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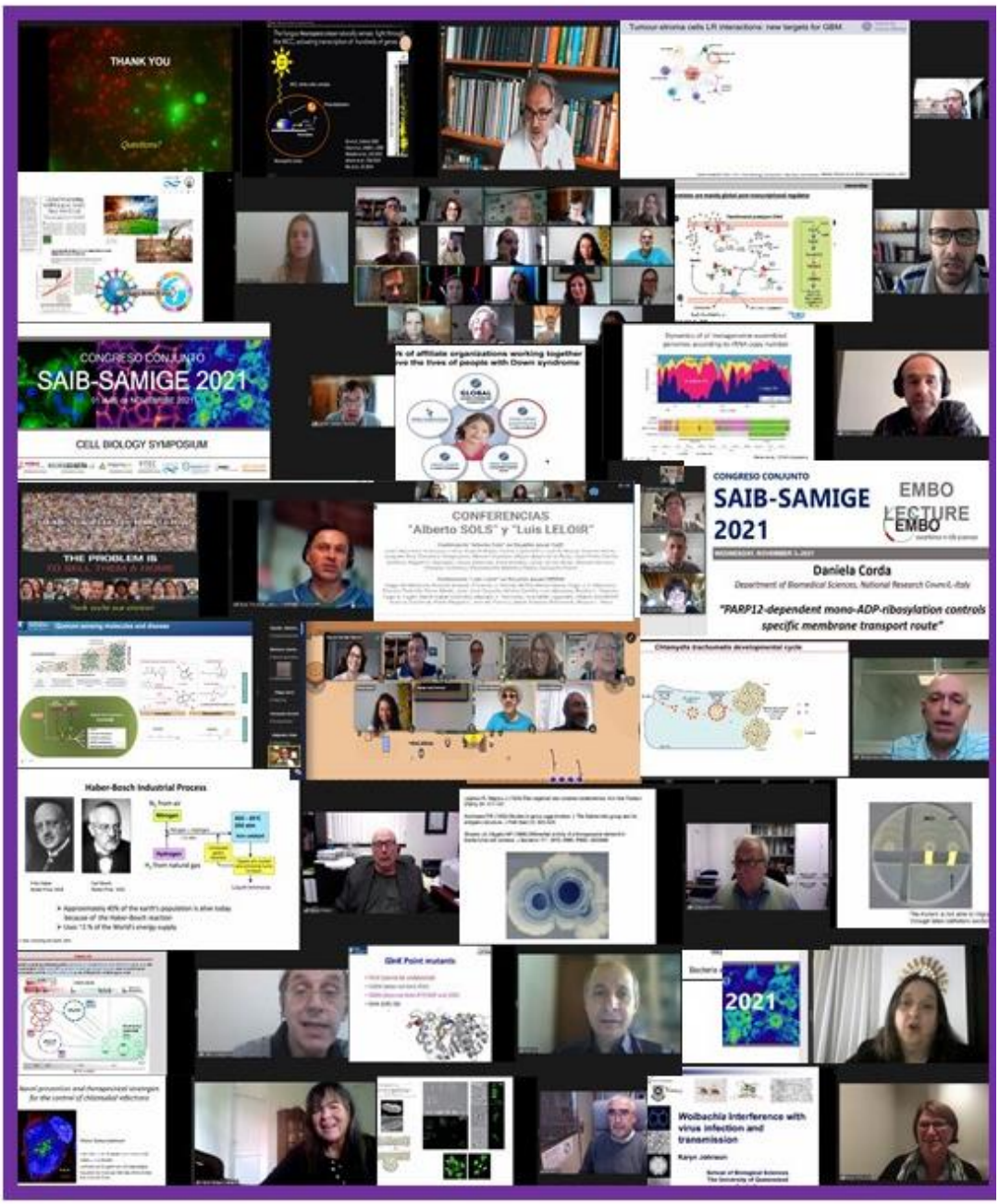

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MI-P014-299
ANTAGONIST ACTIVITY OF LACTIC ACID BACTERIA AGAINST FUNGAL POSTHARVEST PATHOGENS OF CITRUS

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Postharvest diseases caused mainly by green mold (*Penicillium digitatum*) and blue mold (*P. italicum*) led to economic losses in the Argentine citrus production, by affecting the shelf-life and quality of fresh fruits. Several synthetic fungicides are commonly used to control the fungal phytopathogens. Their widespread use has led to the appearance of resistant isolates; thus, there is an urgent need to develop natural and safe strategies to control postharvest diseases and to guarantee fruit conservation through alternative technologies. Biocontrol has received much attention in the last years. Lactic acid bacteria (LAB) are the most promising candidates to be used as fungal antagonists, since they have been reported to have strong antimicrobial properties and are considered harmless to human health. The aim of this study was to evaluate the potential antifungal activity of several LAB strains against *P. digitatum* and *P. italicum*, and to determine the nature of the antifungal metabolites produced. First, inhibitory activities of *Lactobacillus fermentum* CRL 973, *L. paraplantarum* CRL 1905, *L. casei* CRL 1110 and *L. plantarum* Q1 strains were assayed by the overlayed method as a fast preliminary screening. Based on the inhibition halo, all strains showed an antifungal ability against both fungi, exerting a major activity against *P. italicum*. Next, LAB were grown in MRS medium at 37 °C; at 24 and 48 h, cells were removed, and cell-free supernatants (CFS24 and CFS48, respectively) were obtained by filtration. The antifungal activity of each CFS was evaluated in a 96-well polystyrene microtiter plate containing the conidial suspensions adjust to 10^5 CFU/ml. Microplates were incubated during 5 d at 22°C, and conidia germination was evaluated by observation using an inverted light microscope. Additionally, conidia viability after each time incubation was determined. Results showed that CFS24 and CFS48 from CRL 1905 and Q1 strains, and CFS24 from CRL 973 inhibited conidia germination of *P. digitatum* until 5 d of incubation, while CFS of most strains delayed *P. italicum* germination. It is worth to mention that the CFS inhibitory activity seems to be fungistatic, since conidia viability was maintained after treatments. To determine the nature of the antifungal compound, the different CFS were submitted to heat, proteinases treatment or neutralization. It was observed that most CFS lost their antagonistic properties after pH neutralization, suggesting an acidic nature of the antifungal metabolite. *In vivo* assays on lemons are necessary to detect whether CFS has a potential application for the prevention and control of postharvest diseases. Our results showed that LAB could be a promising alternative to be used as natural preservatives in postharvest lemons to control fungal growth.

MICROBIOLOGY – BIOTECHNOLOGY and FERMENTATION

MI-P015-20
CHARACTERIZATION OF LACTIC ACID BACTERIA AS SPOILAGE AND THEIR EFFECTS ON THE SHELF LIFE OF MINIMALLY PROCESSED VEGETABLES

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The increasing demand for fresh vegetables and for convenience foods is causing an expansion of the market share for minimally processed vegetables (MPV). The new technologies for processing and packaging have made possible to obtain a product ready to serve. Nevertheless, the associated risk with pathogens and loss of quality due to microbial spoilage seems to be involved. Low refrigeration temperatures restrain the growth of spoilage microorganisms while the partial or complete exclusion of oxygen inhibits the proliferation of Gram-negative bacteria frequently isolated from spoiled products whilst favoring the growth of Gram positive such as lactic acid bacteria (LAB). Considerable levels of acidification, emission of volatile organic compounds, slime formation have been associated with their metabolic activity as spoilage properties. Recently, microbial spoilage characterized by gas and slime formation in vegetable products became a main concern of the manufacturer. On these bases, this study aims to establish the potential spoilage LAB of vegetable origin and evaluate their effects on the physical-chemical and sensory properties of MPV packaged under aerobic conditions at 4 °C for 15 days. The production of exopolysaccharide, gas, biogenic amines and organic acids from LAB of vegetable origin (19 strains) was qualitatively determined. In order to have a global view the useful features to interpret the LAB spoilage capacity a multiple correspondence analysis was applied. *Leuconostoc mesenteroides* CRL950, CRL742 and *L. citreum* CRL1904 were selected for presenting the highest amount of spoilage characteristics assayed. Carrots or cabbage were washed and cut into thin strips. Samples were inoculated with each strain separately (10^4-10^5 CFU/mL) packed in aerobic conditions and incubated at 4 °C for 15 days. Microbiological counts, pH, and color were evaluated at regular intervals. In both refrigerated vegetables the spoilage strains were able to grow reaching a count of ~10^9 CFU/mL at the end of the incubation period while the native microbiota slightly exceeded 10^7 CFU/mL. The pH values were kept practically constant in the un-inoculated samples and the greatest drop was observed in the samples treated with *L. mesent* CRL742. In addition, total color difference (ΔE) was calculated by using L*, a* and b* values of days 0 and 15. Pronounced ΔE were detected for inoculated samples with *L.*

mesent. CRL950, CRL742 and *L. citreum* CRL1904 (9,19; 9,57; 9,13 in carrots and 9,05; 11,37; 15,58 in cabbage). Control samples maintained a similar visual color of the vegetables with ΔE values of 2.97 and 4.34 in carrots and cabbage, respectively. Species, belonging to the genera *Leuconostoc* were the main spoilage, being able to acidify and change the color of refrigerated vegetables, causing their early deterioration. The findings suggest the need of the microbiological control of the MPV ready-to-use to assure their quality.

MI-P016-21
LACTIC ACID BACTERIA FROM THE REPRODUCTIVE TRACT OF MARES AS POTENTIALLY BENEFICIAL STRAINS TO PREVENT ENDOMETRITIS

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Endometritis is the most frequent inflammatory disease in mares that can cause subfertility and subsequently economic losses in horse breeding. Between 25%-60% of the cases are due to uterine infections, requiring the local and systemic application of antibiotics, ecbolic drugs, uterine lavage, among others. The development of probiotic formulas for the prevention of different infections in animals are consistent with the reduction of the antibiotics use to achieve more sustainable systems. Probiotics are defined as "*live microorganisms that are administered to the host in adequate amounts to produce a beneficial physiological effect*". These microorganisms should be isolated from the host in which they will be applied, based on the host and mucosal specificity of the indigenous microbiota, in order to favor their adaptation and maintenance in the tract. Lactic acid bacteria (LAB) are a heterogeneous group that include different genera, being Lactobacilli the most frequent microorganisms isolated from the indigenous vaginal microbiota of mares. The aim of this work was to isolate, phenotypically identify and evaluate surface-adhesive properties of LAB from mare's reproductive tract. Vaginal swabs samples obtained from 15 healthy mares from Córdoba (Argentina) were seeded on MRS agar pH 5.5 and incubated at 37°C during 24-48 h. Phenotypic identification was performed by morphological and phenotypic characteristics as Gram staining, catalase reaction, nitrate reduction and indol production. Also, surface-adhesive characteristics as hydrophobicity, auto-aggregation, biofilm formation and exopolysaccharide (EPS) production were evaluated. Thirty strains were isolated from the swabs, and on the base of phenotypic results, 96.66% of the microorganisms were included in the LAB group. According to the morphology of the strains, 43.33%, 16.66% and 40% were cocci, coccobacilli and bacilli, respectively. All the strains showed a range of auto-aggregation from medium (36.66%) to low (63.33%), and low degrees of hydrophobicity. The biofilm formation of the strains was performed in different culture media: MRS and LAPTg with and without Tween (-T). In general, an increased biofilm formation was observed in media without the surfactant, being the biofilm formed in LAPTg-T higher than in MRS-T. Also, colonies grown on agar medium with different carbohydrate sources were macroscopically observed, and EPS (+) strains were evidenced by their ropy/mucous phenotype. From the evaluated strains 43.33% were EPS (+) in the media with different source of sugars. These results contribute to advance in the characterization of host and tract-specific beneficial LAB strains for their further selection and inclusion in the design of a probiotic product to prevent equine endometritis.

MI-P017-30
IN VITRO INHIBITION ASSAY OF COPPER SULPHATE AS FUNGICIDE AGAINST WHITE THREAD BLIGHT FUNGAL ISOLATES

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White thread blight is a disease caused by a fungal complex that causes drying of leaves, stems and branches in Yerba mate and tea plants and causes serious losses in the yield of these crops. Conventional synthetic fungicides are largely considered as the most effective and cost-efficient means for disease management. One of the most used broad-spectrum fungicides for the control of foliar diseases is copper sulphate pentahydrate. Sensitivity of the pathogens to copper varies greatly, depending on the product and the fungus. However, to date, no published studies are available on the inhibition/tolerance to copper sulphate concentrations of the white thread blight fungal isolates. This research investigated the grow inhibition of seven isolates (ACK2, AFE1, ASD4, AKD2, ACJ2, ACB1 and APC1) associated with white thread blight disease by the poisoned food method. Czapek agar medium was supplemented with copper sulphate pentahydrate ($CuSO_4.5H_2O$) at concentrations of 100ppm, 500ppm, 1000ppm and 5000ppm. Twenty milliliters of each sterile medium were dispensed into Petri dishes and inoculated with a 5mm disc cut from the periphery of a 7 days-old culture. Each isolate was inoculated onto two plates and incubated at 28°C. Mycelial growth of the isolates was determined by linear measurements of colony diameters with an electronic caliper at four intervals. To determine fungicide or fungistatic effect the discs which concentrations that completely inhibited growth were inoculated in Potato Dextrose Agar (PDA) medium. Three isolates (AKD2, ACB1, AFE1) showed a maximum inhibition of mycelial growth at 500ppm. Additionally, two isolates (ACK2, ACJ2) were inhibited at a concentration of 1000 ppm. Maximum effect of inhibition of growth was observed at the highest concentration at 5000 ppm. In fungicide/fungistatic assay we verified that at 1000 ppm copper sulphate acts as a fungistatic, inhibiting the development of the fungus but without causing its total elimination. These results suggest that white thread blight pathogens are sensitive to copper sulphate-based fungicides.