A synthetic biology approach for the sugar cane industry improvement: Introducing enzyme surface display as an alternative to enzyme immobilization
Brainstorm

- Last year’s Biosensor
- Sulfurafane Metabolic pathway
- Curing Cancer
Different Approach

- Feasable Project
- Social and Economical Impact
- Versatile

New setbacks arise, while old problems are left behind
Mexican Sugar Cane Industry

- 13.5% National agricultural production
- 5.3 million tons of sugar
- 4.8 million annual tons
- 57 sugar mills near 277 cities inhabited by 12 million people
- 450,000 direct jobs
- Benefits 2.2 million
Actual context

- Replacement of sugar by sweeteners like high fructose corn syrup.
- Industry of beverages
- NAFTA 2008 (North American Free Trade Agreement)
- Total cost of sugar production takes 80% of its sales.
Opportunities

Past 2011

sucrose 2011

Past

sucrose

bagasse 11 million tons (51.8% cellulose)

bagasse CO₂ emission

CO₂ emission
Sugar Cane Industry

(12) United States Patent
Serna-Saldivar et al.


(54) PRODUCTION OF INVERT SYRUP FROM SUGARCANE JUICE USING IMMOBILIZED INVERTASE

4,405,715 A 9/1983 Monsan
4,918,016 A 4/1990 Leuba et al.
5,270,177 A 12/1993 Ramos Lazcano et al.
5,314,814 A 5/1994 Harder et al.
5,405,764 A 4/1995 Harder et al.

(73) Inventors: Sergio R. Serna-Saldivar, San Pedro Garza García, N.L. (MX); Marco A. Rito-Palomares, Monterrey N.L. (MX)

(73) Assignee: Instituto Tecnologico y de Estudios Superiores de Monterrey, Monterrey (MX)

Advances

Novel Process: Sugar Cane Juice- Fructose
However there is still room left for optimization...

Enzyme Overproduction

Enzyme
Analysis

- **Economical Impact**

- **Social Impact**

- **Poor handling of byproducts**

- **Expensive downstream processing, specifically purification and immobilization of enzyme**
How can we help?

- If we can provide a synthetic biology approach, to improve the sugar cane industry, then it will gain an added value by manufacturing valuable products.

  - High Fructose Syrup out of Sucrose
  - Biofuels substrates out of bagaze (cellulose)
Main Objective

- Provide as a proof of concept, a genetic construction in a model microorganism (*Escherichia coli*), capable of displaying functional enzymes (invertase and cellulase) outside the cell.
Specific Objectives

1- Selection of a Capable Membrane Proteins
2- Selection of suitable Enzymes
3- Selection of appropriate strains
4- Design of a functional expression cassette
5- Evaluation of the expression of the constructs
6- Measurement of the chimeric enzymes activity
Membrane Proteins

- PhoA SP + EstA Fusion
  - Origin: Pseudomonas aeruginosa
  - Exression Mechanism: Type V
  - PhoA SP: Alkaline Phosphatase
  - Compatibility: Free C-terminus

- Lpp SP + OmpA Fusion
  - Origin: Escherichia coli
  - Compatibility: Free N-Terminus
  - Exression mechanism: Type II
  - Lpp SP: Native Lipoprotein

1- Extracellular Transport Ability
2- Signal Peptide
3- Compatibility

Linker + EstA Membrane Protein
**SacC Invertase**
- Origin: *Zymomonas mobilis*
- Structure: Monomeric Structure
- Characteristics: 20°-40° C, pH 2.5-7.5, 48 kDa
- Free N-Terminus

**CelD Cellulase**
- Origin: *Clostridium thermocellum*
- Structure: Monomeric
- Characteristics: Max 80° C, pH 5-8, 68 kDa
- Free C-Terminus

**Enzyme**

1-Structure
2-Characteristics
3-Active Site

**Extracellular Cellulase**

**Extracellular Sucrase**

**RBS+signal peptide**

**phoA+Cellulase**
Protein Expression Systems

Rosetta Gami

BL21 SI

BL21 Star

XL1Blue

C43

E. coli Strains

Escherichia coli

Characterization AraBAD

BW27783
Expression Cassette Design

Arabinose Induced Constructs
Construct Expression

Arabinose protein induction / sample preparation SDS-PAGE

Pre-inoculum six strains, wild type and transformed. Overnight 37°C.

Inoculum. 0.1 Initial OD, 0.6 to 1.0 final OD.

Arabinose 0.1 micromolar

30°C 24 hours

15°C 48 hours

xTractor buffer kit

Soluble Fraction

Insoluble Fraction

Sonication with water; half initial volume
CelID Expression Results

- Expected MW fusion protein (estA + celD) 102.5 kDa
Device Functionality
CelD + estA Activity

REDUCING SUGARS + DNS → Rosetta Gami

\[ y = 0.3085x - 0.0641 \]
\[ R^2 = 0.982 \]

Calibration Curve

Proportional Colorimetric Concentration
Rosetta Gami

Whole-Cell

Cell Lysate Fraction

Soluble
Insoluble
Whole-Cell Cellulase Activity was determined by IUPAC Filter Paper Assay, with E. coli strain, Rosetta Gami, negative control and transformed.

T-test
Alpha = 0.05
Ho -> rejected  Suggesting
Cellulase Activity of Cell lysates

SOLUBLE FRACTION

INSOLUBLE FRACTION

Glucose Concentration (uM)

Cell Lysate Fractions Activity was determined by IUPAC Filter Paper Assay, with E. coli strain, Rosetta Gami cellular lysate, negative control and transformed with celD+estA

T-test
Alpha = 0.05
Ho -> rejected
We can conclude that ...

- Difference between negative control and estA+celD -> Statistically significant
- Cellulase + estA .... ACTIVE
- Activity in C- .... Background signal
Future Work

- Standarize the IUPAC Filter Paper Assay -> more measurements
- LB media -> M9 media or others
- Different *E. coli* strains
- Another measurement methods *e.g.* Benedict method, HPLC
Construct Expression
SacC Expression Results

- **Expected MW**
  - fusion protein (OmpA + SacC)
  - 62.8 kDa

- **Unclear evidence vector expression** by SDS-PAGE + Coomassie blue
Device Functionality
Whole Cell SacC Activity

- Enzyme assay
  - Enzymatic reaction using sucrose as substrate @ pH 5.0, @ 36°C 30 min
  - Quantification of released fructose
  - Colorimetric assay based on Tetrazole reduction

![Graph showing fructose levels over OD 600 for BL21 SI + pSacCompA and BL21 SI Neg Ctrl]
After 30min Rx .. BL21SI

- Tetrazole ---- Fructose

- **t-Test (2 tails, α=0.05)**
  - Rejects the null hypothesis
  - \( H_0 = \text{The population means are the same} \)
Conclusion

• More Specific Stain
• More measurements
• Different *E. coli* strains
• Another measurement methods e.g. HPLC
Human Practice

- Genes in a Bottle
  Molecular biology workshop

- Ene.Pé notes
  Myths and facts Biotech

- MicroCongress
Augmented Biobricks
Synthetic biology

Real time 3D modeling of your construct
Further Approach

- Comparing Raw Data with analytic tests
- Eukaryotic systems for heterologous expression
- Sustainable high fructose syrup process
- Unit operations
Thank you.