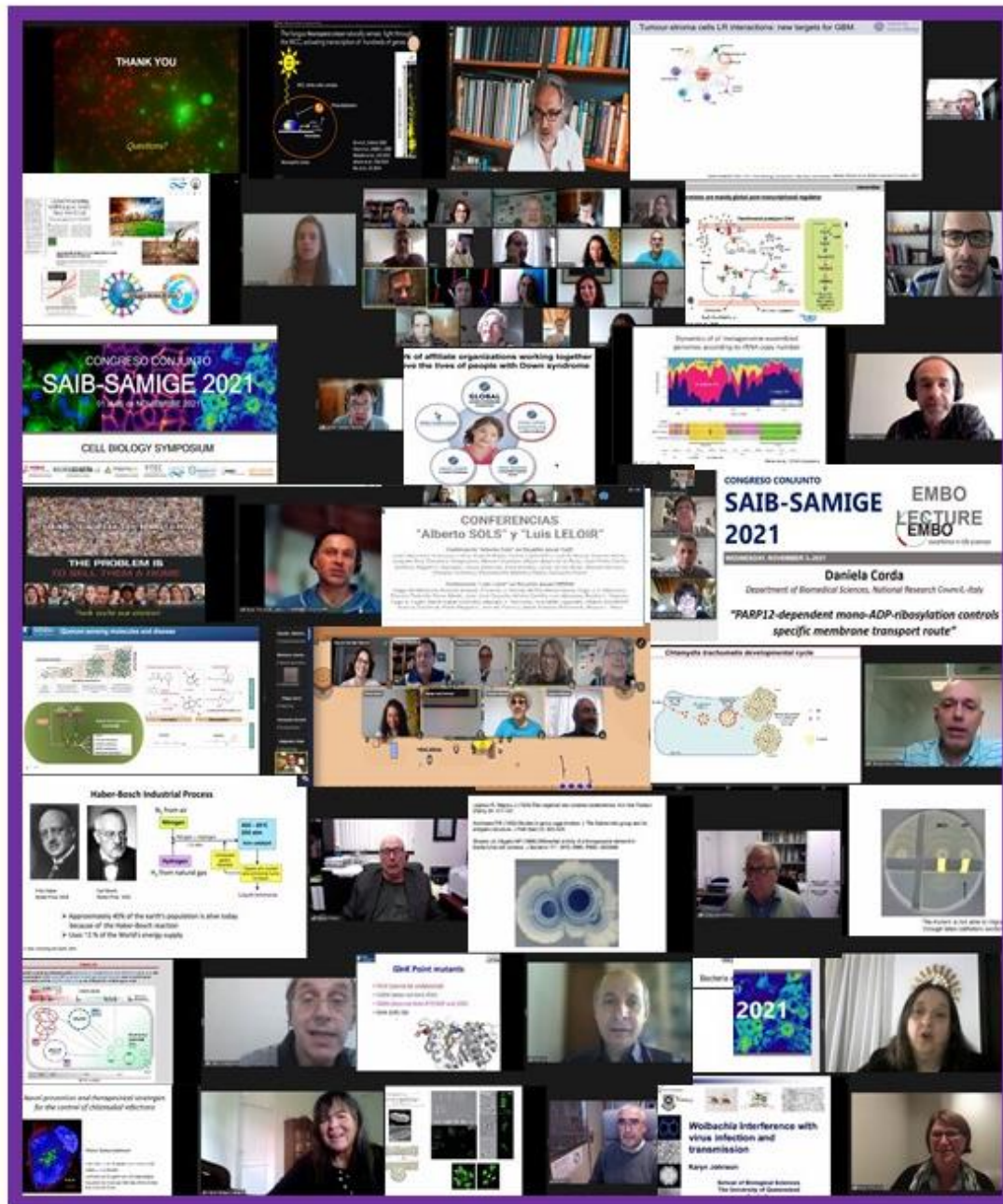


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MI-P018-31

MANNITOL PRODUCTION BY FRUIT-ORIGIN *Fructobacillus* STRAINS USING A FRUCTOSE-RICH SYRUP-BASED MEDIUM

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Mannitol is a low-calorie sweetener used in the food and pharmaceutical industries. *Fructobacillus* species reduce fructose to mannitol thanks to their fructophilic metabolism. In this work, we aimed to study mannitol production by fruit-origin *F. tropaeoli* CRL2034 and *Fructobacillus* sp. CRL2054, using a minimized culture medium (FYP-based) containing fructose-rich syrup as carbon source under optimized culture conditions. Fermentations with a 2-L bioreactor were performed at pH 5.0 and 30 °C under stirring conditions (130 or 200 rpm for CRL2054 or CRL2034, respectively) for 24 h. Two different total saccharide contents (10 and 20%, m/v) were assessed for each strain. Mannitol yield (mannitol production/consumed fructose) was close to 100 % for both strains using a sugar concentration of 10 %; however, higher mannitol concentrations were achieved when 20 % sugar was used (77-79 g/L compared to 47-51 g/L with 10 % carbohydrates). Mannitol crystals were isolated from 24-h fermentation culture supernatants using 20 % sugar. For both producer strains, the physicochemical properties of the mannitol crystals were highly similar to those of high purity commercial mannitol. These results showed that fermentations of fructose-rich syrup-based medium by selected *Fructobacillus* strains at constant pH are an interesting alternative for mannitol production.

MI-P019-33

PARTIAL CHARACTERIZATION OF BIOSURFACTANTS PRODUCED BY HYDROCARBON-DEGRADING *Pseudomonas* spp.

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Availability of hydrophobic compounds is a key factor for their biodegradation. Because of that, the use of surfactants was proposed for Surfactant Enhanced Remediation (SER) or Surfactant Enhanced Oil Recovery (SEOR) and the use of biosurfactants became interesting because of their chemical properties and biodegradability. Previous studies from our group showed that *Pseudomonas extremaustralis* and *Pseudomonas* sp. KA-08 were able to produce biosurfactants of different chemical nature using diesel or kerosene as their sole carbon source. In this work we continued the analysis of those compounds, using different growth conditions, carbon sources and extraction methods to improve their production. *P. extremaustralis* was able to degrade long chain alkanes only when cultures were carried in microaerobiosis, but a recombinant strain carrying a plasmid pGEc47, that contains the *alk* genes from *P. putida* GPo1, allowed the use of medium chain alkanes and to develop in aerobic growth conditions. On the other hand, *Pseudomonas* sp. KA-08 showed to be an excellent xylene and toluene degrader but was unable to use alkanes as carbon source. For *P. extremaustralis* and *P. extremaustralis*/pGEc47 two growth conditions were assayed. Microaerobiosis cultures were carried out in 50 ml E2 minimum medium supplemented with 2% diesel and KNO₃ as electron acceptor, in 100 ml capped bottles without agitation. Aerobic cultures (only for *P. extremaustralis*/pGEc47) were carried out in the same media but using 50 ml of culture in 500 ml bottles and 280 rpm. To analyze if an extra carbon source could enhance surfactant production, 0.05% glucose addition was also tested. For *Pseudomonas* sp. KA-08, cultures were grown in aerobiosis with three different carbon sources: 10% kerosene, 0.1% toluene and 1% xylene. After 7 days, cultures were centrifuged, and the supernatants were separated into two halves. One half was filtered with a 0,22µm pore cellulose ester filter and the second half remained without filtration. All the samples were then acidified up to pH 2, left overnight at 4°C and centrifuged at 12000 rpm, 4°C for 20 minutes. The pellets were resuspended in 1 ml 0.1mM TrisHCl (pH 8), extracted with ethyl acetate and concentrated by Rotavap. Finally, these crude extracts were resuspended in 0.5mL ethyl acetate and analyzed by TLC. *P. extremaustralis* and *P. extremaustralis*/pGEc47 showed similar glycosidic compounds (Molisch staining), but only *P. extremaustralis*/pGEc47 presented also a putative aminoacidic surfactant in the unfiltered samples (Ninhydrin staining). On the other hand, *Pseudomonas* sp. KA-08 showed glycosidic compounds when it was grown with kerosene or toluene as carbon source. In this case, also unfiltered samples showed spots with different R_f than the filtered ones. Glucose addition seems to have no effect on the produced biosurfactants. This work allows us to continue the study of these compounds and to evaluate their potential as biosurfactants.