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Directed evolution of acyltransferases for the improvement of triglyceride production in *Escherichia coli*

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Background

Wax ester synthase/diacylglycerol acyltransferase (WS/DGAT) is a family of enzymes able to perform the esterification of acyl-CoA and diacylglycerol or fatty alcohols to produce triglycerides (TAGs) or wax esters, respectively[1].
Both products can be used for diverse fatty acid-derived products such as biodiesel[2].
WS/DGAT protein from *Thermomonospora curvata* (tDGAT) is able to produce TAGs and waxes in *E. coli*.
TAG production in *E. coli* could be improved through directed evolution of this enzyme.

Objectives

- Improve TAG production in *E. coli*.
- Acquire information about amino acids residues involved in substrate recognition.
- Elucidate possible key interactions between WS/DGAT and lipid droplets.

Results

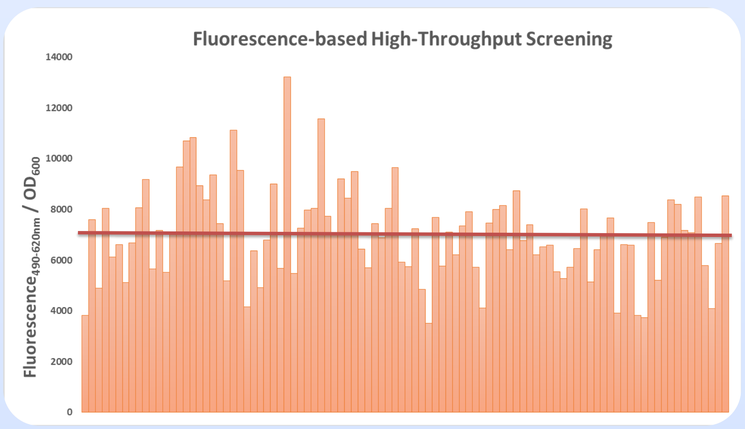

Figure_2. 96-well plates fluorescence (490-620nm) is measured using PerkinElmer's VICTORX3 Multilabel Plate Reader. Red line represents the average of each plate fluorescence after normalized by the OD_{600}. We selected the best mutants of each plate for a second selection step.

In order to confirm that the mutants actually improved TAG accumulation, lipid extraction and further thin layer chromatography were performed.

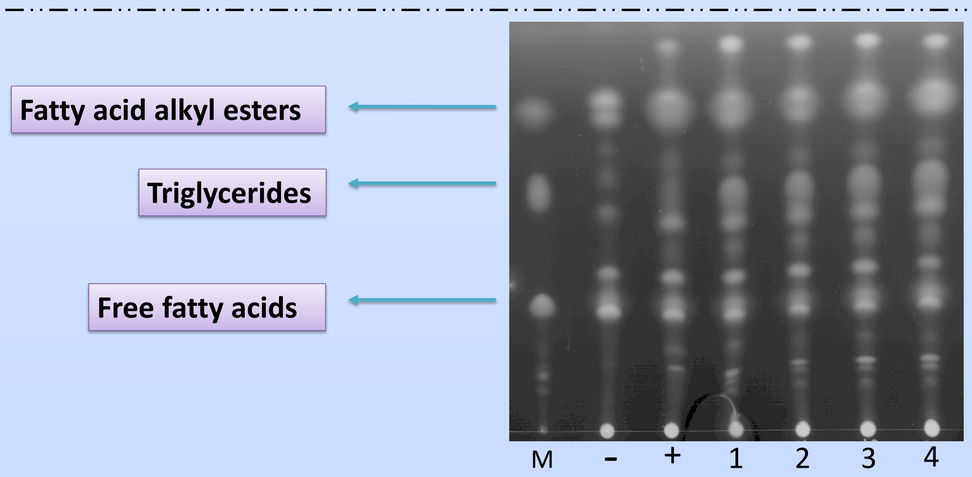

Figure_3. Thin layer chromatography analysis. M) Marker. Lipid fractions were extracted from BW27783 *E. coli* cells containing : -) PBAD, +) PBAD::tDGAT, 1,2,3,4) four selected mutants.

Mutants producing higher amount of TAG are further selected and sequenced. This way we were able to localize in the protein structure mutations that lead to a 2-fold increase in the TAG production.

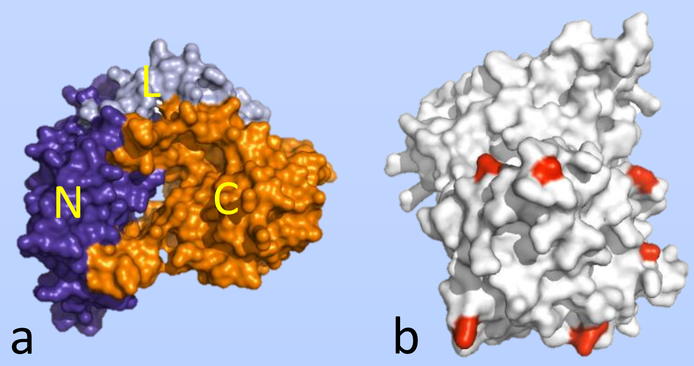

Figure_4. Phyre 2 server was used to generate WS/DGAT 3D models[4]. a) General structure prediction of tDGAT with two domains conected by an helix loop. b) Some of the positive mutants into the N domain appear only on the surface of the protein.

Interestingly, we have found a pattern in the mutation sites that led us to generate a possible model of protein-lipid droplets interaction.

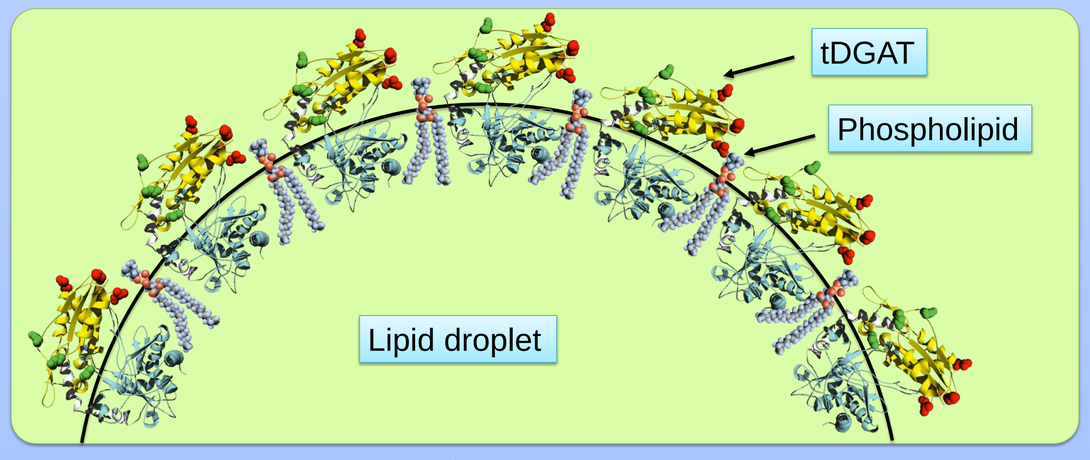

Figure_5. Proposed model for the WS/DGAT proteins assembly surrounding the lipid droplets. Mutations highlighted in green and red could facilitate the settlement of the proteins on the lipid monolayer by protein-protein or protein-lipids interactions.

Methodology

To perform directed evolution we have developed a protocol where we constructed mutant libraries by MEGAWHOP[3] after mutagenic PCR in order to obtain thousands of variants of the protein.

Using Nile Red, a fluorescent dye that binds to neutral lipids, we can select improved variants of tDGAT. A library of ≃12.000 was analysed through a high throughput selection system based on Nile Red fluorimetry using 96-well plates and a Victor fluorometer (Perkin Elmer).

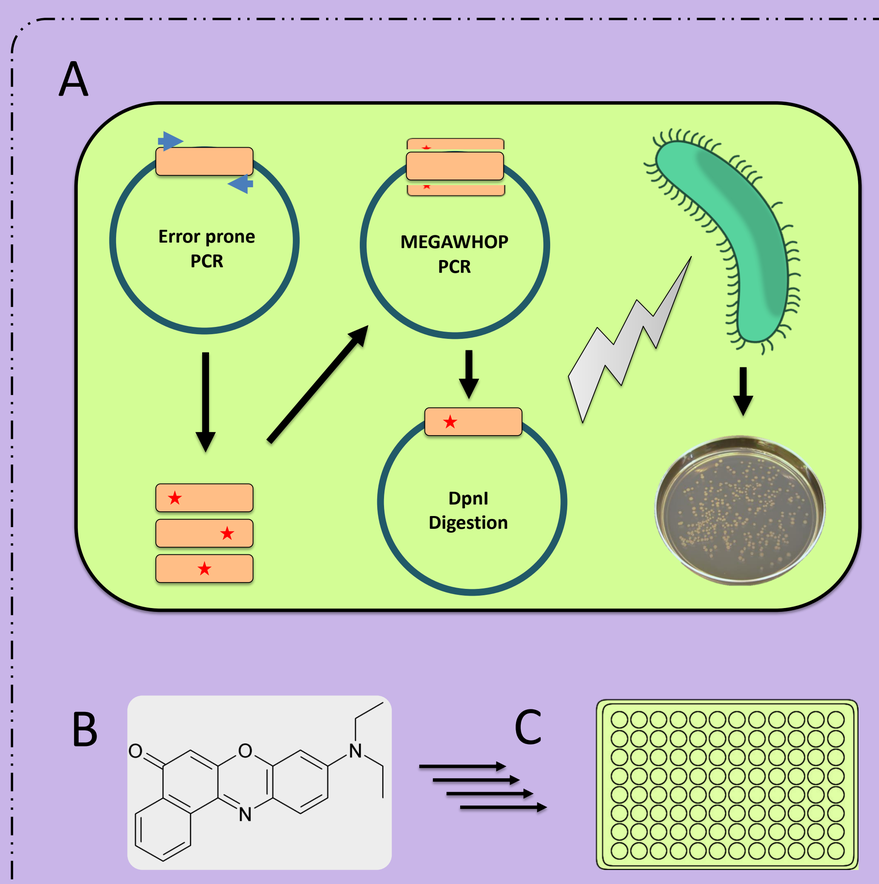

Figure_1. Schematic representation of the procedure for creating mutant libraries. A. Mutagenic PCR product was used as megaprimer for a second PCR step. The plasmid library is screened after subsequent electroporation of the synthetic plasmid collection in E. coli. Cells are further grown and induced in 96-well plates. Nile Red (B) is added directly to the culture plate (C) to measure changes if fluorescence .

References

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Acknowlwdgements

This work was supported by Ministerio de Ciencia e Innovación (BIO2010-240512). We are grateful to the Intergenomics group at IBBTEC for helpful discussions and advice.