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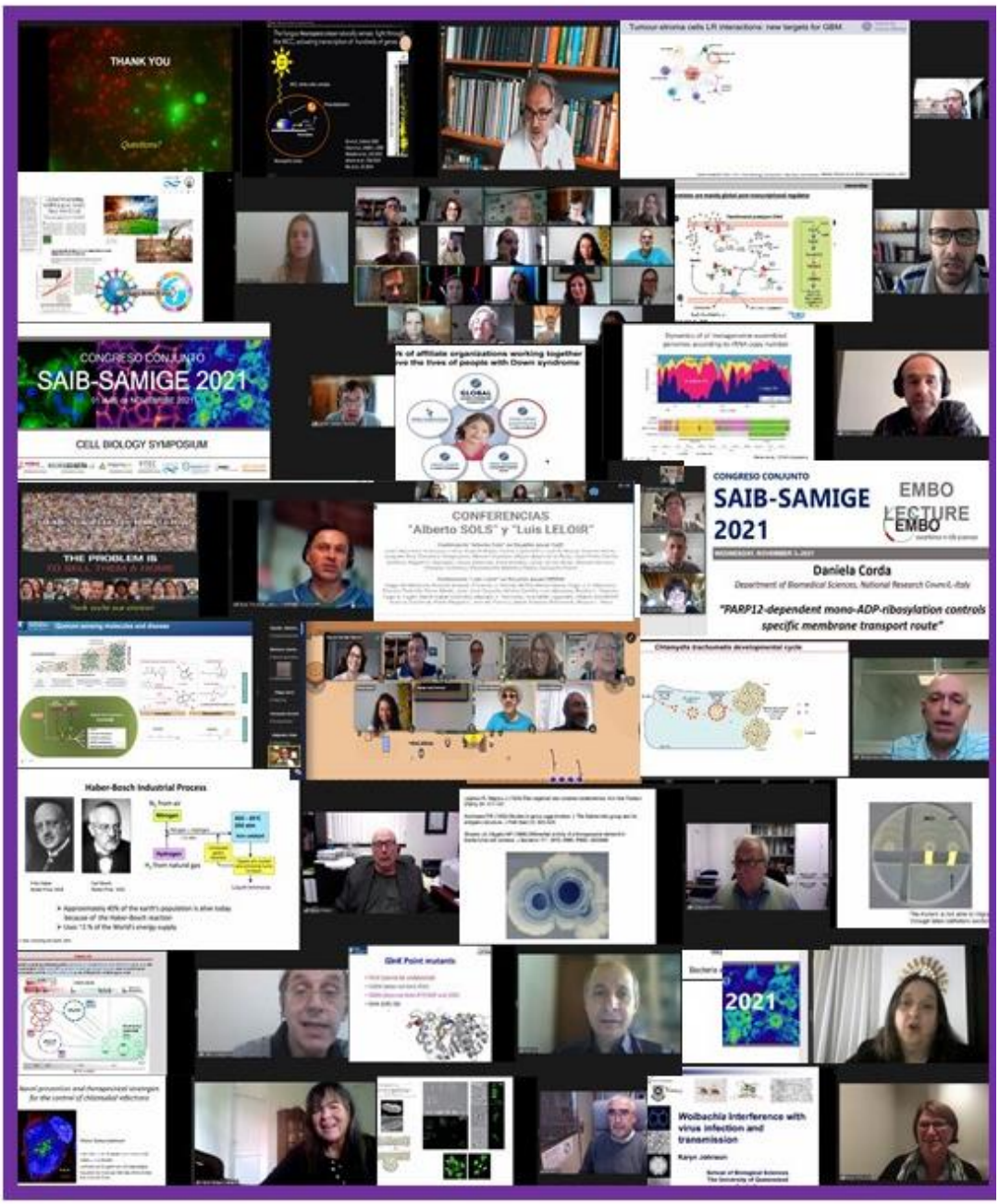

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MI-P066-265
BIOFILM FORMATION CHARACTERIZATION OF *Mannheimina haemolytica* ARGENTINIAN ISOLATES

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Mannheimina haemolytica is a Gram-negative respiratory pathogen frequently isolated in Argentinian feed lots. Symptoms are observed after stress situation like transport or diet changes. Infection by this bacterium causes reduced weight and death in calves. Other authors described a strong correlation between biofilm formation and animal stress. Particularly, the stress induced hormone adrenaline inhibits biofilm formation *in vitro*. This result suggests that biofilm formation and bacteria response to animal stress may be important for *M. haemolytica* pathogenesis. In order to design prevention and palliative strategies to reduce the impact of the disease caused by this pathogen we decided to characterize local isolates. In this work we present five *M. haemolytica* strains isolated from Buenos Aires province area. Laboratory characterization included growth, biofilm formation on plastic surface, macrocolony formation over semi solid media and sensitivity to adrenaline. Interestingly we observed significative differences in growth kinetics in BHI media. Strains Mh1 and Mh2 present a growth velocity of 0.31 and 0.30 h^{-1} respectively. Other isolates (Mh3, Mh4 and MhA) presented lower velocities (0.11, 0.15 and 0.14 h^{-1} respectively). Biofilm formation in plastic 96-well were observed in all strains after 48 and 72 hours in static incubation. However, biofilm phenotype was significantly different between strains. Strains Mh1 and Mh2 presented significantly more biofilm formation compared to other strains. This phenotype correlates with bigger macrocolony formation observed in plaques. Finally, we were not able to observe sensitivity to adrenaline, biofilm formation was not affected by adrenaline in any strain, in the conditions tested (growth in BHI media, 48 h static incubation, 55 µM adrenaline). Further work is needed to elucidate if adrenaline effect, previously observed by other authors, is present in local isolate if other growth conditions are tested. The work present here is the milestone for further characterization of local isolates of *M. haemolytica*. This will permit design experiments to understand how the pathogen induce severe symptoms and finally the death of animals and economical losses.

MI-P067-279
ANTIFUNGAL ACTIVITY OF BIOGENIC SILVER NANOPARTICLES IN COMBINATION WITH AMPHOTERICIN B ON *Candida glabrata*

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Non-albicans Candida (NAC) species cause 35-65% of all candidaemias in the general patient population. Among the NAC species, *Candida glabrata* is considered the second or third most frequent causative agent of superficial (oral, esophageal, urinary, and vaginal) or systemic infections, with a high mortality rate. These infections are difficult to treat for their innate resistance to many azole antifungals (ATF) therapy, especially fluconazole. Nowadays, current advances in nanotechnology constitute a promising alternative in the development of new antimicrobial agents. Silver nanoparticles (AgNPs) are very interesting products currently provided by available nanotechnology to evaluate their antifungal activity. In the present study, the synergism of AgNPs in combination with amphotericin B (AmB) against *C. glabrata* was investigated. Biogenic AgNPs were synthesized by eco-friendly method, and the antifungal activity against *C. glabrata* ATCC 2001 was evaluated through determination of minimum inhibitory concentration (MIC50) and Minimum Fungicidal Concentration (MFC) according to protocol M27-A3 of Clinical and Laboratory Standards Institute (CLSI). The checkerboard microdilution method was used to study the synergistic combinations of AgNPs with AmB. The results were analyzed using the fractionary inhibitory concentration (FIC) indices, a non-parametric model based on the Loewe additivity theory, and by CompuSyn software. CompuSyn is a computer program for quantitation of synergism and antagonism in drug combinations and the determination of IC 50 (drug concentration causing 50% growth inhibition) and ED 50 (dose causing 50% of maximum effect) values. Furthermore, we investigated the effects of the resazurin reduction (alamarBlue) assay, which measured metabolically active cells. The same MIC and MFC values were found for 0.13 pM AgNP and for 2.7 10^5 pM AmB. The FIC index was 0.37 (a FIC index of < 0.5 indicates synergism). This value corresponded to 0.033 pM AgNPs + 3.4 10^4 pM AmB (0.25 CIM AgNP + 0.125 CIM AmB) combination. Resazurin (blue, non-fluorescent) was reduced by metabolically active cells to resorufin (pink, fluorescent) showing the cytotoxic effect, which was visually weighted. The CompuSyn analysis confirmed synergism between biogenic AgNPs and AmB against *C. glabrata*. The analysis also shows that the maximal inhibitory activity of the combination is substantially expanded compared to those of the single agents. The IC 50 was 0.08 CIM AgNPs and 0.06 CIM AmB (0.01 pM and 1.6 10^4 pM, respectively). Isobolograms demonstrate a stark reduction of the AmB dose when used with AgNP to induce 50% inhibition or greater. Combined therapy has the advantage of attacking different targets by combining several active principles with different mechanisms of action. The development of new approaches has great clinical relevance in the treatment of mycoses.