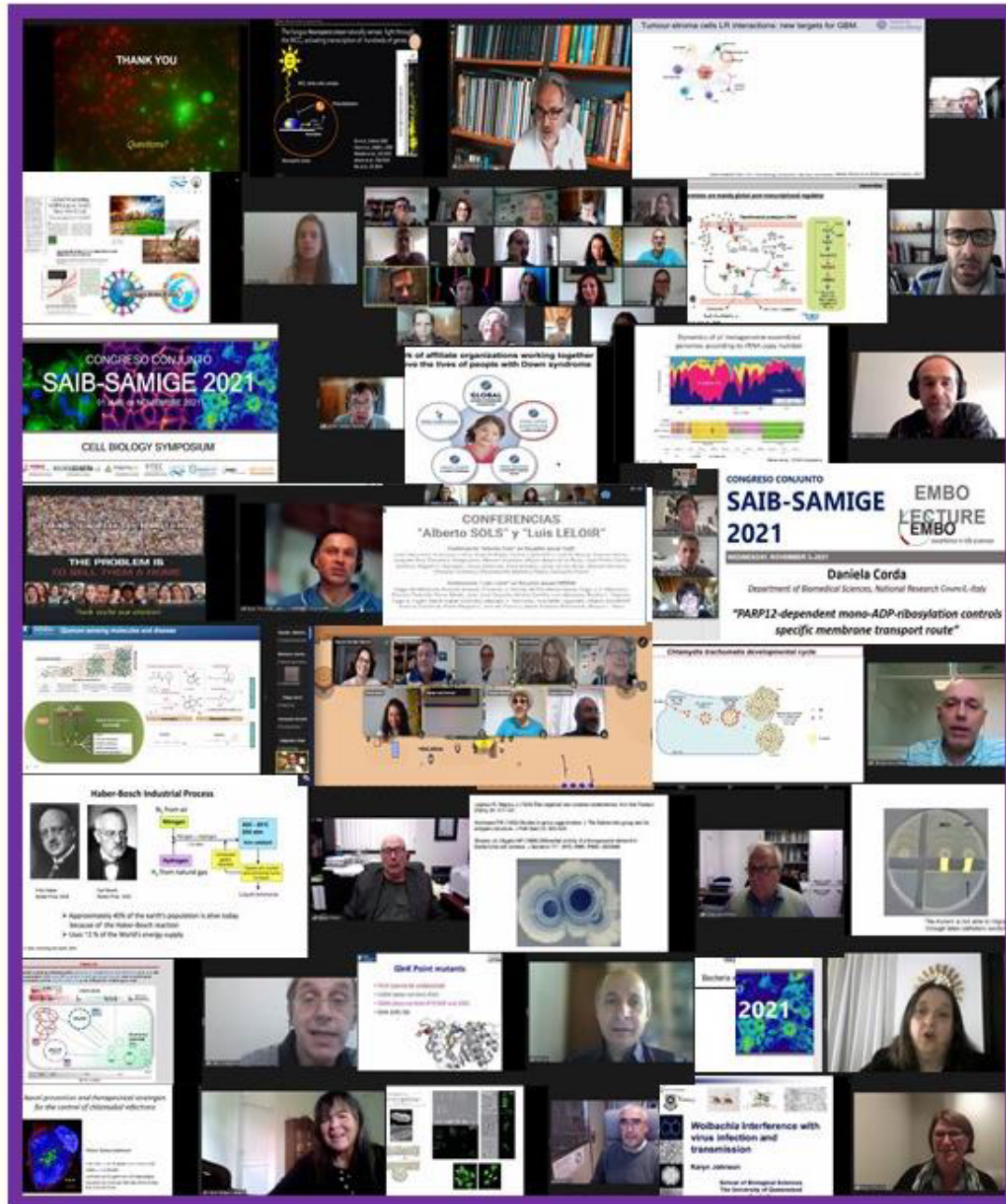


SAIB - SAMIGE Joint meeting 2021 on line



November 1-5, 2021



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

***XVI Annual Meeting of the
Argentinean Society for
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***SAIB - SAMIGE Joint meeting
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organized in polysaccharide utilization loci, in which we identified regulatory regions and putative intracellular glycoside hydrolases, which could be involved in the final degradation of short oligosaccharides. Extracellular extracts from sucrose (SAC) cultures were analyzed as control and only two enzymes were identified: the GH13 alpha- amylase and a GH16 beta-glucanase. These results allowed us to build a model for polysaccharide utilization in *P. xylanivorans* and demonstrated the viability of obtaining extracellular enzymatic extracts with high xylanase activity, for their application in xylan bioprocessing.

MI-P036-266

SYNTHESIS OF SINGLE CELL OIL VIA *DE NOVO* AND *EX NOVO* FROM *Aspergillus niger* MYA 135 UNDER SUBMERGED FERMENTATION: IMPACT OF MICROPARTICLES

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Fungal biofactories are well established in industries. Thus, the synthesis of single cell oil from organic wastes constitutes an attractive topic for research toward a biorefinery concept within the demanded circular economy. As an example, microbial lipids are interesting for biodiesel production due to the independence from seasonal and climatic changes, the fast production rate, the minimal labor requirements, and the easy scale-up for industrial processing. Oleaginous microorganisms are capable of produce oil *via de novo* and *ex novo* pathways using hydrophilic and hydrophobic substrates, respectively. In addition, productivities of filamentous fungi in submerged fermentation are often associated with specific morphological forms. Thus, in order to control fungal morphology several strategies have been reported. One of these allows the control of growth physically by blocking the aggregation of filamentous microorganisms using microparticles such as talc, aluminium oxide, titanium silicom oxide, and forsterite. In the present work, the native *Aspergillus niger* ATCC MYA 135 was used to explore its potential to accumulate lipids in the presence of waste cooking oil or waste glycerol as feedstocks. Shake flask fermentation were conducted with or without supplementation of talc. Firstly, biomass samples withdrawn at periodic intervals were stained using Sudan Black and observed at 100X magnification under a light microscope. Interestingly, we found that microparticles performance depended on the organic waste utilized. Adding talc to culture medium not only favored the single cell oil synthesis under *ex novo* culture condition but also changed the fungal morphology radically. Microparticles modified both size and shape of mycelial objects. On the contrary, the presence of talc decreased the microbial oil accumulation in culture medium formulated with waste glycerol. In relation to the macroscopic fungal morphology, under *de novo* culture condition, microparticles did not significantly alter the mycelial shape observing hairy and irregular structures sometimes growing out of their centers. Secondly, microbial oils obtained from biomass developed from both feedstocks were extracted using the Folch method. It was found that *A. niger* MYA was able to accumulate more than 25% (w/w) of lipids per dry weight. Finally, lipids extracted from biomass grown in culture medium formulated with either hydrophilic or hydrophobic substrate were separated by thin layer chromatography observing the spot corresponding to triacylglycerol. In summary, these findings highlighted that microparticles could impact not only on fungal morphology but also on metabolite production. In addition, our results also showed that medium composition always has to be taken into account when evaluating the impact of microparticles on processes involving filamentous fungi.

MI-P037-268

GENETIC ENGINEERING OF AMMONIUM RELEASE AND COMPETITIVE FITNESS IN THE N₂-FIXING BACTERIUM *Azotobacter vinelandii*

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Food security calls for improved alternatives for the production and management of N-fertilizers. There is an increasing interest in broadening the exploitation of bacterial biological N₂ fixation beyond the inoculation of legumes with symbiotic rhizobia. Copious release of NH₄⁺ was successfully accomplished by different genetic strategies largely surpassing those of the parental strains. However, most of the times this was accompanied by very slow growth, and frequent reversion into non-NH₄⁺-excreting phenotypes, which, in addition to severe regulation of genetically engineered bacteria for its use in field trials, obscures the prospects of developing further this kind of N-biofertilizers. Here we analyzed the bacterial population dynamics of a set of ammonium-excreting *A. vinelandii* strains in long term laboratory experiments regarding their competitive fitness in comparison with their parental strain (*wt*) and the genetic stability of the mutations. NH₄⁺- excreting strains bearing a deletion in *nifL* for constitutive expression of the N₂ fixation genes, a point mutation on *glnA* (D49S) for decreased activity of glutamine synthetase (GS) and impaired conversion of NH₄⁺ into amino acids, or both, were rapidly excluded by competition with the *wt* strain in long-term co-culture experiments. Conversely, other strains expressing only an inducible allele of *glnA*, or in combination with the *nifL* mutation, initially displayed an increased competitive fitness in comparison with the *wt* strain, reducing its population up to 1,000-fold. These strains allowed the accumulation of different levels of GS, which become depleted by cell-division in non-inducing medium. Under these conditions, the mutant strains displayed a dynamic competitive fitness in comparison with the *wt* strain according to the previous strength of induction of *glnA*. At even longer times, the *wt* strain took over and returned to the initial relative levels and begun outcompeting the mutant strains. These genetically engineered bacteria revealed a self-bio-containment behavior which could eventually be improved and considered for their