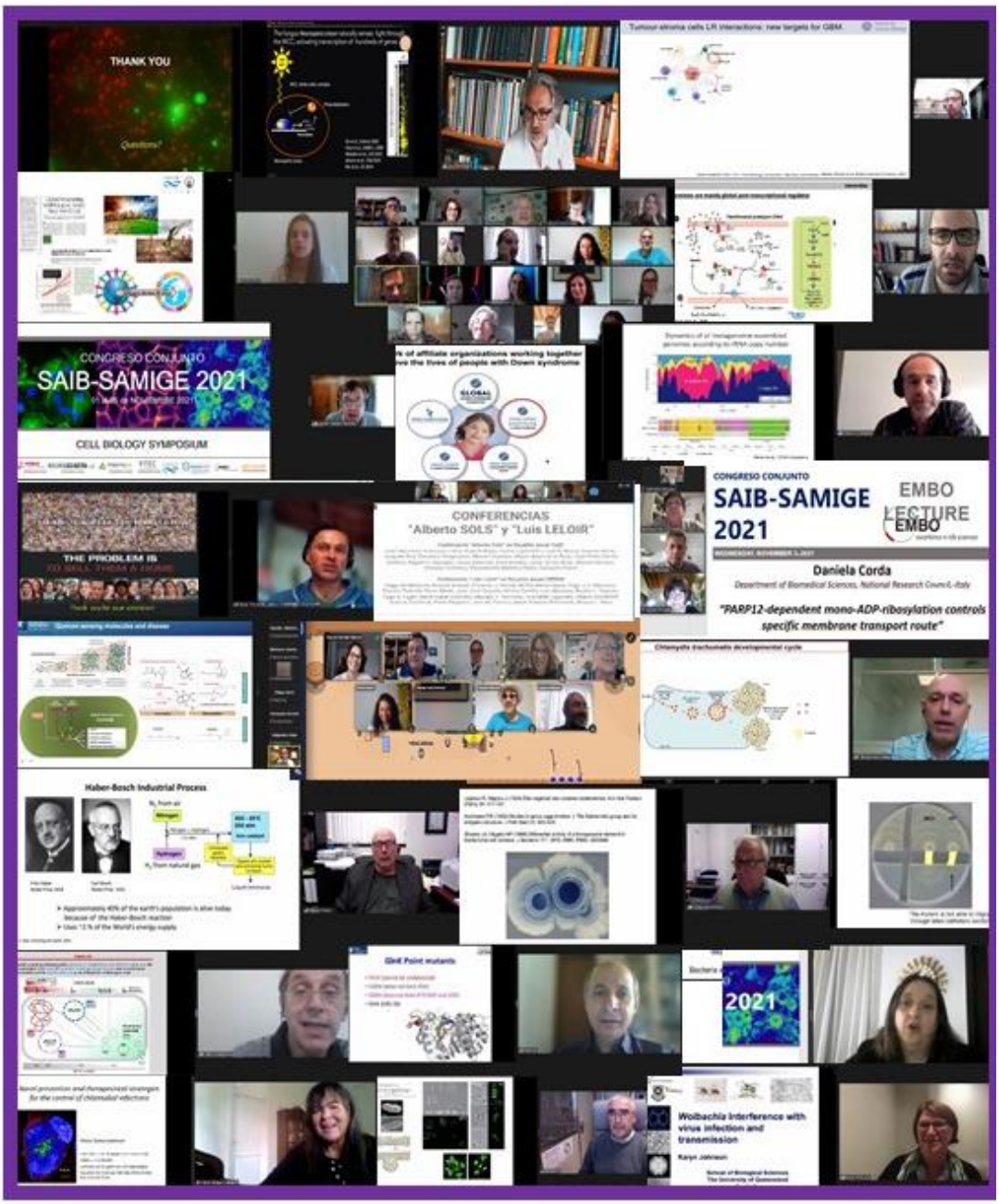
BIOCELL 46 (Suppl. 1), 2022
ISSN 0327- 9545 (printed version)
ISSN 1667-5746 (online version)



# *SAIB - SAMIGE Joint meeting*
# 2021 *on line*




# *November 1-5, 2021*

BIOCELL 46 (Suppl. 1), 2022
ISSN 0327- 9545 (printed version)
ISSN 1667-5746 (online version)





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

# *XVI Annual Meeting of the Argentinean Society for General Microbiology (SAMIGE)*

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BIOCELL 46 (Suppl. 1), 2022
ISSN 0327- 9545 (printed version)
ISSN 1667-5746 (online version)


## MI-P006-19
## INHIBITORY EFFECT OF LACTOCIN AL705 ON *Listeria monocytogenes* BIOFILM UNDER CONTINUOUS FLOW NUTRIENTS CONDITIONS

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*Listeria monocytogenes*, which causes serious foodborne infections and public health problems worldwide, is one of the most important foodborne pathogens. Some strains of *L. monocytogenes* are able to form biofilm facilitating their persistence in the food-processing environments as a chronic source of contamination. Since abundant evidence indicates that the biofilm mode of life leads to increased resistance to antimicrobials/sanitizers, new and effective strategies to control pathogen biofilms as eco-friendly approaches involving lactic acid bacteria (LAB) and/or their bacteriocins have emerged. Therefore, the objective of this work was to evaluate the inhibitory effect of lactocin AL705 produced by *Latilactobacillus curvatus* CRL1579 on *L. monocytogenes* FBUNT biofilms under continuous nutrient flow using microscopic techniques. Continuous-flow biofilms were grown in a flow cell (76 x 18 mm, with 3 channels of 40 x 4 mm) at 10°C. Overnight-grown cultures (18 h at 30°C) were diluted in TSB (1%), and the flow chambers were inoculated. After 2-h bacterial adhesion, lactocin AL705 at a subinhibitory concentration (20 AU/ml) was added and TSB medium was pumped through the flow cell with a flow of 3 ml/h. The biofilms were washed to remove planktonic bacteria, specifically stained live/dead by flushing with a 1:1000 dilution of BacLight staining (SYTO9/propidium iodide) and examined by fluorescence and Confocal Laser Scanning Microscopy (CLSM) at 3 and 6 days of incubation. Fluorescence micrographs of the untreated biofilms on glass surface displayed greater complex multilayered cells and strong adhering ability at 6 days of incubation than at 3. Developing biofilm-treated lactocin AL705 exhibited a structure composed of sparse cells and a greater reduction of live cells at 3 days of incubation. By employing ImageJ software, the thresholding analysis revealed that there was a 43% reduction in cell adhesion at 3 days while 23% at 6 days in the presence of lactocin AL705. The CLSM images analyzed using the program comstat2 (allows quantification of three-dimensional biofilm structure) showed the clumping and complex morphology of *L. monocytogenes* FBUNT biofilm in untreated control surfaces. Lactocin AL705 produced a visible reduction in the biofilm formation, specifically in the biomass, average and maximum thickness of the biofilms. Furthermore, the bacteriocin caused the dispersing and disintegrating clumps along with collapsed microcolonies. In conclusion, anti-listeria bacteriocin from *L. curvatus* CRL1579 may be considered as novel anti-biofilm strategy for the control of persistent *L. monocytogenes* biofilms in the food industry. Unlike bactericidal strategies, the implementation of this approach would not impose any selective pressure for pathogen resistance development.

## MI-P007-60
## IDENTIFICATION AND CHARACTERIZATION OF ALGAE METALLOTHIONEINS FOR USE IN HEAVY METALS BIOREMEDIATION

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Metallothioneins (MTs) constitute a large and heterogeneous superfamily of low molecular mass cytosolic proteins composed of about 30-100 amino acids. Its primary structure is characterized by a high content of cysteine residues (Cys) located in highly conserved CC, CxC and CxxC motifs. This characteristic allows these proteins a great ability to coordinate significant amounts of mono or divalent metal ions through metal-thiolate bonds, thus constituting metal clusters. MTs are usually the main primary response of organisms to an inadequate type/dose of heavy metals, operating by chelation and immobilization. In the case of algae, MTs from only two species have been found and characterized. It is strange that these proteins have not been identified in a greater number of algae, these species being highly resistant to metals, and with a great capacity to accumulate them. For this reason, in this work we used different bioinformatic approaches to uncover new algae MTs. Our objectives were to establish phylogenetic relationships between MTs from the different algae taxons and to characterize some of them for use in heavy metal bioremediation. We identified 124 potential MT sequences from algae: 26 from Chlorophytas, 51 from Rodophytas and 47 from Ochrophytas. The sequences of algal MTs are very heterogeneous. Most of the primary structures of MTs from Rhodophytas and Ochropytas contain Cys domains and intermediate linker regions devoid of these amino acids, similar to higher plants. However, the primary structures of Chlorophytas tend to contain Cys residues throughout the entire sequence or very short linkers. We are currently working on the characterization of four MTs. Two correspond to the brown macroalgae *Ectocarpus siliculosus* (EsilMT1 and EsilMT2), one to a red microalgae *Galdieria sulphuraria* (GsulMT) and one to a green microalgae *Auxenochlorella protothecoides* (AproMT). EsilMT1 has a primary structure similar to higher plants, whereas EsilMT2 has a shorter sequence with fewer Cys residues. GsulMT and AproMT consist of sequences with more than 30% Cys residues distributed throughout their sequences. Complementation assays in MT-deficient yeasts showed that the MTs conferred, to varying degrees, resistance to the presence of hydrogen peroxide, Zn, Cu, and Cd. When these MTs were expressed in *E. coli*, they also provided a better growth performance to the bacteria in high Zn, Cu and Cd media. The characterization by ICP-AES and ESI-MS of the MTs synthesized in *E. coli* showed that they have affinity for metals in different ways. We present here these algae MTs as promising tools for metal bioremediation, with the perspectives