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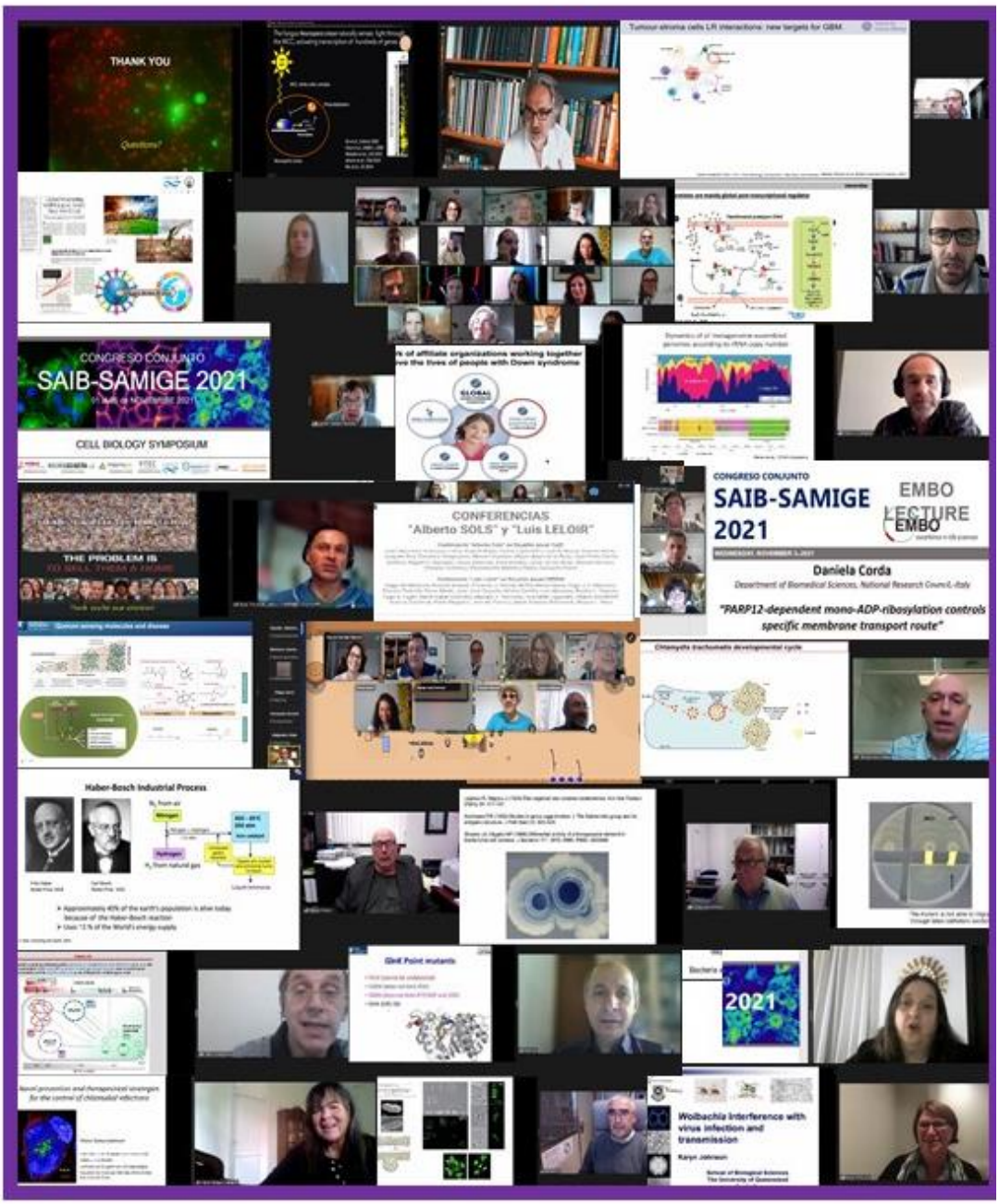
SAIB - SAMIGE Joint meeting
2021 *on line*



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the mutant strains. All of them showed higher levels of biofilm formation on abiotic surfaces, lower motility in semisolid media, and different colony phenotypes in Congo red assay, as compared to WT. These indicate an altered envelope structure or composition in the mutants, leading to the observed phenotypes, and further suggest roles for these OMPs in Ab pathogenesis. The increase in biofilm formation and reductions in cell adherence and invasion supports the notion that Ab can modulate its adhesion properties in order to adapt to diverse environments. Although more work is needed, these results contribute to the understanding of Ab virulence mechanisms, revealing novel possible targets for therapeutic development.
1. Gallagher LA et al. (2015) Journal of Bacteriology, 197(12), 2027–2035. https://doi.org/10.1128/JB.00131-15
2. Giacone L, et al. "Characterization of outer membrane vesicle-carried proteins as pathogenicity factors from *Acinetobacter baumannii*". ISEV 2020 Annual Meeting, Julio 2020.

MI-P054-119
THE POLYAMINE SPERMIDINE REGULATES IRON UTILIZATION AND PYOVERDINE SECRETION IN *Pseudomonas syringae*

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Iron is an essential element for most organisms as it takes part in a wide range of cellular processes. Under iron-limiting conditions, many bacteria synthesize and secrete siderophores such as pyoverdine (PVD) to scavenge this element from their surrounding environment and make it available to the cell. Importantly, it has been shown that PVD synthesis and secretion is essential for virulence in *Pseudomonas aeruginosa*, and that the metabolism of PVD in *P. putida* is regulated by different nitrogenous compounds including amino acids and polyamines (PAs). PAs are a family of polycations derived from ornithine and arginine playing essential functions in all living organisms. With the purpose to corroborate whether this connection also exists in phytopathogenic *Pseudomonas* species, in this work we used the model *P. syringae* pv. tomato DC3000 (Pto) to study the relationship between the metabolism of PAs and iron utilization. When Pto was grown in M9 minimal medium the only PAs detected at the extracellular and intracellular cell environments were putrescine (Put) and spermidine (Spd). Incubation of Pto in an iron-depleted medium supplemented with Spd reduced PVD secretion, whereas this reduction did not occur with Put amendment. These results suggest that siderophore production is negatively regulated by Spd. To analyze in depth the effect of PA depletion on iron utilization, we constructed two mutant strains lacking genes involved in the synthesis of Put (Δ*speA*Δ*speC*) and Spd (Δ*speE*). Both mutant strains were affected on PVD secretion during iron-limiting conditions. Additionally, no significant increments in growth were observed in the Δ*speE* and Δ*speA*Δ*speC* strains in response to iron supplementation, indicating that the utilization of this compound results impaired under PA depletion. However, Spd supplementation promotes the growth of the Δ*speE* mutant in iron-amended media, while no effect in growth was observed in the Δ*speA*Δ*speC* strain after Put addition. Deficiency on PDV secretion and iron utilization in mutant strains were restored by genetic complementation. These contrasting effects of Spd in PDV secretion and iron utilization might be crucial to maintain iron homeostasis, as even though the supply of iron is required for biochemical demand, the intracellular abundance of this element might lead to iron-induced toxicity. Our future research will try to discern the regulatory mechanisms mediated by Spd that operates on iron utilization in bacteria.

MI-P055-123
CHARACTERIZATION OF TWO DGAT ENZYMES IN THE NON-OLEAGINOUS *Rhodococcus fascians* STRAIN F7

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Some species of the *Rhodococcus* genus, such as *R. opacus* and *R. jostii*, are able to accumulate triacylglycerols (TAG) up to 60% or more of their cellular dry weight. For this reason, oleaginous rhodococci are promising microbial cell factories for the production of lipids to be used as fuels and oleochemicals. In agree with their oleaginous phenotype, species with the greatest capacity for TAG synthesis have several copies of *atf* genes coding for potential DGAT enzymes in their genomes. For example, *R. jostii* RHA1 and *R. opacus* PD630 contain up to 16 copies of *atf* genes. This high gene redundancy makes these strains very robust models for TAG accumulation but at the same time, they constitute very complex models to study the individual contribution of DGAT enzymes. In this study, we analyzed the *R. fascians* F7 genome, a non-oleaginous bacterium able to accumulate significantly TAG only under certain conditions (minimal media with glycerol as the sole carbon source). F7 strain possesses only two *atf* copies (*F7_3568* and *F7_4458*) coding for possible DGAT enzymes and then, it constitutes a good model to study the DGAT enzymes and their role not only in TAG biosynthesis but also in its cellular physiology. Bioinformatic analysis revealed that F7_3568 possess the typical HHxxxDG catalytic site, whereas in F7_4458 this site is only partially conserved. RT-PCR analysis demonstrate that *F7_3568* and *F7_4458* genes are induced approximately 2-fold at low nitrogen levels but *F7_3568* expression was higher than *F7_4458* in both nitrogen rich and nitrogen poor culture media. In order to analyze the contribution of each gene in the physiology and lipid metabolism, we also overexpressed both genes under an inducible thiostrepton promoter. The growth profiles in recombinant strains (F7 pTip-QC2 /F7_3568 and F7 pTip-QC2

/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

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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*

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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