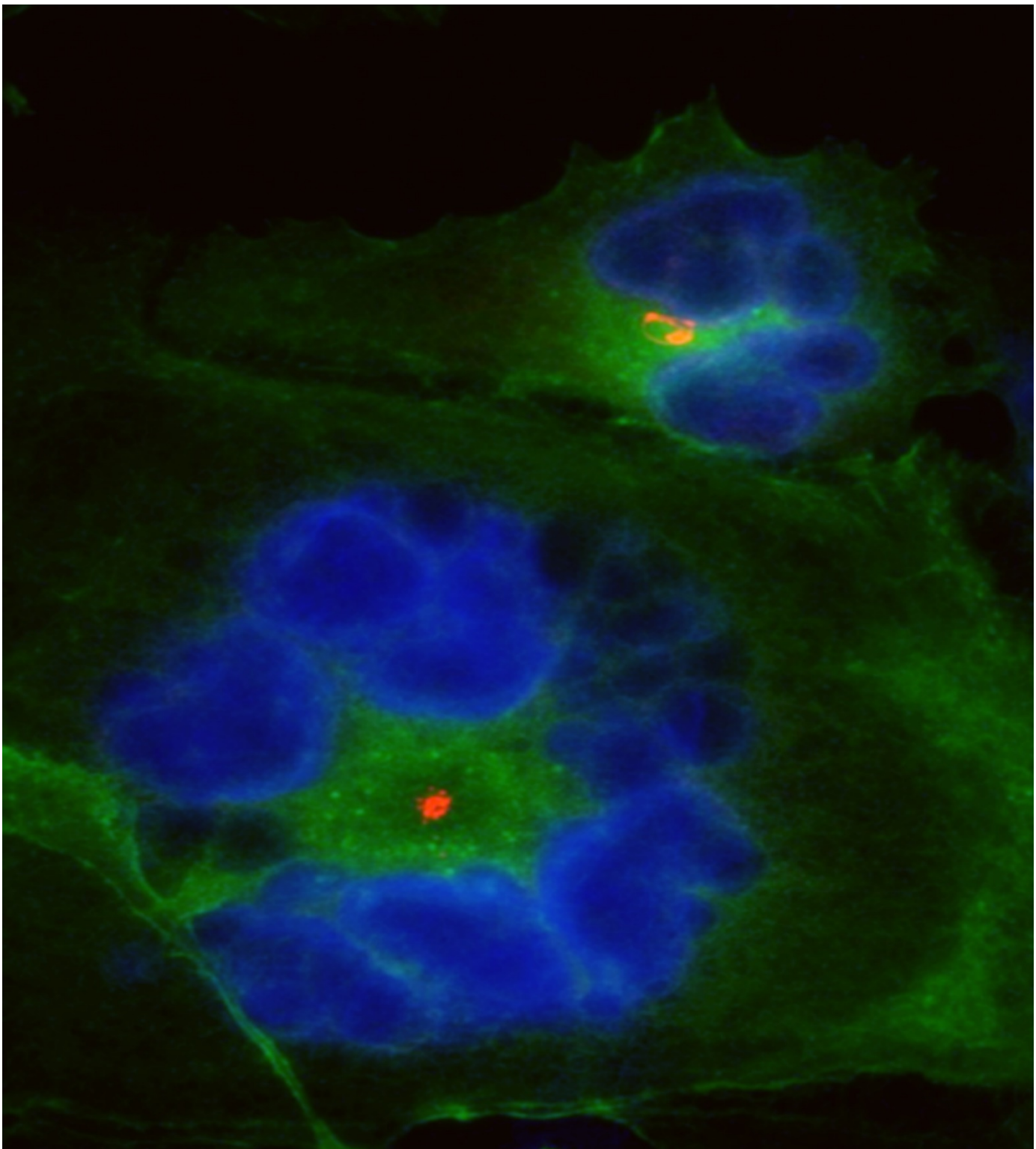
BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)



BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

Cover page: The Synthetic Lethal Rosette

Aberrant mitotic phenotype found in BRCA1-deficient cells treated with the PLK1 inhibitor Volasertib. Cells become giant and multinucleated and acquire a flower shape, with nuclei arranging in a circular disposition around a cluster of centrosomes. Blue (DAPI: nuclei), Green (FITC-phalloidin: actin cytoskeleton), Red (γ-Tubulin: centrosomes).

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BIOCELL 43 (suppl.5), 2019
ISSN 1667-5746 (online version)

MI-P30
A NEW DNA BINDING SITE IN THE MISMATCH REPAIR MUTS PROTEIN INVOLVED IN THE INTERACTION WITH DNA REPLICATION STRUCTURES

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MutS maintains genomic stability by recognizing mispaired nucleotides (MMs) and triggering the Mismatch Repair (MMR) pathway. Findings from our laboratory have demonstrated that the repair factor also contributes to DNA replication fidelity by regulating the access of the mutagenic DNA polymerase IV to replication sites. In this work, we showed for the first time that MutS from Pseudomonas aeruginosa can interact with DNA structures present at replication sites, i.e. primed DNA (pDNA). Moreover, MutS suffered a conformational change upon binding to a pDNA containing a GT mismatch (GT-pDNA), resulting in a more compact and stable protein structure, as determined by native gel electrophoresis, circular dichroism and trypsin digestion assays. This structural rearrangement was not observed when MutS was associated with pDNA and the MMR double-stranded DNA substrates, GT-dsDNA and dsDNA. We hypothesized that the MutS conformational change induced by its association with the mismatched pDNA could result from binding to new protein residues. In fact, using the nucleic acid binding prediction BindUP software and DNA-protein docking analysis, we identified a new DNA binding surface in MutS. Within this novel site, Arg275 appeared to directly contact the 3'-OH end of the pDNA. Mutation of this residue to Glu abolished DNA binding *in vitro* and produced a mutator phenotype *in vivo*, indicating a key role of this novel binding site in the activity of MutS. We are testing if the structural change induced by the mismatched replication substrate and the new DNA binding site are important for MutS gaining access to replication sites. In conclusion, our results reveal a novel DNA interaction site in MutS, which may play an important role in the control of Pol IV access to replication sites by MutS.

MI-P31
DNA REPAIR AND TOLERANCE PATHWAYS INVOLVED IN THE ROCESSING OF A SINGLE LESION ON THE CHROMOSOME OF *ESCHERICHIA COLI*

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The genome is constantly exposed to DNA damaging agents that alter the integrity of the DNA molecule. Most of these lesions are removed by the repair mechanism called Nucleotide Excision Repair (NER). However, some lesions might escape the repair process and perturb DNA synthesis by the replicative DNA Polymerase (Pol), resulting in cell lethality. To survive DNA damage, cells have evolved Damage Tolerance (DT) pathways such as the Translesion Synthesis (TLS), which involves specialized Pols capable of inserting a nucleotide opposite the lesion. In this work, we studied both repair and tolerance pathways implied in the processing of a single alkylation lesion (N^2-furfurylguanine, FF) inserted site-specifically in the chromosome of *Escherichia coli*. The main results were: i-TLS was highly favored over other DT pathways; ii- Pol IV, but not Pol II and Pol V, catalyzed the TLS reaction; iii- Pol IV incorporated the correct nucleotide opposite the FF lesion and thus, the TLS reaction is error-free; iv-the proofreading subunit of the replicative Pol III prevented the Pol IV-catalyzed TLS, indicating a competition between exonucleolytic excision and TLS; v-the FF lesion was not removed by the NER, which is the main repair pathway implicated in the reversion of the alkylating damage; vi- the postreplicative Mismatch Repair (MMR) pathway was able to repair this damage. Finally, no differences in the repair or TLS of the FF lesion were detected when the damaged base was located in the leading or the lagging strand of replication. Our finding showed that Pol IV-catalyzed TLS and MMR become the main tolerance and repair pathways used by *E. coli* cells to deal with FF lesions on the chromosome.

MI-P32
ANTI-INFLAMMATORY EFFECTS OF *LACTOBACILLUS PLANTARUM* CRL 759 SUPERNATANT IN OCULAR INFLAMMATIONS

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Anti-inflammatory effect of probiotic bacteria cell free supernatant was extensively proved as therapy for different inflammatory diseases, but no investigated in ocular inflammatory disorders. Uveitis, an intraocular inflammatory disease, is a common cause of vision loss. Traditional treatments with corticosteroid present several side effects, and alternative therapies are continuously investigated. The aims of this study were to evaluate whether *Lactobacillus plantarum* CRL 759 supernatant (LplS) was able to diminish the inflammatory response triggered by LPS in ARPE-19 cells (human retinal pigment epithelium cell line); in addition, to evaluate *in vivo* its capacity to exert anti-inflammatory effect in uveitis induced by endotoxin in mice. *L. plantarum* CRL 759 was cultured in DMEM medium at 37ºC and 5% CO_2. LplS was obtained by filtration with 0.22 µm membranes. ARPE-19 cells (2.5×10^5) were treated with LplS 4 h; then, the cells were stimulated with LPS (10 µg/mL). Cytokines (by flow cytometry), NO and TBARS (by colorimetric methods) produced by ARPE-19 cells were measured in the culture supernatant. To induce uveitis, 130 µg LPS was injected subcutaneously into C57BL/6 mice. LplS was administered as drops and Prednisolone (P) was used as anti-inflammatory control. The mice were divided into six groups randomly: LPS group (LPS injection + PBS drops); LPS + LplS group (LPS injection + LplS drops); LPS + P group (LPS injection + prednisolone drops) and control groups: treated with PBS, LplS or P drops and a PBS injection. 24 h after stimulation with LPS or PBS, mice were sacrificed. The ocular inflammation was assessed by slit lamp microscopy and clinical scores were determined at the same time. The aqueous humor (AqH) was collected, and total protein (by Bradford assay), TNF-α level (by ELISA), and cell count (by Giemsa coloration) were determined. Eyes were enucleated to histopathologic evaluation. Results showed that LplS reduced the production of IL-6, IL-8, NO and TBARS in LPS-stimulated ARPE-19 cells. *In vivo* studies, the clinical score of mice treated with LplS drops

was significantly lower than the LPS group. LplS also reduced levels of TNF-α and protein concentration in AqH. Histological examination showed reduction of infiltrating inflammatory cells in the posterior segment of LPS + LplS group, however, there was no significant difference in leukocyte count in AqH in all groups. LplS anti-inflammatory effect was similar to that induced by prednisolone. In this study, we showed that LplS as ophthalmic drops attenuates the inflammatory process in an endotoxin-induced uveitis. These effects were comparable to the one achieved by prednisolone and could be proposed as a potential therapy for ocular inflammatory disorders.

MI-P33
SELECTION OF LACTIC ACID BACTERIAL STRAINS ABLE TO MODULATE THE HOST CENTRAL NERVOUS SYSTEM

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Current evidence indicates that modulation of the central nervous system (CNS) by the microbiome occurs primarily through neuroimmune and neuroendocrine mechanisms, often involving the vagus nerve. In fact, the gut-brain axis provides the intestinal bacteria and its metabolites a way to get access to the brain, thus regulating the expression of key effectors. Although lactic acid bacteria (LAB) represent only a small percentage of the total gut-dwelling microorganisms, they are undoubtedly important players. Pro-inflammatory cytokines are naturally upregulated in the elderly and gut microbiota undergoes changes during aging. Therefore, it has been proposed that administration of probiotics may decrease the synthesis of these pro-inflammatory cytokines, hence reducing inflammation and oxidative stress, ameliorating the effects of senescence and the progression of neurodegenerative diseases often associated with aging. These facts strongly suggest that LAB may be an invaluable tool in the treatment of aging-related pathologies such as Alzheimer's disease (AD), where microglia and non-parenchymal macrophages drive the neurodegeneration via neuroinflammation. The aim of the present work was to assess the capacity of different LAB strains metabolites to regulate the secretion of cytokines, inhibit acetylcholinesterase (AChE) and protect cells from the cytotoxic effects of the Aβ oligomers, key features of AD. For that purpose, murine RAW 264.7 macrophages were treated with conditioned media from seven selected LAB strains prior to the LPS stimulation. The mRNA expression levels of the tumor necrosis factor alpha (TNF)-α, interferon-gamma (IFN-*γ)* and interleukin IL-10 were examined by RT-PCR. The results showed that conditioned media from *Lactobacillus delbrueckii subsp. lactis* CRL 581 and *Lactobacillus reuteri* CRL 1098 significantly inhibited TNF-α mRNA expression. In addition, CRL 1098 strain increased IL-10 mRNA expression level in LPS-stimulated RAW 264.7 cells. Then, all conditioned media were evaluated in their ability to inhibit AChE from human erythrocytes (AChE-E) by Ellman's method. AChE-E constitutes a model of the isoform present in the CNS. As controls, polyphenols previously characterized as efficient inhibitors of AChE as well as strong anti-inflammatory agents were used. Conditioned media from *L. delbrueckii subsp. lactis* CRL 581 showed a 40% inhibition of enzymatic activity. Finally, APP-expressing neuroblastoma cells were preincubated with conditioned media and the expression of the APP transgene was induced by butyric acid. Viability of cells were evaluated using alamar blue assay. Results evidenced that conditioned media from *L. rhamnosus* A29 and *Enterococcus mundtii* CRL 35 protected against Aβ induced neuron cytotoxicity. These preliminary data support our ongoing investigations regarding the molecular mechanism of LAB interactions in the gut brain axis.

MI-P34
CHARACTERIZATION OF NEW ANTIMICROBIALS PRODUCED BY CLINICAL ISOLATES FROM FECAL SAMPLES FOR BIOTECHNOLOGICAL APPLICATION

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Foodborne diseases are one of the most widespread problems of the worldwide population and are produced by the ingestion of contaminated water or food. We analyzed the *Shigella* clinical isolates (CI), from patients suffered gastrointestinal infections during the summer period 2013-2017 from Catamarca, Santiago del Estero and Tucumán patients (Northwest Argentina region), in order to select those capable to produce antimicrobial compounds, using the plate's diffusion technique. We found that 11 of the total analyzed samples (371 CI) were able to produce an antimicrobial agent that inhibited the growth of the *Escherichia coli* AB1133 used as an indicator sensitive strain. In addition, in this work we characterized these compounds studying its thermotolerance (100°C), stability to pH (5 and 8) and proteinase treatments, as well its cross immunity against other bacteriocin producer strains, and its spectrum of antimicrobial action, between other characteristics. To complete the proprieties and classification of these compounds, the molecular weight was also estimated by the ability of such agents to diffuse through dialysis membranes with different cut-off size pores, mainly in the range of 10 to 12000 Da. The results indicate that the total numbers of antimicrobials are tolerant to the temperature (100°C) and sensitive to the proteinase K treatment, which are two desirable characteristics for use as food preservatives. In addition, we found that only 2 compounds are affected by pH 8 treatment. The cross-immunity test showed that at least 3 antimicrobials have a different nature and can be classified as different agents. From this study, we select producer strains of these 3 new compounds and analyzed the bacterial growth and the antimicrobial production curve through the time. On the other hand, we investigated if the production of these antimicrobial agents was induced by mitomycin C (0,5 µg/mL), an inducer agent of the SOS system required to activates the synthesis of high molecular weight bacteriocins called colicins. In this work we demonstrated that 3 of the 11 antimicrobials studied presented important characteristics that enable their use and development as new antibiotics or new food preservatives and that at least one of them is a different compound to the typical colicins produced by *Shigella*.