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staining of SDS-PAGE gels, but no secreted protein was detected in medium supernatant by coomassie staining or western blot using anti-his antibodies. Enzyme activity was measured by the DNS method with 1% pullulan solution as substrate, giving 0.282 U/ml of *L. lactis* crude extract. Suggesting that the overexpressed protein has the predicted function, but further optimization will be required to increase its activity.

Keywords: GRAS, protein expression, lactic acid bacteria

(639) **REVEALING TAG SYNTHESIS PATHWAY IN THE GREEN MICROALGA *Haematococcus pluvialis:* A SOURCE OF TRIACYLGLYCERIDES FOR BIODIESEL AND VALUABLE CO-PRODUCTS**

Paola Gabriela Scodelaro Bilbao (1, 2, 3, 4), Andrés Garelli (2, 3, 4), Marina Diaz (1, 3), Gabriela Salvador (2, 3, 4), Patricia Leonardi (1, 3, 4)
(1) CERZOS. (2) INIBIBB. (3) UNS. (4) CONICET.

Haematococcus pluvialis is an oleaginous microalga known as an important source of the red carotenoid astaxanthin. Recently, we proposed *H. pluvialis* as a potential source of triacylglycerides (TAG) for biodiesel production, and of phytosterols with potential nutraceutical properties. In this work, our purpose was to study the metabolic pathways involved in TAG synthesis induced by high-light stress in *H. pluvialis* UTEX 2505. For this end, the strain was grown in Bold's Basal Medium under high-light intensity or control light for 1, 2 and 3 days. Then, the lipid content and the expression of enzymes known to be involved in lipid metabolism in plants were analyzed. High-light stress significantly stimulated TAG and phytosterol synthesis and accumulation ($p<0.01$) in all the conditions tested. Red Nile staining analyzed by confocal microscopy confirmed the presence of cytoplasmic lipid droplets after *H. pluvialis* high-light exposure. The use of propranolol, a lipin inhibitor, significantly diminished ($p<0.01$) TAG synthesis induced by high-light intensity. Western Blot analysis revealed that high-light stress significantly induced the expression of the fatty acid synthase (FAS) complex ($p<0.01$), lysophosphatidic acid acyltransferase (LPAAT) ($p<0.01$) and diacylglycerol acyltransferase (DGAT) ($p<0.01$) enzymes. In addition, qPCR also showed increased mRNA levels of the above-mentioned enzymes. On the other hand, high-light also induced the expression of the monoacylglyceride ($p<0.01$) and diacylglyceride ($p<0.01$) lipases. However, no significant changes ($p>0.05$) were observed in the release of glycerol to the medium after high-light stress. Together, these results suggest that TAG synthesis in *H. pluvialis* occurs *de novo* through stimulation of the fatty acid synthesis pathway and induction of the Kennedy pathway under light stress. Our results constitute the starting point to design molecular strategies for increasing the synthesis of valuable products from *H. pluvialis*.

Keywords: TAG synthesis pathway, *Haematococcus pluvialis*, biodiesel

(737) **BUILDING THE MOLECULAR BLOCKS OF MULTI-ENZYMATIC COMPLEXES FOR THE IMPROVEMENT OF BIOFUEL PRODUCTION**

Natalia Gorojovsky (1), Matias Rubén Iglesias Rando (1), Javier Santos (2), Martín Dodes Traian (1), Vanesa Zylberman (3), Fernando Alberto Goldbaum (4), Patricio Oliver Craig (1)
(1) IQUIBICEN. (2) IQUIFIB. (3) INMUNOVA. (4) Fundación Instituto Leloir.

The enzymatic degradation of lignocellulose generates sugars that upon fermentation produce bioethanol. The enzymes currently used in this process are expensive and have low efficiency. It is important to develop new methods to increase their activity and stability for an economically viable production of bioethanol.

Cellulosomes are multienzymatic complexes that colocalize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. However, the industrial production of natural cellulosomes has serious limitations because of the properties of their scaffolding protein. Our goal is to use the structure of an oligomeric protein that is highly stable and highly expressed in bacteria, as a scaffold for the colocalization of a consortium of cellulolytic enzymes and the development of artificial cellulosomes. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy trough high affinity heterodimeric peptides complementary fused to the oligomeric scaffold and the target enzymes. For this purpose we cloned and expressed in *E.Coli* the catalytic domain of a variety of enzymes, including endoglucanases, exoglucanases, xylanases, beta glucosidases and cellulose binding domains from different organisms, fused to the coupling peptide. The amount and solubility of these multienzyme building blocks were compared to the original isolated domains. The coupling peptide doesn't perturb the solubility of the target proteins. At least one member of each functional category is solubly expressed in significant amounts, encouraging further development of the artificial cellulosomes.

It is expected that these complexes will help to increase the enzymatic lignocellulose degradation, reducing the cost of bioethanol production and favoring fossil fuels substitution.

Keywords: Cellulase, Macromolecular complex, Protein engineering, Biotechnology, Bioethanol

(1129) **DEVELOPMENT OF BIOTECHNOLOGICAL PROCESSES FOR THE PRODUCTION OF RECOMBINANT THERAPEUTIC PROTEINS WITH HIGH SOCIO-ECONOMIC IMPACT**

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The aim of this work is to develop the biotechnological processes for the production of three recombinant proteins for therapeutic use. The α-galactosidase A (α-GAL) and the α-Glucosidase (α-GAA) are used for the treatment of Fabry disease and Pompe disease, respectively. These diseases are ultra-orphan pathologies whose treatments are amongst the most expensive on a cost-per-patient basis (U$S 150.000-3.000.000/year). The DNaseI is used for the treatment of cystic fibrosis, one of the most common lethal inherited genetic diseases in Caucasian population (approximately 1/2500 births). Initially, we analyzed the transient expression of these proteins in Chinese hamster ovary (CHO-K1) cells. Plasmid pCI-neo (Promega) was used as the expression vector, which promote constitutive expression of cloned DNA inserts in mammalian cells. The coding sequence of each protein followed by HA-tag and preceded by the signal peptide were synthesized (GenScript, USA) and cloned into pCI-neo downstream of CMV promoter region. For the three proteins, the sequences of coding regions and signal peptides used in the constructions were identical to the corresponding human sequences. An additional plasmid was constructed for the α-GAL, in which the signal peptide sequence was identical to the corresponding CHO-K1 cell sequences and the coding sequence was optimized for the codon usage in this cell line. CHO-K1 cells were transfected with the plasmids and the expression of the proteins was analyzed by Western blot and by immunostaining using anti-HA antibodies, followed by confocal microscopy observation. All three proteins were successfully expressed. However, the optimized sequence seems to work worse than the non-optimized sequence. Treatment of transfected CHO-K1 cells with tunicamycin (N-glycosylation inhibitor) modified the relative migration of the three proteins indicating that the expressed proteins are glycosylated, requirement for the functionality of these proteins.

Keywords: α-galactosidase A, α-Glucosidase, DNaseI, Biosimilars.

(1370) **PROCESS DEVELOPMENT FOR THE PRODUCTION OF A THERMOSTABLE STERYL GLUCOSIDASE IN *ESCHERICHIA COLI***

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