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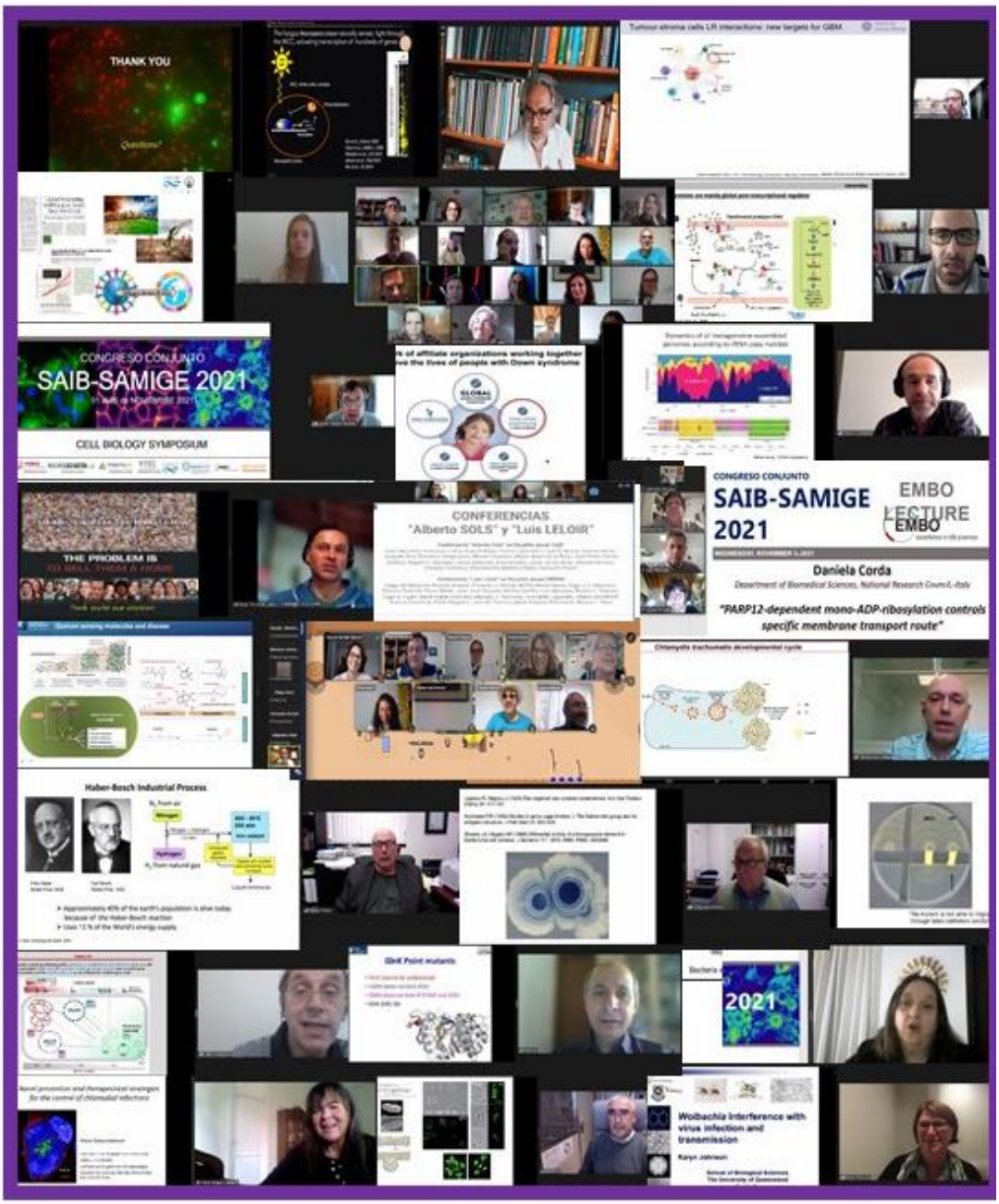

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(CFCS) of this strain. This inhibitory action on curli is mediated by a metabolite whose synthesis in *B. subtilis* requires activation by the 4'-phosphopantetheinyl transferase (PPTase) associated with secondary metabolism, as a PPTase deficient mutant of *B. subtilis* NCIB 3610 and its CFCS showed no inhibitory effect on curli production. Analyses of expression of the *csgBAC* operon, which encodes the curli structural subunits (CsgB and CsgA), and of key regulators of curli biogenesis in *E. coli* cells grown in the presence or absence of the effector metabolite, indicate that the inhibitory effect occurs at post-transcriptional level, affecting either translation of *csgBAC* mRNA or the assembly of CsgB and CsgA into amyloid fibres. In sum, this work provides molecular insights into the mode of action of a microbial compound that targets the major structural component of *E. coli* biofilms.

MI-P050-78
IMPLICATION OF PROBIOTIC BACTERIA ON ACENOCOUMAROL METABOLIZATION

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Probiotic-based foods are becoming highly popular, and their consumption is growing steadily. On the other hand, very little information is available about the interaction of these products with drugs, including anticoagulants, such as the warfarin derivative acenocoumarol (AC). AC is known to interact with some food components. Previous studies by our group demonstrated that incubation of acenocoumarol with two strains of bifidobacteria (*B. bifidum* CIDCA 5310 and *B. adolescentis* CIDCA 5317) reduced the drug concentration after 16 h incubation. In order to deepen our knowledge in this field, different studies were carried out. Moreover, another probiotic bacterium (*Lactobacillus acidophillus* ATCC 314) was included to examine if the effect was genus dependent. Strains were culture in MRS broth at 37°C for 24 h in anaerobic conditions. Then, strains were washed with PBS and incubated with AC 0.16 mg ml^{-1} for 1h. A reduction in AC concentration was observed only for the strain CIDCA 5317 (0.14 mg ml^{-1} ± 8.5 x 10^{-4}). In other experiments, the strains were sonicated for 5 or 8 min and then incubated 1 h with AC 0.16 mg ml^{-1}. The three strains were able to lower the drug concentration and this effect was more evident in samples sonicated for 8 min. The values obtained for strains CIDCA 5310, CIDCA 5317 and ATCC 314 sonicated 8 min were 0.06 mg ml^{-1} ± 0.015, 0.03 mg ml^{-1} ± 2.5x10^{-4} and 0.03 mg ml^{-1} ± 0.017 respectively. Finally, the three strains were grown at different times (16, 18 and 24h) at 37°C in MRS broth, and then incubated 1 h with AC 0.16 mg ml^{-1}. All incubations were carried out in anaerobic conditions. Here we could see that the three strains were able to lower down the anticoagulant concentration. As an example, for strain CIDCA5310 the values obtained were 0.006 mg ml^{-1} ± 5.0x10^{-4} for 16 h-old cultures and 0.004 mg ml^{-1} ± 3.0x10^{-3} for 18 h-old cultures. For 24 h-old cultures, the peak of AC in HPLC was present but not quantifiable. These last results were also observed for strains CIDCA 5317 and ATCC 314. Student t-Test was used for statistical analysis. Results showed here suggest that intracellular factors might play a role in the biomodification of the drug and that the physiologic status of bacteria is relevant for the enzymatic activity altering the drug. The effects observed at short periods of time are significant in the context of physiological interaction between microorganisms and xenobiotics in the gastrointestinal tract. In conclusion, the strains under study were able to modify acenocoumarol.

MI-P051-81
HETEROLOGOUS EXPRESSION OF A GLOBAL TRANSCRIPTIONAL REGULATORY PROTEIN IN A NON-OLEAGINOUS *Rhodococcus erythropolis* STRAIN TO IMPROVE LIPID PRODUCTION

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Lipid accumulation is a well-studied process that occurs in many bacteria, such as those of the genus *Rhodococcus*, due its biotechnological applications. In a previous study, we identified and characterized a pleiotropic transcriptional regulator called NlpR (Nitrogen Lipid Regulator) that simultaneously modulates carbon and nitrogen metabolisms in the oleaginous *R. jostii* strain RHA1 in response to nitrogen limitation. In this study we analyzed the effect of the heterologous expression of NlpR from *R. jostii* RHA1 on lipid accumulation in the non-oleaginous strain; *Rhodococcus erythropolis* ATCC 15960. Bioinformatic analyses demonstrated that *nlpR*orthologous gene is also present in *R. erythropolis*, exhibiting 81% identity (98% query cover) in comparison to the *nlpR* gene of *R. jostii*. In addition, a conserved synteny of *nlpR* locus was observed in genomes of both rhodococcal species. We analyzed the occurrence of putative DNA binding sites for NlpR in *R. erythropolis* genome. "NlpR box" motifs were found in the upstream region of several genes involved in nitrogen and lipid metabolisms, including *nark* (nitrate/nitrite uptake), *nirD* (nitrite reductase small subunit), eukaryotic like-acetyl-CoA carboxylase gene, *fasI* (fatty acid synthase Complex I) and 1-acyl-*sn*-glycerol-3-phosphate acyltransferase (AGPAT), among others. To analyze the possible effect of $NlpR_{RHA1}$ on lipid accumulation in *R. erythropolis* ATCC 15960, the inducible expression vector pTipQC2/*nlpR*$_{RHA1}$ was transferred into the ATCC 15960 cells. Thin layer chromatography analysis of cell lipid extracts demonstrated that the heterologous expression of $NlpR_{RHA1}$ promoted an increase of neutral lipid fractions, including

triacylglycerols (TAG), diacylglycerols and free fatty acids in comparison to the control cells carrying the empty inducible vector. In addition, quantitative gas chromatography analysis revealed an increase of 1.9-fold in total fatty acid content (8.97% CDW) in ATCC15960 pTipQC2/*nlp*R_{RHA1} in comparison to the control cells, after cultivation in minimal salt medium with glucose (1%, w/v) and nitrogenlimiting conditions (0.1 g/L of ammonium). Unexpectedly, the heterologous expression of $NlpR_{RHA1}$ in *R. erythropolis* ATCC 15960 promoted the production of a co-polymer of 3-hydroxybutyrate-*co*-3-hydroxyvalerate (12.04% CDW), whereas the control cells produced only traces of the copolymer. In contrast, *nlpR*$_{RHA1}$ overexpression in *R. jostii* RHA1 increased only the total fatty acid content in cells and neutral lipid fractions (TAG, DAG, MAG), but it did not promote the PHA biosynthesis. These results demonstrated that the pleiotropic transcriptional regulator NlpR can be considered an interesting tool for genetic modification of rhodococcal species to improve lipid production. Deregulation of cell metabolism by NlpR expression can produce differential phenotypic effects among rhodococcal species.

MI-P052-96
CONTRIBUTION OF A SPECIFIC XRE FAMILY TRANSCRIPTIONAL REGULATOR TO THE OLEAGINOUS PHENOTYPE IN RHODOCOCCI

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Oleagenicity is a property attributed to some microorganisms capable of accumulating high levels of intracellular lipids within the so-called lipid droplets (LDs). 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. Although several genes involved in TAG biosynthesis and accumulation have been well described, it is not clear yet how these processes are regulated. Global and specific transcriptional regulators (TRs) contribute to the oleaginous phenotype in *Rhodococcus*. Among specific TRs, a XRE family transcriptional regulator (TR) is associated with the lipid droplet ontogeny through regulation of a structural protein coding gene. In this work, we study the role of this specific TR on lipid metabolism in oleaginous rhodococci at the physiological and molecular level. Bioinformatic analysis revealed the occurrence of this regulator only in actinobacteria. In addition, the occurrence of putative TR boxes into the promoters' regions varied between oleaginous *Rhodococcus* strains and non-oleaginous strains. Docking studies revealed putative interactions of this specific TR with palmitic acid. *In vitro* and *in vivo* assays confirmed that the TR binding capacity to DNA is controlled by long chain fatty acids or their acyl-CoA derivatives. Glutaraldehyde (GT) cross-linker assay and limited proteolysis analysis revealed that long chain fatty acids induce oligomerization and conformational changes of TR, respectively. Furthermore, putative binding sites for this TR within upstream regions of genes coding for a lipase, an acyl-CoA dehydrogenase and the fatty acid synthase complex (FASI) were found and validated by EMSA and RT-PCR assays. Finally, deregulation of the TR levels by overexpression of the corresponding gene was used as a strategy to improve TAG biosynthesis and lipid recovery for biotechnological purposes under rich nitrogen conditions. We propose a model in which the activity of this TR is controlled by fatty acyl-CoA pools in cells according to the nutritional conditions of the environment. In addition, this protein participates in the regulatory network controlling lipid metabolism and lipid droplet formation in oleaginous rhodococci.

MI-P053-113
CONTRIBUTION OF UNCHARACTERIZED GENES TO *Acinetobacter baumannii* ENVELOPE FUNCTIONS

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Acinetobacter baumannii (Ab) is a nosocomial pathogen, of major concern due to its multi-drug resistance (MDR) and the recent appearance of hyper-virulent strains in the clinical setting. The World Health Organization included Ab as a critical priority pathogen for the development of novel antibiotics. Ab pathogenesis is associated with a multitude of potential virulence factors (VF) that remain poorly characterized. It is well known that many bacterial envelope components, such as outer membrane proteins (OMPs) and exopolysaccharides facilitate the establishment of a disease state, the persistence in abiotic surfaces and resistance to antibiotic treatment. We previously reported a bioinformatics prediction of *A. baumannii* AB5075 genes coding for uncharacterized OMPs with putative roles in the pathophysiology of Ab. Analysis of mutants in the corresponding genes (1) revealed that four of them showed reduced A549 cell adherence and invasion (2), thus indicating virulence roles for the corresponding proteins. Here, we further analyze the physiology of these four mutant strains. First, *in silico* analysis of the candidate proteins revealed that two of them share high similarity with bacterial domains related to stress response or involved in protein-protein interaction and degradation, with roles in the maintenance of outer membrane integrity. The third protein shares low similarity with a protein involved in biofilm formation in *Escherichia coli*, while no domain similarity was found for the fourth one. In addition, synteny analysis showed that three of the corresponding genes are in proximity to genes related to stress response or other virulence processes like capsule formation, thus suggesting probable regulatory functions. Based on these analyses, we conducted several assays in order to characterize the surface properties of