A Synthethic Biology Approach for the Sugar Cane Industry Improvement: Introducing Enzyme Surface Display as an Alternative to Enzyme Immobilization

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Abstract

Sugar production is one of the major Mexican industries, but now is facing problems such as substitution by sweeteners and high production costs.

Our project expects to apply synthetic biology to use products of sugar cane. With our new genetic construct, using a cell surface display approach, we will be able to immobilize invertase and cellulase by fusing it to bacterial native transmembranal protein domains.

Results of our work show that there is expression and activity of the chimeric enzymes, however it is needed to do more tests in order to confirm the effectiveness of our system.

Background

Due to economic and social reasons the Mexican sugar industry is now facing a downfall and 12 million Mexicans are suffering the consequences. Currently the low international price of sugar, the beginning of NAFTA, the use of alternate sweetener, added to old manufacturing technology and the lack of usage of byproducts is making a 5.3 million ton product industry, powder to the ground.

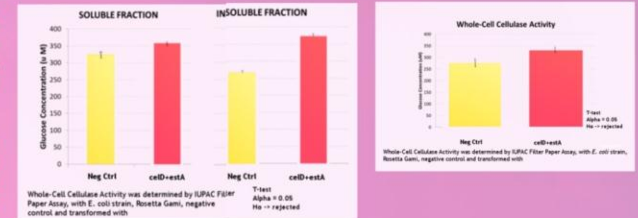
Cell surface display is a technique to exhibit proteins on the surface of bacteria, fungi, or mammalian cells by fusing them to the surface anchoring motifs. When a protein is expressed in the outer membrane of *Escherichia coli*, the cell envelope acts as a matrix, simplifying the process of preparing and purifying the protein.

Using cell surface display technique, fusing enzymes to fragments of membrane proteins such as estA and ompA, we expect successful localization of invertase and cellulase at the external surface of *E. coli*, giving an alternative to employ sugar cane in a profitable way.

Results

The activity of celD+estA was measured with the IUPAC Filter Paper Assay Method. The charts show that there is a statistically significant difference ($\alpha \leq 0.05$) in the glucose concentration between the negative control and celD+estA whole-cells, and between celD+estA insoluble fraction and celD+estA soluble fraction.

However, the presence of activity in the negative controls demands the improvement of our methods to determine cellulose activity. A method for reducing this uncertainty is to change the culture medium (LB) to minimum nutrient medium (M9). A more extensive selection for higher cellulose activity strains and other methods to assay enzyme activity (HPLC, Benedict Assay and others) are *required*.



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

The functionality of the fusion protein ompA + sacC was measured by a commercial colorimetric enzymatic assay kit based on the tetrazole reduction. The higher fructose concentration in the sample comparing with the non-transformed BL21SI C -, indicates sacC activity in the BL21SI +ompAsacC. T-test (2 tails, $\alpha \leq 0.05$) was carried out to reject that the population means are the same.

Protein profile of cell lysates from culture experiments of *E. coli* BL21 SI, C43, XL1 Blue, Rosetta Gami and BW27783.
Soluble and insoluble phases were obtained using Clontech's xTractor buffer kit. Insoluble phases were homogenized with sonication in water with half of the total inicial volume of lysis mix.

TNI - transformed and no induced
WT - wild type
SF - soluble fraction
IF - insoluble fraction
PMWM - protein molecular weight marker

Construct Design

Enzymes

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

SacC Invertase
Origin: *Zymmomonas mobilis*
Structure: Monomeric Structure
Characteristics: 20-40 °C, pH 2.5-7.5, 51 kDa
Free N-terminus available for linking

Membrane proteins and signal peptides:
- Construct lpp+ompA+sacC.
- Signal peptide from native lipoprotein of *E. coli* Periplasm translocation
- Origin: *E. coli*
- Compatibility: free N-terminus
- Excression mechanism: secretion pathway type II

Construct phoA+celD+estA
- Signal peptide from alkalyne Phosphatase native *E. coli* Periplasm translocation
- Origin: *Pseudomonas aeruginosa*
- Compatibility: free C- terminus
- Excression mechanism: secretion pathway type V

Protein Expression Systems
Rosetta Gami
BL21 SI
XL1Blue
C43

Characterization AraBAD
BW27783

Use of biobricks in cassette design
araC - I13458
P_{BAD} - K206000
RBS - B0034

Conclusions

- We built two functional constructs: celD+estA and ompA+sacC, both are novel membrane fusion proteins on *E.coli*
- The cellulose (celD) and invertase (sacC) fusions to transmembrane protein domains (Gram negative bacteria), presented biological activity in whole cells.
- For celD+estA, most of the activity was shown in the insoluble fraction, even though both constructs did not have a translation terminator.
- We determined with a SDS-PAGE the presence of a band which matches the molecular weight of celD+estA.
- This system could be used then to express other enzymes that can complement the process of sugar cane industry. Examples of this are glucose isomerase, which converts glucose to fructose; and exo-beta-glucanase, that converts of cellulose to glucose, allowing the possibility of producing biofuels.