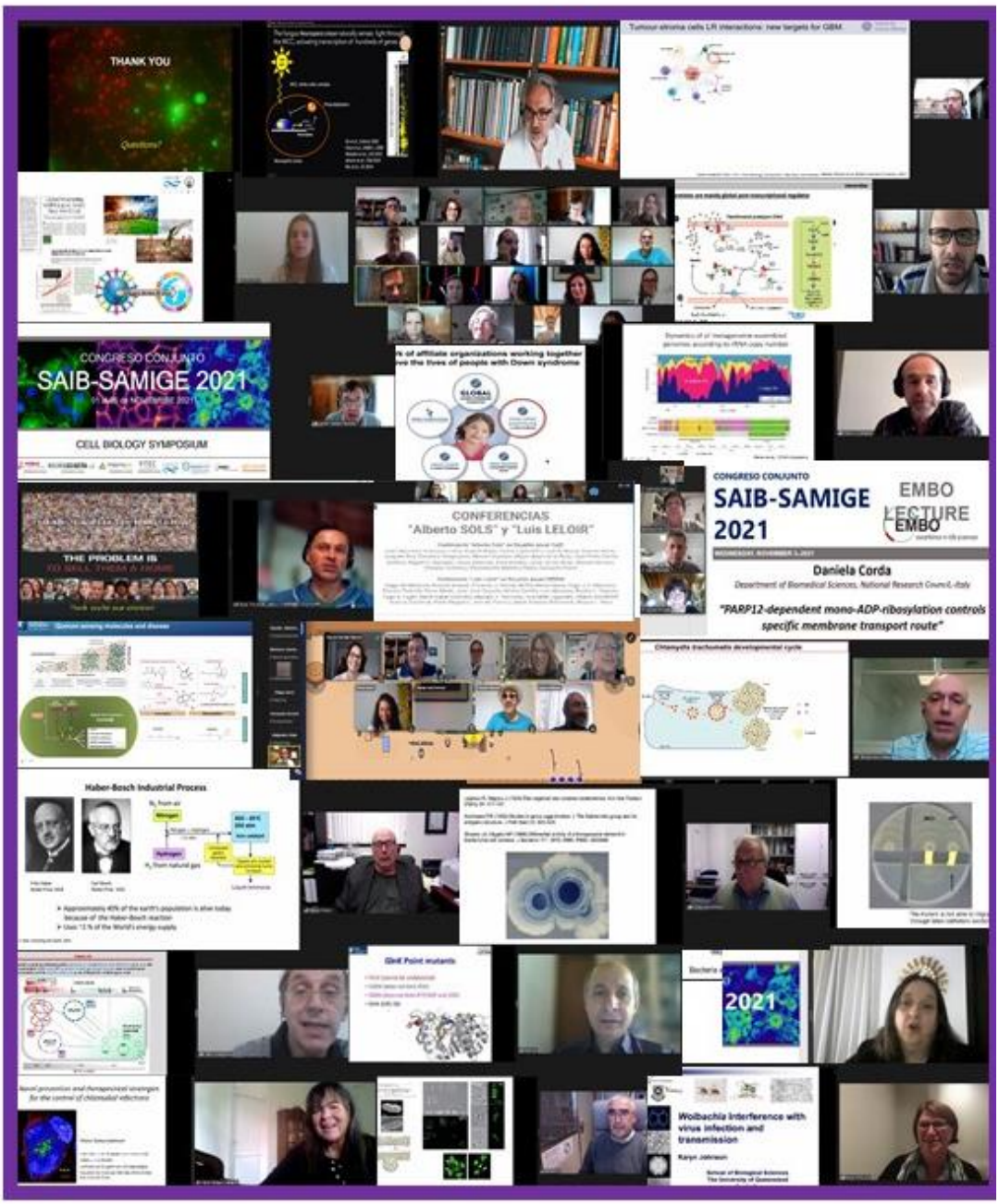
BIOCELL 46 (Suppl. 1), 2022
ISSN 0327- 9545 (printed version)
ISSN 1667-5746 (online version)

SAIB - SAMIGE Joint meeting
2021 *on line*



November 1-5, 2021

BIOCELL 46 (Suppl. 1), 2022
ISSN 0327- 9545 (printed version)
ISSN 1667-5746 (online version)

LVII Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research (SAIB)

XVI Annual Meeting of the Argentinean Society for General Microbiology (SAMIGE)

SAIB - SAMIGE Joint meeting 2021 *on line*

/F7_4458) did not show significant differences with control cells either with fructose or glycerol as the sole carbon sources. On the other side, whereas overexpression of *F7_3568* gene result an increase of TAG content, no significant changes were observed in the case of *F7_4458* gene in comparison with control cells cultivated with same carbon sources. The results obtained in this study suggest that F7 cells possess at least one active DGAT enzyme responsible for TAG biosynthesis. Deciphering the functions of these enzymes is of great importance not only to understand the role of TAG in the physiology and survival of these microorganisms but also as a key target to improve the lipid content in these bacteria for biotechnological purposes.

MI-P056-163
Rhodococcus oleaginous AS A CHASSIS FOR ADIPOSE PROTEIN EXPRESSION AND IN VIVO EFFECT ON GROWTH AND LIPID METABOLISM

Herrera Y, Villalba MS, Alvarado C, Alvarez HM, Hernández MA
Universidad Nacional de la Patagonia San Juan Bosco, Instituto de Biociencias de la Patagonia, INBIOP, UNPSJB-CONICET, Argentina. E-mail: mahernandez@unpata.edu.ar

Oleaginous Rhodococcus are powerful biological systems for the production of several compounds of biotechnological interest, including neutral lipids such as triacylglycerols (TAG) and fatty acids (FA). These bacteria also possess a robust metabolism which in turn permits them to growth from a high range of carbon sources and under diverse stress conditions. Based on these properties, these bacteria are promising chassis for the production of several compounds via both, expression of native proteins as well as proteins from other biological systems. In this work, we demonstrate that these oleaginous bacteria can be used as an alternative host for protein expression from a complex system, such as adipocytes. Fatty acid binding protein type 4 (FABP4) is one of the most abundant protein in adipocytes associated with lipid metabolism and a promising therapeutic target for several metabolic diseases. Here, we expressed FABP4 under a thiostrepton inducible promoter vector (pTipQC2) and analyzed its effect on growth profile and intrinsic lipid metabolism in the oleaginous strain *R. jostii* RHA1. SDS-PAGE analysis demonstrated a positive expression of the recombinant protein under standard culture conditions. Whereas no significant changes on growth profile was observed in recombinant cells growing with glucose, FABP4 expression resulted in a slight enhancement of cell growth under rich nitrogen conditions (MSM1) with palmitate, a native ligand for this protein. By the contrary, the growth of FABP4 overexpressing cells was lower with both, glucose and palmitate under low nitrogen conditions (MSM0.1). These results may suggest that FABP4 may alters the lipid homeostasis and indirectly the growth profile in recombinant cells. Analysis of the lipid profile after growing on glucose or sodium palmitate was also analyzed. As revealed by TLC and GC analysis, total lipids varied in recombinant strain in more or less, depending on the nitrogen levels, the carbon source and cell harvesting time. Under rich nitrogen conditions, TAG fraction increased and decreased in recombinant cells growing with glucose and palmitate, respectively. On the contrary, a decrease tendency of TAG fraction was observed in recombinant cells growing with both glucose and palmitate, under poor nitrogen conditions. According to these results, FABP4 expression may influence the in vivo lipolysis and/or lipogenesis processes in rhodococcal cells. These results are a preliminary proof of concept demonstrating that: (1) oleaginous rhodococci may serve as valuable hosts for expression of eukaryotic proteins involved in lipid metabolism; (2) FABP4 protein from adipocyte was able to functionally engage with the rhodococcal lipid metabolism promoting an alteration of the neutral lipid fractions dynamic in the recombinant cells. This genetic approach may offer a faster and cheaper alternative to *in vivo* evaluate the effect of potential FABP4 inhibitors, which is relevant to medical research.

MI-P057-165
AMIDOTRANSFERASE ACTIVITY AS A TARGET FOR CHEMOTERAPEUTIC DEVELOPMENT AGAINST *Trypanosoma brucei*

Scattolini A, Uttaro AD, Mansilla MC
Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Instituto de Biología Molecular y Celular de Rosario – CONICET. E-mail: scattolini@ibr-conicet.gov.ar

Lipoic acid (LA) is a universally conserved sulfur-containing cofactor involved in one-carbon and oxidative metabolism. LA can be acquired by a salvage pathway, in which it is attached to their cognate enzymes by a lipoate ligase, or *de novo* synthesized by a pathway requiring an octanoyltransferase and a lipoate synthase. A more complex pathway, referred to as "lipoyl-relay", requires two additional proteins, GcvH, the glycine cleavage system H subunit, and the amidotransferase, LipL. Interfering LA synthesis would be a potential chemotherapeutic target against parasites like *Trypanosoma cruzi* and *T. brucei*, due to the essentiality of protein lipoylation for cell viability. By complementation of different mutants of *Bacillus subtilis* we identified TbLipL as the amidotransferase of the parasite. This protein shares most of its N-terminal amino acid sequence with bacterial amidotransferases but it has an additional C-terminal domain. Primary structure of this domain is highly conserved in *Trypanosomas* but differs from those of other eukaryotes. We found that the truncated version of TbLipL, lacking this C-terminal domain, was unable to restore growth of a mutant strain of *B. subtilis* deficient in amidotransferase activity, indicating that it is essential either for catalysis or proper folding. It is remarkable that TbLipL lacks a cysteine residue equivalent to C150 of *B. subtilis*, identified as essential for the amidotransfer reaction, and conserved in bacterial proteins. This seems to be a common characteristic of eukaryotic amidotransferases, which only share the conserved lysine present in the biotin/lipoyl