmid also contains the BioBrick prefix, suffix, a 5' terminator and a uracil autotrophymarker.



Chromoprotein expression in E. coli using our new BioBrick plasmid pSB1A8YL

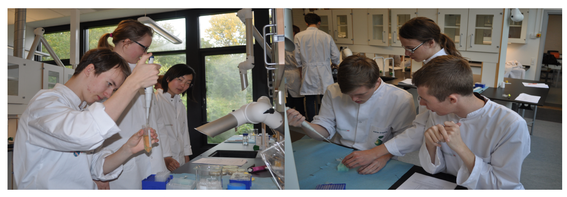
Testing the cloning ability in *E. coli,* we used three chromoproteins:
- amilP (BBa_K592009)
- amilGFP(BBa_K592010)
- and mRFP (E1010)
- Promoter (BBa_K88005)

Biosensor

In Denmark, it is not possible for high school students to work with purification of plasmids due to rules issued by the Danish Ministry of Education. As a consequence, very limited knowledge is passed on to the students about synthetic biology and many will only encounter this exciting field when attending university.

With our Biosensor project, **we made it possible for high school students to work with BioBricks!**

The project has been funded to make it possible to offer kits to **200 Danish high schools** in Denmark, allowing them to create their own customized Biosensors. We have tested the kit at a local high school, and are soon ready to ship the kits to high schools nationwide!

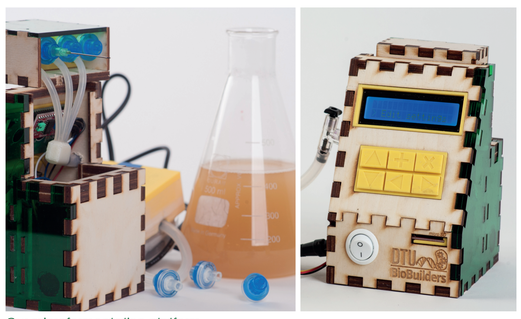


Students testing out our Biosensor kit at Bagsværd High School

Microfermentation

Not every lab is as fortunate as ours, where we have expensive equipment to measure e.g. growth. We therefore started a **hardware project aiming to develop a cheap alternative** to the Hamilton robot, used in our project. A small device that would enable hackerspaces and high schoolers to **easily acquire and compare growth data** for their projects.

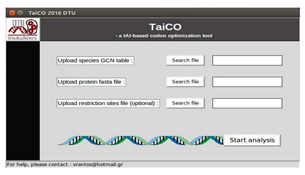
We build a working prototype that:
- can be rebuild at a cost of ~ **USD 150**
- Demonstrates its functionality
- Integrates feedback from prospective users
- Provides all source files for other developers



Our microfermentation platform

TaiCO

TaiCO is a **computional tool** developed by DTU BioBuilders 2016 for performing **codon optimization** for any species with sufficient data available. The code preference is based on the species specific **tRNA Adaption Index (tAI)** and is estimated such that the correlation between protein level and tAI is maximized, heavily inspired by a method proposed by dosReis (2). The final result is a **unique stand-alone application** with fast run-time, available for Windows and Linux and is bundled with a **simplistic graphical user interface** (GUI). The user is offered the option to optimize sequences such that the requested restriction sites are removed during the process.

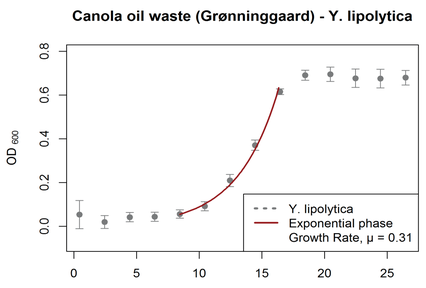


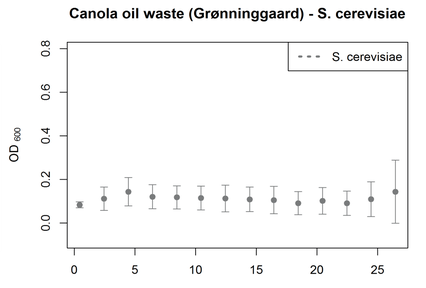
The GUI of TaiCO

Growing Yeast on Danish Waste

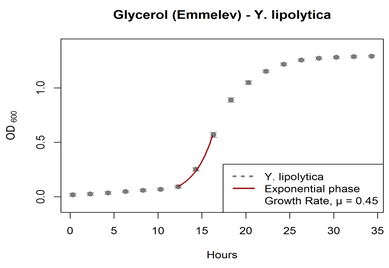
Our substrate should be **cheap**, **available** & **abundant**. It should have **no competing uses** and should be compatible with **fermentation.**

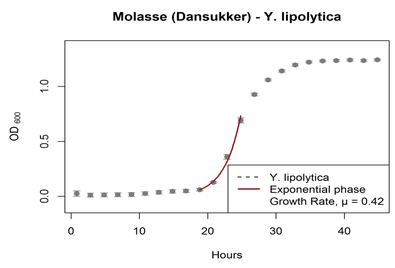
The **canola oil sediment** is a great candidate, as it is abundant in Denmark due to the high production of canola oil. It is not otherwised utilized because it has a high concentration of polyaromatic hydrocarbons.





We did growth experiments with our newly found substrate provided by Grønninggaard, a local canola oil production site. We investigated the **growth rate** of the model organism *Saccharomyces cerevisiae* and the non-conventional yeast *Y. lipolytica*. We knew that *Y. lipolytica* grew well on undefined or complex substrates whereas the *S. cervisiae* would not. Our growth experiments confirmed the **superiority of *Y. lipolytica*** when grown on canola oil.

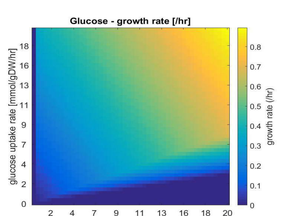


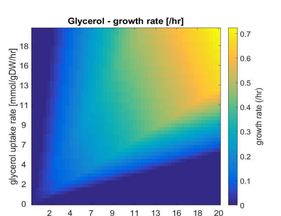


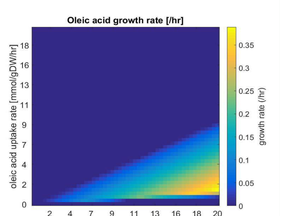
Growing *Y. lipolytica* on three other waste or by-product sources such as **glycerol** from Emmelev and **molasse** from Dansukker showed the capability of *Y. lipolytica* to utilize these carbon sources, demonstrating the very wide substrate range.

Modelling

We used **genome-scale modelling** techniques to optimize beta-cartoene 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 growth and production capacity. We further optimized *Y. lipolytica in silico* for **optimal beta-carotene yield** by using FSEOF to identify gene amplification targets, enforcing prouction and growth to go hand-in-hand.

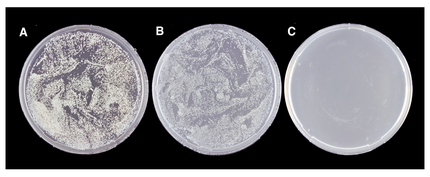




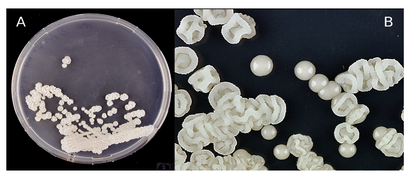


CRISPR-Cas9 & Gene Insertion

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 purpose. We have focused on the integration of an **auxotrophic marker gene**, ***Ura3*** and disruption of the gene ***PEX10*** necessary in peroxisome biogenesis.



The picture shows the insertion of the auxotrophic marker Ura3. A) pIW357+linearized pIW501, B) Linearized pIW501, C) control, into Y. lipolytica PO1f (SC-Ura plates), it was observed that the URA3 gene can be integrated into the genome by CRISPR-induced NHEJ and HR.

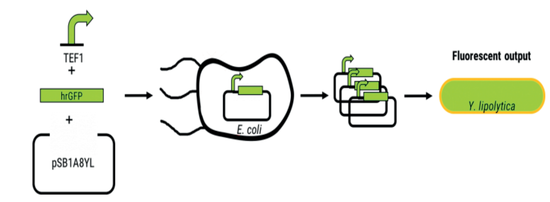


The changed Y. lipolytica morphology from rosey structure to pearl structure, indicates that PEX10 has successfully been knocked out using the CRIPR-Ca9 system.

The successful CRISPR-induced gene disruption and gene insertion opens up for gene integration of any desired product in the future.

Heterologous Protein Expression

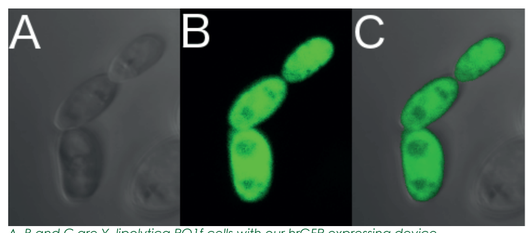
We aimed to create a **versatile cell factory**, which in the future will be able to produce any product. We wanted to demonstrate this versatility of *Y. lipolytica* by producing heterologous proteins such as the humanized *Renilla reinformis* retina green fluorescence protein **hrGFP** and **proinsulin** using our own plasmid pSB1A8YL.



To express HrGFP, we chose to **develop our own BioBricks**. Namely a constitutive TEF promoter (BBa_K2117000) and the hrGFP (BBa_K2117001) previously used succesfully in *Y. lipolytica*. By combining these two parts in a device (BBa_K2117005), the expression can be easily detected due to the fluorescence signal produced.

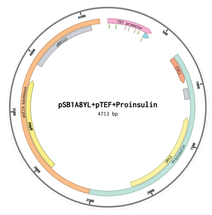
We managed to express the hrGFP. The construct was codon optimised using TaiCO.

Expression of the protein is **groundbreaking** for future use of *Y. lipolytica*.



A, B and C are Y. lipolytica PO1f cells with our hrGFP expressing device (BBa_K2117005) by pSB1A8YL.

Feeling confident we wanted to transform a gene encoding a protein relating to the Danish industry. Therefore, we went for the **human proinsulin** gene sequence. We constructed a new BioBrick for this purpose: BBa_K2117002. We succeded with **assembly, transformation and detection** by cPCR.



Achievements

- ✓ Integrated **inputs from the local industry** in the development of our project
- ✓ Constructed and **characterized BioBricks** for protein production i *Y. lipolytica*
- ✓ Developed an **expression system for** *Y. lipolytica* in accordance with the iGEM standards
- ✓ Distributed knowledge of iGEM and **made synthetic biology available to high schools nation wide**

A Great Thanks to Our Sponsors!

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References

(1) Miljøstyrelsen, Affaldsdatasystemet. Available at: ads.mst.dk [Accessed October 17, 2016].
(2) dos Reis, Mario, Renos Savva, and Lorenz Wernisch. "Solving the riddle of codon usage preferences: a test for translational selection."

iGEM