XIII SIMPÓSIO
Instituto de Ciências Biológicas – IB, UnB
De Biologia Molecular

Programa de Pós-graduação em Biologia molecular

ANAIS

XIII SIMPÓSIO DE BIOLOGIA MOLECULAR

Programa de Pós-graduação

em Biologia Molecular

Brasília – 2025

Anais do XIII Simpósio de Biologia Molecular

Programa de Pós-graduação em Ciências Biológicas (Biologia Molecular)

Universidade de Brasília

Annals of the XIII Molecular Biology Symposium

Graduate Program in Biological Sciences (Molecular Biology)

University of Brasília

Brasília

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Sumário / Summary

Apresentação

O 13º Simpósio de Biologia Molecular do Programa de Pós-Graduação em Ciências Biológicas (Biologia Molecular) (PPGBioMol) da Universidade de Brasília (UnB) foi realizado nos dias 5 e 6 de dezembro de 2024, em Brasília, DF. O simpósio reuniu estudantes, professores e pesquisadores da UnB, além de convidados especiais. Neste ano, as palestras e discussões foram organizadas em várias áreas temáticas: Imunologia – Terapia, Vacinas, Interação Hospedeiro-Patógeno e Genômica, Virologia, Biologia Estrutural e Inteligência Artificial Aplicada à Química Medicinal. O PPGBioMol abrange uma variedade de linhas de pesquisa. Para melhor organizar a diversidade de conteúdos, os resumos submetidos foram agrupados em quatro grandes áreas: (01) Biologia Celular e Imunologia; (02) Bioquímica, Biofísica e Biologia Estrutural; (03) Genética Molecular, Biotecnologia e Genômica; (04) Virologia e Microbiologia Molecular. Além disso, uma quinta área foi criada para avaliar os trabalhos submetidos por estudantes de graduação. No total, foram submetidos 78 resumos de pós-graduandos e 30 de graduandos. Um trabalho de cada área foi selecionado para apresentação oral no dia 6 de dezembro. Estes Anais contêm os resumos dos estudantes e refletem o empenho tanto dos alunos quanto dos docentes no avanço e fortalecimento da pesquisa científica em nossa universidade.

Presentation

The 13th Molecular Biology Symposium of the Graduate Program in Biological Sciences (Molecular Biology) (PPGBioMol) at the University of Brasília (UnB)** was held on December 5th and 6th, 2024, in Brasília, DF. The symposium brought together students, professors, and researchers from UnB as well as invited guests. This year, the lectures and discussions were organized into several thematic areas: Immunology – Therapy, Vaccines, Host-Pathogen Interaction and Genomics, Virology, Structural Biology, and Artificial Intelligence Applied to Medicinal Chemistry. PPGBioMol encompasses a variety of research lines. To better organize the diverse content, the submitted abstracts were grouped into four major areas: (01) Cell Biology and Immunology; (02) Biochemistry, Biophysics, and Structural Biology; (03) Molecular Genetics, Biotechnology, and Genomics; (04) Virology and Molecular Microbiology. Additionally, a fifth area was created to evaluate undergraduate student submissions. A total of 78 abstracts from graduate students and 30 from undergraduates were submitted. One work from each area was selected for an oral presentation on December 6th. These Proceedings contain the students' abstracts and reflect the engagement of both students and faculty in advancing and strengthening scientific research at our university.

Comissão organizadora

Professora do Departamento de Biologia Celular

Dra. Izabela Marques Dourado Bastos

Dra. Andrea Queiroz Maranhão

Pós-doutorandos e pesquisadores colaboradores

Dra. Alexandra Maria dos Santos Carvalho

Dra. Izadora Cristina Moreira de Oliveira

Doutoranda

MSc. Bruno Felix Pimentel Vianna

MSc. Daniela Franco Rosa

MSc. Lucas Silva Rodrigues

MSc. Sabrina Azevedo Machado Doutoranda

MSc. Sarah Pinho Bezerra, Doutoranda

Mestrandos

Andrey Duarte Boava

Felipe da Silva Mendonça de Melo

Júlia Lisboa Beserra e Silva

Pietra Oliveira Guimarães

Renato Rodrigues Oliveira

Comissão avaliadora / Evaluation committee

(01) Cell Biology and Immunology

Dra. Andrea Queiroz Maranhão

Dra. Raquel

(02) Biochemistry, Biophysics, and Structural Biology

Dra. Izabela Marques Dourado Bastos

(03) Molecular Genetics, Biotechnology, and Genomics

Dr(a).

(04) Virology and Molecular Microbiology

Dra. Izadora Cristina Moreira de Oliveira

(5) Graduação

Dra. Alexandra Maria dos Santos Carvalho

Programação / Program

XIII Simpósio Biomol 2024

05/12- Quinta-feira

08:30 – 09:15 – Registration

09:15 – 09:30 – Open Cerimony:

Coordenador do Curso, Prof. João Alexandre Barbosa
Chefe do Departamento de Biologia Celular, Prof. Napoleão Valadares
Diretor do Instituto de Biologia, Prof. Luiz Eduardo Blum

09:30–10:25

Prof. Pedro Manoel Viera (Unicamp) (http://lattes.cnpq.br/6667440528985761)
Metabolic controlo f macrophages in health and disease.

10:25 – 11:10

Dr. Andrey Fabrício Ziem Nascimento. (Laboratório Nacional de Luz Síncroton, Campinas – SP) (http://lattes.cnpq.br/3082338571105417)
Sirius lab and structural biology research

11:10 – 11:25 – Coffee Break

11:25 – 12:00

Prof. Carlos André Ricart (CEL- UnB) (http://lattes.cnpq.br/1271167329803156)
The history of proteomics at University of Brasilia.

12:00- 12:20

Company Talk – Jayme Nunes de Souza Filho (Loccus do Brasil).
Automated Nucleic Acid Extraction: New Frontiers

14:00 – 17:00 Poster Session

06/12 sexta-feira

09:00 – 10:30 - Mesa redonda: Academia e Sociedade

Participantes:

Profª. Thaiane Moreira de Oliveira (UFF-RJ) (http://lattes.cnpq.br/4073806576367509)
Scientific misinformation in crisis

Dr. Matias Eliseo Melendez-(INCA-RJ) (http://lattes.cnpq.br/2746759168721010)
Health innovation: diagnosis, therapy and Inovação em Saúde: Diagnóstico, Terapia and entrepreneurship

Prof. João Paulo Figueiró Longo (GEM-UnB) (http://lattes.cnpq.br/3208175361373924)
Development of a simple, high-efficiency and low-cost praziquantel nanoformulation for the treatment of schistosomiasis

10:30 – 10:45 – Coffee Break

10:45–11:15

Profª. Drª. Bruna Fuga Araújo (CEL-UnB) – (http://lattes.cnpq.br/1891060802570287).
Antimicrobial resistance: Impacts on animal, human, and environmental health

11:15 -11:45

Drª. Maria Beatriz Walter Costa (Friedrich Schiller University Jena, Alemanha) (http://lattes.cnpq.br/7844076733494022):
Association between prokaryotic genomes and environmental abiotic factors with machine learning

11:45 – 12:15

Dr. Elíbio Leopoldo Rech (EMBRAPA – CENARGEN) (http://lattes.cnpq.br/0995074904257272)
Complex and synthetic genomes:

Almoço

14:00 – 14:45 – Honored Professor

14:45 – 17:00– Oral presentation of top research posters, awards ceremony, and closing.

Apresentações orais síncronas/Synchronous oral presentations

A1 – Cell Biology and Immunology

Sylvia Barbosa Pinhate

Título do trabalho: *Saccharomyces boulardii* Expressing Anti-CD3 Attenuates Symptoms in Mice with DSS-Induced Colitis.

A2 – Biochemistry, Biophysics, and Structural Biology

Felipe Diego Medeiros de Sousa

Título do trabalho: Are endogenous antioxidants relevant for tardigrade survival during anhydrobiosis and recovery?

A3 – Molecular Genetics, Biotechnology, and Genomics

Flávia Cabral Netto Resende –

Título do trabalho: Heterologous expression of two chimerical proteins containing antimicrobial peptides from plant genomes in bacteria

A4 – Virology and Molecular Microbiology

Ellen Caroline Feitoza Pires

Título do trabalho: Anti-chikungunya virus activity and isolation of coumarins from aqueous extract of *Erythroxylum suberosum* leaves through bioassay-guided fractionation

Graduação

Hillary da Silva Velame

Título do trabalho: Expression and characterization of a crystallized SARS-CoV-2 membrane (M) protein fused with the C-terminal portion of the Bacillus thuringiensis Cry1C for enhanced stability and cellular response analysis

Menção honrosa

Bruno Matheus Ferreira Paula

Título do trabalho: Purification, dynamic light scattering and antineoplastic activity of enterolobin.

Resumos / Abstracts

BIOLOGIA CELULAR E IMUNOLOGIA (A1)

EFFECT OF ÔMEGA-3 AND PALMITIC ACID IN CARCINOGENIC PARAMETERS AND LIPID DROPLET METABOLISM OF PANCREATIC ADENOCARCINOMA CELLS

RAMON BUSON LIMA PAIVA; NICOLAS SÁ RODRIGUES MAIA; GABRIEL FELIPE GOMES CALIXTO; KELLY GRACE MAGALHÃES

OMEGA-3 IS AN ESSENTIAL FATTY ACID ACQUIRED FROM FISH AND PLANT OILS. AMONG OTHER ÔMEGA-3, DOCOSAHEXAENOIC ACID (DHA) HAS BEEN STUDIED TO PREVENT AND TREAT SEVERAL DISEASES, INCLUDING CANCER. PALMITIC ACID (PA) IS THE MOST COMMON UNSATURATED FATTY ACID, USUALLY RELATED AWORST PROGNOSIS IN CANCER. PANCREATIC ADENOCARCINOMA IS AN AGGRESSIVE PRIMARY MALIGNANCY AND IS RESPONSIBLE FOR MOST OF PANCREAS CANCER–RELATED DEATHS. MIA-PACA-2 CELLS WERE STIMULATED OR NOT WITH DHA OR PA AT DIFFERENT TIMES AND CONCENTRATIONS. MITOCHONDRIAL CELL VIABILITY WAS ASSESSED BY MTT ASSAY AND ANALYZED BY SPECTROPHOTOMETRY. MEMBRANE PORE FORMATION WAS ASSESSED BY PROPIDIUM IODIDE UPTAKE AND ANALYZED BY FLUORESCENCE SPECTROPHOTOMETRY AND BY LDH RELEASE ASSAY ANALYZED BY SPECTROPHOTOMETRY. CELL PROLIFERATION WAS ANALYZED BY CFSE PROBE AND CYTOMETRY. OXYGEN REACTIVE SPECIES PRODUCTION WAS MEASURED BY DCFDA PROBE AND ANALYZED BY SPECTROPHOTOMETRY. LIPID DROPLET BIOGENESIS WAS ANALYZED BY BODIPY AND CYTOMETRY OR BY OIL RED WITH HEMATOXYLIN STAINING AND ANALYZED BY MICROSCOPY. DHA, BUT NOT PA, WAS ABLE TO REDUCE MIA PACA-2 CELLS VIABILITY IN A TIME AND DOSE DEPENDENT WAY. NEITHER DHA OR PA WERE ABLE TO INDUCE LDH RELEASE NOR INDUCE MEMBRANE PORE FORMATION. OXYGEN REACTIVE SPECIES PRODUCTION WAS NOT AFFECTED BY DHA OR PA AT 8 HOURS. DHA REDUCED CELL PROLIFERATION. PA WAS ABLE TO INDUCE LIPID DROPLET ACCUMULATION AT 48 AND 72 HOURS AT A HIGHER CONCENTRATION. BOTH DHA AND PA INCREASED THE NUMBER OF LIPID DROPLETS AT 24 48 AND 72 HOURS. CONCLUSION: OUR DATA SUGGEST THAT OMEGA-3 DHA BUT NOT PA CAN REDUCE CELL VIABILITY OF PANCREATIC CANCER CELLS, BY THE INDUCTION OF A NON-LITHIC CELL DEATH. IN ADDITION, OMEGA-3 DHA AND PA HAD DISTINGUISH EFFECTS ON LIPID DROPLET BIOGENESIS. TAKEN TOGETHER, OUR RESULTS SUGGEST THERE IS AN ANTAGONISM EFFECT OF OMEGA-3 DHA AND PA IN PANCREATIC

CANCER CELLS, DEMONSTRATING THE POTENTIAL ANTINEOPLASTIC OF OMEGA-3 DHA AGAINST THIS CANCER.

EXPLORING THE MATERNAL-FETAL INTERFACE AND SEX-BASED SUSCEPTIBILITY TO DISSECT ZIKA VIRUS PATHOGENESIS IN THE OFFSPRING

HELOISA ANTONIELLA BRAZ DE MELO; SHANSHAN ZHANG; INES ZALONISK; FERNANDA GOMES LAGO; JUN R. HUH AND KELLY GRACE MAGALHAES

DESPITE EXTENSIVE RESEARCH ON ZIKV VERTICAL TRANSMISSION, THE MECHANISMS BY WHICH MATERNAL INFLAMMATION MAY CONTRIBUTE TO THE DEVELOPMENT OF DISABILITIES IN OFFSPRING REMAIN UNCLEAR. THIS IS CRUCIAL GIVEN GROWING EVIDENCE THAT MATERNAL IMMUNE ACTIVATION CAN HAVE LONG-TERM EFFECTS ON OFFSPRING, AS OBSERVED IN VARIOUS NEURODEVELOPMENTAL DISORDERS. CONSIDERING THIS, OUR AIM WAS TO DEMONSTRATE THAT ZIKV-INDUCED MATERNAL INFLAMMATION IS IMPORTANT FOR ITS PATHOGENESIS IN THE OFFSPRING. HETEROZYGOUS HSTAT2 KNOCK-IN (KI) FEMALES, A ZIKV-SUSCEPTIBLE MOUSE MODEL, WERE MATED WITH WILD-TYPE (WT) MALES TO GENERATE MICE EXPRESSING HSTAT2 IN A SUSCEPTIBLE MATERNAL ENVIRONMENT. IN CONTRAST, WT FEMALES (RESISTANT TO ZIKV) WERE MATED WITH KI MALES TO PRODUCE FETUSES EXPRESSING HSTAT2 ONLY IN FETAL TISSUES, CREATING A SUSCEPTIBLE OFFSPRING FROM A RESISTANT ENVIRONMENT. PREGNANT MICE WERE INFECTED WITH ZIKV AT EMBRYONIC DAY 10.5 (E10.5), AND TISSUES WERE COLLECTED AT E17.5 OR 9 DAYS POSTPARTUM. VIRAL LOAD IN PLACENTAS FROM BOTH RESISTANT AND SUSCEPTIBLE MOTHERS WAS COMPARABLE, BUT SUSCEPTIBLE MOTHER-DERIVED FETUSES EXHIBITED HIGHER INFLAMMATORY MARKER EXPRESSION IN THE PLACENTA. THE FEMALE KI OFFSPRING FROM SUSCEPTIBLE MOTHERS SHOWED INCREASED USV INDEX, ELEVATED VIRAL LOAD, AND HIGHER AXL EXPRESSION IN THE PLACENTA, POTENTIALLY CONTRIBUTING TO THEIR INCREASED SUSCEPTIBILITY. POST-BIRTH, THESE FEMALES ALSO SUSTAINED CYTOKINE EXPRESSION IN THE BRAIN AND EXHIBITED HEIGHTENED CD8 T CELL ACTIVATION IN THE SPLEEN, INDICATING LONG-TERM IMMUNE EFFECTS. OUR FINDINGS EMPHASIZE THE IMPORTANCE OF MATERNAL IMMUNE RESPONSES IN ZIKV PATHOGENESIS AND SUGGEST A SEX-SPECIFIC SUSCEPTIBILITY, WITH FEMALE OFFSPRING BEING MORE VULNERABLE TO LONG-TERM EFFECTS.

SACCHAROMYCES BOULARDII EXPRESSING ANTI-CD3 ATTENUATES SYMPTOMS IN MICE WITH DSS-INDUCED COLITIS.

SYLVIA BARBOSA PINHATE; PIETRA OLIVEIRA GUIMARÃES; ISABEL GARCIA SOUSA; MANUELA MARAGNO DO ALMO; RENATO RODRIGUES DE OLIVEIRA; ANDRÉA QUEIROZ MARANHÃO; MARCELO DE MACEDO BRIGIDO.

ULCERATIVE COLITIS (UC) HAS HAD ITS INCIDENCE INCREASE GLOBALLY IN RECENT YEARS. UC IS AN INFLAMMATORY BOWEL DISEASE (IBD) CAUSED BY RECURRENT INFLAMMATION OF THE COLON REGION, RESULTING IN DAMAGE TO THE TISSUE AND IMBALANCE TO THE MICROBIOTA COMPOSITION. THOUGH AVAILABLE TREATMENTS ARE EFFECTIVE, THEY ARE EXPENSIVE AND CAN CAUSE ADVERSE EFFECTS. SACCHAROMYCES BOULARDII IS A PROBIOTIC YEAST COMMONLY USED TO TREAT INTESTINAL DISORDERS DUE TO ITS CHARACTERISTICS AND IS SUGGESTED TO HAVE PROTECTIVE EFFECTS IN UC. IT CAN BE MODIFIED FOR HETEROLOGOUS EXPRESSION OF RECOMBINANT PROTEINS, AND TOLERATE BODY TEMPERATURE AND LOW PH LEVELS, MAKING IT A POTENTIAL VEHICLE FOR ORAL DELIVERY OF MOLECULES TO THE GASTROINTESTINAL TRACT. THEREFORE, THIS WORK EXPLORES THE CAPACITY TO DELIVER AN ANTI-CD3 SCFV FRAGMENT BY S. BOULARDII AND INCREASE ITS PRODUCTION. ALSO, TO OBSERVE THE EFFECTS OF THIS MODEL IN A DEXTRAN SODIUM SULFATE (DSS) INDUCED COLITIS MOUSE MODEL. PLASMID CONSTRUCTIONS TESTED TO MAKE ANTI-CD3 MORE AVAILABLE ON THE YEAST SURFACE RESULTED IN INCREASED DETECTION IN FLOW CYTOMETRY. C57BL FEMALE MICE WERE ORALLY GAVAGED DAILY, WITH EITHER THE DOSE WITH TRANSFORMED YEAST FOR THE TREATMENT GROUP OR 0.9% SALINE FOR THE CONTROL GROUP. UC WAS INDUCED FOR THE NEXT 5 DAYS BY ADDING 3% DSS IN WATER, ALONGSIDE DAILY TREATMENTS. MICE WERE MONITORED DAILY ON WEIGHT, FECAL CONSISTENCY, AND RECTAL BLEEDING TO EVALUATE DISEASE ACTIVITY. THE COLON WAS MEASURED AND WEIGHED AFTER EUTHANASIA. MICE TREATED WITH ANTI-CD3 PRESENTING YEAST SHOWED DISEASE ACTIVITY VALUES CLOSER TO THE HEALTHY GROUP AND SIGNIFICANTLY DIFFERED FROM THE NON-TREATED MICE. WE CONCLUDED THAT THE YEAST CAN PRODUCE AND PRESENT THE ANTIBODY ON ITS SURFACE. IT ALSO HAD IMPROVED PROTECTIVE EFFECTS IN THE UC MODEL AND DELAYED INFLAMMATION PROGRESSION. THIS MODEL HAS GREAT POTENTIAL TO RESHAPE THE IMMUNOTHERAPY FIELD ON IBD.

ENHANCING ANTIBODY DISCOVERY: MINION SEQUENCING FOR COMPREHENSIVE PHAGE DISPLAY ANALYSIS

PEDRO HENRIQUE ARAGÃO BARROS

PHAGE DISPLAY ENABLES THE IDENTIFICATION OF THERAPEUTIC ANTIBODIES BY ALLOWING THE SELECTION OF ANTIBODIES SPECIFIC TO TARGETS, INCLUDING VIRAL PROTEINS. DESPITE THE STRENGTH OF THE TECHNIQUE, THE COMPREHENSIVE ANALYSIS OF ANTIBODIES PRODUCED BY PHAGE DISPLAY REMAINS CHALLENGING DUE TO THE LARGE VOLUME OF DATA AND DUE TO THE LONG SCFV READ LENGTHS. TO ADDRESS THESE ISSUES, WE ARE DEVELOPING SOFTWARE FOR MINION NANOPORE SEQUENCING TO ENABLE FULL-LENGTH SCFV ANTIBODY SEQUENCING, WHILE IMPLEMENTING METHODS TO MANAGE HIGH ERROR RATES. THE GOALS ARE TO ASSEMBLE AND GENERATE HIGH-QUALITY CONSENSUS SEQUENCES FROM SEQUENCING,

ASSESS ANTIBODY ENRICHMENT ACROSS PHAGE DISPLAY SELECTION ROUNDS, AND ANALYSE V-D-J GENE USAGE AT THE FAMILY AND GENE LEVEL, WITH A FOCUS ON IDENTIFYING CANDIDATE ANTIBODIES AGAINST COVID-19 AND ZIKA. THE METHODS INCLUDES MINION PLASMID SEQUENCING COUPLED WITH CUSTOM-BUILT PIPELINES FOR ERROR CORRECTION, SEQUENCE COUNTING, AND ENRICHMENT ANALYSIS. THE SOFTWARE, IMPLEMENTED WITHIN A SHINY INTERFACE (R V4.2.0), PROVIDES VISUALISATION TOOLS FOR TRACKING ENRICHMENT PATTERNS, ANTIBODY DIVERSITY AND V-D-J USAGE, ENABLING ANALYSIS ACROSS SELECTION ROUNDS. PRELIMINARY RESULTS INDICATE THAT THE SOFTWARE EFFECTIVELY ASSEMBLY CONSTRUCTS AND GENERATED ACCURATE CONSENSUS SEQUENCES, REVELING ENRICHMENT DYNAMICS ACROSS ROUNDS. THE TOOL WILL CONTRIBUTE TO THE IDENTIFICATION OF POTENTIAL THERAPEUTIC ANTIBODIES, AS WELL AS THE PROFILING AND INVESTIGATION OF ANTIBODY LIBRARIES.

OPTIMIZATION OF DNA VACCINE FOR ENHANCED ANTIGEN EXPRESSION AND IN VIVO BIOLUMINESCENCE MONITORING

ANDREY DUARTE BOAVA; ALEXANDRA MARIA DOS SANTOS CARVALHO; DANIELA FRANCO ROSA; KAREN STEPHANIE DE SOUZA MANGABEIRA; JULIA LISBOA BESERRA E SILVA; FELIPE DA SILVA MENDONÇA DE MELO; KAUÃ DOS SANTOS SILVA; AISSA LANA OLIVEIRA LIMA; FLÁVIA NADER MOTTA; JOÃO PAULO FIGUEIRÓ LONGO; CARLA ARAÚJO NUNES; JAIME MARTINS DE SANTANA; IZABELA MARQUES DOURADO BASTOS

THE LIMITED EFFECTIVENESS OF CURRENT TREATMENTS AND THEIR ASSOCIATED SIDE EFFECTS HIGHLIGHT THE URGENT NEED FOR ALTERNATIVE THERAPEUTIC AND PREVENTIVE STRATEGIES FOR CHAGAS DISEASE (CD). IN THIS CONTEXT, THE AIM OF THIS STUDY WAS TO CONDUCT A PROOF-OF-CONCEPT FOR THE DEVELOPMENT OF A DNA VACCINE PLATFORM, USING A PLASMID CONTAINING THE REPORTER GENES LUCIFERASE AND MNEONGREEN (OPTV/LUCNEO). THE PLASMID WAS OPTIMIZED WITH 5'UTR SEQUENCES FROM THE HUMAN A-HEMOGLOBIN GENE, THE 3'UTR FROM THE AES GENE, AND A 100-NUCLEOTIDE POLYA TAIL. THE OPTV-LUCNEO PLASMID WAS TRANSFECTED INTO HEK293 CELLS USING LIPOFECTAMINE 3000 AND ANALYZED AT 18, 24, AND 48 HOURS POST-TRANSFECTION BY FLUORESCENCE MICROSCOPY AND ACTIVITY ASSAYS. FEMALE BALB/C MICE WERE IMMUNIZED WITH 50 OR 100 µG OF OPTV/LUCNEO VIA INTRAMUSCULAR (IM), INTRADERMAL (ID), SUBCUTANEOUS (SC), OR INTRAPERITONEAL (IP) ROUTES. IN VIVO EXPRESSION WAS EVALUATED USING BIOLUMINESCENCE. AN ELISA WAS PERFORMED TO MEASURE ANTI-LUCIFERASE IGG ANTIBODIES, WHICH WERE CONFIRMED BY WESTERN BLOT. MNEONGREEN EXPRESSION WAS DETECTED AS EARLY AS 18 HOURS POST-TRANSFECTION, AND LUCIFERASE EXPRESSION WAS ALSO OBSERVED AT 18 HOURS ($P < 0.0001$). MICE IMMUNIZED WITH 100 µG OF OPTV-LUCNEO EXHIBITED BIOLUMINESCENT FOCI AT THE INJECTION SITE AFTER 24 HOURS. ALL INJECTION ROUTES (IP, IM, SC, ID) SHOWED LUMINESCENT SPOTS.

MICE IMMUNIZED WITH 50 µG OF OPTV-LUCNEO VIA IM (P<0.0001) AND IP (P<0.0115) DEVELOPED SIGNIFICANT LEVELS OF ANTI-LUCIFERASE ANTIBODIES. THIS STUDY NOT ONLY EXPLORES THE OPTIMAL CONDITIONS FOR DNA VACCINE ADMINISTRATION BUT ALSO DEMONSTRATES THAT THE VALIDATED PLASMID COULD SERVE AS AN EFFECTIVE PLATFORM FOR DEVELOPING VACCINES TARGETING CHAGAS DISEASE.

MODULATORY EFFECTS OF MELATONIN ON PYROPTOSIS, MITOCHONDRIAL FUNCTION, AND INFLAMMATION IN HUMAN GASTRIC CANCER

SABRINA AZEVEDO MACHADO; JÚLIA PERIN MANCHINE; HELOÍSA ANTONIELLA BRAZ DE MELO; SARAH PINHO BEZERRA; GABRIEL PASQUARELLI-DO-NASCIMENTO; MILENA VERDAM NASCIMENTO; PAULA MARIA QUAGLIO BELLOZI; ANA LUÍSA GOUVÊA DA SILVA, BRUNO MILHOMEM PILATI RODRIGUÊS; ANDREZA FABRO DE BEM; SONIA NAIR BÁO; DARIO SIMÕES ZAMBONI; KELLY GRACE MAGALHÃES

INTRODUCTION: MELATONIN IS A HORMONE WITH NUMEROUS BIOLOGICAL ACTIVITIES. IT IS MAINLY PRODUCED BY THE PINEAL GLAND IN RESPONSE TO DARKNESS. THERE IS AN INCREASING FOCUS ON MELATONIN IN THE FIELD OF ONCOLOGY SINCE IT CAN MODULATE SEVERAL TUMORAL PARAMETERS. HOWEVER, THE ROLE OF MELATONIN IN HUMAN GASTRIC CANCER IS POORLY UNDERSTOOD. THEREFORE, THIS WORK AIMED TO ANALYZE THE ROLE OF MELATONIN IN THE MODULATION OF CARCINOGENIC PARAMETERS, INFLAMMATION, MITOCHONDRIAL FUNCTION, AND OXIDATIVE STRESS IN HUMAN GASTRIC CANCER CELLS. METHODS: AGS CELLS WERE STIMULATED WITH MELATONIN AT CONCENTRATIONS OF 0.625, 2.5, AND 5 MM AT DIFFERENT TIMES. MITOCHONDRIAL VIABILITY AND FUNCTION WERE ASSESSED BY MTT ASSAY, AND HIGH-RESOLUTION RESPIROMETRY, RESPECTIVELY. MEMBRANE PORE FORMATION, LACTATE DEHYDROGENASE (LDH) RELEASE WERE ANALYZED BY SPECTROPHOTOMETRY. CELL DEATH PROFILE, CELL PROLIFERATION, CELL CYCLE AND OXIDATIVE STRESS WERE ASSESSED BY FLOW CYTOMETRY. CASPASE-1 EXPRESSION AND ACTIVITY WAS ASSESSED BY WESTERN BLOT AND CONFOCAL MICROSCOPY, RESPECTIVELY. HMGB1 TRANSLOCATION WAS ASSESSED BY CONFOCAL MICROSCOPY. CYTOKINE LEVELS WERE EVALUATED BY ELISA RESULTS: BOTH MELATONIN AT 2.5 AND 5 MM PROMOTED A REDUCTION IN MITOCHONDRIAL VIABILITY, CELL PROLIFERATION, OXIDATIVE RESPIRATION, AND ROS PRODUCTION. IN ADDITION, THESE CONCENTRATIONS SIGNIFICANTLY INCREASED APOPTOTIC DEATH COMPARED TO UNSTIMULATED CELLS. MOREOVER, MELATONIN WAS ABLE TO REDUCE KEY PARAMETER RELATED TO PYROPTOSIS SUCH AS CASPASE-1 EXPRESSION AND ACTIVITY, HMGB1 TRANSLOCATION, LDH RELEASE, AND MEMBRANE PORE FORMATION. CONCLUSION: OVERALL, OUR DATA INDICATE THAT AT HIGHER CONCENTRATIONS, MELATONIN EXERTS AN ANTITUMOR EFFECT IN AGS GASTRIC CANCER CELLS BY REDUCING

MITOCHONDRIAL VIABILITY, INCREASING CELL DEATH, AND DECREASING OXIDATIVE PHOSPHORYLATION. NOTABLY, OUR FINDINGS SUGGEST THAT MELATONIN CAN INHIBIT PYROPTOSIS IN GASTRIC CANCER CELLS, WHICH MAY BE PIVOTAL FOR ITS DEVELOPMENT IN THERAPEUTIC APPLICATIONS.

ADMINISTRATION OF DNA AND MRNA ENCODING FRAGMENTS OF ANTIBODIES AGAINST FLAVIVIRUS

LUCAS SILVA RODRIGUES, RENATO KAYLAN FRANÇA, ANA CLARA ANTONELLI, ANDREA QUEIROZ MARANHÃO E MARCELO DE MACEDO BRIGIDO

ZIKA AND DENGUE ARE EMERGING DISEASES AND A PUBLIC HEALTH CONCERN. BOTH ARE VIRAL DISEASES PROVOKED BY SSRNA-POSITIVE VIRUSES OF THE FAMILY FLAVIVIRIDAE. THE DENGUE VIRUS (DENV) HAS BEEN PRESENT IN BRAZIL SINCE THE 80S. ZIKA VIRUS (ZIKV) EMERGED IN BRAZIL IN 2015 AFTER AN OUTBREAK OF THE DISEASE OCCURRED IN THE STATE OF BAHIA. RECENTLY, AN ANTI-DENGUE VACCINE WAS APPROVED BY ANVISA IN BRAZIL, ALTHOUGH THERE IS NO TREATMENT AGAINST THE ZIKA VIRUS. MONOCLONAL ANTIBODIES ARE A POWERFUL TOOL IN TREATING MANY DISEASES, SUCH AS CANCERS, AUTOIMMUNE AND INFECTIOUS DISEASES. A FEW VIRAL DISEASES ALREADY HAD A MAB APPROVED FOR TREATMENT, SUCH AS EBOLA, RSV, AND SARS-COV-2. HOWEVER, THERE IS DIFFICULTY IN DEVELOPING THERAPEUTIC MEASURES SPECIFICALLY AGAINST FLAVIVIRUS DUE TO ADE (ANTIBODY-DEPENDENT ENHANCEMENT) ASSOCIATED WITH THE FC PORTION OF ANTIBODIES. MOREOVER, DESPITE THE GREAT POTENTIAL FOR TREATING VARIOUS DISEASES, THE TECHNOLOGY FOR PRODUCING MONOCLONAL ANTIBODIES IS EXPENSIVE, WHICH HAMPERS ITS BROADER USE. PART OF THIS COST IS THE NEED TO PRODUCE ANTIBODIES IN MAMMALIAN CELL CULTURE. ALTERNATIVELY TO ADMINISTERING ANTIBODIES, THERAPIES BASED ON GENE DELIVERY THROUGH MRNA OR DNA MAY BECOME A VIABLE OPTION. IT IS POSSIBLE TO OBTAIN THE EXPRESSION OF RECOMBINANT FC-MODIFIED ANTIBODIES IN VIVO THROUGH GENE DELIVERY. THEREFORE, IN THIS WORK, WE INTEND TO ADMINISTER MRNA ENCAPSULATED IN LIPID NANOPARTICLES OR NAKED DNA TO MICE, EVALUATE THE EXPRESSION OF THESE ANTIBODIES IN VIVO, AND TEST THEIR POTENTIAL PROTECTION CHALLENGING ANIMALS FROM ZIKV. INITIALLY, THE ANTIBODY CODING GENES IN DIFFERENT ANTIBODY FRAGMENT FORMATS WERE CLONED INTO TWO EXPRESSION VECTORS AND TRANSFECTED INTO THE EXPI293 CELL LINE. THE GFP REPORTER GENE EVALUATED THE TRANSFECTION EFFICIENCY. THE ANTIBODIES WERE PURIFIED TO PROTEIN-A AFFINITY, AND BINDING TO THE ANTIGEN WAS TESTED BY ELISA. NEXT, THE DIFFERENT FORMATS OF THE ANTIBODY WILL BE ANALYZED IN TERMS OF BINDING TO VIRAL PARTICLES AND NEUTRALIZATION TESTS BY PRNT (PLAQUE REDUCTION NEUTRALIZATION TEST). THE BEST ANTIBODY FORMAT WILL BE USED IN SUBSEQUENT TESTS.

THE ROLE OF MELATONIN IN MODULATING CARCINOGENIC PARAMETERS AND PYROPTOTIC DEATH IN HUMAN PANCREATIC ADENOCARCINOMA CELLS

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PANCREATIC ADENOCARCINOMA IS THE MOST COMMON MALIGNANT NEOPLASM OF THE PANCREAS AND REPRESENTS ONE OF THE MOST LETHAL FORMS OF CANCER IN THE WORLD, WITH NO EFFECTIVE TREATMENT OPTIONS FOR ADVANCED STAGES. THESE LIMITATIONS HAVE MOTIVATED THE RESEARCH FOR ALTERNATIVE THERAPIES, SUCH AS USE OF SUPPLEMENTS AND NATURAL COMPOUNDS. IN THIS CONTEXT, MELATONIN HAS EMERGED AS A PROMISING ADJUVANT THERAPY, OFFERING THE DUAL BENEFIT OF MITIGATING THE SIDE EFFECTS OF CHEMOTHERAPY AND RADIOTHERAPY WHILE EXHIBITING ANTIPROLIFERATIVE PROPERTIES IN VARIOUS TYPES OF CANCER. HOWEVER, THERE IS A SIGNIFICANT GAP IN KNOWLEDGE ABOUT THE ROLE OF MELATONIN IN CELLULAR PROCESSES IN PANCREATIC CANCER CELLS. THEREFORE, THIS STUDY AIMS TO INVESTIGATE THE EFFECTS OF MELATONIN ON THE MODULATION OF CARCINOGENIC PARAMETERS IN HUMAN PANCREATIC ADENOCARCINOMA CELLS (PANC-1) IN VITRO. PANC-1 CELLS WERE STIMULATED WITH DIFFERENT CONCENTRATIONS OF MELATONIN (0.625MM, 1.25MM, 2.5MM, 3.75MM AND 5MM) FOR DIFFERENT PERIODS. CARCINOGENIC PARAMETERS SUCH AS VIABILITY AND CELL CYCLE, NUCLEAR FRAGMENTATION, PROLIFERATION AND LIPID DROPLETS BIOGENESIS WERE EVALUATED. ANALYSES TO CHARACTERIZE CELL DEATH PROFILE WERE ALSO CARRIED OUT, SUCH AS THE MEMBRANE PORE FORMATION, RELEASE OF THE ENZYME LACTATE DEHYDROGENASE (LDH), ACTIVATION OF CASPASE-1, CYTOKINE SECRETION PROFILE, LYSOSOMAL ACIDIFICATION AND GENERATION OF REACTIVE SPECIES (ROS). OUR RESULTS REVEALED THAT MELATONIN INDUCED CYTOTOXICITY IN A DOSE-AND TIME-DEPENDENT MANNER AND ARRESTING THE CELL CYCLE AT THE G1/G0 PHASE, REDUCING CELL PROLIFERATION AND BIOGENESIS OF LIPIDS DROPLETS. CELL DEATH BY PYROPTOSIS WAS ALSO OBSERVED, EVIDENCED BY REDUCED PLASMA MEMBRANE INTEGRITY, LDH RELEASE AND CASPASE-1 ACTIVATION, AS WELL AS AN INCREASE IN ROS PRODUCTION AND LYSOSOMAL DAMAGE. THIS STUDY HIGHLIGHTS THE POTENTIAL ANTITUMOR EFFECT OF MELATONIN IN VITRO, UNVEILING NEW PERSPECTIVES FOR ITS USE AS AN ADJUVANT IN THE TREATMENT OF PANCREATIC CANCER.

OPTIMIZED DNA VACCINE OF THE 80 KDA PROLYL OLIGOPEPTIDASE OF T. CRUZI MODULATES THE IMMUNE RESPONSE AND DECREASES PARASITEMIA IN A MURINE MODEL

ALEXANDRA MARIA DOS SANTOS CARVALHO; DANIELA FRANCO ROSA; ANDREY DUARTE BOAVA; FELIPE DA SILVA MENDONÇA DE MELO; JULIA LISBOA BESERRA E SILVA; FRÉDÉRIC FÉRCOQ; SÉBASTIEN OLIVIER CHARNEAU; KAUÃ DA SILVA SANTOS; MARIA LUISA SAUNDERS DE FARIAS VIANA; LARISSA YONAHA PEREIRA; RAQUEL NOGUEIRA GUIMARÃES; AISSA LANA OLIVEIRA LIMA; FLÁVIA NADER MOTTA; PHILIPPE GRELLIER; JOÃO PAULO FIGUEIRÓ LONGO; CARLA ARAÚJO NUNES; CORALIE MARTIN; JAIME MARTINS DE SANTANA; IZABELA MARQUES DOURADO BASTOS

CONTROLLING CHAGAS DISEASE IS DIFFICULT DUE TO THE LIMITED EFFECTIVENESS OF CURRENT DRUGS. IT IS THEREFORE ESSENTIAL TO DEVELOP NEW STRATEGIES SUCH AS VACCINES. THEREFORE, THE AIM OF THIS WORK IS TO DEVELOP A PROPHYLACTIC VACCINE WITH THE DNA OF THE 80 KDA PROLYL OLIGOPEPTIDASE PROTEIN FROM T. CRUZI (POPTC80). CLONING OF THE POPTC80 GENE INTO THE OPTIMIZED OPTV VECTOR (PCDNA3.1+ WITH THE ADDITION OF 5' AND 3' UTR'S AND A 100 NUCLEOTIDE POLY-A TAIL); IMMUNIZATION OF BALB/C MICE WITH 25, 50 OR 100 µG; LYMPHOPROLIFERATION ASSAY; ELISA; CYTOKINE ANALYSIS AND HISTOLOGICAL ANALYSIS. IMMUNIZATION WITH OPTV-POPTC80 WAS ABLE TO GENERATE CD4+ AND CD8+ T CELLS SPECIFIC AGAINST POPTC80 ($P < 0.0001$) AND STIMULATED THE PRODUCTION OF ANTI-POPTC80 IGG ANTIBODIES ($P < 0.001$). THE CHALLENGE WITH TRYPOMASTIGOTES SHOWED THAT THE OPTV-POPTC80 VACCINE REDUCED PARASITEMIA ($P < 0.001$) IN MICE CHALLENGED WITH TRYPOMASTIGOTES ON THE 14TH AND 19TH DAY AFTER INFECTION ($P < 0.0001$), BUT DID NOT REDUCE IT DURING THE CHRONIC PHASE OF INFECTION. IN ADDITION, DURING THE ACUTE PHASE IMMUNIZED MICE SHOWED HIGHER LEVELS OF IL10 ($P < 0.0134$) AND LOWER LEVELS OF TNF-A ($P = 0.0020$), THERE WERE NO CHANGES IN RELATION TO IL-17A AND INF-Γ. HISTOLOGICAL ANALYSIS SHOWED NO SIGNIFICANT CHANGES. THE RESULTS SUGGEST THAT DNA IMMUNIZATION IS A PROMISING PLATFORM FOR THE SEARCH FOR NEW TARGETS FOR VACCINE DEVELOPMENT AND THAT IT IS NECESSARY TO RESEARCH OPTIMIZATION METHODS THAT PROVIDE THE VECTOR WITH GREATER IMMUNOGENICITY AND STABILITY.

EXPLORING ZYMOMONAS MOBILIS AS A PROBIOTIC MICROORGANISM FOR ANTI-CD3 DELIVERY IN INFLAMMATORY BOWEL DISEASE

RENATO RODRIGUES OLIVEIRA; MANUELA MARAGNO DO ALMO; LIDIA MARIA PEPE DE MORAES; ANDRÉA QUEIROZ MARANHÃO; MARCELO DE MACEDO BRIGIDO

THE ADMINISTRATION OF PROBIOTIC MICROORGANISMS CAN BENEFIT THE INTESTINE BY ASSISTING THE MAINTENANCE OF INDIVIDUALS MICROBIOTA. THE GRAM-NEGATIVE BACTERIUM ZYMOMONAS MOBILIS SHOWS PROBIOTIC ACTIVITY WITH THERAPEUTIC POTENTIAL. IN BRAZIL, STRAINS AG11 AND CP1 OF THE BACTERIUM Z. MOBILIS HAVE BEEN USED IN TREATING INTESTINAL DISORDERS, PARTICULARLY CHRONIC COLITIS. IN THIS CONTEXT, THE MOLECULAR IMMUNOLOGY LABORATORY AT UNIVERSITY OF BRASÍLIA UTILIZED Z. MOBILIS TO PRODUCE AND EXPRESS ANTI-CD3 ANTIBODIES ON THE BACTERIAL MEMBRANE. OUR GOAL WAS TO EVALUATE THE EFFICACY OF THIS MICROORGANISM IN TREATING ULCERATIVE COLITIS IN A MURINE MODEL. TO ACHIEVE MORE EFFECTIVE TREATMENT RESPONSE, WE REDESIGN A NEW EXPRESSION VECTOR CAPABLE OF REGULATING THE PRODUCTION OF THE TARGET PROTEIN (ANTI-CD3 SCFV) USING THE REVERSE TETRACYCLINE REPRESSOR (REVTETR). A CONSTRUCT CONTAINING EGFP WAS ALSO MADE TO FACILITATE DETECTION TESTS IN HETEROLOGOUS EXPRESSION. TO VERIFY THE FUNCTIONALITY OF THE PLASMID CONTAINING THE SYNTHETIC OPERON WITH EGFP, FLUORESCENCE SPECTROPHOTOMETRY ASSAYS WERE PERFORMED ON Z. MOBILIS TRANSFORMED WITH THE NEW VECTOR, USING A TETRACYCLINE CONCENTRATION GRADIENT. THIS GENE REGULATION SYSTEM PROVED VIABLE AND FUNCTIONAL, AND CAN BE NOW USED TO CONTROL SCFV EXPRESSION FOR IN VIVO USE. THE OTHER PLASMIDS CONTAINING THE SYNTHETIC OPERON FOR ANTI-CD3 SCFV ARE CURRENTLY BEING TESTED AND CHARACTERIZED. THE STUDY DEMONSTRATED THAT THE GENE REGULATION SYSTEM EMPLOYED WAS VIABLE AND FUNCTIONAL, AND CAN BE NOW USED TO CONTROL SCFV EXPRESSION FOR IN VIVO USE. CONSIDERING THE POTENTIAL THERAPEUTIC USE OF PROBIOTIC MICROORGANISMS LIKE Z. MOBILIS TO DELIVER ANTI-CD3 DIRECTLY TO THE INTESTINAL MUCOSA, WHEREAS THAT AREA OF RESEARCH REMAINS LARGELY UNEXPLORED IN THIS MICROORGANISM, THIS WORK COULD CONTRIBUTE VALUABLE INSIGHTS FOR FUTURE RESEARCH EXPLORING MONOCLONAL ANTIBODY PRODUCTION IN THIS MICROORGANISMS.

VACCINE PLATFORM CARRYING THE THIMET OLIGOPEPTIDASE GENE AGAINST TRYPANOSOMA CRUZI

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CHAGAS DISEASE IS A NEGLECTED TROPICAL DISEASE CAUSED BY THE FLAGELLATE PROTOZOAN T. CRUZI, WHICH IS POTENTIALLY FATAL AND ENDEMIC IN AT LEAST 21 LATIN AMERICAN COUNTRIES. IT IS PRIMARILY TRANSMITTED BY A VECTOR, A TRIATOMINE INSECT KNOWN AS THE

"BARBEIRO." THE AVAILABLE DRUGS FOR TREATMENT HAVE LIMITATIONS AND ADVERSE EFFECTS. THUS, PROPHYLACTIC AND/OR THERAPEUTIC VACCINES ARE EMERGING AS ALTERNATIVE CONTROL STRATEGIES.WE PROPOSE THE DEVELOPMENT OF AN ORAL VACCINE PLATFORM USING THE BACTERIUM SALMONELLA SL7207 AS A VECTOR FOR A MODIFIED PLASMID, WITH ANOTHER VERSION OF THE VACCINE DELIVERED INTRAMUSCULARLY. INITIALLY, THE OPTIMIZED VECTOR OPTV-LUCNEON (PCDNA3.1+ WITH THE ADDITION OF 5' AND 3' UTRS AND A 100-NUCLEOTIDE POLY-A TAIL), PURCHASED COMMERCIALLY, WAS DIGESTED WITH THE HINDIII AND XHOI ENZYMES. THE THIMET OLIGOPEPTIDASE (TOP) GENES FROM T. CRUZI WERE AMPLIFIED BY PCR AND CLONED USING T4 LIGASE.FOR THE ORAL FORMULATION, ELECTROCOMPETENCE WAS ACHIEVED USING A 10 MM HEPES AND 10% GLYCEROL PROTOCOL. THE PLASMID WAS INCORPORATED BY ELECTROPORATION, USING 40 µL OF CELLS WITH 2 µL OF PLASMID, FOLLOWED BY GROWTH ON LB AGAR MEDIUM CONTAINING AMPICILLIN FOR SELECTION, AND INCUBATION FOR 18 HOURS AT 37°C. COLONIES WERE ANALYZED BY PCR TO CONFIRM TRANSFORMATION. OF THE THREE TOP COLONIES, ONE WAS CONFIRMED POSITIVE BY COLONY PCR. OF 19 LUCNEON COLONIES, 11 WERE POSITIVE.FOR THE INTRAMUSCULAR FORMULATION, WE EXTRACTED THE PLASMID (MAXIPREP) THAT HAD BEEN PREVIOUSLY CLONED IN E. COLI, AND THE ANIMALS RECEIVED THREE DOSES OF 25 µG AT 14-DAY INTERVALS.THE NEXT STEPS WILL INVOLVE THE DEFINITIVE PRODUCTION OF THE ORAL VACCINE FORMULATION AND BIOLUMINESCENCE ANALYSIS OF THE VACCINATED ANIMALS. THIS VACCINE PLATFORM COULD REPRESENT A SIGNIFICANT ADVANCEMENT IN THE FIGHT AGAINST CHAGAS DISEASE, OFFERING A VIABLE ALTERNATIVE TO CURRENT TREATMENTS AND CONTRIBUTING TO THE CONTROL AND ERADICATION OF THE DISEASE IN ENDEMIC REGIONS.

THE IMPACT OF SINGLE-CHAIN VARIABLE FRAGMENT (SCFV) AGGREGATION ON CHIMERIC ANTIGEN RECEPTOR FUNCTIONALITY

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MULTIPLE MYELOMA (MM) IS A TYPE OF HEMATOLOGICAL CANCER CHARACTERIZED BY THE OVEREXPRESSION OF B-CELL MATURATION ANTIGEN (BCMA) ON PLASMA CELLS. DESPITE ADVANCEMENTS IN TREATMENT, MM REMAINS INCURABLE. IMMUNOTHERAPY USING T CELLS ENGINEERED WITH CHIMERIC ANTIGEN RECEPTORS (CAR-T) REPRESENTS AN IMPORTANT PARADIGM SHIFT AGAINST MM. HOWEVER, CAR-T CELL EXHAUSTION SIGNIFICANTLY LIMITS THE EFFICACY OF THIS APPROACH. FURTHERMORE, OLIGOMERIZATION OF SINGLE-CHAIN VARIABLE FRAGMENTS (SCFV) IN THE EXTRACELLULAR PORTION OF CAR CONTRIBUTES TO T CELL

EXHAUSTION AND REDUCTION OF ANTI-TUMOR ACTIVITY. THEREFORE, THIS WORK AIMS TO DESIGN NOVEL ANTI-BCMA SCFVS, CONSIDERING THEIR HUMANIZATION AND AGGREGATION POTENTIAL, AND TO ANALYZE THEIR BIOPHYSICAL AND BIOLOGICAL FEATURES. A MURINE ANTI-BCMA SCFV WAS RETRIEVED FROM THE LITERATURE, AND TWO HUMANIZED VERSIONS WERE CONSTRUCTED. POTENTIAL AGGREGATION WAS ANALYZED IN SILICO, RESULTING IN THREE SCFVS WITH INCREASING DEGREES OF PREDICTED AGGREGATION: HARMONIZED, HUMANIZED 1 AND HUMANIZED 2. THE ANTIBODIES WERE EXPRESSED IN A SOLUBLE SCFV FORMAT IN ESCHERICHIA COLI AND PURIFIED BY NICKEL AFFINITY CHROMATOGRAPHY. THE SDS-PAGE AND WESTERN BLOTTING CONFIRMED ANTIBODY EXPRESSION IN TRANSFORMED BACTERIA FOLLOWING IPTG INDUCTION, WITH A TIME-DEPENDENT ACCUMULATION. MOLECULAR EXCLUSION ANALYSIS SUPPORTED THE PREDICTED AGGREGATION PROFILES, AS THE HARMONIZED SCFV EXHIBITED UNIQUE ELUTION PATTERNS COMPARED TO THE OTHER CONSTRUCTS. FURTHERMORE, PRELIMINARY FLOW CYTOMETRY DEMONSTRATED THAT ALL SCFVS ARE CAPABLE OF BINDING TO BCMA EXPRESSED ON MAMMALIAN CELLS. FURTHER CHARACTERIZATION OF THE RECOMBINANT PROTEINS WILL BE CONDUCTED USING BIOPHYSICAL TECHNIQUES, SUCH AS DYNAMIC LIGHT SCATTERING. IN CONCLUSION, THESE FINDINGS ADVANCE OUR UNDERSTANDING OF ANTIBODY AGGREGATION BY INVESTIGATING NOVEL SCFVS TARGETING BCMA, AND HOLD SIGNIFICANT POTENTIAL FOR IMPROVING THE DESIGN AND EFFICACY OF CAR-T CELL-BASED IMMUNOTHERAPIES.

DEVELOPMENT OF ANTI-CD19 CAR MOLECULES WITH SPACER REGIONS DERIVED FROM HUMAN IGG4

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IMMUNOTHERAPIES HAVE BEEN ESTABLISHED AS AN EFFECTIVE STRATEGY IN THE TREATMENT OF VARIOUS TYPES OF CANCER. AMONG THESE APPROACHES, T CELL THERAPY USING CHIMERIC ANTIGEN RECEPTORS (CAR-T) THERAPY STANDS OUT FOR ITS ABILITY TO GENETICALLY MODIFY T LYMPHOCYTES, ENABLING THEM TO RECOGNIZE AND ELIMINATE CELLS THAT EXPRESS SPECIFIC ANTIGENS. OF THE SIX PRODUCTS APPROVED FOR CLINICAL USE, FOUR TARGET THE CD19 ANTIGEN. THE CAR USED IN THESE PRODUCTS FEATURES A 2ND GENERATION RECEPTOR STRUCTURE, CONSISTING OF A SCFV FRAGMENT FUSED TO A SPACER REGION (HINGE), A TRANSMEMBRANE DOMAIN, AN INTRACELLULAR CO-STIMULATORY ACTIVATOR DOMAIN, AND A CD3Z SIGNALING DOMAIN. THE DESIGN OF THE CAR STRUCTURE PLAYS A CRITICAL ROLE IN DETERMINING THE EFFECTIVENESS OF T CELLS. IN PARTICULAR, THE SPACER REGIONS CAN AFFECT THE ANTITUMOR ACTIVITY. THIS STUDY PROPOSES TO INVESTIGATE THE IMPACT OF DIFFERENT HINGES

DERIVED FROM HUMAN IGG4 ON NOVEL ANTI-CD19 CAR VARIANTS. WE DESIGNED AND CLONED THE GENES FOR FOUR DIFFERENT CAR CONSTRUCTS, EACH CONTAINING VARIATIONS OF THE IGG4 HINGE, INTO THE PT4 EXPRESSION VECTOR. THIS VECTOR WILL DELIVER THE CAR TRANSGENE TO CELLS USING A TRANSPOSON-BASED SYSTEM. THESE VECTORS WILL THEN BE USED FOR TRANSFECTION VIA ELECTROPORATION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS TO EVALUATE THE ANTITUMOR ACTIVITY OF CAR+ CELLS FOR EACH CONSTRUCT. THE ACTIVATION AND EXHAUSTION PROFILES OF THE CAR-T+ CELLS WILL BE ASSESSED USING FLOW CYTOMETRY. ADDITIONALLY, THE CYTOTOXIC ACTIVITY WILL BE MEASURED THROUGH CELL LYSIS ASSAYS THAT INVOLVE CO-CULTURING CAR-T CELLS WITH TARGET ANTIGEN-EXPRESSING CELL LINES. BY TESTING THE HYPOTHESIS THAT MODIFICATIONS IN THE SPACER REGION OF THE EXTRACELLULAR PORTION OF THE CAR CAN MODULATE THE PERFORMANCE OF CAR-T CELLS, THIS STUDY MAY CONTRIBUTE TO THE UNDERSTANDING OF THE IMPORTANCE OF THE HINGE, CONSIDERING ASPECTS SUCH AS FLEXIBILITY AND THE CAPACITY TO INTERACT WITH THE TARGET ANTIGEN, CONTRIBUTING TO THE DEVELOPMENT OF INNOVATIVE CARS.

NLRP3 INFLAMMASOME ACTIVATION-DEPENDENT PYROPTOSIS EXERTS POTENTIAL ANTITUMOR EFFECT ON HUMAN GASTRIC CANCER CELLS

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INTRODUCTION: PYROPTOSIS, A TYPE OF PROGRAMMED LYTIC CELL DEATH, WAS INITIALLY DISCOVERED IN IMMUNE CELLS BUT HAS GAINED SIGNIFICANT ATTENTION IN ONCOLOGY. IN TUMOR CELLS, CELL DEATH PATHWAYS ARE OFTEN INHIBITED AS AN EVASION MECHANISM. HOWEVER, WHEN PYROPTOSIS IS INDUCED IN CANCER CELLS, IT CAN HAVE DUAL EFFECTS ON TUMOR PROGRESSION, DEPENDING ON THE TUMOR TYPE, REQUIRING DEDICATED STUDIES FOR EACH TYPE. GASTRIC CANCER IS THE FOURTH MOST LETHAL CANCER, WITH LIMITED EFFECTIVE TREATMENTS FOR ADVANCED STAGES, DEMANDING THE EXPLORATION OF NEW THERAPEUTIC APPROACHES. THUS, THIS STUDY AIMS TO ELUCIDATE THE ROLE OF NLRP3 INFLAMMASOME ACTIVATION-DEPENDENT PYROPTOSIS ON THE MODULATION OF TUMORAL PARAMETERS IN HUMAN GASTRIC CANCER CELLS. METHODS: AGS CELLS WERE STIMULATED WITH LIPOPOLYSACCHARIDE (LPS) (1 µG/ML) FOR 24 HOURS AND NIGERICIN (20µM) FOR 2 HOURS, FOLLOWED BY REPLACEMENT WITH CULTURE MEDIUM (SHORT STIMULUS - SS) OR CONTINUOUS STIMULATION (LONG STIMULUS - LS) THROUGHOUT THE ANALYSIS PERIOD. MITOCHONDRIAL CELL VIABILITY, LACTATE DEHYDROGENASE (LDH) RELEASE AND MEMBRANE PORE FORMATION WERE ANALYZED BY SPECTROPHOTOMETRY. CELL DEATH PROFILE, CELL PROLIFERATION CELL CYCLE AND NUCLEAR FRAGMENTATION WERE

INVESTIGATED VIA FLOW CYTOMETRY. RESULTS: IT WAS OBSERVED THAT BOTH SS AND LS REDUCED CELL VIABILITY IN AGS CELLS AND INCREASED LYTIC CELL DEATH, WITH THE EFFECT BEING MORE PROMINENT IN LS. BOTH STIMULI REDUCED AGS CELL PROLIFERATION, WHICH WAS INTENSIFIED IN SS. MOREOVER, MEMBRANE PORE FORMATION WAS INDUCED BY BOTH STIMULI BUT WAS MORE NOTABLE IN LS, WHICH ALSO SHOWED AUGMENTED LDH RELEASE IN AGS CELLS. CONCLUSION: OUR DATA DEMONSTRATED THAT THE INDUCTION OF PYROPTOSIS HAD AN ANTITUMOR EFFECT ON VARIOUS TUMORAL PARAMETERS, INCLUDING REDUCED PROLIFERATION AND CELL VIABILITY, INCREASED LYTIC CELL DEATH AND LDH RELEASE, AND INDUCED PORE FORMATION IN HUMAN GASTRIC CANCER CELLS. THESE FINDINGS PROVIDE NOVEL INSIGHTS INTO POTENTIAL THERAPEUTIC STRATEGIES AGAINST GASTRIC CANCER.

AVALIAÇÃO DE POTENCIAIS MECANISMOS PARA EFEITOS ADVERSOS DE ESTATINAS EM CÉLULAS MICROGLIAIS.

GABRIEL FELIPE GOMES CALIXTO, JÁDER ROMÉRO FERNANDES CARDOSO, KELLY GRACE MAGALHÃES

CARDIOVASCULAR DISEASES WERE RESPONSIBLE FOR 18.5 MILLION DEATHS IN 2019, MAKING THEM THE LEADING CAUSE OF MORTALITY GLOBALLY. STATINS ARE THE PRIMARY CHOICE FOR THE PREVENTION OF CARDIOVASCULAR EVENTS, WITH THE NUMBER OF USERS REACHING 145 MILLION IN 2018. HOWEVER, THEIR USE IS ASSOCIATED WITH NUMEROUS ADVERSE EFFECTS. MUSCLE SYMPTOMS ARE THE MOST COMMON, BUT CENTRAL NERVOUS SYSTEM (CNS)-RELATED EFFECTS, SUCH AS MOOD ALTERATIONS, COGNITIVE CHANGES, AND HEADACHES, HAVE ALSO BEEN REPORTED BY PATIENTS. THE MOLECULAR MECHANISMS UNDERLYING THESE CNS-RELATED EFFECTS REMAIN UNCLEAR, HIGHLIGHTING THE IMPORTANCE OF FURTHER INVESTIGATION. IMMORTALIZED HUMAN MICROGLIAL CELLS WERE USED FOR THE STUDY. SIMVASTATIN, ROSUVASTATIN, AND PITAVASTATIN WERE EMPLOYED AS TREATMENTS. MITOCHONDRIAL VIABILITY WAS ASSESSED USING THE MTT ASSAY. CYTOTOXICITY WAS MEASURED THROUGH THE LDH ASSAY. OXIDATIVE STRESS WAS EVALUATED USING DCFDA, CELLROX DEEP RED, AND GREEN PROBES. THE INFLAMMATORY PROFILE WAS ANALYZED VIA SANDWICH ELISA. THE TYPE OF CELL DEATH WAS DETERMINED USING ANNEXIN V AND PROPIDIUM IODIDE STAINING. NORMALLY DISTRIBUTED DATA WERE ANALYZED USING ONE-WAY ANOVA OR TWO-WAY ANOVA IN GRAPHPAD PRISM SOFTWARE (VERSION 8). LIPOPHILIC STATINS SHOWED A GREATER REDUCTION IN VIABILITY COMPARED TO THE HYDROPHILIC ROSUVASTATIN. NO SIGNIFICANT DIFFERENCES WERE OBSERVED IN THE GENERATION OF REACTIVE SPECIES AT EARLY OR LATE TIME POINTS. THE DATA SUGGEST THE OCCURRENCE OF LYTIC CELL DEATH AND, PREDOMINANTLY, APOPTOTIC CELL DEATH. A MORE PRO-INFLAMMATORY

PROFILE WAS OBSERVED WITH LIPOPHILIC STATINS COMPARED TO THE HYDROPHILIC ONE. UNLIKE ROSUVASTATIN, LIPOPHILIC STATINS CAN CROSS CELLULAR MEMBRANES WITHOUT RELYING SOLELY ON MEMBRANE TRANSPORTERS. THIS MAY LEAD TO INCREASED AVAILABILITY, WHICH COULD HELP EXPLAIN THE OBSERVED HIGHER TOXICITY IN THE DATA AND WHY LIPOPHILIC STATINS ARE MORE PRONE TO CAUSING SIDE EFFECTS RELATED TO THE CENTRAL NERVOUS SYSTEM.

IN VIVO EVALUATION OF THE IMMUNOMODULATORY POTENTIAL OF BETA-GLUCANS IN A MURINE MODEL OF SEPSIS-LIKE INFECTION

JESSE PEREIRA MACHADO VIANA, ANAMELIA LORENZETTI BOCCA, MARCIA CRISTINA GONÇALVES MACIEL B-GLUCANS ARE NATURAL POLYSACCHARIDES FOUND IN THE CELL WALLS OF FUNGI, YEASTS, CEREALS, AND ALGAE, RECOGNIZED FOR THEIR IMMUNOMODULATORY PROPERTIES AND USE IN INFECTION TREATMENT (MURPHY ET AL., 2020). IN THE SEPTIC CONTEXT, THEY ACT AS IMMUNE MODULATORS, SHOWING THERAPEUTIC POTENTIAL FOR INFECTION MANAGEMENT (MASTERSON ET AL., 2020; HARRIET ET AL., 2022). THIS STUDY AIMED TO INVESTIGATE THE IMMUNOMODULATORY POTENTIAL OF A FUNGAL-DERIVED B-GLUCAN IN A MURINE MODEL OF SYSTEMIC INFECTION. SPECIFICALLY, WE ASSESSED THE TREATMENT'S EFFECTS ON THE CYTOKINE PROFILE (IL-6, IL-10, MCP-1, IFN-Γ, TNF-A, AND IL-12), PERIPHERAL BLOOD CELLULARITY, CELLULARITY OF PRIMARY AND SECONDARY LYMPHOID ORGANS, BACTERIAL DISSEMINATION BY QUANTIFYING COLONY-FORMING UNITS (CFU), AND LUNG HISTOPATHOLOGICAL ANALYSIS. THE RESULTS INDICATE THAT B-GLUCAN TREATMENT EXHIBITED BENEFICIAL EFFECTS ON THE IMMUNE SYSTEM, PROMOTING AN INCREASE IN THE TOTAL NUMBER OF PERIPHERAL LYMPHOCYTES AND GRANULOCYTES. FURTHERMORE, A REDUCTION IN CELL NUMBERS IN THE SPLEEN, BONE MARROW, AND PERITONEUM WAS OBSERVED. THE TREATMENT ALSO PROVED EFFECTIVE IN DECREASING CFU COUNTS IN PERIPHERAL BLOOD, REFLECTING IMPROVED MICROBIAL LOAD CONTROL. LASTLY, THE STUDY REVEALED THAT TREATMENT SIGNIFICANTLY REDUCED THE SYSTEMIC PRODUCTION OF PRO-INFLAMMATORY CYTOKINES, CONTRIBUTING TO A MORE BALANCED IMMUNE RESPONSE AND INCREASING THE SURVIVAL OF SEPTIC ANIMALS.

SCREENING THE BIOACTIVITY OF POLYSSACHARIDES EXTRACTED FROM AURICULARIA AURICULA

LUÍSA COUTINHO COELHO, GABRIEL PASQUARELLI DO NASCIMENTO, MARIA CAROLINA BEZERRA DI-MEDEIROS LEAL, ANAMÉLIA LORENZETTI BOCCA

INTRODUCTION
MUSHROOM POLYSACCHARIDES REPRESENT A GROWING MARKET DEMAND, DRIVEN BY THEIR ABILITY TO CONTROL EXACERBATED INFLAMMATION AND

ESTABLISH HOMEOSTASIS BETWEEN PRO- AND ANTI-INFLAMMATORY RESPONSES. THIS CONTROL IS SUGGESTED THROUGH CELL RECEPTORS, WHICH LEAD TO CELL ACTIVATION AND SIGNALING, TRIGGERING AN INNATE AND ADAPTIVE IMMUNE RESPONSE. THESE MECHANISMS' CHARACTERISTICS, HOWEVER, ARE ASSOCIATED WITH THE MOLECULAR AND STRUCTURAL COMPOSITION OF THESE MOLECULES, WHICH MAY VARY. OUR PREVIOUS DATA HAVE SHOWN THAT THE MONOSACCHARIDE COMPOSITION OBSERVED AFTER SAMPLE PROCESSING PROVIDES A POLYSACCHARIDE WITH 48% GALACTOSE, 21% GLUCOSE, 14% MANNOSE, AND LESSER AMOUNTS OF FUCOSE AND XYLOSE. THIS WORK AIMS TO SCREEN PHAGOCYTES' RESPONSES TO STIMULI WITH POLYSACCHARIDES EXTRACTED FROM AURICULARIA AURICULA. METHODS AND RESULTS A. AURICULA POLYSACCHARIDES WERE OBTAINED FROM THE MYCELIAL SUBMERGED CULTURE OF THE MUSHROOM IN POTATO DEXTROSE BROTH AFTER 14 DAYS. UPON RETRIEVAL, WE PROCEEDED WITH HOT WATER EXTRACTION FOLLOWED BY ETHANOL PRECIPITATION. THE OBTAINED EXOPOLYSACCHARIDES WERE FRACTIONED AND DIRECTED FOR IN VITRO ASSESSMENT. BONE-MARROW MACROPHAGES (BMDM) OBTAINED FROM C57BL6/J MICE WERE TREATED WITH 25-200 µG/ML OF THE POLYSACCHARIDE OBTAINED FROM A. AURICULA. AFTER 24 HOURS OF THE INTERACTION COURSE, THE SUPERNATANT WAS COLLECTED FOR CYTOKINE AND NITRIC OXIDE DETECTION. WE OBSERVED THAT THE POLYSACCHARIDE REDUCED TNF-A PRODUCTION OF BMDM CELLS PRE-TREATED WITH LPS, AND THEY'VE SHOWN A TENDENCY TO INCREASE IL-10 PRODUCTION. NO DIFFERENCE WAS OBSERVED IN NITRIC OXIDE PRODUCTION. CONCLUSION IN THIS STUDY, WE OBSERVED THAT AURICULARIA AURICULA POLYSACCHARIDES COULD MODULATE THE CYTOKINE PRODUCTION OF BMDM, LEADING TO FURTHER INVESTIGATION OF WHICH PATHWAYS AND RECEPTOR AGONISTS ARE INVOLVED IN THIS PROCEDURE.

DNA VACCINE BASED ON OLIGOPEPTIDASE B FROM TRYPANOSOMA CRUZI MODULATES TH1 RESPONSE AND REDUCES PARASITES IN MICE

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CHAGAS DISEASE (CD), CAUSED BY THE PROTOZOAN TRYPANOSOMA CRUZI, IS A SIGNIFICANT NEGLECTED TROPICAL DISEASE THAT AFFECTS MILLIONS GLOBALLY, LEADING TO SUBSTANTIAL MORBIDITY AND MORTALITY. DESPITE THE AVAILABILITY OF TWO MAIN TREATMENTS, BENZNIDAZOLE AND NIFURTIMOX, THESE DRUGS ARE ASSOCIATED WITH SEVERE SIDE EFFECTS AND FAIL TO PROVIDE A DEFINITIVE CURE, ESPECIALLY IN THE CHRONIC

PHASE OF THE DISEASE. THE CHALLENGES POSED BY THE LIMITED EFFICACY AND ADVERSE REACTIONS OF CURRENT THERAPIES HIGHLIGHT THE URGENT NEED FOR ALTERNATIVE APPROACHES, SUCH AS VACCINE DEVELOPMENT. DNA VACCINES, WHICH UTILIZE GENETIC MATERIAL TO STIMULATE IMMUNE RESPONSES, HAVE EMERGED AS A PROMISING STRATEGY FOR COMBATING CD. THIS STUDY AIMED TO DEVELOP AND EVALUATE A DNA VACCINE TARGETING T. CRUZI BY INCORPORATING THE GENE FOR OLIGOPEPTIDASE B (OPB), A KEY ENZYME INVOLVED IN THE PARASITE'S CELLULAR INVASION PROCESS. GIVEN ITS CRUCIAL ROLE IN PATHOGENESIS, OPB IS CONSIDERED A PROMISING CANDIDATE FOR VACCINE DEVELOPMENT. THE OPB GENE WAS CLONED INTO AN OPTIMIZED DNA VECTOR, OPTV (A MODIFIED PCDNA3.1+ PLASMID WITH ADDITIONAL 5' AND 3' UNTRANSLATED REGIONS AND A POLY-A TAIL). MICE WERE IMMUNIZED WITH 25 AND 50 μG OF OPTV-OPB, AND A CONTROL GROUP WAS INCLUDED FOR COMPARISON. IMMUNIZATION WITH OPTV-OPB RESULTED IN THE PRODUCTION OF ANTI-OPB IGG ANTIBODIES ($P < 0.001$), STIMULATED THE PROLIFERATION OF OPB-SPECIFIC CD8+ T CELLS ($P = 0.0203$), AND INCREASED INF-Γ PRODUCTION ($P = 0.0348$) WHEN COMPARED TO THE CONTROL GROUP. UPON INFECTION WITH THE CL-BRENNER LUC STRAIN OF T. CRUZI, BIOLUMINESCENCE IMAGING REVEALED A SIGNIFICANT REDUCTION IN PARASITIC LOAD IN THE OPTV-OPB IMMUNIZED GROUP ($P < 0.0001$). THESE FINDINGS INDICATE THAT THE DNA VACCINE FORMULATION BASED ON OPB AS AN ANTIGEN IS CAPABLE OF ELICITING BOTH HUMORAL AND CELLULAR IMMUNE RESPONSES, WITH POTENTIAL FOR REDUCTION OF PARASITEMIA IN T. CRUZI INFECTION.

ANTITUMOR POTENTIAL OF COPPER OXIDE NANORODS IN A MURINE OF MAMMARY CARCINOMA

GIOVANNA DE CARVALHO NARDELI BASÍLIO LÔBO 1, RAQUEL DAS NEVES ALMEIDA 2, RAMON TIAGO ALBUQUERQUE ANDRADE 3, LEONARDO GIORDANO PATERNO 3 AND SÔNIA NAIR BÁO 1

CANCER REMAINS A CRITICAL GLOBAL HEALTH CHALLENGE, RANKING AS THE SECOND LEADING CAUSE OF MORTALITY WORLDWIDE. AMONG WOMEN, BREAST CANCER IS THE MOST LETHAL FORM, ATTRIBUTED TO ITS CLINICAL, MORPHOLOGICAL, AND GENETIC HETEROGENEITY. THESE COMPLEXITIES SIGNIFICANTLY IMPACT TREATMENT EFFICACY AND PATIENT OUTCOMES. ADVANCES IN NANOTECHNOLOGY HAVE INTRODUCED NANOPARTICLES AS TRANSFORMATIVE TOOLS IN ONCOLOGY, ENHANCING THE PHARMACOLOGICAL PROPERTIES OF DIAGNOSTIC AND

THERAPEUTIC COMPOUNDS. COPPER OXIDE NANOPARTICLES (CUO-NR-CIT) HAVE EMERGED AS PROMISING CANDIDATES DUE TO THEIR UNIQUE THERMOPHYSICAL AND BIOLOGICAL PROPERTIES. THIS STUDY HIGHLIGHTS THE ANTITUMOR ACTIVITY OF CUO-NR-CIT NANORODS IN THE 4T1 BREAST TUMOR CELL LINE, A MURINE MODEL OF MAMMARY CARCINOMA. THE NANORODS DEMONSTRATED SELECTIVE CYTOTOXICITY, SIGNIFICANTLY REDUCING 4T1 CELL VIABILITY WHILE SPARING NON-TUMOR NIH-3T3 CELLS, PARTICULARLY AT THREE OF THE FIVE TESTED CONCENTRATIONS. CONFOCAL MICROSCOPY ANALYSES REVEALED THAT CUO-NR-CIT NANOSTRUCTURES DECREASED MITOCHONDRIAL CONTENT IN 4T1 CELLS WITHOUT DETECTABLE ADVERSE EFFECTS ON NIH-3T3 CELLS. FURTHERMORE, A NOTABLE REDUCTION IN LIPID BODY FORMATION—A MARKER OF CELLULAR ACTIVATION—WAS OBSERVED IN TREATED TUMOR CELLS. THESE FINDINGS UNDERSCORE THE POTENTIAL OF CUO-NR-CIT NANOPARTICLES AS A TARGETED THERAPEUTIC APPROACH FOR BREAST CANCER, PAVING THE WAY FOR FURTHER RESEARCH INTO THEIR CLINICAL APPLICATION.

EVALUATION OF DENDRITIC CELLS ACTIVATION THROUGH METHYLENE BLUE ASSOCIATED WITH NANOSTRUCTURES FOR BREAST AND OVARIAN CANCER

ANA LUÍSA G. SILVA 1, CLEBER L. FILOMENO 2, BEATRIZ CAMPOS ARAÚJO 3, LEONARDO G. PATERNO 2, DANIEL MENDES PEREIRA ARDISSON-ARAÚJO 3 AND SÔNIA N. BÁO 1

THE IMMUNE SYSTEM PLAYS A PIVOTAL ROLE IN CANCER THERAPY, DRIVING EXTENSIVE RESEARCH INTO INNOVATIVE IMMUNOTHERAPEUTIC STRATEGIES. BREAST CANCER, THE SECOND MOST COMMON MALIGNANCY AMONG WOMEN, AND OVARIAN CANCER, THE MOST LETHAL CANCER OF THE FEMALE GENITAL SYSTEM, PRESENT SIGNIFICANT THERAPEUTIC CHALLENGES. NANOBIOTECHNOLOGY HAS GAINED PROMINENCE FOR TARGETED DRUG DELIVERY AND IMMUNE RESPONSE ENHANCEMENT, PARTICULARLY VIA DENDRITIC CELL ACTIVATION. THIS STUDY INVESTIGATES MAGHEMITE NANOPARTICLES ASSOCIATED WITH METHYLENE

BLUE (MAGCIT-MB) AS A DUAL THERAPEUTIC APPROACH FOR BREAST AND OVARIAN CANCERS CELL LINES. MAGCIT-MB, WITH A HYDRODYNAMIC DIAMETER OF 60.93 NM, DEMONSTRATED EFFICIENT CELLULAR UPTAKE BY TRANSMISSION ELECTRON MICROSCOPY (TEM), WITCH REVEALED NANOPARTICLE INTERNALIZATION WITHIN 6 HOURS, LOCALIZED IN VESICULAR STRUCTURES RESEMBLING LYSOSOMES AND/OR ENDOSOMES. FLUORESCENCE MICROSCOPY CONFIRMED MITOCHONDRIAL ALTERATIONS POST-TREATMENT, WHILE OXIDATIVE PATHWAYS ASSAYS SHOWED ELEVATED ROS LEVELS AND THE INVOLVEMENT OF ANTIOXIDANT ENZYMES IN CELL DEATH. PANOPTIC STAINING REVEALED MORPHOLOGICAL CHANGES IN TREATED TUMOR CELLS. FLOW CYTOMETRY INDICATED DECREASED MITOCHONDRIAL TRANSMEMBRANE POTENTIAL AND MODULATION OF LIPID DROPLET BIOGENESIS, SUGGESTING METABOLIC DISRUPTION. CELLULAR VIABILITY ASSAYS IN MURINE CELLS, 4T1 AND NIH-3T3 REVEALED CYTOTOXICITY LEVELS COMPARABLE TO HUMAN CELLS, SUPPORTING THE POTENTIAL FOR IN VIVO APPLICATIONS. MAGCIT-MB ALSO DEMONSTRATED IMMUNOSTIMULATORY EFFECTS. SUPERNATANTS FROM TREATED TUMOR CELLS WERE USED TO STIMULATE DENDRITIC CELLS, RESULTING IN INCREASED EXPRESSION OF MATURATION MARKERS CD80, CD86, AND CD11C. LIGHT MICROSCOPY SHOWED CHARACTERISTIC MORPHOLOGICAL CHANGES, INCLUDING FUSIFORM SHAPES AND DENDRITIC EXTENSIONS. THESE FINDINGS HIGHLIGHT MAGCIT-MB AS A NANOTHERAPEUTIC WITH ANTI-TUMOR EFFICACY AND IMMUNE-ENHANCING PROPERTIES, PAVING THE WAY FOR FURTHER PRECLINICAL STUDIES.

EVALUATION OF THE CYTOTOXICITY OF CURCUMIN AND OCELLATIN IN THP-1 CELLS INFECTED WITH THE ZIKA VIRUS USING THE MTT ASSAY

GLAUCO DE OLIVEIRA MACEDO, TATIANA KARLA DOS SANTOS BORGES, MARIANGELA SOUSA DEOLIVEIRA

INTRODUCTION/BACKGROUND: ALTHOUGH THE ZIKA EPIDEMIC AND CASES OF MICROCEPHALY HAVE DECLINED, DATA FROM THE MINISTRY OF HEALTH SHOW A CONSISTENT ANNUAL INCIDENCE AVERAGING 8,093 CASES FROM THE END OF THE EPIDEMIC IN 2017 TO THE END OF 2023 (BRAZIL, 2024). THESE DATA INCLUDE MICROCEPHALY CASES WHERE ZIKA VIRUS (ZIKV) INFECTION IS SUSPECTED. THE CIRCULATION OF NEW VIRUS VARIANTS UNDERSCORES THE IMPORTANCE OF STUDIES LIKE THIS ONE, WHICH USES THE MTT ASSAY TO INVESTIGATE THE IMMUNOPATHOLOGY OF THE VIRUS AND DEVELOP NEW CONTROL STRATEGIES.
METHODS: THP-1 CELLS WERE DETACHED, RESUSPENDED, COUNTED, AND DISTRIBUTED INTO WELLS. THE VIRUS SUSPENSION WAS ADDED AND LEFT TO ADSORB FOR 1 HOUR. SUBSEQUENTLY, SPECIFIC SOLUTIONS (RPMI, DRUGS [OCELLATIN PT-4 AND CURCUMIN], OR DMSO) WERE ADDED AND INCUBATED FOR 24 HOURS. THE PLATES WERE CENTRIFUGED, PART OF THE SUPERNATANT WAS DISCARDED, MTT WAS ADDED, AND THE PLATES WERE INCUBATED FOR 4

HOURS. FINALLY, DMSO WAS ADDED, AND THE READINGS WERE PERFORMED USING A SPECTROPHOTOMETER. STATISTICAL ANALYSIS WAS CONDUCTED USING PRISM.
RESULTS: MOI 0.1 RESULTED IN HIGHER CELL MORTALITY COMPARED TO MOI 1 IN UNTREATED INFECTED GROUPS. THE OCELLATIN DOSE OF 4 MG/ML WAS LESS TOXIC TO CELLS, AS WAS THE CURCUMIN DOSE OF 2.5 µM. OTHER DOSES WERE TOXIC TO THE CELLS.
CONCLUSIONS: THE OPTIMAL DOSE OF OCELLATIN IS 4 MG/ML, AND FOR CURCUMIN, IT IS 2.5 µM, AS THESE CONCENTRATIONS WERE LESS TOXIC. MOI 0.1 WAS SELECTED FOR FUTURE TESTS SINCE IT KILLED MORE CELLS COMPARED TO MOI 1 IN THE ABSENCE OF DRUGS.

ANALYSIS OF THE POTENTIAL ANTITUMOR ACTION OF METFORMIN ALONE OR IN COMBINATION WITH OMEGA-3 DHA IN HUMAN OVARIAN CANCER CELLS

MILENA NASCIMENTO VERDAM DE ARAÚJO, LAÍS BEZERRA DA COSTA, KELLY GRACE MAGALHÃES.

CANCER IS A SET OF CHRONIC GENETIC DISEASES CHARACTERIZED BY ABNORMAL CELL GROWTH, DUE TO MUTATIONS THAT ENABLE PROLIFERATIVE SIGNALING, RESISTANCE TO CELL DEATH, METABOLIC DEREGULATION, AND TISSUE INVASION. OVARIAN CANCER RANKS AS THE 2ND MOST COMMON GYNECOLOGICAL NEOPLASM AMONG TUMORS. METFORMIN (MET) AND OMEGA-3 DHA ARE GAINING INCREASING RELEVANCE WORLDWIDE DUE TO THEIR ANTITUMOR EFFECTS ON SOME TYPES OF NEOPLASIA, NOTABLY FOR MODULATING METABOLISM TO ENHANCE CHEMOTHERAPY SENSITIVITY AND REDUCE TUMOR AGGRESSIVENESS. HOWEVER, THEIR COMBINED POTENTIAL REMAINS UNEXPLORED. WE AIM TO INVESTIGATE THE EFFECTS OF SINGLE AND COMBINED TREATMENT WITH MET AND DHA ON CELL VIABILITY, DEATH, AND PROLIFERATION, FOCUSING ON THE MODULATION OF LIPID DROPLET (LD) BIOGENESIS IN OVARIAN CANCER CELLS, AS THIS ORGANELLE PLAYS A KEY ROLE IN CELLULAR METABOLIC SHIFT AND IS DIRECTLY ASSOCIATED WITH TUMOR AGGRESSIVENESS, CONSIDERED A BIOMARKER OF THIS PHENOTYPE. A2780 CELLS WERE TREATED WITH DIFFERENT DOSES OF MET AND DHA, EITHER ALONE OR IN COMBINATION WITH EACH OTHER. CYTOTOXICITY WAS ASSESSED BY SPECTROPHOTOMETRY USING THE MTT COLORIMETRIC TECHNIQUE. THE CELL DEATH PROFILE WAS EVALUATED BY ANNEXIN/PI STAINING AND FLOW CYTOMETRY ANALYSIS. LD BIOGENESIS ASSAYS WERE PERFORMED USING BODIPY PROBE STAINING AND FLOW CYTOMETRY ANALYSIS, AS WELL AS OIL RED-O DYE STAINING FOLLOWED BY OPTICAL MICROSCOPY AND SPECTROPHOTOMETRY ANALYSIS. OUR DATA SHOW A SIGNIFICANT, TIME- AND DOSE-DEPENDENT REDUCTION IN CELL VIABILITY FOLLOWING TREATMENT WITH BOTH MET AND DHA. TOGETHER, THEY WERE MORE EFFICIENT IN INDUCING CYTOTOXICITY WITH THE SAME PATTERN. IT WAS

ALSO OBSERVED THAT THE CO-TREATMENT DECREASED CELL PROLIFERATION. LP NUMBERS AND MORPHOLOGY ALSO APPEARED TO BE MODULATED IN THIS CELL LINE. TAKEN TOGETHER, OUR RESULTS SUGGEST A SYNERGISTIC EFFECT BETWEEN MET AND DHA, PROMOTING ANTITUMOR EFFECTS IN HUMAN OVARIAN CARCINOMA CELLS.

NANOTECHNOLOGY APPLIED TO SKIN HEALTH: DEVELOPMENT AND CHARACTERIZATION OF LIPID NANOSTRUCTURES BASED ON CO-PRODUCTS FROM THE BRAZILIAN FLORA

FRANCIÉLE DE MATOS DA SILVA, DANIELLE GALDINO DE SOUZA, VICTOR CARLOS MELO DA SILVA, TATHYANA BENETIS PIAU E SÔNIA NAIR BÁO

SUSTAINABLE DEVELOPMENT, ESSENTIAL TO PRESERVE THE ENVIRONMENT FOR FUTURE GENERATIONS, REQUIRES INNOVATIONS WITH GREEN TECHNOLOGIES THAT PROMOTE BIODEGRADABLE PRODUCTS. THIS STUDY ADDRESSES THE APPLICATION OF NANOTECHNOLOGY IN SKIN HEALTH THROUGH NANOSTRUCTURED LIPID CARRIERS (NLCS) FORMULATED FROM NATURAL CO-PRODUCTS, SUCH AS SERIGUELA BARK EXTRACT (SPONDIAS PURPUREA L.), TUCUMÃ BUTTER (ASTROCARYUM VULGARE) AND PRACAXI OIL (PENTACLETHRA MACROLOBA), VALUING BRAZILIAN BIODIVERSITY. IN ALIGNMENT WITH THE UN SUSTAINABLE DEVELOPMENT GOALS AND MISSION 5 OF THE ACTION PLAN FOR NEOINDUSTRIALIZATION 2024-2026, THE STUDY AIMS TO INTEGRATE SUSTAINABLE TECHNOLOGIES INTO THE COUNTRY'S STRATEGIC PRODUCTION CHAINS. THE CHARACTERIZATION OF THE SERIGUELA EXTRACT, PERFORMED BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC), REVEALED BIOACTIVE COMPOUNDS WITH HIGH ANTIOXIDANT POTENTIAL, WHICH MAY BE PROMISING IN THE COSMETICS AREA. NLC FORMULATIONS NAMED NLC-BRANCO AND NLC-BIO WERE DEVELOPED, THE LATTER ENRICHED WITH SERIGUELA EXTRACT. NLC-BIO PRESENTED AN AVERAGE PARTICLE SIZE OF 32.94 NM, PDI OF 0.1 AND ZETA POTENTIAL OF -30.27 MV, INDICATING COLLOIDAL STABILITY. ANTIOXIDANT TESTS (DPPH AND ABTS) CONFIRMED ITS HIGH ANTIOXIDANT CAPACITY, SURPASSING THE EFFICACY OF NLC-BRANCO. TOXICITY TESTS IN ZEBRAFISH (DANIO RERIO) EMBRYOS DEMONSTRATED THE SAFETY OF THE FORMULATIONS, WITH LC50 OF 49.47 µG/ML IN 96 HOURS. STRUCTURAL ANALYSIS BY FTIR AND RAMAN EVIDENCED THE PRESERVATION OF CHEMICAL INTERACTIONS BETWEEN LIPIDS AND BIOACTIVE COMPOUNDS, VALIDATING THE ENCAPSULATION EFFICIENCY AND THERAPEUTIC POTENTIAL. THE SPHERICAL MORPHOLOGY OF THE NANOPARTICLES, CONFIRMED BY TRANSMISSION ELECTRON MICROSCOPY, REINFORCES THE STABILITY AND EFFICACY OF THE ENCAPSULATION. THESE FINDINGS HIGHLIGHT THE POTENTIAL OF NLCS FORMULATED WITH NATURAL

INGREDIENTS FOR TOPICAL SKIN HEALTH APPLICATIONS, DEMONSTRATING THE VALUE OF SUSTAINABLE TECHNOLOGIES IN COSMETICS AND PHARMACEUTICALS.

COMPARATIVE ANALYSIS OF CHEMOTHERAPEUTIC AGENTS IN A MURINE MODEL OF SPONTANEOUS BREAST CANCER: INFLUENCE OF ADIPOSE TISSUE ON TUMOR PROGRESSION

IGOR DE OLIVEIRA SANTOS, LÍVIA PIMENTEL DE SANT'ANA DOURADO, SABRINA AZEVEDO MACHADO, MARTÍN HERNÁN BONAMINO, KELLY GRACE MAGALHÃES

THE CURRENT STANDARD TREATMENT FOR BREAST CANCER IS A COMBINATION OF ANTHRACYCLINES FOLLOWED BY TAXANES. HOWEVER, THE NEOSAMBA CLINICAL TRIAL DEMONSTRATED THAT THE REVERSE ORDER OF ADMINISTRATION IMPROVED DISEASE-FREE SURVIVAL AND OVERALL SURVIVAL IN BREAST CANCER PATIENTS. ADIPOSE TISSUE PLAYS A CRUCIAL ROLE IN THE TUMOR MICROENVIRONMENT (TME), INFLUENCING CANCER PROGRESSION AND RESPONSE TO TREATMENT. THIS STUDY AIMS TO EVALUATE THE IMPACT OF NEOADJUVANT CHEMOTHERAPY ON THE TME, WITH A FOCUS ON THE ROLE OF ADIPOSE TISSUE IN THE CONTEXT OF BREAST CANCER. ADIPOSE CELLS SECRETE SEVERAL FACTORS THAT CAN PROMOTE TUMOR GROWTH AND ANGIOGENESIS. HOWEVER, THE IMPACT OF CHEMOTHERAPY ON ADIPOSE TISSUE IN THE TME IS STILL POORLY UNDERSTOOD. OUR HYPOTHESIS IS THAT CHEMOTHERAPY CAN ALTER THE BEHAVIOR OF ADIPOSE TISSUE, INFLUENCING ITS INTERACTION WITH TUMOR CELLS AND IMMUNE CELLS. THIS STUDY WILL USE THE MMTV-PYMT MOUSE MODEL, WHICH SPONTANEOUSLY DEVELOPS MAMMARY TUMORS, TO INVESTIGATE THE EFFECTS OF DIFFERENT CHEMOTHERAPY REGIMENS ON THE TME. WE WILL ANALYZE CHANGES IN THE COMPOSITION AND FUNCTION OF THE ADIPOSE TISSUE, AS WELL AS ITS INTERACTION WITH OTHER CELLULAR COMPONENTS WITHIN THE TME, INCLUDING IMMUNE CELLS, FIBROBLASTS AND ENDOTHELIAL CELLS. IN ADDITION, WE WILL EXAMINE THE IMPACT OF CHEMOTHERAPY ON THE ADIPOSE TISSUE SECRETOME AND HOW THESE SECRETED FACTORS MAY INFLUENCE TUMOR GROWTH, METASTASIS AND IMMUNE RESPONSE. THE FINDINGS OF THIS STUDY WILL SHED LIGHT ON THE ROLE OF ADIPOSE TISSUE IN BREAST CANCER PROGRESSION AND TREATMENT RESPONSE, WHICH MAY LEAD TO THE DEVELOPMENT OF NEW THERAPEUTIC STRATEGIES TARGETING THE ADIPOSE TISSUE TO IMPROVE PATIENT OUTCOMES.

BIOQUÍMICA, BIOFÍSICA E BIOLOGIA ESTRUTURAL (A2)

HIGH EXTRACELLULAR GLUCOSE CONCENTRATION DRIVES PALMITATE-INDUCED TOXICITY AND METABOLIC DYSFUNCTION IN MICROGLIA CELLS

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MICROGLIA EXHIBIT DIRECTED RESPONSES TO DIFFERENT STIMULI, WHICH CAN VARY DEPENDING ON THE ENVIRONMENTAL CONDITIONS THEY ENCOUNTER. THIS STUDY TESTED WHETHER INFLAMMATORY, METABOLIC AND PHAGOCYTIC RESPONSES OF MICROGLIA TO THE SATURATED FATTY ACID PALMITATE DEPEND ON EXTRACELLULAR GLUCOSE CONCENTRATIONS. BV2 CELLS WERE CULTURED IN LOW GLUCOSE (LG; 5.5 MMOL/L) OR HIGH GLUCOSE (HG; 25 MMOL/L) CONCENTRATIONS, AND EXPOSED TO PALMITATE (100 OR 200 µMOL/L) OR VEHICLE FOR 24 HOURS. UNDER HG, PALMITATE DECREASED CELL VIABILITY, WHICH WAS ACCOMPANIED BY AN INCREASE IN INFLAMMATORY MARKERS ASSOCIATED WITH A DISEASED-PHENOTYPE. ADDITIONALLY, PALMITATE INDUCED HIGHER EXPRESSION OF GENES RELATED TO LIPID METABOLISM IN BOTH LG AND HG, WITHOUT AFFECTING ENZYMES LINKED TO GLUCOSE METABOLISM. HG CONDITION LED TO AN INCREASE IN THE OXYGEN CONSUMPTION RATE (OCR) AND GLYCOLYTIC FLUX (I.E., EXTRACELLULAR MEDIUM ACIDIFICATION) COMPARED TO LG-CULTURED CELLS, WITH PALMITATE REDUCING OCR AND GLYCOLYTIC FLUX IN BOTH CONDITIONS. THE SHORT-CHAIN FATTY ACID BUTYRATE DID NOT PREVENT PALMITATE-INDUCED MITOCHONDRIAL DYSFUNCTION IN BV2 CELLS. IN PRIMARY MICROGLIA, PALMITATE DID NOT AFFECT MITOCHONDRIA AREA AND CARGO METABOLISM. ALTOGETHER OUR RESULTS INDICATE THAT BV2 CELLS ARE PRONE TO PALMITATE-INDUCED STRESS ON VIABILITY ASSAYS UNDER HG BUT NOT LG IN THE MEDIUM.

EFFECT OF AN HYPERLIPIDIC DIET ON METABOLIC AND NEURODEVELOPMENTAL OUTCOMES AT DIFFERENT DEVELOPMENTAL STAGES OF ZEBRAFISH

LETÍCIA ALVES TAVARES , WHITNEY RIBEIRO SANTOS, VICTOR LUNA PICOLO, NATHASHA MARIA CORREA PRADO LOPES, JÚLIA DE ANDRADE ARAÚJO PINTO , MARIA EDUARDA CORREIA CARVELLI, LARISSA NUNES SOUZA, PEDRO HENRIQUE SANTOS SILVA [1], PAULA MARIA QUAGLIO BELLOZI , JAIR TRAPÉ GOULART, CÉSAR KOPPE GRISOLIA , ANDREZA FABRO DE BEM.

UNBALANCED DIETS DISRUPT METABOLISM AND NEURODEVELOPMENT, INFLUENCING HEALTH AND BEHAVIOR THROUGHOUT LIFE. SUCH DIETS CAN

LEAD TO LIPID ACCUMULATION, INSULIN RESISTANCE, AND INFLAMMATORY RESPONSES, COMPROMISING NEURONAL PLASTICITY AND NEUROCHEMICAL BALANCE. TO ASSESS THE IMPACT OF A HYPERLIPIDIC DIET (HD) ON NEURODEVELOPMENT, ZEBRAFISH LARVAE WERE FED EITHER A CONTROL DIET (CD) OR A HD FROM 21 DAYS POST-FERTILIZATION (DPF) UNTIL 36 DPF (LARVAL STAGE) AND IN A SUBSET OF JUVENILES UNTIL 5-6 MONTHS POST-FERTILIZATION (MPF, EARLY ADULT STAGE). FOLLOWING THE DIETARY PROTOCOLS, MORPHO-METABOLIC, BEHAVIORAL, AND BIOENERGETIC PARAMETERS WERE EVALUATED. DURING THE LARVAL PERIOD, A 16-DAY HD REGIMEN SIGNIFICANTLY INCREASED SURVIVAL, BODY LENGTH AND LIPID STAINING INTENSITY WITH OIL RED O. IN BEHAVIORAL ASSESSMENTS, THE NOVEL TANK TEST SHOWED NO SIGNIFICANT DIFFERENCES, WHILE IN THE OPEN FIELD TEST, THE HD GROUP TENDED TO SHOW REDUCED CENTER EXPLORATION. FOR THE SUBSET FED DIETS UNTIL EARLY ADULTHOOD (5-6 MPF), HD SIGNIFICANTLY INCREASED BODY WEIGHT, BODY LENGTH, AND BODY MASS INDEX, ALONG WITH BLOOD GLUCOSE LEVELS COMPARED TO THE CD GROUP, ALTHOUGH SURVIVAL RATES WERE SIMILAR. IN THE NOVEL TANK TEST, THE HD GROUP TENDED TO SHOW REDUCED SPEED AND LESS TIME EXPLORING THE UPPER ZONE, CONFIRMED BY A DECREASE IN THE EXPLORATION INDEX. NO CHANGES WERE OBSERVED IN BRAIN OXYGEN CONSUMPTION BETWEEN GROUPS, AS ASSESSED BY HIGH-RESOLUTION RESPIROMETRY. IN CONCLUSION, THE HD INFLUENCED MORPHO-METABOLIC AND BEHAVIORAL DEVELOPMENT IN BOTH LARVAL AND EARLY ADULT ZEBRAFISH, SUGGESTING LONG-TERM ADVERSE EFFECTS ON METABOLISM AND BEHAVIOR.

INTERGENERATIONAL EFFECTS OF A HIGH-FAT DIET ON METABOLIC AND NEURODEVELOPMENTAL OUTCOMES IN ZEBRAFISH OFFSPRING

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RECENT STUDIES SUGGEST THAT THE METABOLIC AND NEUROLOGICAL IMPACTS OF UNHEALTHY DIETS MAY EXTEND TO SUBSEQUENT GENERATIONS. THIS STUDY INVESTIGATES THE INTERGENERATIONAL EFFECTS OF A HIGH-FAT DIET ON METABOLIC AND NEURODEVELOPMENTAL OUTCOMES IN ZEBRAFISH OFFSPRING. WE USED AN EXPERIMENTAL DESIGN WITH 80 ADULT F0 ZEBRAFISH (BOTH MALES AND FEMALES) THAT WERE FED A CONTROL DIET (CD) OR A HIGH-FAT DIET (HD) FOR 36 DAYS. F0 ZEBRAFISH WERE THEN BRED TO PRODUCE OFFSPRING (F1), WHICH WERE SUBSEQUENTLY ASSESSED, ALONGSIDE F0, FOR BEHAVIORAL, METABOLIC, AND BIOCHEMICAL PARAMETERS. IN PROGENITORS (F0) ZEBRAFISH, HD INTAKE LED TO

INCREASED BODY WEIGHT IN FEMALES AND BODY LENGTH IN MALES ($P < 0.01$). EGG VIABILITY AND LARVAL SURVIVAL DURING THE INITIAL 15 DAYS POST-FERTILIZATION WERE HIGHER IN THE CD GROUP. IN THE NOVEL TANK TEST, IH EVALUATES MOTOR ACTIVITY AND ANXIETY-LIKE BEHAVIOR, HD-FED FEMALES SHOWED REDUCED TOTAL DISTANCE TRAVELED ($P < 0.05$), WITH DECREASED UPPER ZONE EXPLORATION IN BOTH FEMALES ($P < 0.01$) AND MALES ($P < 0.05$), SUGGESTING INCREASED ANXIETY-LIKE BEHAVIOR. NO SIGNIFICANT DIFFERENCES IN BRAIN OXYGEN CONSUMPTION WERE OBSERVED BETWEEN GROUPS. BEHAVIORAL AND METABOLIC ANALYSES OF F1 OFFSPRING ARE ONGOING. IN CONCLUSION, HD INTAKE INDUCED MORPHOMETRIC CHANGES IN F0 ZEBRAFISH, IMPACTED EGG VIABILITY AND EARLY OFFSPRING DEVELOPMENT, AND AFFECTED BEHAVIOR. ZEBRAFISH THUS EMERGE AS A VALUABLE TRANSLATIONAL MODEL FOR STUDYING NEUROLOGICAL EFFECTS ASSOCIATED WITH UNBALANCED DIETS AND INTERGENERATIONAL METABOLIC DISTURBANCES.

EVALUATION OF THE ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF CATHELICIDIN-PP ANALOGS

BRENDA CONCEIÇÃO GUIMARÃES SANTANA1, CARLOS JOSÉ CORREIA DE SANTANA1, DANIEL DASTAN REZABALA PACHECO4, KEVEN WENDER RODRIGUES MACEDO1, BRUNNA DE OLIVEIRA SILVA1, MARLON HENRIQUE CARDOSO3; OCTÁVIO LUIZ FRANDO3; OSMINDO RODRIGUES PIRES JR.1, WAGNER FONTES2 E MARIANA S. CASTRO1,2.

CATHELICIDINS ARE IMMUNE SYSTEM PEPTIDES FOUND IN ANIMALS THAT PLAY AN ANTIMICROBIAL ROLE. CATHELICIDIN-PP, THE FIRST IDENTIFIED IN FROGS OF THE SPECIES POLYPEDATES PUERENSIS, DEMONSTRATED ACTIVITY AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA AND FUNGI, AS WELL AS LOW CYTOTOXICITY, LOW HEMOLYTIC ACTIVITY, AND EXHIBITED ANTI-INFLAMMATORY POTENTIAL. EIGHT ANALOGS DERIVED FROM THIS PEPTIDE WERE PRODUCED AND TESTED IN THIS STUDY. THE PEPTIDES WERE SYNTHESIZED CHEMICALLY, PURIFIED, AND LYOPHILIZED. SUBSEQUENTLY, THEIR ANTIMICROBIAL PROPERTIES AGAINST ANTIBIOTIC-SENSITIVE AND ANTIBIOTIC-RESISTANT BACTERIA AND FUNGI, HEMOLYTIC CAPACITY, CYTOTOXICITY ON HACAT AND HELA CELL LINES, AND ANTIBIOFILM ACTIVITY WERE EVALUATED. FIVE ANALOGS SHOWED ANTIMICROBIAL ACTIVITY AGAINST GRAM-NEGATIVE BACTERIA AND/OR CANDIDA SP. ANALOG 8 EXHIBITED THE BROADEST SPECTRUM OF ACTION, BEING EFFECTIVE AGAINST ESCHERICHIA COLI ATCC 25922, KLEBSIELLA PNEUMONIAE ATCC 13883, PSEUDOMONAS AERUGINOSA ATCC 27853, CANDIDA ALBICANS ATCC 14053, AND C. GLABRATA. ANALOG 10 WAS ACTIVE AGAINST E. COLI, P. AERUGINOSA, C. ALBICANS, AND C. GLABRATA. ANALOG 7 SHOWED ACTIVITY AGAINST P. AERUGINOSA, C. ALBICANS, AND C. GLABRATA. ANALOG 11 WAS ONLY ACTIVE AGAINST E. COLI AND P. AERUGINOSA, WHILE ANALOG

2 SHOWED ACTIVITY EXCLUSIVELY AGAINST P. AERUGINOSA. ALL EIGHT TESTED ANALOGS WERE EFFECTIVE AGAINST CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII, ANALOGS 8 AND 11 AGAINST MULTIDRUG-RESISTANT P. AERUGINOSA, AND ANALOG 8 AGAINST K. PNEUMONIAE CARBAPENEMASE. NONE OF THE ANALOGS DEMONSTRATED CYTOTOXIC ACTIVITY AGAINST TUMOR OR NON-TUMOR CELL LINES, AND ALL HAD HEMOLYTIC ACTIVITY BELOW 5%. ANALOGS 2, 8, 10, AND 11 ALSO EXHIBITED ANTIBIOFILM ACTIVITY AGAINST P. AERUGINOSA, MAKING THEM THE MOST PROMISING CANDIDATES. THESE RESULTS INDICATE THE POTENTIAL OF THESE ANALOGS TO SERVE AS NEW ANTIMICROBIAL AGENTS.

EFFECT OF ESTRADIOL (17A-E2) SUPPLEMENTATION ON AGED FEMALE MICE'S METABOLISM AND COGNITION

NATHASHA M. C. P. LOPES, ANA BEATRIZ SOUSA, WELLINGHTON BARROS, LAYANE DAMASCENO, WEMBLEY VILELA, HENVER BRUNETTA, PAULA BELLOZI, ANGÉLICA AMATO, ANDREZA FABRO DE BEM

AGING IN WOMEN IS OFTEN ACCOMPANIED BY COGNITIVE DECLINE, PARTLY DUE TO THE SUBSTANTIAL REDUCTION IN ESTROGEN LEVELS DURING MENOPAUSE. CLINICAL AND PRECLINICAL STUDIES INDICATE THAT ESTRADIOL 17A-E2 ACTS AS BOTH A METABOLIC MODULATOR AND A NEUROPROTECTIVE AGENT, RAISING QUESTIONS ABOUT THE BENEFITS OF ESTRADIOL SUPPLEMENTATION DURING MENOPAUSE. THIS STUDY AIMED TO EVALUATE WHETHER 17A-E2 COULD COUNTERACT HIGH-FAT DIET (HFD)-INDUCED OBESITY, COGNITIVE DEFICITS, AND HIPPOCAMPAL MITOCHONDRIAL DYSFUNCTION IN AGED FEMALES. TWELVE-MONTH-OLD FEMALE C57BL/6J MICE RECEIVED EITHER A STANDARD DIET (SD) OR HFD, WITH OR WITHOUT 17A-E2, FOR SIX WEEKS. GLUCOSE TOLERANCE TESTS (GTT) WERE PERFORMED, FOLLOWED BY BEHAVIORAL ASSESSMENTS WITH OPEN FIELD (OF) AND OBJECT RECOGNITION TEST (ORT). HIPPOCAMPAL MITOCHONDRIAL FUNCTION WAS ASSESSED USING HIGH-RESOLUTION RESPIROMETRY. RESULTS SHOWED THAT 17A-E2 SUPPLEMENTATION PREVENTED HFD-INDUCED INCREASES IN BODY WEIGHT AND VISCERAL AND SUBCUTANEOUS FAT ACCUMULATION, WHILE ALSO AMELIORATING GLUCOSE INTOLERANCE. ESTRADIOL SUPPLEMENTATION REVERSED COGNITIVE DEFICITS OBSERVED IN AGED FEMALE MICE ON SD AND HFD. NO SIGNIFICANT DIFFERENCES WERE OBSERVED IN TRIGLYCERIDE OR CHOLESTEROL LEVELS ACROSS GROUPS. INTERESTINGLY, 17A-E2 ENHANCED MITOCHONDRIAL BIOENERGETICS IN THE HIPPOCAMPUS, IMPROVING COMPLEX I, I+II, OXPHOS, AND ETS ACTIVITY IN SD-FED FEMALES. THESE FINDINGS SUGGEST THAT ESTRADIOL CAN ENHANCE METABOLIC AND COGNITIVE FUNCTIONS IN AGING FEMALES, SUPPORTING ITS ROLE IN POSTMENOPAUSAL HORMONE REPLACEMENT THERAPY.

PROTEÔMICA INTEGRATIVA DE MARCAÇÃO DE PROXIMIDADE DA INTERRELAÇÃO METABÓLICA ENTRE O APICOPLASTO E A MITOCÔNDRIA DURANTE A ESQUIZOGONIA ERITROCITÁRIA DE PLASMODIUM FALCIPARUM

MARIA EDUARDA PORTELA FERREIRA, LUCAS SILVA DE OLIVEIRA, SÉBASTIEN CHARNEAU

MALARIA, CAUSED BY PLASMODIUM FALCIPARUM, REMAINS ONE OF THE PRIMARY GLOBAL HEALTH THREATS, UNDERSCORING THE URGENT NEED FOR NEW TREATMENTS AND RESEARCH APPROACHES. THIS STUDY PROPOSES A DETAILED INVESTIGATION INTO THE METABOLIC INTERPLAY BETWEEN THE MITOCHONDRION AND APICOPLAST DURING THE ERYTHROCYTIC SCHIZOGONY OF THE PARASITE, EMPLOYING AN INTEGRATIVE PROTEOMIC APPROACH WITH THE APEX2 PROXIMITY-LABELING TECHNIQUE. THE WORK AIMS TO ELUCIDATE HOW THESE ORGANELLES COMMUNICATE AND INTERACT METABOLICALLY—AN INTERACTION THAT IS STILL UNDEREXPLORED BUT CRITICAL TO PARASITE SURVIVAL AND, THEREFORE, TO MALARIA CONTROL. PROJECT STEPS INCLUDE CULTURING THE P. FALCIPARUM 3D7 STRAIN IN HUMAN ERYTHROCYTES, FOLLOWED BY TRANSFECTION WITH PLASMIDS TARGETING MITOCHONDRIAL AND APICOPLAST LABELING. AFTER SELECTING TRANSFECTED PARASITES, WESTERN BLOTTING AND IMMUNOFLUORESCENCE ASSAYS WILL CONFIRM THE EXPRESSION OF APEX2-TAGGED PROTEINS. SUBSEQUENT ELECTRON MICROSCOPY WILL ALLOW DETAILED VISUALIZATION OF THE ORGANELLES, WHILE ENRICHMENT OF BIOTINYLATED PROTEINS AND MASS SPECTROMETRY WILL PROVIDE INSIGHTS INTO THE METABOLIC INTERACTIONS BETWEEN MITOCHONDRION AND APICOPLAST. EXPECTED OUTCOMES INCLUDE IDENTIFYING KEY PROTEINS THAT FACILITATE THIS INTERACTION, CONTRIBUTING TO AN IMPROVED UNDERSTANDING OF PARASITE BIOLOGY AND POTENTIALLY LEADING TO NEW THERAPEUTIC STRATEGIES AGAINST MALARIA. ADDITIONALLY, THE STUDY AIMS TO PROVIDE NOVEL THERAPEUTIC TARGETS FOR MALARIA CONTROL, AS BOTH THE APICOPLAST AND MITOCHONDRION ARE CRUCIAL FOR PARASITE BIOLOGY, UNDERSCORING THEIR RELEVANCE IN DEVELOPING EFFECTIVE TREATMENT STRATEGIES.

UNVEILING TST3, A MULTI-TARGET GATING MODIFIER SCORPION A TOXIN FROM TITYUS STIGMURUS VENOM OF NORTHEAST BRAZIL: EVALUATION AND COMPARISON WITH WELL-STUDIED TS3 TOXIN OF TITYUS SERRULATUS

JOÃO ANTONIO ALVES NUNES, DIOGO VIEIRA TIBERY, DANIEL OLIVEIRA DA MATA, LUIS FELIPE SANTOS MENEZES, ADOLFO CARLOS BARROS DE SOUZA, MATHEUS DE FREITAS FERNANDES-PEDROSA, ELISABETH FERRONI SCHWARTZ, WERNER TREPTOW

STUDIES ON THE INTERACTION SITES OF PEPTIDE TOXINS AND ION CHANNELS TYPICALLY INVOLVE SITE-DIRECTED MUTATIONS IN TOXINS. HOWEVER, NATURAL MUTANT TOXINS EXIST AMONG THEM, OFFERING INSIGHTS INTO HOW THE EVOLUTIONARY PROCESS HAS CONSERVED CRUCIAL SEQUENCES FOR ACTIVITIES AND MOLECULAR TARGET SELECTION. IN THIS STUDY, WE PRESENT A COMPARATIVE INVESTIGATION USING COMPUTATIONAL ANALYSIS BETWEEN TWO ALPHA TOXINS FROM EVOLUTIONARILY CLOSE SCORPION SPECIES OF THE GENUS TITYUS, NAMELY, TST3 AND TS3 FROM T. STIGMURUS AND T. SERRULATUS, RESPECTIVELY. THESE TOXINS EXHIBIT THREE NATURAL SUBSTITUTIONS NEAR THE C-TERMINAL REGION, WHICH IS DIRECTLY INVOLVED IN THE INTERACTION BETWEEN ALPHA TOXINS AND NAV CHANNELS. COMPUTATIONAL ANALYSIS DEMONSTRATED A PREFERENCE FOR THE DOWN CONFORMATION OF VSD4 AND A SHIFT IN THE CONFORMATIONAL EQUILIBRIUM TOWARDS THIS STATE. THIS ILLUSTRATES THAT THE SEQUENCE OF THESE TOXINS RETAINED THE NECESSARY INFORMATION, EVEN WITH ALTERATIONS IN THE INTERACTION SITE REGION. THROUGH COMPUTATIONAL ANALYSES, SCREENING OF THE TST3 TOXIN ON SODIUM ISOFORM REVEALED ITS CLASSIFICATION AS A CLASSIC A-NATX WITH A BROAD SPECTRUM OF ACTIVITY. STRUCTURAL ANALYSIS OF MOLECULAR ENERGETICS AT THE INTERFACE OF THE VSD4-TST3 COMPLEX FURTHER CONFIRMED THE DELAYS FAST INACTIVATION ACROSS ALL TESTED ISOFORMS.

EVALUATION OF SOLID LIPID NANOPARTICLES ASSOCIATED WITH DOCETAXEL IN AN IN VITRO AND IN VIVO MODEL OF CASTRATION-RESISTANT AND MULTIDRUG-RESISTANT PROSTATE CANCER

GIOVANNA AMARAL RODRIGUES; MARINA ARANTES RADICCHI; SÔNIA NAIR BÁO

PROSTATE CANCER (PCA) IS A MAJOR CAUSE OF MALE MORTALITY. THE HIGH MORTALITY RATE IS LINKED TO THE MORE ADVANCED STAGES OF THE DISEASE, ESPECIALLY WHEN PATIENTS WITH CASTRATION-RESISTANT PROSTATE CANCER (CRPC) ACQUIRE RESISTANCE TO DOCETAXEL (DTX) CHEMOTHERAPY. AVAILABLE THERAPIES CONTRIBUTE TO INCREASING SURVIVAL RATES, BUT ITS ADVERSE EFFECTS IMPACT SIGNIFICANTLY PATIENT'S QUALITY LIFE. THUS, NEW THERAPIES MUST BE CONSIDERED FOR THIS CLINICAL SCENARIO. OUR RESEARCH GROUP HAS DEVELOPED A SOLID LIPID NANOPARTICLE ASSOCIATED WITH DOCETAXEL (SLN-DTX) THAT HAS SHOWN PROMISE IN GASTRIC AND BREAST CARCINOMA MODELS. ONE OF ITS COMPONENTS IS THE COPOLYMER PLURONIC® 127, WHICH, ACCORDING TO THE LITERATURE, HAS AN INTERESTING ROLE IN MULTIDRUG-RESISTANT NEOPLASMS, SUCH AS P-GLYCOPROTEIN (P-GP) BLOCKADE, BLOCKING THE GLUTATHIONE/GLUTATHIONE-TRANSFERASE DETOXIFICATION SYSTEM, AND INCREASING LEVELS OF REACTIVE OXYGEN SPECIES (ROS). THEREFORE, THIS

STUDY AIMS TO EVALUATE THE EFFECTS OF SLN-DTX IN AN IN VITRO AND IN VIVO MODEL OF CASTRATION-RESISTANT AND MULTIDRUG-RESISTANT PROSTATE CANCER. THE FIRST STEP IN THIS PROCESS WILL BE THE GENERATION OF THE DTX-RESISTANT DU145 CELL LINE (DU145R). TO EVALUATE NANOPARTICLE INTERNALIZATION, TRANSMISSION ELECTRON MICROSCOPY (TEM) WILL BE PERFORMED. TO VERIFY IF THE TREATMENT WAS ABLE TO ATTENUATE THE RESISTANT PHENOTYPE, CELL VIABILITY ASSAYS, RHODAMINE 123 INTERNALIZATION, AND CELLULAR DETOXIFICATION SYSTEM ASSESSMENTS WILL BE CONDUCTED. THEVALUATIONN OF THE ANTITUMOR EFFECT OF THE TREATMENT WILL BE PERFORMED IN A XENOGRAFT MODEL OF DU145R CELLS. TUMOR VOLUME WILL BE MEASURED, AND HEMATOLOGICAL AND BIOCHEMICAL ANALYSES WILL BE CONDUCTED TO DETERMINE WHETHER THE TREATMENT IS TOXIC TO BONE MARROW, KIDNEY, AND LIVER CELLS. IT IS EXPECTED THAT SLN-DTX WILL ATTENUATE THE MECHANISMS CONFERRING CHEMORESISTANCE TO CANCER CELLS AND REDUCE TUMOR GROWTH IN MICE.

EFEITOS DA TOXINA AP1A ISOLADA DA PEÇONHA DA CARANGUEJEIRA ACANTHOSCURRIA PAULENSIS SOBRE A CONDUTÂNCIA DE CANAIS DE CÁLCIO DEPENDENTES DE VOLTAGEM

JONATHAN MARTINS DO NASCIMENTO, DIOGO VIEIRA TIBERY, ISRAEL FLOR SILVA DE ARAÚJO, ADOLFO CARLOS BARROS DE SOUZA E ELISABETH NOGUEIRA FERRONI

SPIDER VENOMS ARE COMPLEX MIXTURES OF BIOACTIVE MOLECULES, INCLUDING PEPTIDES WITH NOTABLE PHARMACOLOGICAL POTENTIAL. THESE PEPTIDES MODULATE VOLTAGE-GATED ION CHANNELS — PROTEINS THAT FORM PORES IN THE PLASMA MEMBRANE TO ENABLE THE SELECTIVE PASSAGE OF IONS, THEREBY GENERATING ELECTRICAL SIGNALS ESSENTIAL FOR CELLULAR COMMUNICATION AND THE COORDINATION OF PHYSIOLOGICAL PROCESSES. VOLTAGE-GATED CALCIUM CHANNELS (CAV) PLAY A CRITICAL ROLE NOT ONLY IN ELECTRICAL CONDUCTION BUT ALSO IN MUSCLE CONTRACTION, SYNAPTIC VESICLE FORMATION, GENE EXPRESSION REGULATION, ENZYMATIC ACTIVITY, AND VARIOUS OTHER ESSENTIAL BIOCHEMICAL PROCESSES. THE TOXIN AP1A, THE FIRST PEPTIDE PURIFIED FROM THE VENOM OF THE TARANTULA ACANTHOSCURRIA PAULENSIS, CONTAINS 48 AMINO ACID RESIDUES AND HAS A MONOISOTOPIC MASS OF [M + H]+ = 5457.79 DA. AP1A FEATURES THREE DISULFIDE BONDS, FORMING THE STRUCTURAL MOTIF KNOWN AS INHIBITORY CYSTINE KNOT (ICK), COMMON IN TOXINS CAPABLE OF MODULATING ION CHANNELS. THIS TOXIN INDUCES DOSE-DEPENDENT PARALYSIS IN SPODOPTERA FRUGIPERDA LARVAE, HIGHLIGHTING ITS BIOTECHNOLOGICAL POTENTIAL AGAINST AGRICULTURAL PESTS. IT ALSO INDUCES RESPIRATORY FAILURE AND DEATH IN MICE DUE TO SEIZURES, WHILE REDUCING BOTH FIRING FREQUENCY AND AMPLITUDE IN

THE DROSOPHILA MELANOGASTER GIANT FIBER CIRCUIT, SUGGESTING POTENTIAL ACTIVITY IN NEUROTRANSMITTER RELEASE AND INVOLVEMENT AT THE NEUROMUSCULAR JUNCTION. THESE RESULTS POINT TO A POSSIBLE INTERACTION WITH PRESYNAPTIC CAV, INFLUENCING GLUTAMATERGIC NEUROTRANSMITTER RELEASE. ALTHOUGH AP1A HAS BEEN TESTED IN VARIOUS ELECTROPHYSIOLOGICAL ASSAYS ACROSS DIFFERENT TYPES OF IONIC CHANNELS, ITS MOLECULAR TARGET REMAINS UNIDENTIFIED, AND ITS MECHANISM OF ACTION IS STILL UNCLEAR. THIS STUDY AIMS TO CHARACTERIZE THE ELECTROPHYSIOLOGICAL ACTIVITY OF AP1A ON CAV CHANNELS USING THE PATCH-CLAMP TECHNIQUE. BY EXPANDING KNOWLEDGE OF A. PAULENSIS VENOM COMPONENTS, THIS RESEARCH WILL EXPLORE THEIR PHARMACOLOGICAL POTENTIAL AND UTILITY AS MOLECULAR TOOLS.

TARGETING SARS-COV-2 MAIN PROTEASE (MPRO) WITH STRUCTURALLY DIVERSE METAL-BASED COMPLEXES

MATHEUS FERRONI SCHWARTZ, AMANDA SOUZA BERNASOL, LIEM CANET SANTOS, AISEL VALLE GARAY, SONIA MARIA DE FREITAS

WHILE COVID-19 IS NO LONGER CONSIDERED A PUBLIC HEALTH EMERGENCY OF INTERNATIONAL CONCERN, POTENTIAL SARS-COV-2 RESISTANCE TO CURRENTLY AVAILABLE THERAPEUTICS REMAINS A CONCERN FOR THE SCIENTIFIC COMMUNITY. THE MAIN PROTEASE OF SARS-COV-2 (MPRO) IS CONSIDERED THE MAIN TARGET ON THE DEVELOPMENT OF ANTIVIRALS DUE TO ITS ESSENTIAL ROLE IN VIRAL REPLICATION. TO OBTAIN THE MPRO, E.COLI BL21 (DE3) CELLS WERE TRANSFORMED WITH A PLASMID (PGEX-6P1) ENCODING A GST-MPRO-6XHIS FUSION CONSTRUCT WITH PRESCISSION PROTEASE CUT SITES IN BETWEEN MPRO AND THE FUSION TAGS. AFTER EXPRESSION, THE LYSATE WAS PURIFIED BY GST-GLUTATHIONE AND NI-AFFINITY CHROMATOGRAPHY. CLEAVAGE BY PRESCISSION PROTEASE DURING PURIFICATION YIELDED THE MPRO WITH THE NATIVE C- AND N-TERMINI. THE PROTEASE ACTIVITY OF MPRO WAS MEASURED BY KINETIC ASSAYS WITH THE SUBSTRATE MCA-AVLQSGFR-LYS(DNP)-LYS-NH2 AND KINETIC PARAMETERS WERE DETERMINED USING THE MICHAELIS–MENTEN EQUATION BY PLOTTING THE INITIAL VELOCITY OF THE LINEAR PORTION VERSUS THE SUBSTRATE CONCENTRATION TO OBTAIN THE MICHAELIS CONSTANT (KM). THE KM OBTAINED OF 3.54 µM WAS CONSISTENT WITH SIMILAR VALUES REPORTED IN THE LITERATURE. DYNAMIC LIGHT SCATTERING (DLS) EXPERIMENTS WERE PERFORMED TO DETERMINE THE OLIGOMERIC STATES OF MPRO. THE ENZYME WAS FOUND TO BE IN ITS HOMODIMERIC STATE, WHICH IS THE MOST ENZYMATICALLY ACTIVE. NEXT,

METAL COMPLEXES COMPOUNDS HARBORING DIFFERENT METAL IONS IN COMPLEX WITH STRUCTURALLY DIVERSE ORGANIC LIGANDS WERE ASSESSED AS INIHIBITORS. NIRMATRELVIR, A POTENT MPRO INHIBITOR, WAS UTILIZED AS A POSITIVE CONTROL. THE HALF-MAXIMAL INHIBITORY CONCENTRATION (IC50) WAS DETERMINED BY NONLINEAR REGRESSION ANALYSIS OF THE INITIAL VELOCITY VS INHIBITOR CONCENTRATION PLOT. TWO COPPER-SCHIFF BASE COMPLEXES SHOWED A REMARKABLE INHIBITION WITH A IC50 VALUE OF 0.5 μM. TO ACCESS THEIR PROMISCUITY TOWARDS OTHER ENZYMES AND TO INVESTIGATE IF THESE COMPOUNDS COULD BE ACTING AS AGGREGATORS, A TRYPSIN INHIBITION ASSAY WAS PERFORMED. BOTH COMPOUNDS FAILED TO INHIBIT TRYPSIN. FINALLY, TO DETERMINE THE STRUCTURE OF THE MPRO-INHIBITORS COMPLEXES, MPRO CRYSTALLIZATION ASSAYS WERE PERFORMED BY THE SITTING-DROP METHOD. A NEEDLE CLUSTER FORMATION COMPRISED OF MPRO CRYSTALLIZATION COULD BE OBSERVED AFTER A TWO WEEKS GROWTH PERIOD. THIS SEMI-CRYSTAL FORMATION WILL BE UTILIZED IN THE SCREENING BY SEEDING AND IN CO-CRYSTALLIZATION AND/OR SOAKING ASSAYS WITH THE COPPER-SCHIFF INHIBITORS.

ANTIMICROBIAL AND HEMOLYTIC ACTIVITY OF ANTIMICROBIAL PEPTIDES ISOLATED FROM THE SKIN SECRETION OF THE ANURAN PHYSALAEMUS CICADA

NATANAEL SALES SILVA; GISLENE FERREIRA BAPTISTA; CARLOS JOSÉ CORREIA SANTANA; OSMINDO RODRIGUES PIRES JR.; MARIANA DE SOUZA CASTRO.

THE INCREASE IN BACTERIAL RESISTANCE IS A GROWING CHALLENGE FOR PUBLIC HEALTH, MAKING THE ELIMINATION OF THESE MICROORGANISMS INCREASINGLY DIFFICULT. AS A RESULT, THERE IS AN URGENT NEED TO DEVELOP NEW THERAPEUTIC STRATEGIES AND DRUGS CAPABLE OF EFFECTIVELY COMBATING RESISTANT BACTERIA. A PROMISING ALTERNATIVE IS ANTIMICROBIAL PEPTIDES, NATURAL COMPOUNDS KNOWN FOR THEIR ABILITY TO FIGHT BACTERIAL AND FUNGAL INFECTIONS. THIS STUDY AIMED TO EVALUATE THE ANTIMICROBIAL AND HEMOLYTIC ACTIVITY OF FOUR ANTIMICROBIAL PEPTIDES ISOLATED FROM THE SKIN SECRETION OF THE ANURAN PHYSALAEMUS CICADA, AN ENDEMIC SPECIES FROM BRAZIL. THE PEPTIDES, NAMED PC24, PC26.1, PC26.2, AND PC28, WERE IDENTIFIED BASED ON THE PEAKS OBTAINED BY LIQUID CHROMATOGRAPHY. TO ASSESS THEIR ANTIMICROBIAL PROPERTIES, MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAYS WERE PERFORMED AGAINST THE GRAM-NEGATIVE BACTERIUM ESCHERICHIA COLI, THE GRAM-POSITIVE BACTERIUM STAPHYLOCOCCUS AUREUS, AND THE YEAST CANDIDA ALBICANS. THE RESULTS INDICATED GOOD ACTIVITY AGAINST E. COLI, WITH MICS OF 2 μM FOR PC24, PC26.1, AND PC26.2, AND 16 μM FOR PC28. REGARDING C. ALBICANS, PC28 SHOWED EFFECTIVENESS (32 μM), FOLLOWED BY PC26.1 (32 μM) AND PC26.2 (64 μM). NO PEPTIDE SHOWED SIGNIFICANT ACTIVITY AGAINST S. AUREUS AT THE TESTED

CONCENTRATIONS, WITH HIGH MIC (>128 μM). HEMOLYSIS EVALUATION SHOWED THAT THE PEPTIDES CAUSED LESS THAN 5% DESTRUCTION OF HUMAN ERYTHROCYTES AT THE OBTAINED MICS, INDICATING A LOW RISK OF CELLULAR TOXICITY. IN SUMMARY, THE ANTIMICROBIAL PEPTIDES ISOLATED FROM P. CICADA DEMONSTRATED POTENTIAL FOR THE DEVELOPMENT OF NEW THERAPEUTIC AGENTS, ESPECIALLY FOR COMBATING INFECTIONS CAUSED BY E. COLI AND C. ALBICANS, WITH A LOW RISK OF DAMAGING HUMAN CELLS.

ATOMISTIC STUDY OF ION CHANNEL MODULATION BY VOLATILE GENERAL ANESTHETICS

JOÃO VICTOR BADARÓ DE MORAES, WERNER TREPTOW

THE ATOMISTIC STUDY OF HOW VOLATILE GENERAL ANESTHETICS MODULATE ION CHANNELS ADDRESSES A LONG-STANDING MYSTERY IN MEDICINE. DESPITE NEARLY TWO CENTURIES OF CLINICAL USE, THE MECHANISMS BY WHICH THESE ANESTHETICS EXERT THEIR EFFECTS REMAIN UNCLEAR. TWO MAIN HYPOTHESES HAVE EMERGED: THE INDIRECT HYPOTHESIS, SUGGESTING THAT ANESTHETICS ALTER MEMBRANE PROPERTIES, AND THE DIRECT HYPOTHESIS, WHICH POSITS DIRECT MODULATION OF PROTEINS LIKE ION CHANNELS. EVIDENCE FOR BOTH HYPOTHESES INDICATES THEY MAY NOT BE MUTUALLY EXCLUSIVE, NECESSITATING AN INTEGRATED APPROACH THAT CHALLENGES EXISTING FRAMEWORKS. OUR RESEARCH GROUP HAS DEVELOPED A COMPUTATIONAL MODEL THAT TREATS ANESTHETIC INTERACTIONS AS A PARTITION PHENOMENON, RATHER THAN CLASSIC HIGH-AFFINITY LIGAND BINDING. APPLYING THIS MODEL TO THE POTASSIUM CHANNEL KV1.2, WE OBSERVED THAT ANESTHETIC BINDING IS HIGHLY DEPENDENT ON THE CHANNEL'S CONFORMATIONAL STATE, SIGNIFICANTLY IMPACTING ITS EQUILIBRIUM. WE PROPOSE THAT ANESTHETICS ACT THROUGH STATE-DEPENDENT, DEGENERATE INTERACTIONS ON THE SURFACES OF MULTIPLE ION CHANNELS, STABILIZING SPECIFIC CONFORMATIONAL STATES AND DISRUPTING THEIR PHYSIOLOGICAL FUNCTION. TO TEST THIS HYPOTHESIS, WE ARE BUILDING AN ANNOTATED DATABASE OF ION CHANNEL STRUCTURES IN VARIOUS CONFORMATIONAL STATES, INTEGRATING BOTH EXPERIMENTAL DATA AND ADVANCED MOLECULAR MODELING TECHNIQUES. WE WILL SYSTEMATICALLY INVESTIGATE HOW DIFFERENT VOLATILE ANESTHETICS INTERACT WITH ION CHANNELS, ANALYZING THESE INTERACTIONS AS PARTITION PHENOMENA. THIS RESEARCH AIMS TO UNCOVER, AT THE ATOMIC LEVEL, THE MOLECULAR PRINCIPLES UNDERLYING THE ACTION MECHANISM OF VOLATILE GENERAL ANESTHETICS.

APPLICATION OF RAMAN SPECTROSCOPY IN THE CHARACTERIZATION OF BIOMATERIALS FOR 3D BIOPRINTING

GABRIELA MENDES DA ROCHA VAZ E LUCIANO PAULINO DA SILVA

RAMAN SPECTROSCOPY IS A WIDELY USED ANALYTICAL TOOL FOR THE CHARACTERIZATION OF BIOMATERIALS, WITH POTENTIAL APPLICATIONS IN THE FIELD OF 3D BIOPRINTING. BASED ON INELASTIC LIGHT SCATTERING, THIS TECHNIQUE PROVIDES DETAILED INFORMATION ABOUT THE MOLECULAR COMPOSITION, CHEMICAL INTERACTIONS, AND STRUCTURAL ORGANIZATION OF ANALYZED MATERIALS, MAKING IT PARTICULARLY USEFUL IN TISSUE ENGINEERING. IN 3D BIOPRINTING, THE USE OF RAMAN SPECTROSCOPY ENABLES THE EVALUATION OF BIOMATERIAL COMPOSITION, MONITORING OF CHEMICAL CHANGES THAT MAY OCCUR DURING THE BIOPRINTING PROCESS, AND INVESTIGATION OF BIOMARKERS AND CELL-MATRIX INTERACTIONS, AIDING IN THE ANALYSIS OF THE CHEMICAL MICROENVIRONMENTS OF CELLS. THIS APPROACH CONTRIBUTES TO THE OPTIMIZATION OF BIOMATERIALS AND PROCESSES, ENSURING THAT BIOPRINTED CONSTRUCTS HAVE THE DESIRED PROPERTIES FOR THERAPEUTIC AND RESEARCH APPLICATIONS. IN THIS STUDY, DIFFERENT BIOPOLYMERS WERE ANALYZED TO IDENTIFY THEIR CHARACTERISTIC SPECTRA IN ISOLATION, INDEPENDENT OF THE BIOPRINTING PROCESS, WHICH WILL SERVE AS A REFERENCE FOR FUTURE STUDIES. SUBSEQUENTLY, THE BIOMATERIALS WILL BE COMBINED WITH CELLS AND APPLIED TO THE BIOPRINTING PROCESS. THE BIO-STRUCTURES OBTAINED FROM THIS PROCESS WILL BE EVALUATED FOR CHEMICAL CHANGES, THE PRESENCE OF BIOMARKERS, AND THE DISTRIBUTION OF COMPONENTS IN THE 3D MICROENVIRONMENT. THIS APPROACH WILL ALLOW MONITORING OF MOLECULAR ORGANIZATION AND POTENTIAL CELL-MATRIX INTERACTIONS, WHICH ARE FUNDAMENTAL ASPECTS FOR UNDERSTANDING THE FUNCTIONALITY OF THE DEVELOPED BIOMIMETICS. THE RESULTS OBTAINED SO FAR DEMONSTRATE THAT RAMAN SPECTROSCOPY IS A PROMISING TOOL FOR CHARACTERIZING BIOMATERIALS USED IN BIOPRINTING. THIS TECHNIQUE WILL CONTRIBUTE TO VALIDATING THE BIOPRINTING PROCESS AND EXPLORING THE POTENTIAL OF 3D MODELS AS ANALYTICAL AND THERAPEUTIC TOOLS.

MULTISTRESS RESILIENCE AND REDOX METABOLISM IN INTERTIDAL MARINE INVERTEBRATES

MARINA MINARI; DANIEL CARNEIRO MOREIRA; FELIPE DIEGO MEDEIROS DE SOUSA; MARCELO HERMES-LIMA

COASTAL ENVIRONMENTS CHALLENGE INVERTEBRATE BIODIVERSITY WITH DAILY FLUCTUATIONS IN TEMPERATURE AND UV RADIATION, STRESS FROM AERIAL EXPOSURE DURING LOW TIDES, SEASONAL VARIATIONS IN SALINITY, PH, ORGANIC MATTER, WATER TURBIDITY, AND PHYSICAL DISTURBANCES LIKE WAVES AND WINDS. MOREOVER, THEY COPE WITH POTENTIAL POLLUTANTS NEAR URBAN AREAS. INTERTIDAL ANIMALS HAVE DEVELOPED PHYSIOLOGICAL ADAPTATIONS FOR SURVIVAL. COASTAL ENVIRONMENTS

THUS REPRESENT A MULTI-STRESSOR SCENARIO, WHERE METABOLIC RESPONSES MAY DEPEND ON THE SIMULTANEOUS OCCURRENCE OF MULTIPLE FLUCTUATIONS. UNDERSTANDING THEIR RESPONSES REQUIRES EMPLOYING THE NATURAL EXPERIMENT METHODOLOGY, IN WHICH RESEARCHERS DO NOT INTERFERE WITH THE PHYSICAL/ABIOTIC VARIABLES. OUR RESEARCH FOCUSED ON THE REDOX RESPONSES OF THREE DISTINCT INTERTIDAL SPECIES IN THE NATURAL ENVIRONMENT: SUN SPONGES HYMENIACIDON HELIOPHILA, MUSSELS BRACHIDONTES SOLISIANUS, AND ADULT SEA URCHINS ECHINOMETRA LUCUNTER. RESPONSES VARIED AMONG SPECIES. SPONGES SHOWED A DYNAMIC REDOX RESPONSE WITH INCREASED CATALASE AND TBARS AFTER 10 MINUTES OF REOXYGENATION, I.E., UPON THE RETURN OF THE HIGH TIDE, FOLLOWING 2H OF AERIAL EXPOSURE. MUSSELS AND SEA URCHINS EXHIBITED THE “PREPARATION FOR OXIDATIVE STRESS” (POS) STRATEGY, INCREASING ANTIOXIDANTS DURING ENVIRONMENTAL STRESS TO PROTECT AGAINST OXIDATIVE DAMAGE DURING REOXYGENATION. FOR MUSSELS, A HIGH UV INCIDENCE WAS NECESSARY TO TRIGGER THE POS RESPONSE WITH THE UPREGULATION OF GLUTATHIONE DURING AERIAL EXPOSURE. CONVERSELY, AERIAL EXPOSURE PROVED TO BE THE VARIABLE MOST SIGNIFICANTLY INFLUENCING THE METABOLIC REDOX RESPONSE FOR SEA URCHINS, WITH THE INCREASE OF INTESTINAL GLUTATHIONE TRANSFERASE DURING LOW TIDE. THESE FINDINGS HIGHLIGHT THE IMPORTANCE OF CONDUCTING STUDIES IN NATURAL SETTINGS TO UNDERSTAND THE REAL METABOLIC RESPONSES OF ADAPTED ANIMALS TO STRESSORS. RESEARCH LICENSED BY ICMBIO AND SISBIO (PERMIT NUMBERS 61407-1; 20030-9 AND 28917-1).

STATISTICAL INVESTIGATION OF TRUE-POSITIVE PROTEIN PARTNERS IN COEVOLUTIONARY APPROACHES

JOSÉ ANTONIO FIOROTE, JOÃO ALVES, WERNER TREPTOW

PHYSICAL INTERACTIONS IN PROTEINS ARE MAINTAINED THROUGHOUT EVOLUTION VIA COMPENSATORY MUTATIONS. AS EXTENSIVELY INVESTIGATED IN RECENT YEARS, THE COEVOLUTIONARY SIGNAL IS CONSIDERED HIGHLY RELEVANT FOR THE AB INITIO RESOLUTION OF SPECIFIC PROTEIN PARTNERS BASED ON MULTIPLE SEQUENCE ALIGNMENTS (MSAS). DESPITE RECENT ADVANCES IN THE FIELD, PRIMARILY ROOTED IN MUTUAL INFORMATION I CORRELATION ANALYSIS, THE PREDICTIVE PROBLEM OF PROTEIN PARTNERS REMAINS UNSOLVED FOR SEQUENCE ENSEMBLES IN GENERAL. THIS IS PRIMARILY BECAUSE THERE IS NO EFFECTIVE NON-DEGENERATE HEURISTIC TO SEARCH FOR THE CORRECT SET OF PROTEIN PARTNERS ACROSS THE IMMENSE SPACE OF POSSIBILITIES INHERENT IN THIS TYPE OF PROBLEM. IN RECENT PUBLICATIONS, WE SHOWN GENETIC ALGORITHM SIMULATIONS THAT START FROM MINIMUM MUTUAL INFORMATION FAIL AT PAIRING NATIVE SEQUENCES CORRECTLY IN A SYSTEM

WITH TWO MSAS AFTER MUTUAL INFORMATION MAXIMIZATION. THESE ERRORS ARISING FROM MISMATCHES AMONG (I) SIMILAR AND (II) NON-SIMILAR SEQUENCES. HOWEVER, A QUANTITATIVE DESCRIPTION IS LACKING IN THE SCIENTIFIC COMMUNITY. THUS, TRYING TO ELUCIDATE DEGENERATION OF I IN PROTEIN INTERACTION SPACE, WE CONTRIBUTE HERE A STATISTICAL FRAMEWORK TO DESCRIBE THE PROBABILITY DISTRIBUTION OF INTERACTION MODELS OF PROTEINS A AND B FOR A LARGE NUMBER OF SEQUENCES M THAT FEATURE A UNIQUE “NATIVE” ARRANGEMENT (‘) AT A MAXIMUM MUTUAL INFORMATION CONTENT. OUR SPECIFIC AIM IS THE QUANTITATIVE DESCRIPTION OF THE MUTUAL INFORMATION SIMULATED NATIVE→SCRAMBLED→NEAR-NATIVE.

BIOPROSPECTING OF FUNGAL ENZYMES FOR APPLICATION IN ANIMAL NUTRITION

VINÍCIUS ROCHA CARDOZO DA SILVA; ELIANE FERREIRA NORONHA; BRENDA RABELLO DE CAMARGO; YASMIN FOLHA; TAVARES BETÂNIA FERRAZ QUIRINO; DASCIANA DE SOUSA RODRIGUES

BRAZIL OFFERS IDEAL CONDITIONS FOR DEVELOPING ITS BIOECONOMY DUE TO ITS VAST MICROBIAL BIODIVERSITY, WHICH SERVES AS A SOURCE FOR NUMEROUS BIOPRODUCTS. THE MICROBIAL DIVERSITY OF THE CERRADO, THE PREDOMINANT BIOME IN BRAZIL'S CENTRAL REGION, HAS BEEN STUDIED TO IDENTIFY COMMERCIALLY RELEVANT BIOINPUTS. AMONG THESE, LIGNOCELLULOLYTIC ENZYMES FROM FILAMENTOUS FUNGI ISOLATED FROM CERRADO SOIL HOLD POTENTIAL AS ZOOTECHNICAL ADDITIVES (ENZYME COCKTAILS) FOR RUMINANTS. THIS STUDY FOCUSED ON SCREENING THE BEST LIGNOCELLULOLYTIC SECRETOMES OF 21 FUNGAL ISOLATES FROM CERRADO SOIL FOR APPLICATION IN THE ANIMAL NUTRITION INDUSTRY. ENZYMATIC ACTIVITIES, INCLUDING ENDOGLUCANASE, XYLANASE, B-GLUCOSIDASE, AND FPASE, WERE ANALYZED TO IDENTIFY ELITE ORGANISMS FOR HYDROLYZING BRACHIARIA BRIZANTHA CV. MARANDU, A GRASS WIDELY USED IN GRAZING SYSTEMS FOR RUMINANTS. THE SELECTION PROCESS FOR THE ENZYMATIC CHASSIS INCLUDED COMPARISONS WITH TWO EMBRAPA AGROENERGIA FUNGAL STRAINS (147T AND 79(11A)), EVALUATIONS OF COMMERCIAL ENZYME COCKTAILS, CULTIVATION IMPROVEMENT TESTS, AND PROTEASE ACTIVITY ASSESSMENTS OF THE SECRETOMES. AMONG THE 23 ORGANISMS TESTED, ISOLATE 19 (TALAROMYCES) AND TRICHODERMA HARZIANUM SHOWED SUPERIOR PERFORMANCE IN THE PROPOSED EXPERIMENTS. THESE ISOLATES ARE SET FOR FURTHER EVALUATION THROUGH IN VITRO RUMEN DIGESTION ASSAYS IN COLLABORATION WITH EMBRAPA GADO DE CORTE. FINALLY, MOLECULAR IDENTIFICATION OF THE ELITE ISOLATES (5, 7, 19 AND 29) FROM THE UNIVERSITY OF BRASÍLIA COLLECTION WAS CONDUCTED USING ITS, B-TUBULIN, AND CALMODULIN GENE MARKERS. THIS WORK HIGHLIGHTS THE

POTENTIAL OF CERRADO-DERIVED FUNGAL ISOLATES AS BIORESOURCES FOR SUSTAINABLE SOLUTIONS.

TRANSFORMATION, CLONE SELECTION, AND HETEROLOGOUS EXPRESSION OF SERINE PROTEASES FROM THE VENOMS OF ANIMALS IN THE TOXICOFERA CLADE

ISABELA FERNANDES REZENDE, JÚLIA FREITAS DALTRO VIDAL, JOÃO ALEXANDRE RIBEIRO GONÇALVES BARBOSA

DESPITE THE EXTENSIVE KNOWLEDGE ABOUT SNAKES AND THEIR VENOMS, INFORMATION REGARDING LIZARD VENOMS REMAINS LIMITED, AS THEIR DISCOVERY IS RELATIVELY RECENT. IT IS KNOWN THAT LIZARD VENOMS EXHIBIT A TOXICOLOGICAL PROFILE SIMILAR TO THAT OF SNAKES, INCLUDING THE PRESENCE OF SERINE PROTEASES. THESE ENZYMES HAVE VAST BIOTECHNOLOGICAL POTENTIAL DUE TO THEIR ABILITY TO INTERACT WITH VARIOUS POINTS OF THE BLOOD COAGULATION CASCADE. THIS OCCURS BECAUSE, DESPITE THEIR CONSERVED STRUCTURE, VARIATIONS IN AMINO ACID SEQUENCES ALLOW THEM TO INTERACT SPECIFICALLY WITH DIFFERENT SUBSTRATES. IN THIS CONTEXT, THE PRESENT PROJECT AIMS TO STRUCTURALLY AND ENZYMATICALLY CHARACTERIZE THREE SERINE PROTEASES FROM VENOMS: AN ANCESTRAL SVSP (SNAKE VENOM SERINE PROTEASE) FROM THE VIPERIDAE FAMILY, ANV_TOX, CONSTRUCTED THROUGH ANCESTRAL SEQUENCE RECONSTRUCTION; HS_TOX1, FROM THE VENOM OF THE GILA MONSTER; AND VK_TOX1, FROM THE VENOM OF THE KOMODO DRAGON. STRUCTURAL OR ENZYMATIC DESCRIPTIONS OF THESE PROTEINS ARE NOT YET AVAILABLE IN THE LITERATURE. CELLS OF THE X-33 STRAIN OF KOMAGATAELLA PHAFFII WERE TRANSFORMED VIA ELECTROPORATION WITH PPICZA VECTORS CONTAINING THE CODING GENE FOR HS_TOX1 OR VK_TOX1. THE TRANSFORMANTS WERE PLATED ON YPD MEDIUM CONTAINING ZEOCIN, USED AS A SELECTION MARKER. A TOTAL OF 96 TRANSFORMANT COLONIES WERE OBTAINED FOR EACH GENE. SCREENING ASSAYS ARE CURRENTLY UNDERWAY TO SELECT THE CLONE WITH THE HIGHEST PRODUCTION YIELD. K. PHAFFII CELLS HAD BEEN PREVIOUSLY TRANSFORMED WITH THE CODING GENE FOR ANV_TOX IN EARLIER EXPERIMENTS. IN THE CURRENT STUDY, THESE CELLS WERE USED FOR MG-SCALE EXPRESSION OF THE ENZYME, WHICH WAS SUBSEQUENTLY PURIFIED THROUGH IMMOBILIZED METAL ION CHROMATOGRAPHY USING A NICKEL COLUMN, FOLLOWED BY DESALTING. THE PURIFIED ENZYME WAS DEGLYCOSYLATED TO PREPARE IT FOR CRYSTALLIZATION ASSAYS.

ARE ENDOGENOUS ANTIOXIDANTS RELEVANT FOR TARDIGRADE SURVIVAL DURING ANHYDROBIOSIS AND RECOVERY?

FELIPE DIEGO MEDEIROS DE SOUSA; MARINA MINARI ROCHA DE CARVALHO; DANIEL CARNEIRO MOREIRA; CARLOS ANDRE ORNELAS RICART; MARCELO HERMES LIMA

TARDIGRADES, KNOWN FOR THEIR RESILIENCE TO ENDURE EXTREME CONDITIONS, CAN ENTER A CRYPTOBIOTIC (TUN) STATE TO WITHSTAND HARSH ENVIRONMENTS. DURING RECOVERY FROM TUN, THE GENERATION OF REACTIVE OXYGEN SPECIES (ROS) MAY INCREASE, REQUIRING EFFECTIVE ANTIOXIDANT SYSTEMS TO ENSURE SURVIVAL AGAINST OXIDATIVE DAMAGE. HOWEVER, THE MECHANISMS BY WHICH TARDIGRADES REGAIN ACTIVITY AFTER TUN REMAIN POORLY DESCRIBED. THIS STUDY ASSESSED THE IMPORTANCE OF ANTIOXIDANT SYSTEMS INVOLVING CATALASE (CAT) AND GLUTATHIONE (GSH) FOR THE SURVIVAL OF THESE ORGANISMS, USING SPECIFIC INHIBITORS TO SUPPRESS THESE PATHWAYS AND ANALYZE THE IMPACTS ON THE RETURN TO METABOLIC ACTIVITY. AMINOTRIAZOLE (ATZ), A CAT INHIBITOR, AND BUTHIONINE SULFOXIMINE (BSO), WHICH DEPLETES GSH LEVELS, WERE USED TO UNDERSTAND HOW THE SEVERE INHIBITION OF THESE ANTIOXIDANT SYSTEMS AFFECTS THE REDOX BALANCE. ROS FLUORESCENCE WAS ANALYZED USING THE DCFDA PROBE IN CONFOCAL MICROSCOPY AND MONITORED KINETICALLY WITH THE CELLYTE X. A HIGHER ACCUMULATION OF ROS WAS OBSERVED IN THE ATZ-TREATED GROUPS, ESPECIALLY DURING RECOVERY, INDICATING THAT CAT IS CRUCIAL FOR MITIGATING OXIDATIVE STRESS. HOWEVER, GSH DEPLETION CAUSED EVEN MORE PRONOUNCED OXIDATIVE STRESS, EVIDENCED BY INCREASED FLUORESCENCE AND HIGHER POST-RECOVERY LETHALITY. THESE RESULTS INDICATE THAT THE SUCCESSFUL RECOVERY OF TARDIGRADES FROM THE TUN DEPENDS ON THE INTERPLAY OF MULTIPLE ANTIOXIDANTS. GSH HAS AN IMPORTANT ROLE IN MAINTAINING REDOX BALANCE AS A REDOX BUFFER, WHILE CAT IS ESSENTIAL FOR DECOMPOSING H_2O_2. THESE DATA SUPPORT THE THEORY OF PREPARATION FOR OXIDATIVE STRESS (POS) FOR INVERTEBRATE SURVIVAL UNDER DEHYDRATION, SHOWING THAT SUPPRESSING ANTIOXIDANT ACTIVITY IRREVERSIBLY COMPROMISES SURVIVAL UNDER EXTREME STRESS CONDITIONS. THIS STUDY CONTRIBUTES TO UNDERSTANDING THE MOLECULAR MECHANISMS UNDERLYING CRYPTOBIOSIS IN TARDIGRADES AND POTENTIAL APPLICATIONS IN DEVELOPING ANTIOXIDANT STRATEGIES FOR ORGANISMS EXPOSED TO SEVERE ENVIRONMENTAL CONDITIONS.

IN SILICO ASSAYS OF THE ASPERGILLUS FUMIGATUS AND CANDIDA AURIS SMTS AND TRANSFORMATION OF E. COLI FOR HETEROLOGOUS EXPRESSION OF THE CRYPTOCOCCUS NEOFORMANS SMT

LUANA FERNANDES ROSA CAVALCANTE OLIVEIRA1; GIDEANE MENDES DE OLIVEIRA1; MARIA SUELI SOARES FELIPE2; JOÃO ALEXANDRE RIBEIRO GONÇALVES BARBOSA1

INVASIVE FUNGAL INFECTIONS (IFDS) ARE ESTIMATED TO AFFECT 300 MILLION PEOPLE ANNUALLY, CAUSING AROUND 1.6 MILLION DEATHS, AND ARE CONSIDERED BY THE WORLD HEALTH ORGANIZATION TO BE A RISING GLOBAL HEALTH CONCERN. THE EMERGENCE OF IFDS CORRELATES TO A RISE OF THE AT-RISK POPULATIONS AND POSES A SERIES OF CHALLENGES INVOLVING THEIR DIAGNOSIS AND TREATMENT. THESE CIRCUMSTANCES CALL FOR A GREATER EFFORT TO BE DIRECTED TOWARDS THESE DISEASES, INCLUDING THE DEVELOPMENT OF NEW ANTIFUNGAL DRUGS. CONSIDERING THIS SCENARIO, THIS WORK AIMS TO CHARACTERIZE STEROL C-24 METHYLTRANSFERASE ENZYMES (SMTS), A PUTATIVE PHARMACEUTICAL TARGET FOR WHICH NO SOLVED STRUCTURES ARE AVAILABLE, FROM THREE SPECIES OF MEDICAL IMPORTANCE: ASPERGILLUS FUMIGATUS (AFSMT), CANDIDA AURIS (CAUSMT) AND CRYPTOCOCCUS NEOFORMANS (CNSMT). E. COLI BL21 (DE3) CELLS TRANSFORMED WITH PET28A VECTORS CONTAINING THE ERG6 GENE CODING FOR AFSMT OR CAUSMT WERE USED IN THE HETEROLOGOUS EXPRESSION OF THESE PROTEINS IN SELF-INDUCING MEDIA. PROTEINS WERE PURIFIED THROUGH IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY IN NICKEL COLUMNS. AFSMT AND CAUSMT WERE ASSAYED FOR THEIR STABILITY IN CRYO-EM IMAGING IN BOTH THEIR FREE AND LIGAND-BOUND STATES WITH SAM. RESULTS SHOWED A POSSIBLE DISSOCIATION OF THE PROTEIN OLIGOMERS IN THE CONCENTRATIONS TESTED. AN IN SILICO MODEL OF CAUSMT COMPLEXED WITH SAM WAS USED FOR MOLECULAR DYNAMICS SIMULATIONS TO EVALUATE CONFORMATIONAL CHANGES ON BOTH PROTEIN AND LIGAND, AND INTERACTION OF KEY RESIDUES TO SAM. CONSIDERABLE ROTATION WAS OBSERVED ON THE DIHEDRAL ANGLES ALONG THE METHIONINE MOIETY OF SAM. THE MODEL WAS ALSO USED TO SELECT RESIDUES FOR MUTATION TO ANALYZE CHANGES IN SUBSTRATE SPECIFICITY. PREVIOUS EXPERIMENTS HAD SHOWN CNSMT EXPRESSION IN BL21 (DE3) CELLS TO BE CONTAINED IN THE INSOLUBLE FRACTIONS, SO TRANSFORMATION PROTOCOLS WERE REPEATED FOR CNSMT-PET28A IN BL21, PLYSS, PLYSE, PGRO, LEMO21 AND ARCTIC EXPRESS E. COLI STRAINS GROWN IN MINIMAL MEDIUM.

BIOINFORMATICS WORKFLOWS FOR EXTRACHROMOSOMAL CIRCULAR DNA ANALYSIS

JÚLIA ALVES LUZ, GEORGIOS JOANNIS PAPPAS JUNIOR

EXTRACHROMOSOMAL CIRCULAR DNAS (ECCDNAS) ARE DOUBLE STRANDED, NUCLEAR, CIRCULARISED DNA MOLECULES FOUND IN ALL EUKARYOTIC ORGANISMS INVESTIGATED SO FAR. SINCE THEIR DISCOVERY IN THE 1960S, CERTAIN CHARACTERISTICS OF ECCDNAS HAVE BEEN ILLUMINATED; THEIR INDEPENDENT REPLICATION OUTSIDE OF CHROMOSOMES, VARIATION IN SIZE AND QUANTITY, AND DIVERSITY OF GENETIC CONTENT - SOME EVEN HARBOURING PROTEIN CODING GENES AND DRIVING EXPRESSION AMPLIFICATION. THESE CHARACTERISTICS HAVE LED TO ECCDNAS GAINING THE SPOTLIGHT AS POTENTIAL CANCER AND AGEING RESEARCH TARGETS DUE TO THEIR APPARENT CORRELATION WITH GENOMIC INSTABILITY. NEXT-GENERATION SEQUENCING STRATEGIES HAVE RECENTLY BEEN USED TO CHARACTERISE ECCDNAS IN DIFFERENT SPECIES AND CELL TYPES, YET SO FAR, FEW PROGRAMS HAVE BEEN PUBLISHED TO ANALYSE ECCDNAS FROM NANOPORE SEQUENCING DATA. EACH PROGRAM IS UNIQUE IN TERMS OF INSTALLATION REQUIREMENTS, EXECUTION PARAMETERS AND OUTPUT FORMATS, MAKING USABILITY, TOOL COMPARISON AND DATA INTERPRETATION DIFFICULT FOR THE FINAL USER. OUR OBJECTIVE IS TO DEVELOP A PIPELINE THAT AUTOMATES THE EXECUTION OF FOUR DIFFERENT ECCDNA DETECTION AND PROCESSING TOOLS: FLEC, ECC_FINDER, CYRCULAR-CALLING AND CRESIL. WE UTILISED THE NEXTFLOW DOMAIN-SPECIFIC LANGUAGE TO COORDINATE THE EXECUTION AND PROCESSING OF RESULTS FOR EACH PROGRAM AND LEVERAGED COMPUTATIONAL CONTAINERS TO ENSURE SEAMLESS INSTALLATION. WE TESTED THE PIPELINE USING NANOPORE SEQUENCING DATA FROM HUMAN FIBROBLAST SAMPLES. UNDER DEFAULT SETTINGS, PRELIMINARY RESULTS SHOW THE NUMBER OF PREDICTED ECCDNAS VARIED AMONG THE PROGRAMS. FURTHER COMPARISON AND CONSOLIDATION OF THESE PREDICTIONS RESULTED IN A CONSENSUS SET OF ECCDNAS FOR EACH SAMPLE. THESE SETS CAN BE USED FOR ANNOTATION AND FURTHER BIOLOGICAL INTERPRETATION. ADDITIONALLY, WE ARE IN THE PROCESS OF GENERATING GRAPHICAL REPORTS THAT VISUALISE THE ANNOTATIONS AND GENOMIC CONTEXTS, WHICH WILL AID IN THE INTERPRETATION OF ECCDNA-RELATED RESEARCH.

ISOLATION AND CHARACTERIZATION OF ANAEROBIC LIGNIN-CONVERTING BACTERIA

ROSÁLIA LORIANO DE SANTANA, BRENDA RABELLO DE CAMARGO, ELIANE FERREIRA NORONHA

THE GLOBAL CRISIS OF WASTE ACCUMULATION HIGHLIGHTS THE NEED FOR A SUSTAINABLE APPROACH TO UTILIZING INDUSTRIAL BYPRODUCTS, SUCH AS KRAFT LIGNIN, A BYPRODUCT OF PULP AND PAPER INDUSTRIES. LIGNIN IS A PHENOLIC MACROMOLECULE, AND ITS DECONSTRUCTION CAN GENERATE VARIOUS AROMATIC COMPOUNDS OF INDUSTRIAL INTEREST FOR

PHARMACEUTICAL AND CHEMICAL APPLICATIONS. PREVIOUS STUDIES HAVE REPORTED THAT THE BOVINE RUMEN HOSTS A MICROBIOTA SPECIALIZED IN LIGNOCELLULOSE DEGRADATION. THEREFORE, THE USE OF MICROORGANISMS IN LIGNIN DECONSTRUCTION COULD BE HIGHLY VALUABLE FOR ADDING VALUE TO THIS MATERIAL. THE OBJECTIVE IS TO IDENTIFY AND CHARACTERIZE ANAEROBIC BACTERIA CAPABLE OF DECONSTRUCTING KRAFT LIGNIN TO ENHANCE ITS VALUE. NINETEEN BACTERIAL ISOLATES FROM THE CONSORTIUM WERE ANALYZED FOR THEIR DISCOLORATION AND DEGRADATION RATES OVER FOUR DAYS. THE HIGHEST DISCOLORATION VALUES FOR KRAFT LIGNIN WERE ACHIEVED BY ISOLATES IY 2-3, IL 2-2, AND ICE 6, WITH RATES OF 66.6%, 54.35%, AND 54.46%, RESPECTIVELY. ALL ISOLATES WILL BE IDENTIFIED THROUGH SEQUENCING OF THE 16S RDNA GENE. ISOLATE IY 2-3, WHICH SHOWED THE HIGHEST DISCOLORATION AND DEGRADATION RATES, WAS SELECTED, AND THE 24-HOUR CULTURE SUPERNATANT WAS USED TO ESTABLISH THE METHOD FOR PROTEIN EXTRACTION. THIS WILL BE ANALYZED BY MASS SPECTROMETRY TO OBTAIN A PROTEOMIC MAP. FURTHER ANALYSES ARE PLANNED TO VISUALIZE AND QUANTIFY THE MODIFICATION AND/OR HYDROLYSIS OF THE LIGNIN MOLECULE AND ITS PRODUCTS USING TECHNIQUES SUCH AS FTIR (FOURIER-TRANSFORM INFRARED SPECTROSCOPY), SCANNING ELECTRON MICROSCOPY (SEM), AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS). WE HYPOTHESIZE THAT THE MICROBIAL CONSORTIUM COLLECTED FROM THE BOVINE RUMEN EXHIBITS CONFIRMED LIGNIN DEGRADATION ACTIVITY, AND THE STEPS INVOLVED IN THIS PROCESS WILL BE ELUCIDATED. THESE FINDINGS WILL PROVIDE CRUCIAL INSIGHTS INTO LIGNIN DEGRADATION MECHANISMS BY THESE ORGANISMS AND THEIR POTENTIAL INDUSTRIAL-SCALE APPLICATIONS FOR PRODUCING HIGHER-VALUE COMMERCIAL PRODUCTS.

COMBINED EFFECTS OF INTERMITTENT FASTING WITH SWIMMING-BASED HIGH INTENSITY INTERMITTENT EXERCISE TRAINING ON WHITE ADIPOSE TISSUE METABOLISM AND FEEDING BEHAVIOR IN MALE WISTAR RATS

EVERTON LUIS DOS SANTOS CARDOSO, FÁBIO CAHUÊ, IORDAN EMANUEL FERREIRA MIRANDA, MARCELO DE LIMA SANT'ANNA, CHERLEY BORBA VIEIRA DE ANDRADE, RAIANA ANDRADE QUINTANILHA BARBOSA, TANIA MARIA ORTIGA-CARVALHO, MÁRIO VAISMAN, VERÔNICA SALERNO PINTO.

INTRODUCTION: MODERN LIFESTYLE CONTRIBUTES TO THE INCREASE IN OBESITY RATES WORLDWIDE AND LITERATURE HAS SOUGHT NON-MEDICINAL ALTERNATIVES TO HELP COMBAT THIS EPIDEMIC. IN THIS SCENARIO, HIGH-INTENSITY INTERVAL TRAINING (HIIT) AND INTERMITTENT FEEDING (AI) EMERGE AS POSSIBLE ALLIES WITH SIGNIFICANT RESULTS WHEN OBSERVED SEPARATELY. THE STUDY AIMS TO OBSERVE THE INTERACTION OF THESE TWO PROTOCOLS AND THEIR EFFECTS ON THE MORPHOLOGY AND BIOCHEMISTRY OF ADIPOCYTES, AS WELL AS THE FEEDING BEHAVIOR OF WISTAR MALE RATS.

METHODOLOGY:74 WISTAR MALE RATS AGED 60 DAYS WERE USED AND RANDOMLY DIVIDED INTO 4 GROUPS: (C) SEDENTARY, (EX) EXERCISED, (AI) INTERMITTENT FEEDING, AND (AIEX) INTERMITTENT FEEDING + EXERCISE. GROUPS UNDERWENT 8 WEEKS OF TREATMENT. FEEDING BEHAVIOR, ADIPOCYTE MORPHOLOGY, AND ALSO THEIR BIOCHEMISTRY MARKERS WERE ANALYZED. RESULTS: AS EXPECTED, THE AI GROUP DEVELOPED HYPERPHAGIC BEHAVIOR AND ALTHOUGH TRAINING ALONE ATTENUATED FOOD INTAKE, WHEN ASSOCIATED WITH INTERMITTENT FEEDING, THIS BENEFIT WAS LOST (C: 9.22 G ± 1.98; EX: 6.44 G ± 1.74; AI: 15.11 G ± 2.71; AIEX: 13.55 G ± 3.24). THE EXERCISED GROUPS SHOWED A GREATER AMOUNT OF FAT CELLS IN THE SAME AREA (C: 306.75 ± 57.86; EX: 733 ± 102.37; AI: 404.25 ± 21.17; AIEX: 809.5 ± 178.09), MOREOVER, THE EXERCISED GROUPS, ESPECIALLY AIEX THAT SHOWED GREATER MARKING OF VESSEL FORMATION (C: 0. 0162 ± 0.001; EX: 0.032 ± 0.003; AI: 0.0147 ± 0.002; AIEX: 0.0647 ± 0.009). UCP1 WAS QUANTIFIED AND THE EXERCISED GROUPS SHOWED HIGHER LEVELS, ESPECIALLY THE AIEX GROUP. CONCLUSION: TRAINING HAS A SIGNIFICANT IMPACT ON MORPHOLOGY AND BIOCHEMISTRY OF ADIPOCYTES AND THE ASSOCIATION BETWEEN PROTOCOLS HAS A POTENTIATING EFFECT ON THESE FINDINGS. HIIT INDUCED THE BROWNING, WHICH WAS DETERMINED BY INCREASED ANGIOGENESIS AND UCP1 LEVELS. THIS PROCESS WAS ENHANCED BY THE ASSOCIATION BETWEEN THE PROTOCOLS. EATING BEHAVIOR WAS MODIFIED BY AI AND ATTENUATED BY HIIT, HOWEVER THE ASSOCIATION BETWEEN PROTOCOLS DID NOT REDUCE HYPERPHAGIA.

THE ROLE OF GUT MICROBIOTA IN COLD-DRIVEN METABOLIC AND COGNITIVE OUTCOMES IN MICE FED A HIGH-FAT DIET

HENRIQUE TAMANINI SILVA MOSCHEN; WELLINGHTON DE MEDEIROS BARROS; WEMBLEY RODRIGUES VILELA; NATHASHA MARIA CORREA PRADO LOPES; DANIEL MUNIZ DOMINGUES; ANA BEATRIZ DA SILVA SOUSA; PAULA MARIA QUAGLIO BELLOZI; JAIR TRAPÉ GOULART; ANGÉLICA AMORIM AMATO; ANGELICA THOMAZ VIEIRA; ANDREZA FABRO DE BEM

BROWN ADIPOSE TISSUE (BAT) PLAYS A CENTRAL ROLE IN ENERGY HOMEOSTASIS, PRIMARILY THROUGH ITS THERMOGENIC FUNCTION MEDIATED BY UNCOUPLING PROTEIN 1 (UCP1). RECENT STUDIES HAVE PROPOSED INTERMITTENT AND CHRONIC COLD EXPOSURE AS AN EFFECTIVE STRATEGY TO STIMULATE BAT ACTIVITY, OFFERING A NON-PHARMACOLOGICAL APPROACH TO AMELIORATE METABOLIC AND COGNITIVE IMPAIRMENTS INDUCED BY HIGH-FAT DIETS (HFD). THE GUT MICROBIOTA HAS EMERGED AS A KEY REGULATOR OF BAT FUNCTION AND IS ESSENTIAL FOR UCP1-DEPENDENT THERMOGENESIS. HOWEVER, ITS ROLE IN COLD-INDUCED THERMOGENESIS AND BROADER METABOLIC EFFECTS REMAINS UNCLEAR. THIS STUDY AIMS TO INVESTIGATE WHETHER INTERMITTENT AND CHRONIC COLD EXPOSURE CAN IMPROVE METABOLIC AND COGNITIVE OUTCOMES IN MICE FED AN HFD, EVEN IN THE ABSENCE OF

GUT MICROBIOTA. TO EVALUATE THIS, WE WILL ESTABLISH FOUR EXPERIMENTAL GROUPS: CONTROL DIET WITHOUT ANTIBIOTICS (CD), CONTROL DIET WITH ANTIBIOTICS (CD+ATB), HFD WITHOUT ANTIBIOTICS (HFD), AND HFD WITH ANTIBIOTICS (HFD+ATB). MICROBIOTA MODULUATION WILL BE ACHIEVED VIA AN ANTIBIOTIC COCKTAIL (AMPHOTERICIN-B, AMPICILLIN, NEOMYCIN, METRONIDAZOLE, AND VANCOMYCIN) ADMINISTERED THROUGH DRINKING WATER. THE MICE WILL BE MAINTAINED ON THEIR RESPECTIVE DIETS FOR FIVE WEEKS PRIOR TO MICROBIOTA MODULATION AND COLD EXPOSURE. ALL GROUPS WERE THEN EXPOSED TO INTERMITTENT AND CHRONIC COLD (4°C, 4 HOURS/DAY) FOR AN ADDITIONAL FIVE WEEKS. WE WILL ASSESS METABOLIC PARAMETERS, BAT'S UCP1-DEPENDENT THERMOGENIC CAPACITY, AND COGNITIVE PERFORMANCE. ALL STATISTICAL ANALYSES WILL BE CONDUCTED USING GRAPHPAD PRISM 10 ®.

GENÉTICA MOLECULAR, BIOTECNOLOGIA E GENÔMICA (A3)

COMPARISON OF ANTIFUNGAL DRUG SUSCEPTIBILITY BETWEEN CONIDIA AND MURINE CELLS OF CLINICAL ISOLATES AND RNA SEPARATION FOR TRANSCRIPTOME ANALYSIS OF THE GENUS FONSECAEA

NATHALIA DE ALMEIDA MORAIS AMANDA AMARAL ANAMÉLIA LORENZETTI BOCA LARISSA FERANANDES MATOS

THESE FUNGI HAVE A DIMORPHIC CHARACTERISTIC AND PRESENT PATHOGENIC FORMS THAT CAN BE CALLED MURIFORM CELLS. THESE ARE MELANIZED STRUCTURES THAT CAN CAUSE VARIOUS DISEASES, SUCH AS CHROMOBLASTOMYCOSIS. IT IS A NEGLECTED TROPICAL DISEASE, ACCORDING TO THE WHO. IN BRAZIL, ITS PREDOMINANCE OCCURS IN THE AMAZON REGION. THE TREATMENT OF THIS DISEASE CAN BE HIGHLY COMPLEX DUE TO THE USE OF ANTIFUNGALS AS WELL AS SURGICAL EXCISION, CRYOTHERAPY, THERMOTHERAPY, AMONG OTHERS. DUE TO THE COMPLEXITY OF THE TREATMENT, THE GENERAL OBJECTIVE WILL BE TO COMPARE THE SUSCEPTIBILITY OF CONIDIA AND MURIFORMS TO DIFFERENT ANTIFUNGAL DRUGS SUCH AS ITRACONAZOLE AND TERBINAFINE OF 10 ISOLATES OF FONSECAEA TO EVALUATE THEIR MINIMUM INHIBITORY CONCENTRATION (MIC). FOR CELLULAR DEVIABILITY ANALYSIS, THE RESAZURIN REDUCTION METHOD WILL BE USED. IN ADDITION TO THESE ANALYSES, RNA WILL BE EXTRACTED FROM CONIDIA AND MURIFORMS FOR SUBSEQUENT USE OF THE RNASEQ TECHNIQUE FOR AN INITIAL TRANSCRIPTIONAL RESPONSE TO CONIDIA UNDERGOING A DIMORPHIC TRANSITION IN VITRO WITH THE CULTURE MEDIUM PROTOCOL ESTABLISHED BY THE RESEARCH GROUP.

REMODELAÇÃO DA ARQUITETURA ACIMA DO SOLO DE TOMATE POR EDIÇÃO MEDIADA POR CRISPR/CAS9 DE UM ÚNICO GENE SEMELHANTE AO TILLER ANGLE CONTROL 1 (TAC1)

PEDRO BRÍCIO BRITO FERNANDES, FRANCISCO JOSÉ LIMA ARAGÃO, MARIA ESTER FONSECA, MATIAS GONZÁLEZ-ARCOS, LEONARDO SILVA BOITEUX.

AMONG THE MAIN FACTORS ASSOCIATED WITH HIGHER AGRICULTURAL YIELD, STANDS OUT TOLERANCE TO WATER STRESS EVENTS, A HIGHER FLOWER AND FRUIT COUNT, AS WELL AS STRATEGIES THAT ALLOW INCREASING THE NUMBER OF PLANTS PER CULTIVATED AREA, DEPENDING ON THE PLANT ARCHITECTURE. THE ERECT LEAF PHENOTYPE STANDS OUT AS AN ARCHITECTURAL PROFILE OF HIGH AGRICULTURAL VALUE FOR IMPROVING LIGHT CAPTURE, REDUCING WATER LOSS, OPTIMIZING SPACE UTILIZATION, AND FACILITATING CHEMICAL AND BIOLOGICAL CONTROL OF ARTHROPODS AND PATHOGENS, ESPECIALLY THE ONES INFESTING/INFECTING ABAXIAL LEAF SURFACES. THIS PHENOTYPE HAS BEEN ASSOCIATED WITH TILLER ANGLE CONTROL 1 (TAC1)–LIKE GENES ACROSS MANY HERBACEOUS AND TREE SPECIES. WE HAVE PREVIOUSLY CARRIED OUT GENOMIC AND GENETIC ANALYSES OF THE ERECT LEAF PHENOTYPE IN TOMATO (SOLANUM LYCOPERSICUM L.) INDICATED ITS CONTROL BY A SEMI-DOMINANT (ERL) LOCUS AT CHROMOSOME 10. WE DISCOVERED THAT THIS PHENOTYPE WAS IN TIGHT LINKAGE WITH A CANDIDATE LOSS-OF-FUNCTION MUTATION IN THE SOLYC10G009320 GENE, WHICH IS AN ORTHOLOG OF THE TAC1–LIKE GENES. THEREFORE, EDITING THIS GENE MIGHT CONFIRM ITS FUNCTION AND MAKE A FINE-TUNED MANIPULATION OF THE ABOVE-GROUND TOMATO PLANT ARCHITECTURE ACCESSIBLE. HEREIN, WE VERIFIED (VIA A CRISPR-CAS9 GENOME EDITING SYSTEM) A COMPLETE GENETIC ASSOCIATION OF THE ERECT LEAF PHENOTYPE IN TOMATO BY KNOCKING OUT SOLYC10G009320 IN THE CULTIVAR ‘MICRO-TOM’. IN ADDITION, WE PROVIDE INFORMATION ON THE EFFECTS OF THIS GENE EDITION ON THE OVERALL PLANT PHENOTYPE AS WELL AS PHYSIOLOGICAL AND AGRONOMIC PERFORMANCE. OUR RESULTS INDICATED THAT EDITING SOLYC10G009320 ALLELES IN TOMATO WILL BE THE FOUNDATION FOR THE LARGE-SCALE GENERATION OF SUPERIOR GENOTYPES, PAVING THE WAY FOR THE DEVELOPMENT OF ELITE CULTIVARS DISPLAYING THIS TRAIT.

GENETIC TRANSFORMATION OF COMMON BEAN AND TOMATO FOR RNAI-MEDIATED SILENCING OF WHITEFLY (BEMISIA TABACI) GENES

MATHEUS DA COSTA MOURA[1], PATRÍCIA VALLE PINHEIRO[2], FRANCISCO JOSÉ LIMA ARAGÃO[3]

THE COMMON BEAN (PHASEOLUS VULGARIS L.) AND TOMATO (SOLANUM LYCOPERSICUM L.) ARE CRUCIAL CROPS IN BRAZIL, BUT THEY ARE VULNERABLE TO VARIOUS VIRAL DISEASES TRANSMITTED BY THE WHITEFLY BEMISIA TABACI, RESULTING IN SIGNIFICANT LOSSES IN PRODUCTION. THIS PROJECT AIMS TO DEVELOP BEAN AND TOMATO LINES RESISTANT TO WHITEFLIES BY SILENCING THE ACETYLCHOLINESTERASE (ACHE) AND ECDYSONE RECEPTOR (ECR) GENES OF THE INSECT, USING RNA INTERFERENCE (RNAI) TECHNOLOGY. THE OBJECTIVE IS TO REDUCE THE SURVIVAL RATES OF WHITEFLY NYMPHS AND ADULTS, CONTRIBUTING TO MORE SUSTAINABLE AND RESILIENT CROP MANAGEMENT PRACTICES. PLASMIDS CONTAINING PARTIAL SEQUENCES OF THE WHITEFLY'S ACHE AND ECR GENES WERE CONSTRUCTED FOR GENETIC TRANSFORMATION. IN BEANS, TRANSFORMATION WAS CARRIED OUT THROUGH BIOLISTIC METHODS, WHILE AGROBACTERIUM TUMEFACIENS WAS USED FOR TOMATO TRANSFORMATION. THESE PLASMIDS INCLUDED HERBICIDE RESISTANCE MARKERS FOR GLYPHOSATE OR ISOXAFLUTOLE, ENABLING EFFECTIVE SELECTION OF TRANSFORMED PLANTS. HERBICIDE SELECTION TESTS CONFIRMED SUCCESSFUL TRANSFORMATIONS, WITH FOUR TRANSGENIC LINES OBTAINED IN BEANS AND FIVE IN TOMATOES. THE NEXT STEPS INVOLVE BIOASSAYS WITH WHITEFLIES TO ASSESS THE EFFICACY OF RNAI IN REDUCING INSECT SURVIVAL ON THE GENETICALLY MODIFIED BEAN AND TOMATO PLANTS.

HETEROLOGOUS EXPRESSION OF TWO CHIMERICAL PROTEINS CONTAINING ANTIMICROBIAL PEPTIDES FROM PLANT GENOMES IN BACTERIA

FLÁVIA CABRAL NETTO RESENDE1,2, ANDRÉ MELRO MURAD2, CARLOS BLOCH JR.2 E FERNANDO ARARIPE G. TORRES1

PROTEINS POTENTIALLY CONTAIN INTERNAL PEPTIDE SEQUENCES WITH A WIDE RANGE OF BIOLOGICAL ACTIVITIES. SEVERAL SEQUENCES OF ENCRYPTED BIOACTIVE PEPTIDES 'HIDDEN' WITHIN LARGER PROTEINS IN THE GENOMES OF LIVING BEINGS HAVE ALREADY BEEN PUBLISHED AND A GREAT AMOUNT OF THEM REMAINS TO BE EXPLORED. ON THE OTHER HAND, AMONG THE NATIVE BIOACTIVE MOLECULES THERE IS A NOTORIOUS PRESENCE OF ANTIMICROBIAL PEPTIDES (AMP), PART OF ORGANISM'S DEFENSE SYSTEM. ONE OF THE MOST STUDIED CLASSES ARE THE ALPHA-HELICAL AMP, WHICH ARE MEMBRANOLYTIC, CATIONIC, AND AMPHIPHILIC. A PREVIOUS STUDY CARRIED BY OUR GROUP DISCOVERED, PRODUCED, AND VALIDATED THE ACTIVITY OF SOME ENCRYPTED AMP FOUND IN COCOA, COTTON, AND

ARABIDOPSIS THALIANA GENOMES. THE HYPOTHESIS OF THE PRESENT WORK IS THAT IT WOULD BE POSSIBLE TO 'RE-ENCRYPT' THESE PEPTIDES INSIDE OF CHIMERIC PROTEIN STRUCTURES, PREVENTING THEIR IMMEDIATE FOLDING INTO AN ALPHA-HELIX, SILENCING THEIR ACTIVITY. TWO CHIMERAS, EACH ONE CONTAINING THREE OF THE AMP AFOREMENTIONED, WERE RECOMBINANTLY PRODUCED IN ESCHERICHIA COLI. THE PROTEINS WERE DETECTED BY WESTERN BLOT TARGETING THEIR HIS-TAG. INTERESTINGLY, WE FOUND THAT BOTH CHIMERAS, TO DIFFERENT DEGREES, PRESENTED ANTIMICROBIAL ACTIVITY. INSUFFICIENT MATERIAL WAS OBTAINED FOR SEQUENCING. THEREFORE, A NEW VERSION OF THE FIRST CHIMERA FUSED TO GST WAS PRODUCED. IT ALSO PRESENTED ANTIMICROBIAL ACTIVITY, BUT THIS TIME IT WAS POSSIBLE TO SEQUENCE FRAGMENTS FROM THE N-TERMINAL GST-TAG AND FROM THE LINKER IMMEDIATELY PRECEDING THE FIRST AMP. THIS INDICATES THE PRESENCE OF SOME UNELUCIDATED RECOGNITION MECHANISM OF POTENTIALLY TOXIC SEQUENCES IN BACTERIAL CELLS. FURTHERMORE, CC00, AN ENCRYPTED PEPTIDE FROM COFFEE, WAS ALSO PRODUCED. IT IS PUTATIVELY BIOACTIVE FOR ANOTHER BIOLOGICAL ACTIVITY, AND IT WAS USED AS A POSITIVE CONTROL OF THE EXPRESSION SYSTEM. CC00 AVERAGE MOLECULAR MASS OF 9086.85 DA WAS DETERMINED BY MALDI-TOF/MS. THE SAMPLE WAS TRYPSINIZED AND FRAGMENTS WERE SEQUENCED BY MS/MS DATA.

DARK FERMENTATION IBE IN CLOSTRIDIUM ACETOBUTYLLICUM

BRUNO VIANNA

THE ABE FERMENTATION, TYPICAL OF CLOSTRIDIUM ACETOBUTYLICUM, PRODUCES THE SOLVENTS ACETONE, BUTANOL, AND ETHANOL, OF WHICH ONLY THE LATTER TWO CAN BE USED AS BIOFUELS, AS ACETONE HAS CORROSIVE EFFECTS ON ENGINES. THE IBE FERMENTATION, ALSO KNOWN AS "DARK FERMENTATION," IS CONSIDERED AN ATTRACTIVE ALTERNATIVE, AS IT GENERATES A MIXTURE OF ALCOHOLS WITH HIGHER ENERGY POTENTIAL: ISOPROPANOL, BUTANOL, AND ETHANOL. IN THIS PROJECT, WE PROPOSE TO PRODUCE ISOPROPANOL IN C. ACETOBUTYLICUM BY EXPRESSING THE GENE ENCODING THE ENZYME SADH (SECONDARY ALCOHOL DEHYDROGENASE) FROM CLOSTRIDIUM BEIJERINCKII, WHICH CONVERTS ACETONE INTO THIS ALCOHOL. INITIALLY, ESCHERICHIA COLI XL10 GOLD WILL BE TRANSFORMED WITH THE PUC19 VECTOR (WHICH CONTAINS SELECTION MARKER FOR AMPICILLIN), INTO WHICH A GENE ENCODING AN ENZYME THAT METHYLATES DNA SEQUENCES HAS BEEN CLONED. THESE SEQUENCES WILL BE USED TO TRANSFORM C. ACETOBUTYLICUM TO PROTECT THEM FROM THE BACTERIUM'S RESTRICTION SYSTEM. THE SADH GENE WILL BE SYNTHESIZED WITH REGULATORY SIGNALS AT ITS ENDS FOR EXPRESSION IN C. ACETOBUTYLICUM, INCLUDING A CONSTITUTIVE PROMOTER AND A TRANSCRIPTION TERMINATOR REGION. THIS CASSETTE WILL BE CLONED INTO

THE PMTL8000 VECTOR, WHICH CONTAINS A TETRACYCLINE RESISTANCE MARKER. THIS VECTOR WILL BE USED TO TRANSFORM THE E. COLI STRAIN ALREADY CONTAINING METHYLATION GENES, AND SELECTION WILL BE PERFORMED IN A MEDIUM WITH TETRACYCLINE AND AMPICILLIN. THE PLASMIDS WILL BE EXTRACTED AND QUANTIFIED, FOLLOWED BY ELECTROPORATION OF C. ACETOBUTYLICUM AND SELECTION IN YTA MEDIUM CONTAINING TETRACYCLINE IN AN ANAEROBIC CHAMBER AT 33°C UNTIL COLONIES APPEAR. INDIVIDUAL CLONES WILL BE EVALUATED FOR THE PRESENCE OF PLASMIDS BY PCR USING PRIMERS THAT ANNEAL TO THE SADH GENE. IN A SUBSEQUENT PHASE, THE LDH GENE (LACTATE DEHYDROGENASE)—NON-ESSENTIAL AND RESPONSIBLE FOR LACTATE FORMATION—WILL BE DELETED TO REDUCE THE CARBON FLOW TOWARDS OTHER METABOLITES. FOR THIS, UPSTREAM AND DOWNSTREAM SEQUENCES (1 KB EACH) OF THE LDH GENE WILL BE CLONED INTO THE PMTL-MAZF VECTOR, WHICH WILL BE DEMETHYLATED THROUGH THE PREVIOUSLY DESCRIBED PROCESS. ALCOHOL PRODUCTION WILL BE ASSESSED BY HPLC.

PRODUCTION OF BIOMASS-DECONSTRUCTING ENZYMES FOR ANIMAL FEED

LUÍSA DE MIRANDA BASTO SILVA; KAMILA LOURRANE CARVALHO ALENCAR; VICTOR SOBRINHO ROCHA FERREIRA; FERNANDO KAHRIN CARDOSO DA COSTA; DASCIANA DE SOUSA RODRIGUES; ELIANE FERREIRA NORONHA; BETANIA FERRAZ QUIRINO

CATTLE RAISING IS AN IMPORTANT ACTIVITY IN BRAZIL, AND BIOLOGICAL ADDITIVES CAN ENHANCE DIGESTIVE PROCESSES OF RUMINANTS. ENZYMES USED IN ADDITIVES ACCOUNT FOR A LARGE PART OF THE COSTS INVOLVED IN CATTLE-RAISING. FARMERS COULD REDUCE OPERATION COSTS BY USING ENZYMES PRODUCED IN BRAZIL TO REPLACE IMPORTED ONES FOR BREAKING DOWN BRACHIARIA BRIZANTHA, THE MAIN GRASS CONSUMED BY CATTLE IN BRAZILIAN PASTURES. THIS STUDY AIMED TO PRODUCE AND CHARACTERIZE HYDROLYTIC ENZYMES FROM FILAMENTOUS FUNGI FOR BREAKING DOWN BRACHIARIA BRIZANTHA FOR USE IN RUMINANT FEED ADDITIVES. THREE FUNGAL ISOLATES WERE SCREENED FOR THEIR ABILITY TO PRODUCE CELLULASES, ENDOGLUCANASES, AND B-GLUCOSIDASES USING B. BRIZANTHA AND SUGARCANE BAGASSE AS CARBON SOURCES. ONE FUNGAL ISOLATE SHOWED THE HIGHEST ENZYMATIC ACTIVITIES. ENZYME PRODUCTION BY THIS ISOLATE WAS EVALUATED UNDER DIFFERENT CONDITIONS. VARIABLES EVALUATED INCLUDED ENZYME STABILITY UNDER DIFFERENT STORAGE CONDITIONS, MEDIUM SUPPLEMENTATION, AND THE CULTIVATION TIME. ENZYMATIC EXTRACTS SHOWED STABILITY FOR UP TO 29 DAYS WHEN STORED AT -20°C. HYDROTHERMAL PRETREATMENT OF B. BRIZANTHA SLIGHTLY ENHANCED ENZYME PRODUCTION, HOWEVER THIS EFFECT WAS NOT STATISTICALLY SIGNIFICANT. INTERESTINGLY, THIS ENZYMATIC EXTRACT DEMONSTRATED COMPETITIVE PERFORMANCE IN B.

BRIZANTHA HYDROLYSIS ASSAYS COMPARED TO COMMERCIAL ENZYMES, WITH PEAK HYDROLYSIS OCCURRING BETWEEN 6 AND 12 HOURS. THESE RESULTS SUGGEST THAT THIS FUNGAL ISOLATE HAS GREAT POTENTIAL FOR THE DEVELOPMENT OF ENZYME-BASED FEED ADDITIVES FOR RUMINANT NUTRITION, CONTRIBUTING TO THE ADVANCEMENT OF THE BRAZILIAN BIOECONOMY THROUGH LOCAL ENZYME PRODUCTION.

GENE DELETION OF POLYKETIDE SYNTHASE TYPE I (PKS-1) PRODUCES AN ALBINO MUTANT OF FONSECAEA PEDROSOI

ELIAS EL-SHADDAI DOS SANTOS NERY NUNES RIBEIRO; LARISSA FERNANDES MATOS

FONSECAEA PEDROSOI IS A MELANISED FILAMENTOUS FUNGUS AND THE MAJOR CAUSE OF CHROMOBLASTOMYCOSIS (CBM). CBM IS A CHRONIC AND RECALCITRANT SUBCUTANEOUS MYCOSIS CAUSED BY TRAUMATIC IMPLANTATION AND IS CONSIDERED A NEGLECTED TROPICAL DISEASE (NTD). THE MELANIN IS SYNTHESISED VIA THE DHN-MELANIN (1,8-DIHYDROXYNAPHTHALENE) PATHWAY AND COULD BE CHARACTERIZED BY PROPERTIES SUCH AS: HIGH MOLECULAR WEIGHT, VERY STABLE, NEGATIVELY CHARGED, RESISTANT TO DEGRADATION BY ACIDS, SUSCEPTIBLE TO DEGRADATION BY STRONG BASES, HYDROPHOBIC AND INSOLUBLE IN ORGANIC SOLVENTS. THIS PATHWAY STARTS WITH THE POLYKETIDE 1,3,6,8-TRIHYDROXYNAPHTHALENE (THN), FOLLOWED BY REDUCTION TO SCYTALONE, WHICH IS REDUCED TO VERMELONE AND DEHYDRATED TO DHN. AFTER THIS STAGE, DHN IS POLYMERISED TO FORM DHN MELANIN. THEREFORE, IN ORDER TO CHARACTERIZE THE DHN-MELANIN PATHWAY IN F. PEDROSOI, THE GENE FOR THE PROTEIN POLYKETIDE SYNTHASE TYPE I (PKS-1), WHICH IS RESPONSIBLE FOR THE FIRST STEP OF THE DHN BIOSYNTHETIC PATHWAY, WAS DELETED BY BIOLYSIS. DELETION CASSETTES WERE CONSTRUCTED BY DOUBLE-JOINT POLYMERASE CHAIN REACTION (DJ-PCR), A TECHNIQUE FOR CONSTRUCTING A RECOMBINANT DNA FRAGMENT USING THE PCR METHODOLOGY FOR FUSION OF DIFFERENT FRAGMENTS TO OBTAIN A CASSETTE TO DELETE THE TARGET GENE. WE OBTAINED TWENTY-FIVE TRANSFORMANTS AND TWO CONFIRMED MUTANTS ΔPKS-1 BY PCR AND GROWTH IN SELECTIVE MEDIUM PLATES. WE THEN CARRIED OUT A NEW ASSAY TO RECONSTITUTE PKS-1 SYNTHESIS, AS A PROOF OF CONCEPT. WE OBTAINED SEVENTY-FOUR TRANSFORMANTS, BUT NO CONFIRMED MUTANTS YET.

EXPRESSION OF THE SYNTHETIC PG-PGIP GENE IN SOYBEAN AIMING FOR GREATER TOLERANCE TO THE FUNGUS SCLEROTINIA SCLEROTIORUM.

RENAN MIGUEL DOS ANJOS 1; JULIO CARLYLE MACÊDO RODRIGUES 2; JÉSSICA CARRIJO DE SOUZA 1; MÔNICA TERESA VENEZIANO LABATE 5; FRANCISCO JOSÉ LIMA ARAGÃO 3; FELICE CERVONE 6; CARLOS ALBERTO LABATE 4; GIOVANNI RODRIGUES VIANNA 3

SOYBEAN (GLYCINE MAX), ONE OF THE WORLD'S MAIN CROPS, IS WIDELY USED FOR BOTH ANIMAL AND HUMAN NUTRITION. HOWEVER, IT IS SUSCEPTIBLE TO PATHOGENS THAT CAUSE DAMAGE AND REQUIRE PESTICIDE APPLICATIONS, INCREASING PRODUCTION COSTS AND ENVIRONMENTAL HARM. THUS, THE DEVELOPMENT OF MORE PATHOGEN-TOLERANT PLANTS IS A DEMAND OF THE AGRIBUSINESS. IN THIS CONTEXT, WE DEVELOPED TRANSGENIC SOYBEAN PLANTS OVEREXPRESSING OLIGOGALACTURONIDES (OGS), MOLECULES PRODUCED FROM PECTIN DEGRADATION THAT ACT AS DAMAGE-ASSOCIATED MOLECULAR PATTERNS (DAMPS). PREVIOUS STUDIES SHOWED THAT A CHIMERIC GENE, FORMED BY A FUNGAL POLYGALACTURONASE (PG) FROM FUSARIUM PHYLLOPHILUM FUSED WITH A PG-INHIBITING ENZYME FROM PHASEOLUS VULGARIS (PGIP), CAN PRODUCE OGS AND INDUCE RESISTANCE TO DIFFERENT PATHOGENS IN ARABIDOPSIS. TO TEST THE HYPOTHESIS THAT THIS SYNTHETIC GENE CAN INDUCE RESISTANCE IN SOYBEAN PLANTS, A VECTOR CONTAINING THE GENE UNDER THE CONTROL OF THE CONSTITUTIVE PROMOTER CAMV35S WAS CONSTRUCTED, AND WAS USED TO OBTAIN TRANSGENIC SOYBEAN PLANTS THROUGH BIOLISTIC TRANSFORMATION. A TRANSGENIC EVENT NAMED PME-C WAS SELECTED (T2), AND ANALYSES BY PCR AND SOUTHERN BLOT CONFIRMED THE PRESENCE OF THE TRANSGENE. TO EVALUATE THE EFFECTS OF PGIP-PG GENE EXPRESSION ON INCREASED RESISTANCE TO FUNGAL INFECTION, TWO DISTINCT EXPERIMENTS WERE CONDUCTED. IN THE FIRST, DETACHED LEAVES FROM 9 PROGENIES OF THIS EVENT (T4) WERE INOCULATED WITH FUNGUS SCLEROTINIA SCLEROTIORUM, THE CAUSAL AGENT OF WHITE MOLD DISEASE. THE LESIONS WERE PHOTOGRAPHED AT THREE DIFFERENT TIME POINTS (24, 48, AND 72 H). IN THE SECOND, CONDUCTED IN VIVO, S. SCLEROTIORUM WAS INOCULATED ON TWO LEAFLETS OF THE SAME PLANTS FROM THE PREVIOUS ASSAY. THE LESIONS WERE PHOTOGRAPHED EVERY 24 H FOR 14 DAYS. THE IMAGES WERE USED TO CALCULATE THE INFECTED AREA (IMAGEJ SOFTWARE). PRELIMINARY RESULTS INDICATED THAT THE TRANSGENIC PLANTS SHOWED REDUCED DISEASE PROGRESSION COMPARED TO THE CONTROL, WITH MORE THAN A 50% REDUCTION IN INFECTION DEVELOPMENT.

METAGENOME SEQUENCING AND RECOVERY OF METAGENOME-ASSEMBLED GENOMES FROM A MICROBIAL CONSORTIUM SELECTED FOR LIGNIN DEGRADATION UNDER ANAEROBIC CONDITIONS.

JOVANE DE OLIVEIRA DANTAS, ELIANE FERREIRA NORONHA

LIGNIN IS A PHENOLIC MACROMOLECULE THAT IS COVALENTLY ASSOCIATED WITH HEMICELLULOSE, PROVIDING STRENGTH AND IMPERMEABILITY TO THE PLANT CELL WALL, AND IS ONE OF THE MAIN CONTRIBUTORS TO THE RECALCITRANCE OF PLANT BIOMASS. ITS REMOVAL IS A KEY STEP IN INDUSTRIAL PROCESSES. FROM LIGNIN, VALUABLE PRODUCTS SUCH AS VANILLIN, GUAIACOL, PHENOL, AND OTHER AROMATIC COMPOUNDS CAN BE OBTAINED; HOWEVER, THERE ARE STILL ECONOMIC AND TECHNOLOGICAL CHALLENGES FOR THE INDUSTRIAL PRODUCTION OF THESE PRODUCTS. WHILE LIGNIN IS STRUCTURALLY RESISTANT, IT IS DEGRADED IN NATURE THROUGH THE SYNERGISTIC ACTION OF VARIOUS MICROORGANISMS. THEREFORE, THE METAGENOMIC APPROACH TO EXAMINE MICROBIAL COMMUNITIES COLLECTIVELY IS PROMISING, AS IT ALLOWS FOR THE ANALYSIS OF THEIR GENES AND POTENTIAL METABOLIC PATHWAYS. MOST CURRENT RESEARCH ON LIGNIN METABOLISM HAS FOCUSED ON FUNGI AND AEROBIC ENVIRONMENTS, AND THE METABOLISM IN ANAEROBIC ENVIRONMENTS BY FUNGI AND BACTERIA IS STILL POORLY UNDERSTOOD. IN THIS PROJECT, WE PROPOSE TO ASSEMBLE THE METAGENOME AND RECOVER MAGS (METAGENOME-ASSEMBLED GENOMES) FROM SHOTGUN SEQUENCING OF A LIGNIN-DEGRADING MICROBIAL CONSORTIUM IN DIFFERENT GROWTH PHASES. THIS CONSORTIUM, DERIVED FROM BOVINE RUMEN, WAS SELECTED THROUGH PASSAGES IN AN ANAEROBIC REDUCING MEDIUM WITH LIGNIN AS THE SOLE CARBON SOURCE. THESE GENOMES WILL BE ANALYZED FOR MICROBIAL DIVERSITY, PHYLOGENY, AND POTENTIAL METABOLIC PATHWAYS ANALYSIS. WITH THE GENERATED DATA, WE AIM TO ADVANCE THE UNDERSTANDING OF ANAEROBIC LIGNIN DEGRADATION, WHICH COULD HAVE SIGNIFICANT IMPLICATIONS FOR THE BIOREMEDIATION OF PHENOLIC COMPOUNDS AND BIOTECHNOLOGICAL APPLICATIONS IN THE REUSE OF LIGNOCELLULOSIC BIOMASS.

DECIPHERING THE GENETIC DIVERSITY OF THE BRAZILIAN MAIZE CORE COLLECTION WITH GENOTYPING OF DNA BULKS

BIANCA S. ALCANTARA1,2, FLAVIA F. TEIXEIRA3, GUILHERME F. SIMIQUELI1 AND DARIO GRATTAPAGLIA1,2

ONE OF THE CHALLENGES OF GENOMIC ANALYSIS OF GERMPLASM COLLECTIONS COMPOSED BY LARGE NUMBERS OF GENETICALLY HETEROGENEOUS ACCESSIONS (POPULATIONS, OUTBRED VARIETIES) IS HOW TO DEAL WITH THE GENETIC VARIATION WITHIN ACCESSIONS. GENOTYPING SEVERAL INDIVIDUALS PER ACCESSION MAKES THE ENDEAVOR TIME-CONSUMING AND EXPENSIVE. ALLELOTYPING THE ACCESSION INSTEAD, BY ESTIMATING THE ALLELE FREQUENCIES FROM A POOLED SAMPLE OF INDIVIDUALS OF AN ACCESSION, REPRESENTS AN ELEGANT ALTERNATIVE FOR HIGH PRECISION AND AT LOW COST GENETIC ANALYSIS. A SET OF 3526

GENOME-WIDE SNPS INTERROGATED ON THE EMBRAPA 65K MULTISPECIES CHIP WERE USED IN THIS GENETIC STUDY. TO ASSESS THE PRECISION OF THE METHOD, THREE POOL ASSEMBLY METHODS WERE EVALUATED FOR FOUR DIFFERENT ACCESSIONS BY COMPARING THE ALLELE FREQUENCIES ESTIMATES OBTAINED BY THE NORMALIZED ALLELIC INTENSITY RATIO OF THE TWO SNP ALLELES IN 16-PLANTS POOLS PER ACCESSION, THREE REPLICATES, WITH THE ESTIMATE FROM THE SAME INDIVIDUALLY GENOTYPED PLANTS. THREE METHODS WERE TESTED: 1. EQUIMOLAR POOL OF INDIVIDUALLY EXTRACTED NANODROP MEASURED DNA; 2. SAME AS, BUT PICOGREEN MEASURED DNA; 3. DNA EXTRACTION FROM A BULKED LEAF TISSUE SAMPLE WEIGHED TO THE 0.1GRAM. THE BULK LEAF EXTRACTION METHOD SHOWED THE LOWEST ROOT MEAN SQUARE DEVIATION (5.7%) AND THE HIGHEST CORRELATION (0.98) OF ALLELE FREQUENCIES. USING THIS METHOD DNA POOLS OF 24 PLANTS EACH OF THE 287 BRAZILIAN MAIZE CORE COLLECTION AND 184 INDIGENOUS MAIZE ACCESSIONS WERE ALELLOTYPED WITH 64 REPLICATES. REPRODUCIBILITY (FST= 0.0047) INDICATED ACCURATE ESTIMATES WITH MINIMAL SAMPLING ERROR BETWEEN BIOLOGICAL REPLICATES. GENETIC DISTANCE AND STRUCTURE ANALYSES WERE CARRIED OUT FOR THE 471 ACCESSIONS TOGETHER WITH SNP DATA FROM THE LITERATURE OF TEOSINTE AND CIMMYT MAIZE LINES REPRESENTATIVE OF TROPICAL, SUBTROPICAL AND TEMPERATE GERMPLASM. SEVEN GENETIC GROUPS WERE IDENTIFIED, WITH SIGNIFICANT OVERLAPPING AMONG THEM, INDICATING COMMON ORIGINS THAT ARE NOW BEING THOROUGHLY INVESTIGATED.

BIOTECHNOLOGICAL ADVANCES FOR REDUCING SOYBEAN SUSCEPTIBILITY TO ROOT-KNOT NEMATODES.

NÁTTANY SOUZA COSTA, RAÍRE DOS SANTOS CAVALCANTE, NAYARA SABRINA DE FREITAS ALVES, LORENA SOUSA DE LOIOLA COSTA, MARIA EUGÊNIA LISEI-DE-SÁ, CAROLINA VIANNA MORGANTE, MARIA FÁTIMA GROSSI DE SÁ

SOYBEANS ARE THE MOST IMPORTANT CROP IN BRAZIL AND AMONG THE CHALLENGES IN ITS PRODUCTION, THE PARASITISM BY ROOT-KNOT NEMATODES (RKN) OF THE GENUS MELOIDOGYNE STANDS OUT. THIS ENDOPARASITE FEEDS ON THE ROOTS OF ECONOMIC CROPS AND PRODUCES GIANT CELL CLUSTERS CALLED GALLS THAT INTERFERE WITH THE PLANT'S UPTAKE OF WATER AND SOIL NUTRIENTS. GIVEN THAT THE MOST USED PHYTONEMATODES MANAGEMENT PRACTICES ARE INEFFICIENT FOR THEIR CONTROL, AND THAT TOLERANT SOYBEAN CULTIVARS ARE DERIVED FROM A SINGLE GENETIC SOURCE, THE USE OF BIOTECHNOLOGICAL APPROACHES TO INCORPORATE NEW SOURCES OF RESISTANCE IS PROMISING. HERE, TWO BIOTECHNOLOGICAL STRATEGIES WERE USED SIMULTANEOUSLY TO RKN CONTROL IN SOYBEAN: (1) OVEREXPRESSION OF THE ADEXLB8 GENE; (2) RNAI

MEDIATED SILENCING OF NEMATODE GENES INVOLVED IN ITS PRIMARY METABOLISM OR PLANT INFECTION PROCESS, SUCH AS THOSE ENCODING CYSTEINE PROTEASE, ISOCITRATE LYASE, SPLICING FACTOR, AND THE EFFECTOR 16D10. GENETICALLY MODIFIED (GM) PLANTS WERE OBTAINED USING THE AGROBACTERIUM-BASED TRANSFORMATION METHOD. PLANTS WERE SCREENED BY TRANSGENE AMPLIFICATION BY PCR AND ELISA TO THE MOLECULAR CHARACTERIZATION. PLANTS FROM THREE INDEPENDENT TRANSFORMATION EVENTS AT T2 GENERATION WERE SELECTED FOR CHALLENGE ASSAYS AGAINST M. INCOGNITA. FIFTEEN-DAY-OLD PLANTS WERE INOCULATED WITH 1000 J2 JUVENILES OF M. INCOGNITA. AFTER 60 DAYS, THE GM PLANTS SHOWED A SIGNIFICANT REDUCTION IN THE NUMBER OF GALLS PER GRAM OF ROOT (22.0-34.0%), IN THE NUMBER OF EGG MASS PER GRAM OF ROOTS (46.0-50.0%), IN THE NUMBER OF EGGS PER GRAM OF ROOTS (59.0-59.6%), AND IN NEMATODE REPRODUCTION FACTOR (30.0-50.0%) COMPARED TO WILD-TYPE PLANTS. ADDITIONALLY, THE EXPRESSION OF THE ADEXLB8 TRANSCRIPT WAS HIGHER IN THE ROOTS OF TRANSFORMED PLANTS COMPARED TO THE NT CONTROL. SO FAR, THE PYRAMIDING STRATEGY APPEARS EFFECTIVE IN CONTROLLING M. INCOGNITA AND CAN BE APPLIED TO SOYBEAN BREEDING PROGRAMS AS A COMPLEMENTARY SOURCE OF RESISTANCE TO RKN.

GENOMIC INSIGHTS AND GROWTH OPTIMIZATION OF ALCALIGENES SP. FOR ENHANCED BIOPOLYMER PRODUCTION AND PLASTIC BIODEGRADATION

MARIANA PORTUGAL MATTIOLI; RICARDO HENRIQUE KRUGER; JULIANNA PEIXOTO TREPTOW

SYNTHETIC POLYMERS ARE WIDELY UTILIZED DUE TO THEIR VERSATILITY AND DURABILITY, BUT THEIR ENVIRONMENTAL PERSISTENCE HAS LED TO SEVERE ECOLOGICAL CHALLENGES. CONVENTIONAL WASTE MANAGEMENT STRATEGIES, SUCH AS INCINERATION AND LANDFILLING, HAVE PROVEN INADEQUATE AND ENVIRONMENTALLY HARMFUL, UNDERSCORING THE URGENT NEED FOR SUSTAINABLE ALTERNATIVES. ALCALIGENES SP. HAS EMERGED AS A PROMISING MICROORGANISM CAPABLE OF DEGRADING SYNTHETIC POLYMERS AND SYNTHESIZING POLYHYDROXYALKANOATES (PHAS), BIODEGRADABLE BIOPOLYMERS WITH SIGNIFICANT BIOTECHNOLOGICAL POTENTIAL. THIS STUDY AIMS TO OPTIMIZE THE CULTIVATION PARAMETERS OF ALCALIGENES SP., EVALUATE ITS CAPACITY TO DEGRADE POLYAMIDE, AND ELUCIDATE THE GENOMIC MECHANISMS UNDERLYING THESE PROCESSES. EXPERIMENTS WERE DESIGNED TO DETERMINE OPTIMAL GROWTH CONDITIONS BY VARYING PARAMETERS SUCH AS PH, TEMPERATURE, AND AGITATION. GENOMIC ANALYSES WERE PERFORMED USING A HYBRID SEQUENCING APPROACH, INTEGRATING ILLUMINA AND PACBIO DATA TO ACHIEVE A HIGH-QUALITY GENOME ASSEMBLY. EXPERIMENTAL DATA INCLUDE GROWTH CURVES IN

MICROPLATES AND BENCH-TOP BIOREACTORS, ALONGSIDE MORPHOLOGICAL AND GENETIC CHARACTERIZATIONS. PRELIMINARY FINDINGS CONFIRM THE VIABILITY OF ALCALIGENES SP. FOR BIOTECHNOLOGICAL APPLICATIONS, ALTHOUGH ANALYSES OF PHA PRODUCTION, POLYAMIDE DEGRADATION, AND COMPREHENSIVE GENOMIC INSIGHTS ARE ONGOING. THESE FUTURE INVESTIGATIONS WILL ENHANCE THE UNDERSTANDING OF METABOLIC PATHWAYS AND VALIDATE THE POTENTIAL OF ALCALIGENES SP. AS A SUSTAINABLE SOLUTION FOR SYNTHETIC POLYMER WASTE MANAGEMENT. THE DEVELOPMENT OF THIS BIOPROCESS REPRESENTS A SIGNIFICANT STEP TOWARD ADVANCING THE CIRCULAR ECONOMY, LEVERAGING SCIENTIFIC INNOVATION TO ADDRESS THE ENVIRONMENTAL CHALLENGES POSED BY SYNTHETIC POLYMERS.

DEVELOPMENT OF BIOLUMINESCENT LEISHMANIA STRAINS AS A TOOL FOR MONITORING IN VIVO PARASITIC EVOLUTION.

FELIPE DA SILVA MENDONÇA DE MELO[1]; ALEXANDRA MARIA DOS SANTOS CARVALHO[1]; ANDREY DUARTE BOAVA[1]; KAUÃ DOS SANTOS SILVA[1]; LUCAS GABRIEL SOARES DOS SANTOS[1]; DANIELA FRANCO ROSA[1]; JÚLIA LISBOA BESSERA E SILVA[1]; MARIA LUÍSA SAUNDERS DE FARIAS VIANA[1]; CARLA NUNES ARAÚJO[1]; JAIME DE MARTINS SANTANA[1]; IZABELA MARQUES DOURADO BASTOS[1].

CURRENTLY, THE SCREENING OF INFECTION BY THE LEISHMANIA SPP. PARASITE IN MURINE MODELS IS BASED ON ESTIMATING PARASITIC LOAD IN ORGANS SUCH AS THE SPLEEN, LIVER, AND TISSUE, AS WELL AS THE AMPLIFICATION OF PARASITE DNA THROUGH QPCR. HOWEVER, THESE TECHNIQUES REQUIRE THE SACRIFICE OF A LARGE NUMBER OF MICE. A PROMISING ALTERNATIVE IS THE USE OF REPORTER GENES, SUCH AS LUCIFERASE AND MNEONGREEN, WHICH MAKE THE PARASITES BIOLUMINESCENT AND FLUORESCENT, ALLOWING FOR THE DETECTION OF EVEN LOW PARASITEMIA BOTH IN VIVO AND IN VITRO. IN THIS STUDY, THE COMMERCIALLY SYNTHESIZED CHIMERIC LUCIFERASE-MNEONGREEN GENE WAS CLONED INTO THE PLEXSY-I-BLECHERRY3 VECTOR, WHICH WAS THEN DIGESTED WITH THE RESTRICTION ENZYME SWA I TO RELEASE THE INTEGRATION CASSETTE INTO THE LEISHMANIA SPP. GENOME. THE CASSETTES WERE PURIFIED AND TRANSFECTED INTO L. AMAZONENSIS OR L. INFANTUM PROMASTIGOTES USING THE NUCLEOFECTOR T LYMPHOCYTE KIT AND THE AMAXA N2B EQUIPMENT WITH THE U33 PROGRAM. THE TRANSFECTED PARASITES WERE SELECTED WITH THE HYGROMYCIN ANTIBIOTIC, CLONED IN 96-WELL PLATES, AND MONITORED BY FLUORESCENCE MICROSCOPY. AFTER CLONING IN BOTH SPECIES, A WESTERN BLOT WAS PERFORMED, CONFIRMING THE EXPRESSION OF THE CHIMERIC PROTEIN. THE USE OF REPORTER GENES COULD REPRESENT A SIGNIFICANT ADVANCEMENT IN LEISHMANIASIS RESEARCH, ENABLING NOT ONLY MORE

ACCURATE MONITORING OF IN VIVO INFECTION BUT ALSO CONTRIBUTING TO THE DEVELOPMENT OF NEW PROPHYLACTIC AND THERAPEUTIC THERAPIES.

PROTEOMIC ANALYSIS OF THE COFFEE LEAF MINER (LEUCOPTERA COFFEELLA): AN ECONOMICALLY RELEVANT PEST AFFECTING COFFEE PRODUCTION

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THE MANAGEMENT OF PESTS THAT ATTACK THE COFFEE CROP REPRESENTS A SIGNIFICANT CHALLENGE FOR PRODUCERS. LEUCOPTERA COFFEELLA (GUÉRIN-MÈNEVILLE), ALSO KNOWN AS THE COFFEE LEAF MINER (CLM), IS AN ECONOMICALLY RELEVANT PEST THAT CAUSES SIGNIFICANT LOSSES IN COFFEE PRODUCTION. IN THE LARVAL STAGE, THE CLM FEEDS ON THE PALISADE PARENCHYMA, THEREBY REDUCING THE PHOTOSYNTHETIC CAPACITY OF THE PLANT. THE CURRENT CONTROL METHOD FOR THE CLM IS THE USE OF SYNTHETIC INSECTICIDES. HOWEVER, THE INDISCRIMINATE USE OF THESE CHEMICALS HAS RESULTED IN ADVERSE EFFECTS ON THE ENVIRONMENT, HUMAN HEALTH, AND THE EVOLUTION OF RESISTANT PESTS. IN THIS CONTEXT, THE OBJECTIVE OF THIS STUDY IS TO IDENTIFY THE PROTEINS INVOLVED IN THE L. COFFEELLA METABOLISM, WITH THE AIM OF PROMOTING THE USE OF THIS INFORMATION TO GENERATE NEW FORMS OF CONTROL. TO ACHIEVE THIS OBJECTIVE, LARVAE OF L. COFFEELLA WERE OBTAINED FROM LEAVES OF PLANTS EXHIBITING SYMPTOMS OF THE COFFEE LEAF MINER (CLM) DISEASE. SAMPLES WERE COLLECTED AT THREE DISTINCT DEVELOPMENTAL STAGES (INSTARS L2, L3, L4). THE TOTAL PROTEIN EXTRACT WAS SUBJECTED TO ANALYSIS BY LC MS/MS. THE RESULTS DEMONSTRATED THAT IN THE COMPARISON BETWEEN THE L2 AND L3 INSTARS, 54 PROTEINS EXHIBITED DIFFERENTIAL ABUNDANCE, INCLUDING 14 PROTEINS THAT WERE INCREASED AND 40 PROTEINS THAT WERE DECREASED. IN THE COMPARISON BETWEEN L2 AND L4, 252 PROTEINS WERE FOUND TO BE DIFFERENTIALLY ABUNDANT, INCLUDING 155 THAT WERE INCREASED AND 97 THAT WERE DECREASED. IN THE L3/L4 COMPARISON, 18 PROTEINS WERE IDENTIFIED AS DIFFERENTIALLY ABUNDANT, INCLUDING 17 INCREASED PROTEINS AND 1 DECREASED PROTEIN. THIS STUDY HAS LED TO THE IDENTIFICATION OF POTENTIAL PROTEINS THAT PLAY AN IMPORTANT ROLE IN THE ADAPTATION OF THE COFFEE LEAF MINER DURING COFFEE INFESTATION. THE RESULTS OBTAINED IN THIS STUDY CAN CONTRIBUTE TO THE DEVELOPMENT OF CONTROL STRATEGIES THAT AIM TO REDUCE THE USE OF PESTICIDES IN CROP PRODUCTION.

EVALUATION OF THE MCIPA PROTEIN AS AN ENHANCER OF FUNGAL CELLULASE ACTIVITY IN THE DECONSTRUCTION OF LIGNOCELLULOSIC SUBSTRATES

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BRAZIL HAS SIGNIFICANT POTENTIAL TO BOOST ITS BIOECONOMY, PARTICULARLY THROUGH THE EXPLORATION OF MICROBIAL BIODIVERSITY FOR THE PRODUCTION OF BIOLOGICAL INPUTS. IN THE CONTEXT OF ANIMAL NUTRITION, ENZYMES PLAY A CRUCIAL ROLE IN OPTIMIZING DIGESTIVE PROCESSES, ENHANCING LIVESTOCK HEALTH AND PRODUCTIVITY. AMONG MICROORGANISMS INVOLVED IN LIGNOCELLULOSE DEGRADATION, THE BACTERIUM ACETIVIBRIO THERMOCELLUS STANDS OUT FOR ORGANIZING ITS ENZYMES INTO COMPLEXES CALLED CELLULOSOMES, WHICH PROVIDE HIGH EFFICIENCY IN THE PROCESS. THIS STUDY INVESTIGATES THE SYNERGY BETWEEN THE MCIPA PROTEIN, DERIVED FROM A. THERMOCELLUS AND EXPRESSED IN ESCHERICHIA COLI BL21 (DE3), AND THE SECRETOMES OF THE FUNGI TRICHODERMA REESEI RUT-C30 AND TRICHODERMA HARZIANUM TR274 IN THE DECONSTRUCTION OF LIGNOCELLULOSIC SUBSTRATES. ADDITIONALLY, IT AIMS TO IDENTIFY WHICH MCIPA DOMAINS CONTRIBUTE TO ENHANCING FUNGAL SECRETOME ACTIVITY. THE CENTRAL HYPOTHESIS IS THAT THE PRESENCE OF THE CARBOHYDRATE-BINDING MODULE FAMILY 3 (CBM3) AND TWO COHESIN I DOMAINS IN MCIPA AMPLIFIES THE CELLULOLYTIC ACTIVITY OF THE SECRETOMES, PARTICULARLY IN THE DECONSTRUCTION OF BRACHIARIA, AN IMPORTANT CARBON SOURCE IN ANIMAL NUTRITION, AS WELL AS OTHER LIGNOCELLULOSIC SUBSTRATES. THE HETEROLOGOUS PROTEINS WERE CONSTRUCTED USING THE DIGESTION-LIGATION TECHNIQUE WITH THE PET-21A(+) VECTOR. THE EXPRESSION OF MCIPA, CBM3, AND COHESIN I WAS INDUCED BY AUTOINDUCTION, FOLLOWED BY PROTEIN ANALYSIS THROUGH SDS-PAGE AND WESTERN BLOTTING, AND QUANTIFICATION USING THE BCA ASSAY. PROTEIN PURIFICATION AND ITS DOMAINS WERE PERFORMED VIA AFFINITY CHROMATOGRAPHY. MEANWHILE, FUNGAL SECRETOMES WERE OBTAINED THROUGH LIQUID CULTURE IN MINIMAL MEDIUM CONTAINING BRACHIARIA AS THE CARBON SOURCE. HYDROLYSIS ASSAYS USING BRACHIARIA, AVICEL, MICROCRYSTALLINE CELLULOSE, AND CMC WERE CONDUCTED TO EVALUATE THE SYNERGY BETWEEN THE PURIFIED HETEROLOGOUS PROTEINS AND THE FUNGAL SECRETOMES.

MELHORIA DA DIGESTIBILIDADE DA SOJA PARA ALIMENTAÇÃO VIA EDIÇÃO DE GENOMA

MATEUS MEIRA DOS SANTOS, MARIA EUGENIA LISEI DE SÁ, NAYARA SABRINA DE FREITAS ALVES, ANA CRISTINA PINTO JUHAZ, MARIA FÁTIMA GROSSI DE SÁ.

SOYBEAN (GLYCINE MAX) IS A SIGNIFICANT CROP FOR HUMAN AND ANIMAL NUTRITION. HOWEVER, ITS DIGESTIBILITY IS HINDERED BY THE PRESENCE OF THE BOWMAN-BIRK INHIBITOR (BBI), A PROTEIN THAT INTERFERES WITH DIGESTIVE ENZYMES. THIS STUDY AIMS TO IMPROVE SOYBEAN DIGESTIBILITY BY KNOCKING OUT THE BBI GENE USING CRISPR/CAS9 TECHNOLOGY, SPECIFICALLY EMPLOYING THE HIGHLY EFFICIENT CRISPR ACT3.0 SYSTEM. INITIAL IN SILICO ANALYSES IDENTIFIED 14 LOCI ENCODING BBI, WITH EXPRESSION PATTERNS ASSESSED DURING SEED DEVELOPMENT. AMONG THESE, GMBBI11 SHOWED THE HIGHEST EXPRESSION. GUIDE RNAS (SGRNAS) WERE DESIGNED TO TARGET GMBBI11 AND VALIDATED THROUGH COMPUTATIONAL TOOLS AND SEQUENCING. EXPERIMENTAL APPROACHES INVOLVE QPCR TO MEASURE BBI EXPRESSION IN SOYBEAN SEEDS, CONSTRUCTION OF CRISPR/CAS9 VECTORS VIA GATEWAY TECHNOLOGY, AND VALIDATION OF SGRNA EFFICIENCY USING AGROBACTERIUM RHIZOGENES. THE MOST EFFECTIVE SGRNA WILL THEN BE EMPLOYED FOR STABLE SOYBEAN TRANSFORMATION USING AGROBACTERIUM TUMEFACIENS. THE MOLECULAR CHARACTERIZATION OF TRANSFORMED PLANTS INCLUDES DNA EXTRACTION, PCR, AND SEQUENCING TO CONFIRM INDEL MUTATIONS IN THE TARGET GENE. EDITED T1 PLANTS WILL BE CULTIVATED, AND T2 SEEDS ANALYZED FOR BIOCHEMICAL AND PHYSICAL PROPERTIES. PROTEIN EXTRACTION AND QUANTIFICATION WILL ASSESS THE RESIDUAL BBI LEVELS, AND ENZYMATIC ASSAYS WILL EVALUATE THE REMAINING INHIBITORY ACTIVITY. THE STUDY SUCCESSFULLY DEMONSTRATES THE DESIGN AND VALIDATION OF SGRNAS, RECOMBINANT VECTOR CONSTRUCTION, AND PRELIMINARY TRANSFORMATIONS, PAVING THE WAY FOR GENERATING GENETICALLY MODIFIED SOYBEAN LINES WITH IMPROVED DIGESTIBILITY FOR HUMAN AND LIVESTOCK CONSUMPTION.

DE NOVO GENOME ASSEMBLY AND ANNOTATION OF FUSARIUM OXYSPORUM F. SP. CUBENSE SUBTROPICAL RACE 4 INFECTING CAVENDISH BANANAS IN BRAZIL

RUTIANE MOREIRA DE JESUS COSTA, ERICA DE CASTRO COSTA, LUCAS SANTOS BASTOS, PRISCILA GRYNBERG, ROBERTO COITI TOGAWA, ROBERT NEIL GERARD MILLER

BANANA (MUSA SPP.) IS ONE OF THE MOST CONSUMED FRUITS WORLDWIDE, WITH BRAZIL CURRENTLY THE 4TH LARGEST PRODUCER, YIELDING APPROXIMATELY 6.8 MILLION TONS ANNUALLY. FUSARIUM WILT, CAUSED BY FUSARIUM OXYSPORUM F. SP. CUBENSE (FOC), IS THE MAIN DISEASE AFFECTING BANANA, WITH THE PATHOGEN PRESENT IN APPROXIMATELY 47% OF GLOBAL BANANA-PRODUCING AREAS. FOC IS CLASSIFIED INTO FOUR RACES BASED ON PATHOGENICITY: RACE 1 AFFECTS GROS MICHEL (AAA), APPLE, AND PRATA (AAB) CULTIVARS; RACE 2 AFFECTS BLUGGOE (ABB); AND RACE 4 AFFECTS CAVENDISH BANANAS. THE LATTER RACE IS FURTHER DIVIDED INTO TROPICAL RACE 4 (TR4) AND SUBTROPICAL RACE 4 (STR4), WITH

STR4 LESS AGGRESSIVE AND CAUSING DISEASE ONLY IN SUBTROPICAL CLIMATES. THE STR4 VCG 0120 IS THE MOST WIDELY DISTRIBUTED RACE IN BRAZIL. DESPITE ITS AGRICULTURAL IMPACT, GENOMIC DATA FOR FOC STR4 REMAINS LIMITED. IN THIS STUDY, WHOLE GENOME SEQUENCING WAS CONDUCTED ON FOC STR4 ISOLATE 218A CNPMF, ISOLATED FROM SÃO PAULO ON 'NANICA' (CAVENDISH CULTIVAR). DNA WAS EXTRACTED USING THE DNEASY PLANT MINI KIT (QIAGEN), AND SEQUENCING PERFORMED USING DNBSEQ PE 150 BP TECHNOLOGY (BGI GENOMICS, CHINA). QUALITY ANALYSIS WAS PERFORMED USING FASTQC (>30), WITH CONTIG ASSEMBLY CONDUCTED USING PLATANUS, BWISE, AND MASURCA, FOLLOWED BY BUSCO VALIDATION. PROTEIN ANNOTATION WAS PERFORMED USING FUNANNOTATE. THE GENOME SIZES AFTER ASSEMBLY WERE 43.4, 55.4, AND 43.5 MB FOR PLATANUS, BWISE, AND MASURCA, RESPECTIVELY, EACH PLATFORM GENERATING 1448, 1273, AND 861 CONTIGS, WITH BUSCO GENE COMPLETENESS VALUES OF 97.4%, 98.2%, AND 98.4%. A TOTAL OF 12,214, 15,858, AND 11,689 PROTEIN SEQUENCES WERE ANNOTATED IN THE THREE ASSEMBLIES. PACBIO SEQUENCING IS ONGOING TO FURTHER REFINE THE GENOME. COMPARATIVE ANALYSIS OF ORTHOLOGOUS GENES CONFIRMED IDENTITY AS FOC STR4 RACE. GENOMIC AND PROTEOMIC DATA WILL SUPPORT COMPARATIVE STUDIES OF FOC RACES AND PROVIDE INSIGHTS INTO EFFECTOR INVOLVED IN HOST INTERACTIONS.

IN SILICO STUDY OF THE NOTCH GENE AS A CANDIDATE FOR DSRNA SILENCING IN LEUCOPTERA COFFEELLA (LEPIDOPTERA: LYONETIIDAE)

ERICK SANTOS LUSTOSA DE QUEIROZ 1,2; ÁGUEDA GONÇALVES TAVARES 3; LEONARDO DE AMORIM VIDAL 2; ALINE ARROKELLAS HOLANDA DE MELO 2; GABRIEL BORGES RAIMUNDO 4; NATALIA FLORENCIO MARTINS 5; ÉRIKA VALÉRIA SALIBA ALBUQUERQUE FREIRE 2; ANDREA QUEIROZ MARANHÃO 6.

THE COFFEE LEAF MINER (LEUCOPTERA COFFEELLA) IS A MONOPHAGOUS MOTH (LEPIDOPTERA) CAPABLE OF CAUSING LOSSES TO COFFEE CROPS UP TO 87% IN PRODUCTIVITY. THIS PEST IS CONTROLLED BY CONVENTIONAL AGROCHEMICALS., ALTHOUGH CONTINUOUS APPLICATIONS MAY CONFER RESISTANCE TO INSECTICIDES. THUS, THERE IS A HIGH DEMAND TO SEARCHING OTHER CONTROL METHODS, SUCH AS THE SILENCING MECHANISM BY RNA INTERFERENCE (RNAI), WHICH USES DSRNA FOR TOPIC APPLICATION OR IN PLANTA EXPRESSION, AS A MORE SUSTAINABLE SOLUTION TO REDUCE CHEMICAL INSECTICIDE APPLICATIONS IN THE FIELD. THE NOTCH GENE, WHICH ENCODES THE NEUROGENIC LOCUS NOTCH PROTEIN, THAT IS INVOLVED IN SEVERAL METABOLIC PROCESSES, FROM DEVELOPMENT TO CELL DEATH, IS ALSO AN EFFECTIVE TARGET FOR GENE SILENCING. FROM TRANSCRIPTOMIC DATA, WE SELECTED THE BEST ANNOTATED SEQUENCE AND CONFIRMED THE TARGET GENE IDENTITY BY ADDITIONAL ANALYSES IN SOME DATABASES, SUCH AS NCBI AND UNIPROT, THROUGH ALIGNMENTS WITH ORTHOLOGOUS SEQUENCES OF MEMBERS OF THE LEPIDOPTERA CLADE, AND THEN PROCEEDED TO A REAL-TIME RT-PCR

PRIMER DESIGN. THEN, IN ORDER TO VALIDATE THE GENE EXPRESSION FOR FURTHER USE IN DSRNA APPLICATIONS, WE PERFORMED RNA EXTRACTIONS WITH DIFFERENT STAGES OF INSECT DEVELOPMENT: FOUR LARVAL INSTARS (L1, L2, L3, AND L4), PUPAE, AND ADULTS (MALE AND FEMALE). A PROPORTIONALITY OF THE NUMBER OF INDIVIDUALS AMONG THE SAMPLES WAS PREVIOUSLY ESTABLISHED TO REACH APPROXIMATELY 20 µL OF MACERATED MATERIAL. AFTER EXTRACTION, PURIFICATION, AND QUANTIFICATION, APPROXIMATELY 1 µG OF TOTAL RNA WAS CONVERTED TO CDNA. BY RT-QPCR, THE EXPRESSION WAS OBSERVED IN ALL STAGES OF DEVELOPMENT, WITH HIGH FOLD CHANGE VALUES (FC>2) IN SAMPLES FROM L4, PUPA, MALE AND FEMALE INDIVIDUALS. THIS GENE SEQUENCE WAS ALSO VALUABLE TO SYNTHESIZE DSRNA AND MAY BE CONSIDERED AS A VIABLE CANDIDATE FOR SILENCING VIA RNAI TO PERFORM IN LATE LARVAE AND MATURE CLM STAGES.

A NOVEL STRATEGY FOR ENHANCING GENE TRANSLATION VIA CRISPR/CAS9: THE GMPR10 GENE AS A CASE STUDY FOR TOLERANCE TO MELOIDOGYNE INCOGNITA IN SOYBEAN

LORENA S LOIOLA COSTA1,2, NAYARA S FREITAS-ALVES2,3, CLÍDIA E MOREIRA PINTO2,4, LILIAN H FLORENTINO3, FABRÍCIO B M ARRAES2,4, ELIBIO L RECH-FILHO2, CAROLINA V MORGANTE2,4,5, MARIA F GROSSI-DE-SA2,4,6.

ROOT-KNOT NEMATODES (RKN), MELOIDOGYNE SPP., ARE MAJOR PATHOGENS AFFECTING SOYBEAN YIELD AND QUALITY. A PREVIOUS OMICS STUDY COMPARING CONTRASTING SOYBEAN GENOTYPES IN TERMS OF SUSCEPTIBILITY TO MELOIDOGYNE INCOGNITA IDENTIFIED THE CLASS 10 PATHOGENESIS-RELATED PROTEIN (GMPR10) AS ASSOCIATED WITH TOLERANCE TO THIS PHYTONEMATODE. GMPR10 INTERFERES WITH NEMATODE DIGESTIVE ENZYMES AND CUTICLE INTEGRITY. THE GMPR10 GENE WAS OVEREXPRESSED IN TRANSGENIC TOBACCO PLANTS, RESULTING IN REDUCTIONS IN GALL NUMBER (51.6–57.8%), EGG NUMBER (41.9–43.5%), AND NEMATODE REPRODUCTION FACTOR (40.4–48.7%) COMPARED TO WILD-TYPE PLANTS. THESE RESULTS VALIDATE THE ROLE OF GMPR10 IN ENHANCING TOLERANCE TO M. INCOGNITA. RECENTLY, EDITING UPSTREAM OPEN READING FRAMES (UORFS) HAS EMERGED AS A STRATEGY FOR GENE OVEREXPRESSION USING CRISPR/CAS9 TECHNOLOGY, AS UORFS CAN NEGATIVELY REGULATE THE TRANSLATION OF PRIMARY ORFS (PORFS). IN THIS STUDY, WE IDENTIFIED TWO PUTATIVE UORFS IN THE 5'-UTR SEQUENCE OF GMPR10 IN SILICO. THIS REGION WAS CLONED INTO A DUAL-REPORTER SYSTEM CONTAINING LUCIFERASE/RENILLA (LUC/REN), AND THE FIRST ATGS OF THE UORFS WERE MUTATED. RESULTS FROM LUC REPORTER PROTEIN EXPRESSION IN PROTOPLASTS SHOWED THAT SINGLE MUTATIONS IN EITHER UORF1 OR UORF2 DID NOT CAUSE SIGNIFICANT DIFFERENCES COMPARED TO THE UNMUTATED SEQUENCE. HOWEVER, SIMULTANEOUS MUTATIONS IN BOTH UORFS INCREASED LUC ACTIVITY BY APPROXIMATELY 3.5 TIMES,

INDICATING THAT BOTH UORFS NEED TO BE MUTATED TO ENHANCE GMPR10 PROTEIN LEVELS. A STRATEGY WAS THEN DEVELOPED FOR THE SIMULTANEOUS MUTATION OF UORFS 1 AND 2 OF GMPR10 IN PLANTS VIA CRISPR/CAS9. THE SGRNAS WERE CLONED INTO CRISPR2.0 SYSTEM VECTORS AND ARE CURRENTLY BEING VALIDATED IN SOYBEAN HAIRY ROOTS. THE UORF DELETION STRATEGY USING CRISPR/CAS9 TO ENHANCE GENE TRANSLATION IS INNOVATIVE FOR SOYBEAN AND ENABLES THE PRODUCTION OF NON-TRANSGENIC PLANTS TOLERANT TO RKNS.

DEVELOPMENT OF MEMBRANE-LESS SYNTHETIC ORGANELLES AS A TOOL FOR CONTROLLING CELLULAR FUNCTIONS IN PLANTS

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EUKARYOTIC CELLS ORGANIZE THEIR COMPONENTS INTO ORGANELLES, WHICH CAN BE EITHER MEMBRANOUS OR NON-MEMBRANOUS, CREATING CELLULAR COMPARTMENTALIZATION ESSENTIAL FOR BIOLOGICAL FUNCTIONS. MANY OF THESE ORGANELLES ARE COMPOSED OF PROTEIN AND NUCLEIC ACID CONDENSATES, WHOSE ROLES IN PLANT CELLULAR FUNCTIONS REMAIN POORLY UNDERSTOOD. SPIDROINS, SPIDER SILK PROTEINS, ARE CAPABLE OF PROMOTING THE FORMATION OF THESE CONDENSATES. THEREFORE, THIS STUDY AIMS TO DESIGN SYNTHETIC SPIDROINS (SS) WITH PROPERTIES SIMILAR TO NATURAL ONES FOR THE FORMATION OF MEMBRANE-LESS SYNTHETIC ORGANELLES IN NICOTIANA TABACUM PROTOPLASTS. TO ACHIEVE THIS, THREE GENETIC CONSTRUCTS WERE DESIGNED: TWO CONTAINING DNA SEQUENCES FOR THE PRODUCTION OF SCAFFOLDING PROTEINS AND THE FORMATION OF FUNCTIONAL SYNTHETIC CONDENSATES (1 - JAZ_GFP, A CONTROL FOR CONDENSATE FORMATION, AND 2 - SS_GFP), AND ANOTHER CONTAINING THE PROTEIN TO BE SEQUESTERED (3 - CLIENT_MCHERRY) BY THE SYNTHETIC ORGANELLE THROUGH PROTEIN-PROTEIN INTERACTION DOMAINS. THE ALPHAFOLD 2 ALGORITHM WAS USED TO PREDICT THE THREE-DIMENSIONAL STRUCTURES OF THE PROTEINS. THE PROTOCOL FOR N. TABACUM CULTIVATION, AS WELL AS THE PROCEDURES FOR PROTOPLAST ISOLATION AND TRANSFORMATION, WERE ALSO OPTIMIZED.
SO FAR, THE SYNTHESIS AND EVALUATION OF CONSTRUCT 3 HAVE BEEN COMPLETED. FLUORESCENCE TESTS ON TRANSFORMED PROTOPLASTS WERE POSITIVE FOR THE EXPRESSION OF THE CLIENT_MCHERRY PROTEIN. NEXT STEPS INCLUDE TRANSFORMING PROTOPLASTS WITH THE OTHER TWO CONSTRUCTS AND EVALUATING SYNTHETIC PROTEIN EXPRESSION THROUGH WESTERN BLOTTING AND FLOW CYTOMETRY. THE FORMATION OF CONDENSATES WILL BE CONFIRMED USING FLUORESCENCE AND CONFOCAL MICROSCOPY.
THE USE OF SPIDER-DERIVED SS CAPABLE OF GENERATING PRECISE CELLULAR

RESPONSES IN PLANTS, THROUGH THE FORMATION OF ORGANELLE-LIKE STRUCTURES, HAS POTENTIAL APPLICATIONS IN METABOLIC PATHWAY MODIFICATION AND GENE EXPRESSION REGULATION IN PLANTS.

GENE EDITING IN TOMATO TO OBTAIN PLANTS TOLERANT TO THE HERBICIDE METRIBUZIN

CAMILLA SOARES FARIAS 1; NATÁLIA FAUSTINO CURY 2; THAIS DE MOURA CIPRIANO 2; DÉBORA ALMEIDA ALCANTARA DA SILVA 1; DANIEL CANTARIN SOUZA 3 ; FRANCISCO JOSÉ LIMA ARAGÃO 4

TOMATO (SOLANUM LYCOPERSICUM) IS ONE OF THE MOST IMPORTANT CROPS GLOBALLY, HOWEVER, THE CULTIVATION OF THIS PLANT FACES CONSTANT CHALLENGES, ESPECIALLY COMPETITION WITH SPECIFIC PLANTS THAT CAN COMPROMISE ITS PRODUCTIVITY. METRIBUZIN IS AN EFFECTIVE HERBICIDE FOR CONTROLLING THESE INVADERS, BUT ITS APPLICATION IN TOMATO PLANTS IS LIMITED BY THE SENSITIVITY OF THE CROP, WHICH CAN CAUSE PHYTOTOXICITY. IN THIS CONTEXT, CRISPR-CAS9 TECHNOLOGY EMERGES AS AN INNOVATIVE APPROACH FOR DEVELOPING HERBICIDE-TOLERANT STRAINS, ALLOWING THE PRECISE EDITING OF GENES RELATED TO SUSCEPTIBILITY. THIS WORK AIMED TO EDIT GENES ASSOCIATED WITH TOLERANCE TO METRIBUZIN IN TOMATO PLANTS OF THE MICRO-TOM CULTIVAR, USING GENETIC TRANSFORMATION MEDIATED BY AGROBACTERIUM TUMEFACIENS. THE TRANSFORMATION WAS CARRIED OUT IN SIX INDEPENDENT EXPERIMENTS. IN THE FIRST FOUR EXPERIMENTS, A TOTAL OF THE 348 EXPLANTS WERE SUBJECTED TO THE PROTOCOL, RESULTING IN 192 REGENERATIVE EFFECTS IN SELECTIVE MEDIA, INDICATING A REGENERATION RATES GREATER THAN 50%. FIVE SEEDLINGS ARE IN THE ROOTING PHASE, AND THE LAST TWO EXPERIMENTS ARE IN THE INITIAL SELECTION STAGE. DURING THE PROCESS, TWO DIFFERENT PATTERNS WERE OBSERVED: NON-VIABLE EXPLANTS SHOWED NECROSIS DUE TO SELECTIVE PRESSURE, WHILE REGENERATIVE EXPLANTS SHOWED

VIGOR AND THE FORMATION OF REGENERATIVE SHOOTS. THE PLANTS IN DEVELOPMENT WILL BE SUBJECTED TO MOLECULAR ANALYSES, SUCH AS PCR, IMMUNOCHROMATOGRAPHY TESTS (NPTII IMMUNOSTRIP®), AND SEQUENCING TO CONFIRM THE GENE EDITS. PRELIMINARY, THE RESULTS DEMONSTRATED THE EFFICIENCY OF THE TRANSFORMATION PROTOCOL, INDICATING THAT THE APPROACH CAN GENERATE TOMATO LINES THAT WILL BE TOLERANT TO THE HERBICIDE METRIBUZIN. THIS STUDY HIGHLIGHTS THE RELEVANCE OF GENE EDITING IN IMPROVING CROPS, CONTRIBUTING TO THE SUSTAINABLE MANAGEMENT OF HERBICIDES, AND INCREASING PRODUCTION EFFICIENCY.

DEVELOPMENT OF A MOLECULAR TOOL FOR GENETIC ENGINEERING OF THE MINIMAL CELL JCVI-SYN3B BY CRISPR/CAS9

MARIANA MATHIAS CONROY ARAUJO; JOHN GLASS; DANIELA MATIAS DE C. BITTENCOUR; ELIBIO RECH

MINIMAL CELLS ARE ORGANISMS WITH REDUCED GENOMES IN WHICH ALL GENES ARE ESSENTIAL FOR HOMEOSTASIS, REPLICATION AND SURVIVAL. THE OBJECTIVE OF CREATING A MINIMAL ORGANISM IS TO UNDERSTAND THE REQUIREMENTS FOR LIFE AND TO BUILD NEW PROGRAMMABLE CELLS. FROM THE MINIMAL NATURAL GENOME OF MYCOPLASMA MYCOIDES AND FURTHER ADVANCES IN MOLECULAR BIOLOGY, A SYNTHETIC ORGANISM WAS DEVELOPED, THE JCVI-SYN3.0 CELL, THE SMALLEST GENOME EVER REPORTED, WITH ONLY 438 GENES IN 531 KB. HOWEVER, AROUND 17% OF GENES ARE STILL UNKNOWN. TO BETTER UNDERSTAND ITS GENE CONTENT, NEW MOLECULAR TOOLS ARE NECESSARY SINCE, AS ITS NATURAL PRECURSOR CELL, THE JCVI-SYN3.0 HAS LIMITED KNOWN MUTAGENIC MECHANISMS. OUR PROPOSAL TO CIRCUMVENT THIS PROBLEM IS THE INTRODUCTION OF A SYSTEM CAPABLE OF CAUSING GUIDED MODIFICATIONS, THROUGH THE INSERTION OF CRISPR/CAS9 MACHINERY COUPLED TO THE HOMOLOGOUS RECOMBINATION SYSTEM, RECET. FOR THE INTRODUCTION OF CAS9 AND RECET, AN INTEGRATIVE PLASMID WAS DESIGNED AS AN OPERON REGULATED BY THE TETRACYLLINE PROMOTER, TO THE JCVI-SYN3B GENOME, A STRAIN THAT HAS CRE/LOX RECOMBINATION SITES. ONCE THE STRAIN CAPABLE OF EXPRESSING CAS9-RECET IS ESTABLISHED, WE WILL SILENCE A GENE WITH AN ALREADY KNOWN PHENOTYPE (JCVISYN3B_0004, KGSA) TO CONFIRM THE ACTIVITY OF THE SYSTEM. THE SUICIDE PLASMID CARRYING THE GUIDE RNA AND A HOMOLOGY SEQUENCE WILL BE TRANSFORMED INTO BACTERIA WITH TWO STRATEGIES: DELETION OF THE GENE SEQUENCE AND REPLACEMENT OF A

REPORTER GENE. BOTH MUTATIONS WILL BE CONFIRMED BY SEQUENCING OF TRANSFORMING CLONES, RESISTANCE ASSAY TO THE ANTIBIOTIC KASUGAMYCIN AND FOR THE REPORTER GENE, FLUORESCENCE EXPRESSION ANALYSIS AND THE EFFICIENCY OF THE SYSTEM WILL BE EVALUATED THROUGH PCR. THE INTRODUCTION OF THIS MECHANISM INTO THE MINIMAL CELL MIGHT BE USED FOR GUIDED MUTAGENESIS, ALLOWING THE STUDY OF STILL UNKNOWN GENES AND THEIR FUNCTIONS, CONTRIBUTING TO THE DEVELOPMENT OF PROGRAMMABLE CELLS.

GENOMIC INSIGHTS AND GROWTH OPTIMIZATION OF ALCALIGENES SP. FOR ENHANCED BIOPOLYMER PRODUCTION AND PLASTIC BIODEGRADATION

MARIANA PORTUGAL MATTIOLI ; JULIANNA PEIXOTO TREPTOW; RICARDO HENRIQUE KRUGER

SYNTHETIC POLYMERS ARE WIDELY UTILIZED DUE TO THEIR VERSATILITY AND DURABILITY, BUT THEIR ENVIRONMENTAL PERSISTENCE HAS LED TO SEVERE ECOLOGICAL CHALLENGES. CONVENTIONAL WASTE MANAGEMENT STRATEGIES, SUCH AS INCINERATION AND LANDFILLING, HAVE PROVEN INADEQUATE AND ENVIRONMENTALLY HARMFUL, UNDERSCORING THE URGENT NEED FOR SUSTAINABLE ALTERNATIVES. ALCALIGENES SP. HAS EMERGED AS A PROMISING MICROORGANISM CAPABLE OF DEGRADING SYNTHETIC POLYMERS AND SYNTHESIZING POLYHYDROXYALKANOATES (PHAS), A BIODEGRADABLE BIOPOLYMERS WITH SIGNIFICANT BIOTECHNOLOGICAL POTENTIAL. THIS STUDY AIMS TO OPTIMIZE THE CULTIVATION PARAMETERS OF ALCALIGENES SP., EVALUATE ITS CAPACITY TO DEGRADE POLYAMIDE, AND ELUCIDATE THE GENOMIC MECHANISMS UNDERLYING THESE PROCESSES. EXPERIMENTS WERE DESIGNED TO DETERMINE OPTIMAL GROWTH CONDITIONS BY VARYING PARAMETERS SUCH AS PH, TEMPERATURE, AND AGITATION. EXPERIMENTAL DATA INCLUDE GROWTH CURVES IN MICROPLATES AND BENCH-TOP BIOREACTORS, ALONGSIDE MORPHOLOGICAL AND GENETIC CHARACTERIZATIONS. GENOMIC ANALYSES WERE PERFORMED USING A HYBRID ASSEMBLY APPROACH, INTEGRATING ILLUMINA AND PACBIO DATA TO ACHIEVE A HIGH-QUALITY GENOME ASSEMBLY. ANALYSES OF PHA PRODUCTION, POLYAMIDE DEGRADATION, AND COMPREHENSIVE GENOMIC INSIGHTS ARE ONGOING. HOWEVER, PRESENT FINDINGS CONFIRM THE VIABILITY OF ALCALIGENES SP. FOR GROWTH IN BIOREACTOR SETTINGS FOR FUTURE BIOTECHNOLOGICAL APPLICATIONS. THESE FUTURE INVESTIGATIONS WILL ENHANCE THE UNDERSTANDING OF METABOLIC PATHWAYS AND VALIDATE THE POTENTIAL OF ALCALIGENES SP. AS A SUSTAINABLE SOLUTION FOR SYNTHETIC POLYMER WASTE MANAGEMENT. THE DEVELOPMENT OF THIS BIOPROCESS REPRESENTS A SIGNIFICANT STEP TOWARD ADVANCING THE CIRCULAR ECONOMY, LEVERAGING SCIENTIFIC INNOVATION TO ADDRESS THE ENVIRONMENTAL CHALLENGES POSED BY SYNTHETIC POLYMERS.

RNAI-MEDIATED GENE SILENCING AS A SOURCE OF RESISTANCE TO MELOIDOGYNE INCOGNITA PARASITISM IN SOYBEAN CROPS

PAULA DARLINY SILVA FERREIRA, CAROLINA VIANNA MORGANTE, MARIA FÁTIMA GROSSI DE SÁ

THE GENUS MELOIDOGYNE IS FORMED BY OBLIGATE PLANT PARASITIC NEMATODES RESPONSIBLE FOR THE FORMATION OF SO-CALLED ROOT GALLS, WHICH RESULT FROM MORPHOLOGICAL AND PSYCHOLOGICAL CHANGES THAT PREVENT HOSTS FROM EFFECTIVELY ABSORBING WATER AND NUTRIENTS FROM THE SOIL. THE SPECIES MELOIDOGINE INCOGNITA IS A PROMINENT MEMBER OF THIS GENUS, CAUSING SIGNIFICANT ECONOMIC LOSSES DUE TO ITS HIGH PARASITIC EFFICIENCY AND DIRECTLY DAMAGING THE ROOTS OF MORE THAN 3,000 HOST PLANT SPECIES. THIS SPECIES IS HIGHLY POLYPHAGOUS, AND THIS COSMOPOLITAN ACTIVITY IS ATTRIBUTED TO A COCKTAIL OF EFFECTOR PROTEINS SECRETED TO ESTABLISH ITS FEEDING SITE, WHICH IS ESSENTIAL FOR THE COMPLETION OF ITS LIFE CYCLE. IN THIS CONTEXT, UNDERSTANDING THE ACTION OF THESE EFFECTORS AND THEIR SYNERGISTIC INTERACTIONS ALLOWS FOR THE ADOPTION OF GENE INTERVENTION STRATEGIES VIA RNA INTERFERENCE (RNAI) TARGETING TRANSCRIPTS INVOLVED IN THE TRANSLATION OF THESE PROTEINS, AS WELL AS THE IDENTIFICATION OF GENES ASSOCIATED WITH THE RESISTANCE OF NON-HOST PLANTS. STUDIES CONDUCTED BY OUR RESEARCH GROUP HAVE IDENTIFIED THE GENES MINC03328 AND MINC16803 AS PROMISING TARGETS FOR RNAI-MEDIATED GENE SILENCING, SHOWING A CONSIDERABLE REDUCTION IN M. INCOGNITA INFECTION LEVELS IN TRANSGENIC ARABIDOPSIS THALIANA PLANTS. THESE GENES WERE DEMONSTRATED TO BE LINKED TO THE INDUCTION AND MAINTENANCE OF GIANT CELLS IN HOST PLANTS. THEREFORE, THIS STUDY AIMS TO CONSTRUCT TRANSFORMATION GENE CASSETTES DESIGNED TO GENERATE TRANSGENIC CROP PLANTS OF COMMERCIAL INTEREST, PARTICULARLY SOYBEANS, FROM SUSCEPTIBILITY TO M. INCOGNITA ATTACKS.

SOLANUM LYCOPERSICUM EXPRESSING CRY34AB1 AND CRY35AB1 FOR THE CONTROL OF TUTA ABSOLUTA

DOUGLAS BRAGA FÉLIX, DÉBORA ALMEIDA ALCÂNTARA DA SILVA, NATÁLIA FAUSTINO CURY, FRANCISCO JOSÉ LIMA ARAGÃO

SOLANUM LYCOPERSICUM IS ONE OF THE MOST IMPORTANT CROPS WORLDWIDE, IT IS WIDELY CULTIVATED FOR ITS NUTRITIOUS FRUITS. TUTA ABSOLUTA IS A LEPIDOPTERAN INSECT RESPONSIBLE FOR PRODUCTIVITY LOSS IN TOMATOES, IT LAYS EGGS THAT TURN INTO LARVAE THAT FEED ON THE TISSUE LEAVING OPEN WOUNDS. CHEMICAL CONTROL HAS HISTORICALLY BEEN THE MAIN APPROACH, ALTHOUGH RESISTANT

POPULATIONS ARE ON THE RISE. THE USE OF LIMITED SUBSTANCES IS HARMFUL TO THE ENVIRONMENT AND POSES A THREAT TO NON-TARGET SPECIES. BACILLUS THURINGIENSIS IS KNOWN FOR PRODUCING INSECT-KILLING PROTEINS CALLED "CRY". AMONG THESE PROTEINS, CRY34AB1 AND 34AB1 HAVE NOT BEEN TESTED FOR THE CONTROL OF THE TOMATO LEAF-MINER YET. THE AIM OF THIS PROJECT IS TO TEST THE CONTROL EFFICIENCY OF THIS PEST BY EXPRESSING CRY34/35AB1 ON TOMATOES. GENETICALLY MODIFIED PLANTS CONTAINING THE TRANSGENES OF INTEREST WERE GENERATED BY A. TUMEFACIENS TRANSFORMATION PROTOCOL, WHERE COTYLEDON SECTIONS ARE EXPOSED TO A MEDIUM ENRICHED WITH TRANSGENIC BACTERIAS CONTAINING THE GENES OF INTEREST. THE EXPLANTS WERE THEN CULTIVATED ON A SELECTION MEDIUM TO ONLY ALLOW THE SURVIVAL OF THE CELLS THAT WERE SUCCESSFULLY TRANSFORMED. THESE CELLS WERE CULTIVATED ON ELONGATION AND ROOTING MEDIUM TO PRODUCE INDEPENDENT PLANTS FOR ACCLIMATION AND PROGENY PRODUCTION. SIXTY GENETICALLY MODIFIED PLANTS WERE GENERATED AND TWENTY PLANTS WERE ACCLIMATED. MOLECULAR ASSAYS WERE USED TO DETERMINE THE PRESENCE OF THE TRANSGENES. PCR WAS USED TO DETERMINE THE PRESENCE OF AMPLICONS OF CRY34 AND CRY35 AND LATERAL FLOW IMMUNOASSAY FOR DETECTING NPTII PROTEIN USED AS A SELECTION MARKER. ALL ACCLIMATED PLANTS HAVE THE PRESENCE OF THE TRANSGENE OF INTEREST AND ARE EXPRESSING THE NPTII PROTEINS. THE T1 GENERATION WILL BE USED FOR AN BIOESSAY AGAINST T. ABSOLUTA TO VERIFY THE EFFICIENCY OF CONTROL. IF EFFECTIVE, THESE PROTEINS WILL HAVE THE POTENTIAL TO BECOME AN ALTERNATIVE CONTROL FOR T. ABSOLUTA WHICH IS EFFECTIVE AND POSES LITTLE THREAT TO WILDLIFE AS CURRENT METHODS.

DEVELOPMENT OF A MOLECULAR TEST FOR NEONATAL SCREENING TO QUANTIFY TREC AND KREC

THAÍS LIMA DE SENA, DANIEL MENDES PEREIRA ARDISSON DE ARAÚJO, MIGUEL DE SOUZA ANDRADE

SEVERE COMBINED IMMUNODEFICIENCY (SCID) IS A RARE GENETIC DISEASE THAT COMPROMISES THE FUNCTIONALITY OF T, B, AND/OR NK LYMPHOCYTES, MAKING NEWBORNS SUSCEPTIBLE TO PERSISTENT AND RECURRENT INFECTIONS. REDUCED LEVELS OF T-CELL RECEPTOR EXCISION CIRCLES (TREC) AND KAPPA-DELETING RECOMBINATION EXCISION CIRCLES (KREC) ARE CRUCIAL MARKERS FOR DIAGNOSING SCID AND OTHER INBORN ERRORS OF IMMUNITY (IEI). EARLY DIAGNOSIS IS ESSENTIAL DUE TO THE SEVERITY OF THE DISEASE AND ITS IMPACT ON SURVIVAL. THIS STUDY AIMS TO VALIDATE A MULTIPLEX QPCR TEST FOR NEONATAL SCID SCREENING. PLASMIDS WERE USED AS QUANTIFIED POSITIVE CONTROLS, AND BIOLOGICAL SAMPLES WERE INCLUDED FOR ANALYTICAL AND CLINICAL VALIDATION. THE ANALYSIS INVOLVED 50 SAMPLES WITH NORMAL TREC AND KREC LEVELS

(NEWBORNS) AND 20 SAMPLES WITH REDUCED LEVELS (HEALTHY ADULTS). DNA WAS EXTRACTED FROM 3 MM FILTER PAPER DISCS USING THE GENERATION DNA KIT – QIAGEN. QPCR WAS STANDARDIZED ON THE CFX96 THERMOCYCLER (BIO-RAD) USING SPECIFIC PRIMERS FOR TREC AND KREC SYNTHESIZED BY IDT, WITH RNASEP AS THE INTERNAL CONTROL. OPTIMIZED CONDITIONS INCLUDED PRIMERS AND PROBES AT 0.8 MM AND 0.4 MM, RESPECTIVELY, AND THE FOLLOWING AMPLIFICATION STEPS: 50 °C/120S, 95 °C/10MIN, 45 CYCLES OF 95°C/5S, 55°C/15S, AND 65°C/45S. METHOD VALIDATION DEMONSTRATED REACTION EFFICIENCY EXCEEDING 98%, A DETECTION LIMIT OF 6 COPIES PER REACTION, AND A COEFFICIENT OF DETERMINATION ($R^2 \geq 0.99$). PRECISION TESTS SHOWED EXCELLENT REPEATABILITY AND REPRODUCIBILITY. ANALYTICAL PRECISION AND CLINICAL VALIDATION ASSAYS ARE BEING FINALIZED, AS WELL AS THE DEFINITION OF REFERENCE VALUES. THIS STUDY WAS APPROVED BY THE ETHICS COMMITTEE, CAAE: 80346424.8.0000.0257. THE IMPLEMENTATION OF THIS TOOL CAN ENHANCE NEONATAL SCREENING, ENABLING FASTER AND MORE EFFECTIVE CLINICAL INTERVENTIONS, WITH THE POTENTIAL TO SIGNIFICANTLY IMPROVE THE PROGNOSIS OF PATIENTS WITH SCID.

PFAM SELECT: AN EFFICIENT APPROACH FOR PROTEIN FAMILY SELECTION

DEBORAH BAMBIL, PATRÍCIA VERDUGO PASCOAL, RAYANE NUNES LIMA, LUISA MAYUMI ARAKE DE TACCA, MARCO ANTÔNIO DE OLIVEIRA, ELÍBIO RECH

PROTEIN FAMILIES DATABASE (PFAM) IS A COMPREHENSIVE AND WIDELY USED RESOURCE THAT CATEGORIZES PROTEIN SEQUENCES INTO FAMILIES BASED ON SEQUENCE SIMILARITY AND FUNCTIONAL ANNOTATION. DESIGNED TO SUPPORT COMPARATIVE GENOMICS, FUNCTIONAL ANNOTATION, AND EVOLUTIONARY STUDIES, PFAM PROVIDES A STRUCTURED REPOSITORY WHERE PROTEINS ARE GROUPED INTO FAMILIES AND ASSIGNED UNIQUE IDENTIFIERS, KNOWN AS PFAM IDS.
PFAM SELECT IS A PYTHON-BASED PROGRAM DEVELOPED TO SELECT SPECIFIC PFAM SEQUENCES (PROTEIN FAMILY SEQUENCES) USING THE FAMILY ID INFORMATION. EQUIPPED WITH AN INTUITIVE GRAPHICAL INTERFACE, IT RUNS ON OPERATING SYSTEMS SUCH AS WINDOWS AND LINUX DISTRIBUTIONS, PROVIDED PYTHON IS INSTALLED. THIS PROGRAM WAS CREATED TO SIMPLIFY THE SEARCH AND SELECTION OF PFAM SEQUENCES, ESPECIALLY CONSIDERING THAT THE CURRENT PFAM VERSION (37) HAS A DATABASE SIZE OF 12 GB, MAKING MANUAL SEQUENCE SELECTION HIGHLY COMPLEX OR EVEN UNFEASIBLE.
PFAM SELECT OFFERS A USER-FRIENDLY INTERFACE THAT ENABLES EFFICIENT AND PRECISE SEQUENCE SELECTION. USERS CAN INPUT SPECIFIC IDS TO SEARCH FOR AND EXTRACT SEQUENCES FROM THE PFAM DATABASE. THE OUTPUT PROVIDES THE SELECTED SEQUENCES CORRESPONDING TO THE

SPECIFIED IDS IN A CLEAR AND ORGANIZED MANNER. THIS TOOL IS PARTICULARLY VALUABLE FOR RESEARCHERS AND PROFESSIONALS WORKING WITH LARGE SEQUENCE DATABASES LIKE PFAM. IT STREAMLINES THE SELECTION PROCESS, SAVING TIME AND RESOURCES. PFAM SELECT IS AN ESSENTIAL TOOL FOR EXTRACTING SPECIFIC SEQUENCES FROM THE PFAM DATABASE. ITS INTUITIVE INTERFACE AND ABILITY TO HANDLE LARGE DATA VOLUMES MAKE IT INDISPENSABLE FOR RESEARCHERS SEEKING AN EFFICIENT AND PRACTICAL SOLUTION FOR INTEREST SEQUENCE SELECTION. WITH SUPPORT FOR BOTH WINDOWS AND LINUX OPERATING SYSTEMS, IT IS ACCESSIBLE AND EASY TO USE, AS LONG AS PYTHON IS INSTALLED ON THE SYSTEM.

GENE SILENCING BY RNAI IN TOMATO AS A STRATEGY FOR RESISTANCE TO ORTHOTOSPOVIRUS

DÉBORA ALMEIDA ALCANTARA DA SILVA; NATÁLIA FAUSTINO CURY; DOUGLAS BRAGA FÉLIX; CAMILLA SOARES FARIAS; DANIEL CANTARIN SOUZA; GABRIELA CANTARINO SÁ; FRANCISCO JOSÉ LIMA ARAGÃO

THE TOMATO PLANT (SOLANUM LYCOPERSICUM L.) IS ONE OF THE MOST IMPORTANT VEGETABLES IN THE WORLD. VIRUSES FROM THE GENUS ORTHOTOSPOVIRUS ARE AMONG THE MOST HARMFUL PLANT VIRUSES GLOBALLY, CAUSING SEVERE DAMAGE TO MANY IMPORTANT CROPS. SOME ORTHOTOSPOVIRUS SPECIES ARE KNOWN TO CAUSE THE SPOTTED WILT DISEASE, INCLUDING TOMATO SPOTTED WILT VIRUS (TSWV), TOMATO CHLOROTIC SPOT VIRUS (TCSV), GROUNDNUT RINGSPOT VIRUS (GRSV), AND CHRYSANTHEMUM STEM NECROSIS VIRUS (CSNV). ORTHOTOSPOVIRUSES ARE TRANSMITTED BY SEVERAL SPECIES OF THRIPS. IT HAS BEEN SHOWN THAT THE GENE SW-5B CONFERS BROAD-SPECTRUM RESISTANCE TO SEVERAL TOSPOVIRUSES (INCLUDING TSWV, GRSV, AND TCSV). HOWEVER, THE RESISTANCE MEDIATED BY SW-5B HAS EXERTED SELECTIVE PRESSURE ON TSWV, LEADING TO THE EMERGENCE OF RESISTANT STRAINS, WHICH HAVE BEEN REPORTED IN SEVERAL COUNTRIES. THE AIM OF THIS RESEARCH IS TO OBTAIN A TOMATO PLANT LINEAGE RESISTANT TO VARIOUS TOSPOVIRUS SPECIES PREVALENT IN BRAZIL USING THE RNA INTERFERENCE (RNAI) STRATEGY TO SILENCE VIRAL GENES. INTRON-HAIRPIN TYPE VECTORS WERE DESIGNED TO SILENCE GENES PRESENT IN THE M SEGMENT OF THE ORTHOTOSPOVIRUS GENOME. THE VECTORS WERE TRANSFERRED TO AGROBACTERIUM TUMEFACIENS BY ELECTROPORATION. FOURTEEN TRANSFORMATIONS OF TOMATO COTYLEDON EXPLANTS WERE CARRIED OUT USING THE PROTOCOL OF SUN ET AL. (2006). THE EXPLANTS ARE BEING MAINTAINED ON SELECTION MEDIUM CONTAINING KANAMYCIN. SOME COTYLEDONS FORMED REGENERATING SHOOTS THAT DIFFERENTIATED INTO STEMS AND LEAVES. CURRENTLY, THREE PLANTS ARE IN ROOTING MEDIUM, AND FOLIAR SAMPLES WILL BE COLLECTED TO CONFIRM GENETIC TRANSFORMATION ONCE THEY GROW LARGER. DNA EXTRACTION, PCR, AND

GEL ELECTROPHORESIS ANALYSIS WILL BE CONDUCTED ON THE FOLIAR SAMPLES. DETECTION OF THE PROTEINS OF INTEREST WILL BE PERFORMED THROUGH IMMUNODETECTION STRIPS FOR THE NEOMYCIN PHOSPHOTRANSFERASE PROTEIN NPTII.

O SILENCIAMENTO GÊNICO E GENE DE BT USADOS PARA CONTROLE DO "BICUDO DO ALGODOEIRO", AFETAM AS ABELHAS?

RANGEL DE FREITAS ALVES, DANIEL DAVID NORIEGA VASQUEZ E MARIA FÁTIMA GROSSI-DE-SÁ

THE COTTON BOLL WEEVIL (CBW) IS ONE OF THE PESTS LIMITING THE EXPANSION OF COTTON IN BRAZIL. TO OVERCOME THIS CHALLENGE, GENETICALLY MODIFIED (GM) COTTON RESISTANT TO CBW IS BEING DEVELOPED USING RNA INTERFERENCE (RNAI) AND BT (BACILLUS THURINGIENSIS) TECHNOLOGY. HOWEVER, GM CROPS MAY HAVE A NEGATIVE IMPACT ON BIODIVERSITY AND NONTARGET ORGANISMS, SUCH AS BEES, WHICH ARE IMPORTANT POLLINATORS. THEREFORE, BIOSAFETY TESTS ARE NECESSARY TO RISK ASSESSMENT BEFORE THE COMMERCIALIZATION OF A NEW GM ORGANISM. THIS STUDY AIMS TO INVESTIGATE THE LETHAL AND SUBLETHAL EFFECTS OF DOUBLE-STRANDED RNAS (DSRNA: DSCHS2 AND DSVG) AND ENTOMOTOXIN (CRY23/37AA) PRESENT IN GM COTTON POLLEN AND INGESTED BY LARVAE AND ADULTS OF TWO BEE SPECIES: SCAPTOTRIGONA POSTICA (NATIVE) AND APIS MELLIFERA (EXOTIC). WORKER AND PRINCESS BEES (IMMATURE AND ADULT) WILL BE EVALUATED UNDER CONTROLLED CONDITIONS AT THE PLANT-PEST MOLECULAR INTERACTION LABORATORY (LIMPP) OF EMBRAPA CENARGEN. FOR LARVAE, THE TESTS CONSIST OF THREE TREATMENTS (TRIPLICATES): LARVAL DIET (LD) + DSRNA OR CRY; LD + DISTILLED AND AUTOCLAVED WATER; AND PURE LD. THESE TESTS WILL BE REPEATED FOR EACH DSRNA AND CRY. THERE WILL BE 20 LARVAE PER PLATE (96 WELLS AND NATURAL FOOD) FOR S. POSTICA AND 20 LARVAE (48 WELLS PLATE AND ARTIFICIAL FOOD) FOR A. MELLIFERA, TOTALING 1080 LARVAE FOR EACH BEE SPECIES. FOR ADULTS, THE TESTS CONSIST OF THE SAME TREATMENTS, BUT INSTEAD OF LD, SYRUP (WATER + SUGAR) WILL BE USED, IN QUADRUPLICATES, WITH 10 BEES IN EACH ONE-LITER CIRCULAR CONTAINER. A TOTAL OF 720 ADULT BEES OF EACH SPECIES WILL BE USED. THE EFFECTS EVALUATED IN LARVAE WILL INCLUDE MORTALITY, DEVELOPMENTAL TIME OF IMMATURES, AND IN NEWLY EMERGED BEES, BODY MASS, CEPHALIC CAPSULE WIDTH, AND INTERTEGULAR DISTANCE. THE EFFECTS EVALUATED IN ADULTS WILL INCLUDE MORTALITY AND LOCOMOTION ACTIVITY. WITH THIS WORK, WE HOPE TO CONTRIBUTE TO ACHIEVING SUSTAINABLE AGRICULTURE WITH GENETICALLY MODIFIED ORGANISMS IN BRAZIL.

META HUNTER: AN INNOVATIVE PIPELINE FOR PROTEINS FAMILY IDENTIFICATION IN METAGENOMES

RAYANE NUNES LIMA, DEBORAH BAMBIL, PATRÍCIA VERDUGO PASCOAL, MARCO ANTÔNIO DE OLIVEIRA, LUISA MAYUMI ARAKE DE TACCA, ELIBIO RECH

METAHUNTER IS A PROGRAM DEVELOPED IN C/C++ WITH CONTROL FLOW MANAGED VIA SHELL SCRIPT TO IDENTIFY SEQUENCES HOMOLOGOUS TO FAMILIES IN THE PROTEIN FAMILIES DATABASE (PFAM), A WIDELY RECOGNIZED RESOURCE THAT CATEGORIZES PROTEIN SEQUENCES INTO FAMILIES BASED ON SEQUENCE SIMILARITY AND FUNCTIONAL ANNOTATION, USING METAGENOMIC FILES AS INPUT. METAGENOMES REPRESENT THE COLLECTIVE GENETIC MATERIAL OF MICROBIAL COMMUNITIES EXTRACTED DIRECTLY FROM ENVIRONMENTAL SAMPLES, PROVIDING INSIGHTS INTO THE DIVERSITY AND FUNCTIONS OF ORGANISMS THAT CANNOT BE EASILY CULTURED IN THE LABORATORY. THE PROGRAM EFFICIENTLY SEARCHES FOR HOMOLOGOUS SEQUENCES TO PROTEIN FAMILIES. INPUTS CAN INCLUDE METAGENOMES OR GENOMES, AND THE TOOL PROCESSES MULTIPLE FILES SIMULTANEOUSLY, AS LONG AS THEY ARE LOCATED IN THE DIRECTORY SPECIFIED BY THE USER. ANALYSES ARE EXECUTED IN PARALLEL, SIGNIFICANTLY REDUCING PROCESSING TIME COMPARED TO SEQUENTIAL METHODS. METAHUNTER OFFERS SIMPLE AND INTUITIVE COMMANDS, ENABLING USERS TO IDENTIFY HOMOLOGOUS SEQUENCES EFFICIENTLY AND ACCURATELY. ITS KEY FUNCTIONALITIES INCLUDE: A COMMAND-LINE INTERFACE COMPATIBLE WITH UBUNTU OPERATING SYSTEMS, FLEXIBILITY TO SPECIFY THE NUMBER OF THREADS, THE PATH TO THE FILE CONTAINING THE TARGET FAMILY (E.G., SEQUENCE.FA), AND THE DIRECTORY WHERE THE METAGENOMIC FILES ARE STORED. THIS TOOL IS PARTICULARLY VALUABLE FOR RESEARCHERS AND PROFESSIONALS WORKING WITH LARGE SEQUENCE DATABASES SUCH AS METAGENOMES. METAHUNTER IS AN EFFICIENT AND PRACTICAL SOLUTION FOR SEARCHING HOMOLOGOUS SEQUENCES WITHIN LARGE DATABASES OF METAGENOMES AND GENOMES. ITS PARALLEL PROCESSING CAPABILITY ENABLES SIGNIFICANT TIME SAVINGS, MAKING IT IDEAL FOR RESEARCHERS HANDLING LARGE DATASETS. WITH SIMPLE AND INTUITIVE COMMANDS, METAHUNTER SIMPLIFIES PROTEIN SEQUENCE ANALYSIS, CONTRIBUTING TO ADVANCEMENTS IN BIOINFORMATICS AND METAGENOMICS. IT PROVIDES A ROBUST AND ACCESSIBLE SOLUTION FOR EXPLORING PROTEIN FAMILIES ACROSS DIVERSE BIOLOGICAL ENVIRONMENTS.

A ANALYSIS OF TRICHODERMA REESEI RUT C-30 AND PLEUROTUS CITRINOPILEATUS COCULTURES FOLLOWING GROWTH ON LIGNOCELLULOSIC CARBON SOURCES

RUTIANE MOREIRA DE JESUS COSTA, BRUNA PENA SOLLERO, MAQUIR ALMIRANTE CARDOSO, PRISCILA GRYNBERG, ROBERTO COITI TOGAWA, TAÍSA GODOY GOMES, ROBERT NEIL GERARD MILLER

BIOECONOMY DRIVES SUSTAINABLE PRODUCTION BY REDUCING ENVIRONMENTAL IMPACTS. BRAZIL IS A LEADER IN BIOETHANOL AND BIODIESEL PRODUCTION, WITH SIGNIFICANT POTENTIAL FOR EXPANDING 2G ETHANOL USING AGRICULTURAL RESIDUES SUCH AS SUGARCANE BAGASSE. THIS STUDY AIMS TO ANALYZE DIFFERENTIAL GENE EXPRESSION IN TRICHODERMA REESEI RUT C-30 AND PLEUROTUS CITRINOPILEATUS ASSOCIATED WITH THE BIODEGRADATION OF SUGARCANE BAGASSE, IN BOTH MONOCULTURE AND COCULTURE SYSTEMS. FOLLOWING FUNGAL GROWTH ON SUGARCANE BAGASSE AND GLUCOSE CONTROLS OVER A SIX-DAY PERIOD, RNA EXTRACTION AND PURIFICATION WERE PERFORMED USING A RELIAPREP MIRNA CELL AND TISSUE MINIPREP SYSTEM, WITH SEQUENCING CARRIED OUT USING ILLUMINA NEXTSEQ TECHNOLOGY. QUALITY ASSESSMENT WAS CONDUCTED VIA FASTQ AND MAPPING WAS PERFORMED USING CONCATENATED REFERENCE GENOMES OF EACH FUNGUS USING STAR. HTSEQ-COUNT WAS EMPLOYED TO COUNT THE NUMBER OF MAPPED READS, SEPARATING THE BAM FILES WITH THE CORRESPONDING GFF FILE FOR EACH FUNGUS. THE PIPELINE FOR ANALYSIS OF READ COUNTS FROM RNA-SEQ EXPERIMENTS OF EACH SPECIES APPLIED THE ‘VOOM’ METHOD IMPLEMENTED IN THE EDGER PACKAGE. THE OVERALL MAPPED ALIGNMENT RATE ON THE CONCATENATED REFERENCE GENOME WAS OVER 80% FOR ALL SAMPLES. ACCORDING TO THE CONTRASTS IN A TWO-WAY FACTORIAL DESIGN (COCULTIVATION AND MONOCULTURE ON BAGASSE AND GLUCOSE AS CARBON SOURCES), A TOTAL OF 7,417 DEGS (DIFFERENTIALLY EXPRESSED GENES) WERE IDENTIFIED FOR TRICHODERMA, IN THE INTERACTION BETWEEN CULTIVATION TYPES (MONO OR CO) AND CARBON SOURCE. A TOTAL OF 388 UNIQUE GENES BETWEEN CARBON SOURCES, AND 407 DEGS BETWEEN TYPES OF CULTIVATION WERE IDENTIFIED. FOR PLEUROTUS, A TOTAL OF 3,418, 2,662 AND 1,546 DEGS WERE OBSERVED IN THE SAME RESPECTIVE COMPARISONS. IN THE CONTEXT OF FUNCTIONAL ANALYSIS AND GENE REGULATORY NETWORK INFERENCE, CANDIDATE GENES ASSOCIATED WITH LIGNOCELLULOSIC BIOMASS DEGRADATION WILL BE SELECTED BASED ON THE RESULTS OBTAINED FROM THE COMPARATIVE TRANSCRIPTOME ANALYSIS.

SOYBEAN TRANSFORMATION FOR EXPRESSION OF THE MDODHN11 GENE INVOLVED WITH ABIOTIC STRESSES

JULIANE COSTA CABRAL 1,2 LAYZA MIRANDA ALVES LEITE 1,4 NATÁLIA LIMA DE SOUSA1; LUÍS FERNANDO REVERS 3 FRANCISCO JOSÉ LIMA ARAGÃO

SOYBEAN (GLYCINE MAX (L.) MERRILL) PRODUCTION IS HIGHLY SENSITIVE TO DROUGHT; PROLONGED DROUGHT REDUCES PRODUCTIVITY, CAUSING SUBSTANTIAL FINANCIAL LOSSES TO THE SOYBEAN PRODUCTION CHAIN. THE

DEVELOPMENT OF NEW CULTIVARS TOLERANT TO PERIODS OF WATER STRESS CAN BE PRODUCED THROUGH GENETIC ENGINEERING TECHNIQUES. DEHYDRINS (DNHS) ARE PROTEINS RELATED TO PROTECTION AGAINST ABIOTIC STRESS IN APPLE (MALUS × DOMESTICA BORKH). STUDIES WITH TRANSGENIC ARABIDOPSIS PLANTS EXPRESSING THE MDODHN11 GENE, SUBJECTED TO SEVERE WATER STRESS, CONFIRMED THE PROTECTIVE IMPORTANCE OF DHNS IN PERIODS OF DROUGHT. GIVEN THE IMPORTANCE OF SOYBEAN FOR WORLD AGRICULTURE AND THE RISKS THAT CHANGES IN PRECIPITATION PATTERNS POSE TO THE CROP, THE OBJECTIVE OF THIS WORK WAS TO GENETICALLY TRANSFORM SOYBEAN TO EXPRESS THE MDODHN11 GENE IN ORDER TO INCREASE TOLERANCE TO WATER STRESS. A TRANSFORMATION VECTOR WAS CONSTRUCTED TO EXPRESS MDODHN11 UNDER THE CONTROL OF THE 35S PROMOTER (CAULIFLOWER MOSAIC VIRUS - 35 SCAM); THE ATAHAS GENE, WHICH CONFERS RESISTANCE TO IMIDAZOLINONE HERBICIDES, WAS USED AS A SELECTIVE CONTROL. THE BIOLISTIC SYSTEM WAS USED FOR DIRECT TRANSFORMATION OF SOYBEAN EMBRYOS. AFTER TRANSFORMATION AND SELECTION, PCR ANALYSES CONFIRMED THE PRESENCE OF THE DHN11 TRANSGENE IN REGENERATED SEEDLINGS. PROGENY ANALYSIS CONFIRMED THE PRESENCE OF THE INSERT IN THE T1 GENERATION, SEGREGATING IN A 3:1 MENDELIAN RATIO. T2 PLANTS WITH THE PRESENCE OF THE DHN11 TRANSGENE AND NON-TRANSGENIC PLANTS WERE GROWN IN POTS IN A GREENHOUSE. AFTER 45 DAYS OF GERMINATION, THE PLANTS WERE SUBJECTED TO A PRELIMINARY WATER STRESS TEST. WILD-TYPE PLANTS WERE UNABLE TO RECOVER AFTER THE PERIOD OF WATER STRESS, WHILE THOSE EXPRESSING THE MDODHN11 TRANSGENE WERE ABLE TO SURVIVE, SUGGESTING A PROTECTIVE EFFECT OF THE GENE AGAINST DROUGHT.

METAGENOMIC ANALYSIS OF THE FERNANDO DE NORONHA MARINE NATIONAL PARK ECOSYSTEM: GENETIC AND BIOTECHNOLOGICAL INSIGHTS

CARLOS ALEXANDRE XAVIER DE AZEVEDO, ELIBIO RECH, LUÍSA MAYUMI ARAKE DE TACCA, RAYANE NUNES LIMA, PATRÍCIA VERDUGO PASCOAL, MARCO ANTÔNIO DE OLIVEIRA, DEBORAH RIBEIRO BAMBIL

THE FERNANDO DE NORONHA MARINE NATIONAL PARK (PNMFN) IS A UNIQUE INSULAR ECOSYSTEM CHARACTERIZED BY VOLCANIC ORIGINS AND DIVERSE COASTAL HABITATS, INCLUDING CORAL REEFS, BEACHES, AND ROCKY ISLANDS. THIS RESEARCH FOCUSES ON CHARACTERIZING THE MICROBIAL DIVERSITY WITHIN ITS SOILS AND IDENTIFYING GENETIC RESOURCES WITH BIOTECHNOLOGICAL POTENTIAL. SOIL SAMPLES WERE COLLECTED FROM VARIOUS REGIONS OF THE ARCHIPELAGO, PRIORITIZING MINIMAL ANTHROPOGENIC INFLUENCE AND HIGH ECOLOGICAL SIGNIFICANCE. DNA WAS SUCCESSFULLY ISOLATED AND PREPARED FOR SEQUENCING, ENABLING FURTHER ANALYSIS. THE STUDY EMPLOYS METAGENOMICS TO ANALYZE THE

GENETIC MATERIAL FROM THESE SAMPLES, BYPASSING THE NEED FOR CULTURING. THIS APPROACH ALLOWS THE EXPLORATION OF MICROBIAL COMMUNITIES' TAXONOMIC DIVERSITY AND FUNCTIONAL CAPACITIES, REVEALING ENZYMES RESILIENT TO HIGH TEMPERATURES AND SALINITY, WHICH ARE OF SIGNIFICANT INDUSTRIAL AND AGRICULTURAL INTEREST. THE DATA CONTRIBUTE TO CREATING A COMPREHENSIVE METAGENOMIC DATABASE AND DEVELOPING ALGORITHMS POWERED BY ARTIFICIAL INTELLIGENCE FOR GENOME ASSEMBLY AND GENE ANNOTATION. INSIGHTS FROM THIS RESEARCH SUPPORT CONSERVATION EFFORTS, ENHANCE OUR UNDERSTANDING OF MICROBIAL ECOLOGY, AND FOSTER INNOVATIVE BIOTECHNOLOGICAL APPLICATIONS. THIS PROJECT BRIDGES ENVIRONMENTAL CONSERVATION AND MOLECULAR INNOVATION, LEVERAGING CUTTING-EDGE TECHNOLOGIES TO UNCOVER THE MICROBIAL DYNAMICS OF PNMFN'S SOILS AND THEIR POTENTIAL TO ADDRESS GLOBAL CHALLENGES IN SUSTAINABILITY AND BIOTECHNOLOGY. THE STUDY HAS THE COLLABORATION WITH THE JOHN CRAIG VENTER INSTITUTE, ENHANCING THE ANALYTICAL DEPTH AND TECHNOLOGICAL ADVANCEMENTS.

VIROLOGIA E MICROBIOLOGIA MOLECULAR (A4)

"EMERGENCE OF POTATO VIRUS Y OUTBREAKS IN TOMATOES IN BRAZIL, THE DISEASE AND SPREAD"

VÍVIAN LUCENA, ERICH NAKASU, JOSÉ PEREIRA, CAMILA RÊGO-MACHADO, CRISTIANO RODRIGUES BERNARDO UENO, IVAIR MORAIS, ALICE INOUE-NAGATA

THE EMERGENCE OF 'MEXICAN FIRE' DISEASE IN BRAZILIAN TOMATO FIELDS, ATTRIBUTED TO POTATO VIRUS Y (PVY), HAS RAISED CONCERNS. CHARACTERIZED BY SEVERE NECROSIS ON MEDIAN LEAVES, THE DEFINITIVE ETIOLOGICAL AGENT OF THIS DISEASE REMAINED UNVERIFIED DESPITE PVY DETECTION IN SYMPTOMATIC PLANTS. OUR STUDY AIMED TO ELUCIDATE THE CAUSAL AGENT, OCCURRENCE, SPREAD, AND SYMPTOMATOLOGY OF MEXICAN FIRE. DEEP SEQUENCING OF TOMATO LEAVES WITH TYPICAL NECROTIC SYMPTOMS CONFIRMED THE ASSOCIATION WITH PVY, REINFORCING
ITS ROLE AS THE CAUSAL AGENT. SEROLOGICAL TESTS WITH A PVY-SPECIFIC POLYCLONAL ANTIBODY CONSISTENTLY CORRELATED SYMPTOMS WITH VIRUS PRESENCE IN A FRESH MARKET TOMATO FIELD, WITH HIGHER PVY INCIDENCE NEAR OLDER TOMATO AND MAIZE PLANTS. NECROTIC LEAF DISTRIBUTION ANALYSIS REVEALED A PREDOMINANT OCCURRENCE IN MEDIAN LEAVES, PROGRESSING UPWARDS. DEEP SEQUENCING OF SYMPTOMATIC FIELD SAMPLES EXCLUSIVELY DETECTED PVY, REAFFIRMING

ITS ROLE IN SYMPTOM INDUCTION. IMPORTANTLY, PVY INOCULATION UNDER FIELD AND GREENHOUSE CONDITIONS FULFILLED KOCH'S POSTULATES, TRIGGERING LEAF NECROSIS. OUR FINDINGS UNEQUIVOCALLY ESTABLISH PVY AS THE CAUSAL AGENT OF MEXICAN FIRE DISEASE, SHEDDING LIGHT ON ITS ETIOLOGY, INCIDENCE, SPREAD, AND SYMPTOM EXPRESSION, CRUCIAL FOR EFFECTIVE DISEASE MANAGEMENT STRATEGIES.

DEVELOPING RNA BIOSENSORS FOR AVIAN INFLUENZA DIAGNOSIS THROUGH SYNTHETIC BIOLOGY

MARIELE DE ARAÚJO PALMEIRAS; RAYANE NUNES, DANIELLE PEDROLLI, GRACIA ROSINHA E ELIBIO RECH

AVIAN INFLUENZA (AI) IS A VIRAL DISEASE AFFECTING DOMESTIC AND WILD BIRDS, CAUSED BY THE ORTHOMYXOVIRIDAE FAMILY VIRUS. DUE TO ITS HIGH PATHOGENICITY, PARTICULARLY THE H5N1 SUBTYPE, AI POSES A SIGNIFICANT RISK TO ANIMAL AND PUBLIC HEALTH. ADDITIONALLY, THE DISEASE HAS SIGNIFICANT ECONOMIC IMPACT, RESULTING IN MILLIONS OF ANIMAL CULLING, AFFECTING TRADE AND QUALITY OF LIFE IN VARIOUS COUNTRIES. THIS STUDY AIMS TO DEVELOP AND VALIDATE RNA-BASED BIOSENSORS TO DETECT THE AVIAN INFLUENZA VIRUS. TO ACHIEVE THIS GOAL, TARGET RNA SEQUENCES WERE SELECTED USING THE NCBI DATABASE, FOLLOWED BY MULTIPLE SEQUENCE ALIGNMENT USING MAFFT SOFTWARE. SUBSEQUENTLY, RNA SWITCHES WERE DESIGNED USING NUPACK SOFTWARE, AND TRIGGER RNAS WERE SYNTHESIZED USING THE RIBOMAX LARGE SCALE RNA PRODUCTION KIT (PROMEGA). PLASMIDS CONTAINING THE TARGET REGION AND REPORTER GENE WERE CONSTRUCTED AND SUBJECTED TO CELL-FREE REACTIONS USING THE T7 S30 EXTRACT SYSTEM FOR CIRCULAR DNA KIT (PROMEGA). LUMINESCENCE READINGS OF THE REPORTER GENE WERE PERFORMED ON A 96-WELL WHITE AND OPAQUE MICROPLATE. CURRENTLY, WE ARE OPTIMIZING REACTIONS TO DEVELOP A SWITCH CAPABLE OF DETECTING LOW LEVELS OF SYNTHETIC RNA, AIMING TO SIMULATE INITIAL VIRAL INFECTION CONDITIONS.

CHARACTERIZATION OF TWO ALPHABACULOVIRUS ISOLATED FROM SOYBEAN LOOPER, CHRYSODEIXIS INCLUDENS AND THE IDENTIFICATION OF A CYPOVIRUS IN MIXED INFECTION

LUCAS DE ARAÚJO ANDRADE; MARIELE DE SOUSA GOMES; WILLIAM SIHLER; ROGÉRIO BIAGGIONI LOPES; DANIEL SÓSA-GOMEZ; MARLINDA LOBO DE SOUZA; DANIEL ARDISSON-ARAÚJO;

THE SOYBEAN LOOPER (CHRYSODEIXIS INCLUDENS) IS AN AGRICULTURAL PEST THAT AFFECTS SEVERAL CROPS. TRADITIONAL CONTROL MEASURES OFTEN RELIES ON CHEMICAL INSECTICIDES, WHICH CAN HARM THE

ENVIRONMENT AND PROMOTE SELECTION OF RESISTANT INSECT. ALTERNATIVELY, NATURAL PRODUCTS BASED ON THE BACULOVIRUS CHRYSODEIXIS INCLUDENS NUCLEOPOLYHEDROVIRUS (CHINNPV) ARE BOTH SAFER AND WIDELY USED IN BRAZIL. BACULOVIRUSES ARE ENVELOPED VIRUSES WITH CIRCULAR DSDNA, ENCLOSED WITHIN PROTEINACEOUS CRYSTALS KNOWN AS OCCLUSION BODIES (OBS). THIS STUDY AIMS TO CHARACTERIZE TWO CHINNPV ISOLATES: THE REFERENCE ISOLATE CNPSO168 (C168) AND THE TABATINGA ISOLATE (TB), TO IDENTIFY THE ISOLATE THAT IS MOST LETHAL AND FAST-ACTING FOR FIELD APLICATION. ORIGINAL ISOLATES WERE AMPLIFIED IN CATERPILLARS, SEMI-PURIFIED, AND QUANTIFIED. VIRAL DNA FROM THE AMPLIFIED ISOLATES WAS EXTRACTED, ANALYZED ON AGAROSE GEL, AND SUBJECTED TO LETHAL DOSE ASSAYS USING THE ORIGINAL STOCK. SURVIVAL CURVES AT A 1X107 OB/ML DILUTION WERE ALSO CONDUCTED FOR 10 DAYS. TRANSMISSION ELECTRON MICROSCOPY (TEM) WERE PERFORMED ON BOTH ISOLATES. DNA ANALYSIS OF THE AMPLIFIED VIRUSES REVEALED BANDS CORRESPONDING TO THE CHINNPV GENOME AND RNA SEGMENTS TYPICAL OF CYPOVIRUSES, CONFIRMING CO-INFECTION AFTER AMPLIFICATION. THIS CO-INFECTION WAS VERIFIED BY PCR FOR A CYPOVIRUS SPECIFIC GENE. CYPOVIRUS CO-INFECTION CAN SIGNIFICANTLY REDUCE THE NUMBER OF BACULOVIRUS OCCLUSION BODIES (OBS) AND DECREASE INSECT LETHALITY, POSING A MAJOR CHALLENGE FOR BACULOVIRUS MANUFACTURING. CONSEQUENTLY, WE REVERTED TO THE ORIGINAL STOCK FOR FURTHER TESTING. IN THE LETHAL DOSE ASSAY WITH THE ORIGINAL SAMPLES, THE TB ISOLATE SHOWED A MORTALITY RATE OF 33%, WHILE THE C168 ISOLATE SHOWED 65% AT A 1X106 DILUTION. TEM CONFIRMED THAT BOTH ISOLATES CONTAINED SINGLE NUCLEOPOLYHEDROVIRUS (SNPV) AND CYPOVIRUS. THE COMPLETE GENOMES OF THESE ISOLATES, INCLUDING THOSE WITH CYPOVIRUS CO-INFECTION, WILL BE SEQUENCED.

ANALYSIS OF THE APOPTOTIC EFFECTS OF ANKYRIN AND PROTEIN TYROSINE PHOSPHATASES FROM COTESIA FLAVIPES BRACOVIRUS IN INSECT CELLS.

ANDREWS ALEXANDER FREDÉRIC MONVOISIN SANTOS FISCH; BERGMANN MORAIS RIBEIRO

BRACOVIRUSES ARE A GENUS OF SEGMENTED DSDNA VIRUSES THAT ARE SYMBIOTICALLY ASSOCIATED WITH THE BRACONIDAE FAMILY OF PARASITOID WASPS. THE COTESIA FLAVIPES WASP IS WIDELY USED AS A BIOLOGICAL CONTROL AGENT OF THE DIATRAEA SACCHARALIS LARVAE IN SUGAR CROPS. DURING OVIPOSITION THERE IS A RELEASE OF A LARGE AMOUNT OF VIRAL PARTICLES INTO THE CATERPILLAR BODY THAT WILL INFECT THE HOST HEMOCYTES. THIS LEADS TO AN INDUCED IMMUNOSUPPRESSION IN THE HOST WHICH INCREASES FITNESS OF THE WASP`S PROGENIES. IN THIS WORK, WE CONSTRUCTED PLASMIDS CONTAINING

SEVERAL VIRAL GENES DERIVED FROM THE CFBV GENOME, PROTEIN TYROSINE PHOSPHATASE AND ANKYRIN, FOR TRANSIENT EXPRESSION IN INSECT CELLS. THE PLASMIDS WERE TRANSFECTED INTO TRICHOPLUSIA NI CELLS (TN5B) AND WE CHECKED AT 72 HOURS POST TRANSFECTION FOR MORPHOLOGICAL CHANGES, VIABILITY AND EFFECTOR CASPASE ACTIVITY. CELLS UNDERGOING APOPTOSIS WERE OBSERVED IN TRANSFECTIONS WITH ANK-8, PTP-A, PTP-O, PTP-OMEGA, PTP-Q, PTP-W, PTP-N AND PTP-T GENES. EFFECTOR CASPASE ACTIVITIES WERE VARIED BUT POSITIVE COMPARED TO NEGATIVE CONTROLS. THESE RESULTS DEMONSTRATE THAT THE ISOLATED ANK AND PTP PROTEINS ARE SUFFICIENT TO INDUCE APOPTOSIS IN VITRO AND AFFECT CELLULAR VIABILITY. THE FUNCTIONAL REDUNDANCY WITHIN THIS SET OF GENES CORRELATES WELL WITH THE PRESENCE OF CONSERVED DOMAINS AND TOPOLOGIES, BUT IN CONTRAST THE LOW OVERALL IDENTITIES MAY POINT TO DISTINCT MECHANISMS OF APOPTOSIS INDUCTION BY EACH PROTEIN WHICH REQUIRES IN-DEPTH ASSESSMENTS.

ANTI–CHIKUNGUNYA VIRUS ACTIVITY AND ISOLATION OF COUMARINS FROM AQUEOUS EXTRACT OF ERYTHROXYLUM SUBEROSUM LEAVES THROUGH BIOASSAY–GUIDED FRACTIONATION

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CHIKUNGUNYA VIRUS (CHIKV), TRANSMITTED BY AEDES MOSQUITOES, BELONGS TO THE TOGAVIRIDAE FAMILY AND THE ALPHAVIRUS GENUS. CLINICALLY, THE DISEASE CAUSED BY CHIKV RESEMBLES DENGUE WITH SYMPTOMS LIKE FEVER, HEADACHE, AND ARTHRALGIA. IT SIGNIFI-CANTLY IMPACTS PUBLIC HEALTH IN MANY NEOTROPICAL REGIONS, CAUSING FREQUENT OUTBREAKS. A PROMISING ANTIVIRAL STRATEGY INVOLVES EXPLORING PLANT–BASED COMPOUNDS FOR DISRUPTING VIRAL INFECTION CYCLES. THE ETHNOMEDICINAL PLANT ERYTHROXYLUM SUBEROSUM (ERYTHROXYLACE-AE) IS A COMMON SHRUB IN THE BRAZILIAN CERRADO, WHOSE AQUEOUS EXTRACT SHOWED SIGNIFI-CANT IN VITRO ANTI–CHIKUNGUNYA VIRUS ACTIVITY. CURRENTLY, THERE IS NO DRUG IN CLINICAL USE SPECIFICALLY FOR THE TREATMENT OF THIS VIRUS; THEREFORE, IN THIS WORK, THE ANTIVIRAL AND CYTO-TOXIC PROPERTIES OF THE AQUEOUS EXTRACT, FRACTIONS, AND COMPOUNDS WERE EVALUATED THROUGH BIOASSAY–GUIDED FRACTIONATION. THE CYTOTOXICITY PROFILE OF ERYTHROXYLUM SUBEROSUM (ES) EXTRACT, FRACTIONS, AND COMPOUNDS ON VERO CELLS WERE DETERMINED THROUGH LYSOSOMAL VIA-BILITY ANALYSIS, BY NEUTRAL RED ASSAY. SIMULTANEOUSLY, THE ANTIVIRALS POTENTIALS WERE DETER-MINED THROUGH PLAQUE ASSAYS AT DIFFERENT TREATMENT STEPS. IN THE THIRD AND FINAL FRACTIONA-TION (ESE2F1), WE ISOLATED TWO COUMARINS (ESE2F1–A AND ESE2F1–D) THAT SHOWED ANTI–CHIKUNGUNYA ACTIVITY AT THE CO–TREATMENT STEP. AT 250 NG/ML, THE

MOLECULES REDUCED THE NUMBER OF LYSIS PLAQUES BY 38.21% ± 6.909 AND 56.7% ± 1.389, RESPECTIVELY. WHEN COMPARING THE VIRAL TITER OF 1000 NG/ML OF ESE2F1-A WITH THE VIRAL CONTROL (CHIKV), A STATISTICAL DIFFERENCE WAS NOTED, REDUCING THE VIRAL CONTROL TITER FROM 3.71X10^5 PFU/ML TO 1.49X10^5 PFU/ML. THEREFORE, ERYTHROXYLUM SUBEROSUM EMERGES AS A POTENTIAL SOURCE OF ANTIVIRAL COMPOUNDS AGAINST CHIKV INFECTIONS. ONGOING STUDIES FURTHER INVESTIGATE THEIR ANTIVIRAL ACTIVITY.

INSIGHTS INTO THE HORIZONTAL GENE TRANSFER OF GLYCOPROTEINS BETWEEN ORTHOMYXOVIRUS AND BACULOVIRUSES: DISCOVERY OF A NEW VIRUS AND FUNCTIONAL ASPECTS

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THE BACULOVIRIDAE FAMILY INCLUDES INSECT-INFECTING VIRUSES WITH LARGE CIRCULAR DOUBLE-STRANDED DNA GENOMES. WITHIN THIS FAMILY, THE ALPHABACULOVIRUS GENUS INFECTS LEPIDOPTERAN SPECIES AND IS DIVIDED INTO TWO GROUPS: GROUP I AND GROUP II. THESE GROUPS ARE CHARACTERIZED BY DISTINCT MAJOR ENVELOPE GLYCOPROTEINS—GP64 IN GROUP I AND THE F PROTEIN IN GROUP II—BOTH OF WHICH ARE ESSENTIAL FOR VIRAL BUDDING AND CELL ENTRY. GP64 SHARES STRUCTURAL AND SEQUENCE SIMILARITIES WITH THE ENVELOPE GLYCOPROTEIN (GP) OF THOGOTOVIRUS, A VIRUS FROM THE ORTHOMYXOVIRIDAE FAMILY (NEGATIVE-SENSE SSRNA VIRUSES), SUGGESTING A POTENTIAL HORIZONTAL GENE TRANSFER (HGT) EVENT FROM AN ANCESTRAL ORTHOMYXOVIRUS. TO INVESTIGATE THIS, WE REPLACED THE GP64 GENE IN ACMNPV BACULOVIRUS WITH THE GP FROM APIS THOGOTOVIRUS 1 (ATHOV-1), SEQUENCED FROM HONEYBEE SAMPLES. IN VITRO ASSAYS (VIRAL PASSAGE, VIRUS TITRATION, WESTERN BLOT, AND QPCR) SHOWED THAT THE HETEROLOGOUS GP RESTORED BACULOVIRUS INFECTIVITY IN LEPIDOPTERAN CELL LINES. ADDITIONALLY, THE THOGOTOVIRUS GP ACTED AS A FUSOGEN, AS CONFIRMED BY A PH-SENSITIVE SYNCYTIA FORMATION ASSAY. FLUORIMETRY AND FLOW CYTOMETRY ASSAYS REVEALED THAT THIS GP ENHANCED BACULOVIRUS ENTRY AND GENE TRANSDUCTION IN MOSQUITO CELL LINES. CRYO-ELECTRON MICROSCOPY OF RECOMBINANT VIRUSES SHOWED DIFFERENTIAL INCORPORATION OF GPS INTO THE VIRAL ENVELOPE, SUGGESTING PROTEIN ENVELOPE ANCHORING AS A POSSIBLE DETERMINANT FOR FUNCTIONAL HOMOLOGY BETWEEN THESE PROTEINS. WE ALSO CHARACTERIZED A NEW THOGOTOVIRUS FROM A LEPIDOPTERAN INSECT, IDENTIFIED VIA SRA DATABASE MINING, WHICH CLUSTERS WITH BACULOVIRUS GP64 IN A GP-BASED PHYLOGENY. PHYLODYNAMIC ANALYSIS SUGGESTS THAT THE ACQUISITION OF GP64 OCCURRED ABOUT 330 TO 130 MILLION YEARS AGO. DESPITE ITS CLOSE PHYLOGENETIC RELATIONSHIP WITH

BACULOVIRUS GP64, THE THOGOTOVIRUS GP DID NOT RESTORE INFECTIVITY IN THE ABSENCE OF GP64.

EXPRESSION AND OCCLUSION OF DENGUE VIRUS NS1 PEPTIDES INSIDE POLYHEDRA OF A CYPOVIRUS FOR SEROLOGICAL DIAGNOSIS.

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THE DENGUE VIRUS (DENV) IS AN ARBOVIRUS OF GLOBAL HEALTH CONCERN. THE CHALLENGES OF EXISTING SEROLOGICAL DIAGNOSTIC KITS IN THE MARKET INCLUDE DELAYED DETECTION OF IGG/IGM ANTIBODIES AND SUSCEPTIBILITY TO CROSS-REACTIVITY WITH OTHER SEROTYPES AND DIFFERENT ARBOVIRUSES. CYPOVIRUSES, MEMBERS OF THE SPINAREOVIRIDAE FAMILY, STAND OUT FOR THEIR ABILITY TO FORM HIGHLY STABLE AND PROTEASE-RESISTANT CRYSTALLIZED PROTEIN STRUCTURES KNOWN AS POLYHEDRA. THESE STRUCTURES HOLD PROMISE FOR THE DEVELOPMENT OF SEROLOGICAL DIAGNOSTIC TESTS BY INCORPORATING RECOMBINANT PROTEINS WITHIN THEM. THIS STUDY AIMS TO EXPRESS BOTH MULTI-EPITOPE AND INDIVIDUAL EPITOPES FROM THE NS1 REGION OF THE FOUR DENGUE VIRUS SEROTYPES ENCLOSED WITHIN POLYHEDRA. TO ACHIEVE THIS, A CHIMERIC GENE ENCOMPASSING MULTIPLE EPITOPES WAS DESIGNED, INCORPORATING SEGMENTS OF THE NS1 PROTEIN FROM ALL FOUR DENV SEROTYPES AND SUBSEQUENTLY SYNTHESIZED. INDIVIDUAL EPITOPES OF EACH SEROTYPE WERE AMPLIFIED USING PCR. THESE CONSTRUCTS WERE INDIVIDUALLY CLONED INTO A PLASMID CONTAINING THE CYPOVIRUS POLYHEDRIN, CONTROLLED BY A BACULOVIRUS PROMOTER AND THE SEQUENCE OF THE ALPHA-HELIX 1 (H1) OF POLYHEDRIN. THE H1 HAS BEEN DEMONSTRATED TO ASSOCIATE WITH THE POLYHEDRIN PROTEIN TO FORM POLYHEDRA. FOLLOWING CLONING, THE GENES WERE FUSED TO THE H1. RECOMBINANT BACULOVIRUSES CONTAINING THESE VARIOUS CONSTRUCTS WERE GENERATED AND USED TO INFECT INSECT CELLS. INFECTED INSECT CELLS EXHIBITED THE PRESENCE OF POLYHEDRAL CRYSTALS. THE EXPECTED MOLECULAR MASS OF THE RECOMBINANT PROTEINS WERE ANALYZED AND CONFIRMED BY SDS-PAGE AND WESTERN-BLOT TECHNIQUES. THESE RECOMBINANT CRYSTALS WILL BE USED AS DIAGNOSTICS INPUTS EMPLOYING SERUM FROM PATIENTS PREVIOUSLY DIAGNOSED WITH DENGUE BY RT-PCR AND/OR VIRAL ISOLATION.

PHENOTYPIC PLASTICITY OF FONSECAEA SP. ISOLATES

VITORIA MERÇON DIAS, MÁRCIA CRISTINA GONÇALVES MACIEL, CLARA LUNA MARINA, TATIANA SOBIANSKI HERMAN3, FILIPE DOS SANTOS TIMBONI, JESSE PEREIRA MACHADO VIANA, LUISA COUTINHO COELHO, AMANDA AMARAL, SOFIA SILVA NOBRE MENDES, HIOLANDA LÊDO DA SILVA, PEDRO HENRIQUE MIRANDA BURGEL, AMABEL FERNANDES CORREIA, MARIA SUELI SOARES

FELIPE, ANAMELIA LORENZETTI BOCCA, MARCUS DE MELO TEIXEIRA, LARISSA FERNANDES MATOS

CHROMOBLASTOMYCOSIS (CBM), ONE OF THE DISEASES ON THE LIST OF NEGLECTED TROPICAL DISEASES FROM THE WORLD HEALTH ORGANIZATION, IS CAUSED BY TRAUMATIC INOCULATION OF FILAMENTOUS DEMATIACEOUS FUNGI, AMONG WHICH FUNGI OF THE GENUS FONSECAEA SPP. ARE THE MAIN CAUSES, WITH FONSECAEA PEDROSOI AS THE MAIN AGENT. SPECIES WITHIN THE FONSECAEA GENUS SHARE HIGH MORPHOLOGICAL SIMILARITIES, WHICH CAN LEAD TO DIFFICULTIES IN IDENTIFICATION USING MORPHOLOGY, MAKING IT NECESSARY TO USE MOLECULAR METHODS FOR GREATER ACCURACY. THIS WORK IS AIMED IN THE UNDERSTANDING OF THE PATHOBIOLOGY OF CBM THROUGH THE MOLECULAR IDENTIFICATION AND EVALUATION OF VIRULENCE ATTRIBUTES IN 18 CLINICAL ISOLATES FROM BRAZIL, MORPHOLOGICALLY IDENTIFIED AS FONSECAEA SP. OR F. PEDROSOI. THE SAMPLES WERE IDENTIFIED USING PCR OF THE ITS AND CDC42 REGIONS. SOME SAMPLES WERE WHOLE GENOME SEQUENCED, THIS WAY WE COULD IDENTIFY THE MATING TYPE LOCUS. VIRULENCE ATTRIBUTES WERE EVALUATED BY ASSESSING THE ABILITY OF THE ISOLATES TO DIFFERENTIATE INTO MURIFORM CELLS — CELLS WITH DIFFERENT MORPHOLOGY THAT PATHOGNOMONIC OF THE DISEASE —, SECRETION OF MELANIN, ABILITY TO GROW AT 37ºC, AND RESISTANCE TO OXIDATIVE STRESS. THE RESULTS SHOWED THAT 37.5% WERE NOT F. PEDROSOI, WITH 4 BEING F. MONOPHORA, 2 F. NUBICA, AND 1 RHINOCLADIELLA AQUASPERSA. IN ADDITION, WE FOUND THAT ALL ISOLATES DISPLAYED MORPHOLOGICAL FEATURES CONSISTENT WITH MURIFORM CELLS, MELANIN SECRETION AND GROWTH AT 37ºC WAS OBSERVED FOR ALL SAMPLES WITH DIFFERENT RATES AMONG THE ISOLATES. LASTLY, RESISTANCE TO OXIDATIVE STRESS SEAMS TO VARY ACCORDING TO THE ISOLATE. WE ALSO PERFORMED AN IN VIVO ASSAY WITH 10 ISOLATES, ANALYZING CYTOKINE SECRETION, FUNGAL LOAD AND THE ASPECT OF THE LESION. BASED ON THIS DATA, IT WAS CONCLUDED THAT THE FONSECAEA GENUS ISOLATES EXHIBIT AN ASSORTMENT OF VIRULENCE PHENOTYPES IN VITRO. WE ARE FURTHER INVESTIGATING IF THE PHENOTYPIC PLASTICITY OF FONSECAEA ISOLATES CORELATES WITH THE SEVERITY IN IN VIVO INFECTION MODEL.

OCCURRENCE OF ALLEXIVIRUS IN BRAZILIAN

VANILLA GUSTAVO P. FELIX, ANDREZA H. VIDAL, TAYARA COLINS, MARÍLIA PAPPAS1, DANIEL ARDISSON-ARAÚJO; SIMONE G. RIBEIRO

VANILLA (VANILLA SPP.) IS AN ORCHID SOLD WORLDWIDE DUE TO ITS AROMATIC AND HIGH-VALUE FRUITS. OF THE MORE THAN 100 DESCRIBED SPECIES OF THE GENUS VANILLA, 30 OCCUR IN BRAZIL, OF WHICH 15 HAVE AROMATIC FRUITS. HOWEVER, BRAZIL DOES NOT HAVE A TRADITION IN VANILLA CULTIVATION DESPITE THE MARKET BEING VERY ATTRACTIVE AND

HAVING HIGH COMMERCIAL POTENTIAL. EMBRAPA HAS AN ACTIVE GERMPLASM BANK WITH MORE THAN 70 ACCESSIONS OF DIFFERENT SPECIES OF THE GENUS VANILLA. ONE OF THE MAIN PHYTOSANITARY PROBLEMS IN VANILLA IS DISEASES CAUSED BY VIRUSES THAT CAN CAUSE SIGNIFICANT DAMAGE TO THE CROP. THERE ARE CURRENTLY NO RECORDS OF VIRUS OCCURRENCE IN VANILLA SPP. IN BRAZIL. THE PRESENT WORK AIMS TO INVESTIGATE THE OCCURRENCE OF RNA VIRUSES IN VANILLA COLLECTED IN BRAZIL USING THE HIGH-THROUGHPUT SEQUENCING (HTS) METHOD. WE ANALYZED SAMPLES OF VANILLA COLUMBIANA, COLLECTED IN EMBRAPA'S VANILLA GERMPLASM BANK. TOTAL RNA WAS EXTRACTED FROM V. COLUMBIANA LEAVES AND SUBSEQUENT HTS SEQUENCING WAS PERFORMED USING THE ILLUMINA PLATFORM. THE CLC GENOMICS® PROGRAM WAS USED TO PERFORM THE DE NOVO ASSEMBLY, AND FROM THE ASSEMBLED CONTIGS, A LOCAL SEARCH WAS CARRIED OUT IN A REFSEQ BANK OF RNA VIRUSES WITH THE GENEIOUS® PROGRAM USING THE BLAST TOOL. IN BLASTX SEARCHES, A CONTIG OF 8091 NT SHOWED HIGH IDENTITY WITH ALLEXIVIRUSES. MOREOVER, THE GENOME ORGANIZATION FOR THIS PUTATIVE VIRUS WAS SIMILAR TO VIRUSES BELONGING TO THE ALLEXIVIRUS GENUS. BLASTX (63,59%) AND BLASTN (71,98%) FOR THE PUTATIVE COAT PROTEIN (CP) BELOW THE SPECIES DEMARCATION CRITERIA REGION FOR MEMBERS OF THE GENUS ALLEXIVIRUS, INDICATING THAT IT IS A POSSIBLE NEW SPECIES. EXPERIMENTS ARE UNDERWAY TO COMPLETE MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF THIS VIRUS.

THE GUT RNA VIROME OF THE TERMITE SYNTERMES WHEELERI HARBOR A DIVERSE VIRAL COMMUNITY THAT MAY INFLUENCE THE HOST

RAFAEL DA SILVA OLIVEIRA

METAGENOMICS AND METATRANSCRIPTOMICS HAVE SIGNIFICANTLY ADVANCED OUR UNDERSTANDING OF VIRAL EVOLUTION, DIVERSITY, AND FUNCTIONAL POTENTIAL ACROSS VARIOUS ENVIRONMENTS. INVERTEBRATES, INCLUDING TERMITES, HOST DIVERSE VIRUSES IN THEIR MICROBIOMES, OFFERING OPPORTUNITIES TO DISCOVER NEW VIRUSES AND STUDY VIRAL DYNAMICS. HOWEVER, TERMITE VIROMES REMAIN LARGELY UNEXPLORED DESPITE THEIR ECOLOGICAL AND ECONOMIC SIGNIFICANCE. IN THIS STUDY, WE CHARACTERIZED THE GUT VIROME OF THE BRAZILIAN CERRADO TERMITE SYNTERMES WHEELERI USING METATRANSCRIPTOMIC DATA TO UNCOVER ITS DIVERSITY AND ECOLOGICAL INTERACTIONS. A TOTAL OF SEVEN RNA SEQUENCES LIBRARIES FROM THE P1 HINDGUT SECTION (P1-1, P1-2 AND P1-3), P3 HINDGUT SECTION (P3-3), AND WHOLE GUT (WG-1, WG-2, WG-3) WERE OBTAINED. VIRAL GENOMES WERE DETECTED VIA SEQUENCE HOMOLOGY, AND CHECKV ASSESSED THEIR COMPLETENESS AND QUALITY. TAXONOMIC ASSIGNMENT WAS BASED ON RDRP SEQUENCE ALIGNMENT, WHILE VIRAL AND AUXILIARY METABOLIC GENES (AMGS) WERE ANNOTATED USING VOGDB AND PFAM AS REFERENCES. ALSO, ABSOLUTE AND RELATIVE

ABUNDANCES OF RELEVANT TAXA WERE ESTIMATED. MITOVIRIDAE WAS THE MOST ABUNDANT VIRAL FAMILY IN ALL SAMPLES, EXCEPT FOR P1-2 AND WG-2 IN WHICH KOLMIOVIRIDAE WAS THE MOST PREVALENT TAXA. MITOVIRUS IS USUALLY ASSOCIATED WITH FUNGI, AND IT POSSIBLY INFECTS TERMITES SYMBIONTS. OTHER TAXA, SUCH AS PARTITIVIRIDAE AND NARNAVIRIDAE, WERE ALSO IDENTIFIED ABOVE 1%. OVERALL, MOST SEQUENCES WERE PREDICTED AS UNKNOWN PROTEINS OR VIRAL GENES. YET, GENOME ANNOTATION REVEALED BACTERIOPHAGE GENES AND POSSIBLE AMGS RELATED TO SULFUR CYCLING (DSRC), PHOSPHORUS CYCLING (PHOU), HYDROGEN METABOLISM (HUPH), IRON REGULATION (ISDB), AND OXIDATIVE STRESS PROTECTION (FBIC AND MUTT). THESE GENES INDICATE A POSSIBLE INFLUENCE OF THE VIRAL COMMUNITY IN THE HOST AND ITS MICROBIOME THROUGH METABOLIC REPROGRAMMING AND HORIZONTAL GENE TRANSFER.

GRADUAÇÃO

BIOLOGIA CELULAR E IMUNOLOGIA

AVALIAÇÃO DO PAPEL IMUNOMODULADOR DO BUTIRATO NA INFECÇÃO DE LINHAGENS DE MACRÓFAGOS POR PARACOCCIDIOIDES BRASILIENSIS

EULLER FERNANDES LOPES, ALDO HENRIQUE TAVARES, AIME STEFANY ALVES DA FONSECA

THE MICROBIOTA, COMPOSED OF BACTERIA, ARCHAEA, FUNGI, AND VIRUSES, INTERACTS WITH THE HOST IN A SYMBIOTIC RELATIONSHIP, PERFORMING ESSENTIAL FUNCTIONS SUCH AS FIBER FERMENTATION, WHICH GENERATES NUTRIENTS AND SHORT-CHAIN FATTY ACIDS (SCFAS). AMONG THE SCFAS, BUTYRATE HAS IMMUNOMODULATORY EFFECTS, ACTING ON GPCRS AND INHIBITING EPIGENETIC ENZYMES, PROMOTING A BALANCE BETWEEN INFLAMMATORY RESPONSES. EVIDENCE SUGGESTS THAT BUTYRATE ENHANCES THE MICROBICIDAL ACTIVITY OF MACROPHAGES AND INHIBITS PATHOGENIC FUNGI. IN THIS STUDY, WE INVESTIGATED THE EFFECT OF BUTYRATE ON THE INNATE IMMUNE RESPONSE AGAINST PARACOCCIDIOIDES BRASILIENSIS, EXPLORING ITS THERAPEUTIC POTENTIAL. METHODOLOGY: THE PB18 ISOLATE WAS CULTURED FOR 7 DAYS AT 37°C IN SEMI-SOLID 4% BHI-DEXTROSE MEDIUM. RAW264.7 MACROPHAGES AND BMDMS WERE INFECTED AND TREATED WITH BUTYRATE (10-20 MM) AND LPS (1 MG/ML) AS A CONTROL. AFTER 24H AND 48H, THE CYTOKINES IL-1ß, TNF-A, AND IL-10 WERE QUANTIFIED BY ELISA, AND THE FUNGICIDAL ACTIVITY WAS ASSESSED BY CFU. TO ANALYZE PHAGOLYSOSOMAL ACIDIFICATION, BAFILOMYCIN A1 (0.5 MM) WAS ADDED BEFORE INFECTION. RESULTS: BUTYRATE REDUCED TNF-A LEVELS IN MACROPHAGES STIMULATED WITH LPS AS WELL AS IN THOSE INFECTED WITH P. BRASILIENSIS, INDICATING AN ANTI-INFLAMMATORY EFFECT. IN BMDMS, BUTYRATE REDUCED IL-10 AND INCREASED IL-1B, SUGGESTING INFLAMMASOME ACTIVATION. THE MICROBICIDAL CAPACITY WAS ENHANCED BY BUTYRATE, AN EFFECT REVERSED BY BAFILOMYCIN, SUGGESTING INTRACELLULAR ACIDIFICATION AS A MICROBICIDAL MECHANISM. CONCLUSION: THESE RESULTS SUGGEST THE IMMUNOMODULATORY POTENTIAL OF BUTYRATE AGAINST P. BRASILIENSIS, DEMONSTRATED BY A REDUCTION IN TNF-A AND IL-10 LEVELS, ALONGSIDE AN

INCREASE IN IL-1B EXPRESSION. THE OBSERVED REDUCTION IN COLONY-FORMING UNITS (CFUS) FURTHER UNDERSCORES THE ROLE OF BUTYRATE IN FACILITATING INTRACELLULAR COMPARTMENT ACIDIFICATION. THESE FINDINGS POINT TO BUTYRATE AS A PROMISING STRATEGY IN THE CONTROL OF FUNGAL INFECTIONS.

PREDICTION OF OLIGOPEPTIDASE B AND THIMET OLIGOPEPTIDASE EPITOPES FROM TRYPANOSOMA CRUZI AS A VACCINE DEVELOPMENT STRATEGY

KAUÃ DA SILVA SANTOS; ANDREY DUARTE BOAVA; FELIPE DA SILVA MENDONÇA DE MELO; ALEXANDRA MARIA DOS SANTOS CARVALHO; DANIELA FRANCO ROSA; JULIA LISBOA BESERRA E SILVA; AISSA LANA OLIVEIRA LIMA; IZABELA MARQUES DOURADO BASTOS.

THE IDENTIFICATION OF HIGH-AFFINITY EPITOPES FOR B AND T LYMPHOCYTES ENABLES THE DESIGN OF VACCINES CAPABLE OF INDUCING A ROBUST AND SPECIFIC IMMUNE RESPONSE. IN CHAGAS DISEASE, THE SELECTION OF EPITOPES THAT ARE NOT CONSERVED IN HUMANS IS ESSENTIAL TO ENSURE THE EFFECTIVE RECOGNITION AND ELIMINATION OF THE PATHOGEN BY THE IMMUNE SYSTEM. THUS, THE PREDICTION AND VALIDATION OF THESE EPITOPES EMERGE AS FUNDAMENTAL TOOLS IN THE DEVELOPMENT OF SAFE AND EFFECTIVE VACCINES CAPABLE OF TRIGGERING A PROTECTIVE AND LASTING IMMUNE RESPONSE. FOR PREDICTION, THE SEQUENCES C3747_105G153 (THIMET OLIGOPEPTIDASE) AND C3747_16G242 (OLIGOPEPTIDASE B) WERE USED, ALONG WITH MHC CLASS I AND II AND B LYMPHOCYTE PREDICTION PROGRAMS (NETMHCPAN 4.1, NETMHCIIPAN 4.1, BEPIPRED 2.0, AND IEDB). THE EPITOPES WERE RANKED, AND THOSE WITH THE BEST SCORES WERE SELECTED. THE IDENTIFIED EPITOPE SEQUENCES WERE ALIGNED WITH THE BALB-C MOUSE GENOME. AN ALIGNMENT IN CLUSTAL OMEGA WAS PERFORMED TO ASSESS THE CONSERVATION OF EPITOPES IN OTHER T. CRUZI STRAINS. THE LOCATION OF THE EPITOPES IN THE 3D STRUCTURE OF THE PROTEIN WAS EVALUATED USING ALPHAFOLD AND PYMOL. FOUR EPITOPES WERE FOUND FOR C3747_105G153 AND FOUR FOR C3747_16G242. IN SUMMARY, THESE DATA WILL BE USED FOR THE FORMULATION OF VACCINE COMPOUNDS AGAINST T. CRUZI, AND FROM THESE VACCINE FORMULATIONS, IN VITRO AND IN VIVO TESTS WILL BE CONDUCTED IN THE FUTURE.

PRODUCTION OF CRYSTALIZED BACULOVIRUS ENHANCIN PROTEINS FOR THE BIOLOGICAL CONTROL OF AGRICULTURAL AND FOREST PESTS

FERNANDA COELHO DE MAGALHÃES, BRUNO MILHOMEM PILATI RODRIGUES E PATRICIA DE SOUZA DA SILVA E BERGMANN MORAIS RIBEIRO

ENHANCINS ARE METALLOPROTEASES CAPABLE OF FACILITATING BACULOVIRAL INFECTIONS BY DEGRADING THE INSECT'S PERITROPHIC MATRIX. THE GENERA ALPHABACULOVIRUS AND BETABACULOVIRUS IN THE BACULOVIRIDAE FAMILY ENCODE ENHANCINS. THE AIM OF THE PRESENT STUDY IS TO PRODUCE BACULOVIRUS DERIVED ENHANCIN PROTEINS, UTILIZING THE BAC-TO-BAC EXPRESSION SYSTEM, UNDER A NOVEL CRYSTALLIZATION STRATEGY THAT ALLOWS A MORE STABLE FORMULATION AND USE AS AN ADJUVANT FOR BACULOVIRUS BASED BIOPESTICIDES. THE GENETIC FUSION OF PROTEINS WITH AN ALPHA HELIX OF THYRINTEINA ARNOBIA CYPOVIRUS (THARCPV) POLYHEDRIN GENE, CO-EXPRESSED WITH THE FULL POLYHEDRIN GENE, ALLOWS FOR ITS CRYSTALIZATION. PRIMARILY, WE HAVE EXTRACTED THE GENOMIC DNA OF THE SPODOPTERA FRUGIPERDA GRANULOVIRUS (SFGV) FROM THE OCCLUSION BODIES OF A VIRAL ISOLATE. SPECIFIC OLIGONUCLEOTIDES WERE DESIGNED AND USED TO AMPLIFY AND ISOLATED THE ENHANCIN 1 AND 2 GENES OF SFGV BY POLYMERASE CHAIN REACTION (PCR). PURIFIED INTEREST GENES ARE BEING CLONED IN A MODIFIED PLASMID VECTOR, GENETICALLY FUSED WITH THE CRYSTALIZATION DOMAIN OF THARCPV. THE WORK AIMS TO PRODUCE SUCH PROTEINS IN INSECT CELLS THROUGH THE CONSTRUCTION OF RECOMBINANT BACULOVIRUS VECTORS BY THE BAC-TO-BAC EXPRESSION SYSTEM. FINALLY, WE AIM TO PRODUCE AND PURIFY POLYHEDRIN CRYSTALS CONTAINING SFGV ENHANCINS AND CONDUCT TESTS THAT MEASURE THEIR ABILITY TO ENHANCE THE LETHALITY AND EFFICACY OF BACULOVIRUS AGAINST INSECTS, ATTEMPTING TO INCREASE THEIR BIOPESTICIDE CAPABILITY.

BIOFÍSICA, BIOQUÍMICA E BIOLOGIA ESTRUTURAL

ANÁLISE DO SINERGISMO ENTRE ENZIMAS ATIVAS SOBRE CARBOIDRATO VISANDO DESENVOLVIMENTO DE MISTURAS ENZIMÁTICAS DE INTERESSE BIOTECNOLÓGICO

MARIA CLARA COSTA REIS, VINÍCIUS ROCHA CARDOZO DA SILVA, JOÃO BATISTA HOLANDA LOPES, BETÂNIA FERRAZ QUIRINO, ELIANE FERREIRA NORONHA

ENZYMES ARE KNOWN FOR THEIR CAPABILITY OF ACCELERATING CHEMICAL CATALYSIS REACTIONS BESIDES BEING A BIOLOGICAL METHOD MINUS THE NEGATIVE IMPACT ON THE ENVIRONMENT. THEREFORE, THE PRESENT STUDY INVESTIGATES THE SYNERGISM BETWEEN DIFFERENT CLASSES OF CELLULASES ACTING ON BRACHIARIA, WHICH IS A KIND OF GRASS ESSENTIAL TO CATTLE FEEDING, IN ORDER TO ASSIST BOVINE DIGESTION. THE ENZYMATIC EXTRACTS ARE OBTAINED FROM THREE FUNGI SPECIES: TRICHODERMA HARZIANUM AND ISO 19 (NON-IDENTIFIED ISOLATED 19). THE FUNGI ARE GROWN IN MINIMAL MEDIUM SUPPLEMENTED WITH BRACHIARIA AS THE CARBON RESOURCE. FOLLOWING THAT, THEIR SECRETOME IS EXTRACTED AND TESTED FOR FPASE, CMCASE AND XYLANASE ASSAYS AS WELL AS PROTEIN QUANTIFICATION. FURTHERMORE, BIOCHEMICAL CHARACTERIZATION WAS CARRIED OUT, SUCH AS DEFINING THE BEST PH AND TEMPERATURE OF THE SECRETOMES. PRELIMINARY RESULTS SHOW A SIGNIFICANT ENZYMATIC ACTIVITY OF THE TESTED SECRETOMES, VARYING PH AND TEMPERATURE. THUS, THE STUDY AIMS THE FORMULATION OF AN ENZYMATIC COCKTAIL WELL BALANCED TO UPGRADE ANIMAL NUTRITION, MAKING GOOD USE OF THE SYNERGIC INTERACTIONS BETWEEN CELLULASES DERIVED FROM DIFFERENT FUNGI ORIGINS. FUTURE STEPS OF THIS STUDY INCLUDE IMPROVING AN ENZYMATIC COCKTAIL WITH PROPORTIONAL AMOUNTS OF EACH ENZYME, INCREASING ITS EFFICIENCY TO ACT AS A BIOPRODUCT ON ANIMAL NUTRITION.

IMPLEMENTAÇÃO DE UM SISTEMA DE MARCAÇÃO PROTEÔMICA ORGANELAR POR PROXIMIDADE

MARIA FERNANDA VOGADO DE MESQUITA, PAULA MARIAN VIEIRA GOULART, IZABELA MARQUES DOURADO BASTOS CHARNEAU, FRÉDÉRIC FERCOQ, CORALIE MARTIN FREDER, PHILIPPE GRELLIER, SÉBASTIEN OLIVIER CHARNEAU.

THE TRYPANOSOMATIDS, BELONGING TO THE KINETOPLASTIDA CLASS AND INCLUDING THE GENERA TRYPANOSOMA AND LEISHMANIA, ARE AGENTS CAUSING NEGLECTED TROPICAL DISEASES THAT IMPACT MORE THAN 1.7 BILLION PEOPLE WORLDWIDE, ACCORDING TO WHO DATA. THESE PARASITES POSSESS UNIQUE BIOLOGICAL CHARACTERISTICS, SUCH AS SPECIALIZED ORGANELLES AND NUCLEI WITH PECULIAR PROTEIN COMPOSITIONS. AMONG THESE ORGANELLES, THE GLYCOSOME STANDS OUT FOR ITS DIVERSE ENZYMATIC REPERTOIRE, ESSENTIAL FOR PROCESSES SUCH AS THE PENTOSE PHOSPHATE PATHWAY, GLUCONEOGENESIS, AND LIPID AND STEROL BIOSYNTHESIS. GLYCOSOMAL ENZYMES, FUNDAMENTAL IN THESE PATHWAYS, ARE PROMISING TARGETS FOR DRUG DEVELOPMENT. TO DATE, PROTEOMIC ANALYSES OF THESE ORGANELLES HAVE BEEN LIMITED BY CONVENTIONAL ENRICHMENT METHODS, RESULTING IN LIMITED COVERAGE OF IDENTIFIED PROTEINS. THEREFORE, THIS STUDY PROPOSED THE USE OF THE APEX2-BASED PROXIMITY LABELING STRATEGY FOR DETAILED PROTEIN MAPPING OF THE GLYCOSOMES AND NUCLEUS OF LEISHMANIA INFANTUM AND TRYPANOSOMA CRUZI. THE PROCESS INVOLVED THE CLONING OF THE APEX2 GENE SEQUENCE, FUSED TO THE PTS1 AND NLS, WHICH ARE SIGNAL PEPTIDES FOR GLYCOSOME AND NUCLEAR TARGETING, RESPECTIVELY, IN THE COMMERCIALLY ACQUIRED PLEXSY (LEISHMANIA) AND PTREX (T. CRUZI) VECTORS, RESULTING IN FOUR DISTINCT CONSTRUCTS. AFTER TRANSFECTION AND SELECTION OF RESISTANT PARASITES, PROTEIN EXPRESSION AND ACTIVITY WERE EVALUATED. HIGH APEX2 EXPRESSION AND SPECIFIC BIOTINYLATION WERE OBSERVED IN THE GLYCOSOMES AND NUCLEUS OF L.

INFANTUM, INDICATING THAT THE TECHNIQUE IS FEASIBLE FOR STUDY IN L. INFANTUM. AS A PERSPECTIVE, WE AIM TO COMPLETE THE PROTEOMIC STUDIES AND APPLY THEM TO T. CRUZI. PROTEOMIC COMPARISON BETWEEN THESE SPECIES MAY REVEAL ADAPTIVE MECHANISMS OF THIS GROUP AND IDENTIFY PHARMACOLOGICAL TARGETS FOR THE TREATMENT OF RELATED DISEASES.

PRODUCTION AND ANALYSIS OF THE CARBOHYDRATE-BIDING-MODULE MCIPA AND ITS SYNERGISM WITH CELLULASE

MARIA CLARA COSTA REIS, JOÃO BATISTA HOLANDA LOPES, BETÂNIA FERRAZ QUIRINO E ELIANE FERREIRA NORONHA

CARBOHYDRATE-BINDING-MODULES ARE KNOWN FOR INCREASING THE ACTIVITY OF CELLULASE TACKLING THE INTERACTION OF THE ENZYME WITH THE SUBSTRATE. THE MCIPA IS A MODIFIED CBM ORIGINALLY FROM ACETOVIBRIOS THERMOCELLUM, OBTAINED BY HETEROLOGOUS EXPRESSION IN ESCHERICHIA COLI. IN THIS STUDY, WE PRODUCED THE PROTEIN AND TESTED THE BEST MEDIUM FOR PROTEIN EXPRESSION, PURIFIED USING LIQUID CHROMATOGRAPHY AND TESTED THE PROTEIN FOR SYNERGISM WITH FUGI CELLULASES. THE OBJECTIVE IS TO USE THIS PROTEIN FOR ANIMAL NUTRITION, ALONG SIDE ENZYMES, TO HELP COW DIGESTION THE AND THE PRODUCTIVITY OF BOVINES THROUGHOUT BRASIL.

GENÉTICA MOLECULAR, BIOTECNOLOGIA.

METHOD FOR DETECTION OF THE NS3 GENE FOR DENGUE BASED ON THE USE OF RIBOZYMES AND FLUORESCENCE TRANSFER BY HYBRIDIZATION CHAIN REACTION

THIAGO RABELO LIMA, SOPHIA GARCIA DE RESENDE, PEDRO FELIPE QUEIROZ, ERNESTO BRECIANI TAVEIRA, MIGUEL CUNHA NUNES, SONIA MARIA DE

FREITAS, IZADORA CRISTINA M. DE OLIVEIRA, AMANDA SOUZA BERNASOL, AISEL VALLE GARAY, FERNANDO TORRES ARARIPE, CÍNTIA MARQUES COELHO

CURRENTLY, DENGUE IS THE MOST PREVALENT AND FASTEST-SPREADING ARBOVIRUS AMONG HUMANS. IN BRAZIL, AS OF NOVEMBER 2024, 6,565,224 PROBABLE CASES OF DENGUE HAVE BEEN RECORDED, WITH 5,806 CONFIRMED DEATHS RESULTING FROM THE DISEASE. GIVEN THE INCREASE IN CASES, THE POTENTIAL SEVERITY, AND THE SIMILARITY OF SYMPTOMS WITH OTHER ARBOVIRUSES, IT IS OF UTMOST IMPORTANCE THAT DIAGNOSTICS ARE PERFORMED QUICKLY AND ACCESSIBLY ACROSS THE COUNTRY. DIAGNOSTIC TESTS COMMONLY USED, SUCH AS RT-QPCR AND ELISA, SHARE THE REQUIREMENT FOR A WELL-EQUIPPED LABORATORY WITH HIGH-COST EQUIPMENT, THE USE OF REFRIGERATORS, AND SPECIALIZED PERSONNEL. THE OBJECTIVE OF THIS PROJECT IS TO DEVELOP A DIAGNOSTIC METHOD FOR THE DENGUE VIRUS THAT SURPASSES THESE EXISTING BOTTLENECKS. FOR THIS PURPOSE, RIBOZYMES ARE EMPLOYED TO RECOGNIZE AND CLEAVE THE VIRAL RNA, AND DNA HAIRPINS CONTAINING FLUOROPHORES ARE USED FOR A HYBRIDIZATION CHAIN REACTION, RESULTING IN A POLYMER OF FLUOROGENIC MOLECULES WITH THE POTENTIAL TO TRIGGER FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET-HCR). INITIAL RESULTS INDICATE THAT THE TARGET RNA VIRUS APPEAR DETECTABLE USING UNIQUE AND MULTIPLEX DNA HAIRPINS COUPLED WITH FLUOROPHORES, FACILITATING THE DETECTION OF DENV VIA A RESONANT ENERGY TRANSFER MECHANISM (FRET). NOTABLY, OUR FINDINGS SUGGEST A HIGH DEGREE OF SPECIFICITY, AS THE DNA HAIRPINS EXHIBIT MINIMAL HYBRIDIZATION WITH RNA FROM OTHER ARBOVIRUSES SUCH AS ZIKA AND CHIKUNGUNYA. THIS APPROACH OFFERS THE POTENTIAL FOR A SIMPLE, COST-EFFECTIVE, AND ENZYME-FREE DIAGNOSTIC METHOD FOR DENGUE.

DETECTION METHOD FOR AVIAN INFLUENZA (H5N1) BASED ON FRET-HCR AND RIBOZYME ACTIVITY

MIGUEL CUNHA NUNES, PEDRO FELIPE QUEIROZ, SOPHIA GARCIA DE RESENDE, ERNESTO BRECIANI TAVEIRA, THIAGO RABELO LIMA, SONIA MARIA DE FREITAS, IZADORA CRISTINA M. DE OLIVEIRA, AMANDA SOUZA BERNASOL, AISEL VALLE GARAY, FERNANDO TORRES ARARIPE, CÍNTIA MARQUES COELHO

AVIAN INFLUENZA, CAUSED BY VIRUSES FROM THE ORTHOMYXOVIRIDAE FAMILY, REPRESENTS A SIGNIFICANT THREAT TO PUBLIC AND ANIMAL

HEALTH, ESPECIALLY WITH HIGHLY PATHOGENIC SUBTYPES SUCH AS H5N1. THIS SUBTYPE HAS CAUSED GLOBAL OUTBREAKS SINCE 2003, AND MORE RECENTLY, THERE HAVE BEEN SUSPICIONS OF HUMAN-TO-HUMAN TRANSMISSION. THE MOST RECENT EXAMPLE INVOLVED A YOUNG CANADIAN WHO TESTED POSITIVE FOR AVIAN FLU SUBTYPE H5N1. BRAZIL, AS THE SECOND-LARGEST PRODUCER OF CHICKEN MEAT, FACES THE CHALLENGE OF PROTECTING ITS POULTRY INDUSTRY FROM THE SOCIOECONOMIC IMPACTS OF THIS VIRUS. THUS, LABORATORY DIAGNOSIS BECOMES NECESSARY AS A CONTAINMENT MEASURE TO PREVENT THE SPREAD OF THE VIRUS. HOWEVER, THE MOST USED METHODS LIKE ELISA AND PCR ARE LIMITED DUE TO HIGH COSTS, THE NEED FOR SPECIALIZED EQUIPMENT AND PERSONAL, AND THE REQUIREMENT FOR PROPER REFRIGERATED STORAGE, WHICH RESTRICTS THEIR WIDESPREAD USE. THEREFORE, THIS STUDY PROPOSES THE DEVELOPMENT OF A NEW DIAGNOSTIC METHOD BASED ON THE ACTIVITY OF HAMMERHEAD RIBOZYMES TO IDENTIFY AND CLEAVE THE HA AND NA H5N1 TARGET GENES, COMBINED WITH HYBRIDIZATION CHAIN REACTION (HCR) AMPLIFICATION USING THE FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) TECHNIQUE. THE RESULTS OBTAINED SO FAR DEMONSTRATE THAT THE RIBOZYMES WERE ABLE TO RECOGNIZE AND CLEAVE THE TARGET VIRAL RNA, AND THE RESULTING FRAGMENTS WERE SUCCESSFULLY DETECTED IN FRET-HCR ASSAYS, BOTH IN SIMPLE AND MULTIPLEXED TESTS. ADDITIONALLY, THE ASSAYS SHOWED SPECIFICITY FOR THE TARGET SUBTYPE AND A LOWER DETECTION LIMIT COMPARED TO SARS-COV-2. THIS METHOD PROMISES TO BE A RAPID, COST-EFFECTIVE, AND ENZYME-INDEPENDENT ALTERNATIVE. FUTURE PERSPECTIVES INCLUDE EVALUATING THE RECYCLING OF THE INITIATING FRAGMENT TO FURTHER ENHANCE THE METHOD'S SENSITIVITY.

METHOD FOR DETECTION OF THE NS5 GENE FOR DENGUE BASED ON THE USE OF RIBOZYMES AND FLUORESCENCE TRANSFER BY HYBRIDIZATION CHAIN REACTION

ERNESTO BRECIANI DOS SANTOS MARQUES TAVEIRA, SOPHIA GARCIA DE RESENDE, PEDRO FELIPE QUEIROZ1, THIAGO RABELO LIMA, MIGUEL CUNHA NUNES, SONIA MARIA DE FREITAS, IZADORA CRISTINA M. DE OLIVEIRA, AMANDA SOUZA BERNASOL, AISEL VALLE GARAY, FERNANDO TORRES ARARIPE3, CÍNTIA MARQUES COELHO

DENGUE IS THE MOST PREVALENT AND RAPIDLY SPREADING ARBOVIRUS INFECTION IN HUMANS. BRAZIL IS THE COUNTRY MOST AFFECTED BY DENGUE CASES WORLDWIDE. UP TO 2024, OVER 6,000,000 PROBABLE CASES HAVE BEEN REPORTED. WHEN NOT ASYMPTOMATIC, DENGUE SHARES SIMILAR SYMPTOMS WITH INFECTIONS CAUSED BY OTHER VIRUSES LIKE ZIKA AND CHIKUNGUNYA. THIS SYMPTOM SIMILARITY OFTEN PREVENTS PATIENTS FROM RECEIVING THE APPROPRIATE TREATMENT FOR THEIR CONDITION. THE MOST COMMONLY USED DIAGNOSTIC METHODS, ELISA AND RT-QPCR, SUFFER FROM HIGH RATES OF FALSE-POSITIVE RESULTS AND ISSUES RELATED TO EQUIPMENT COSTS, THE NEED FOR SPECIALIZED PERSONNEL, AND CONSTANT REFRIGERATION OF COMPONENTS, RESPECTIVELY. THUS, THE PRESENT STUDY AIMED TO DEVELOP A RAPID, SENSITIVE, ENZYME-FREE DIAGNOSTIC METHOD. THIS METHOD UTILIZES (1) THE CATALYTIC ACTIVITY OF HAMMERHEAD RIBOZYMES TO RECOGNIZE AND CLEAVE VIRAL RNA, GENERATING A TRIGGERING FRAGMENT, AND (2) FLUOROPHORES ASSOCIATED WITH DNA HAIRPINS TO INITIATE A HYBRIDIZATION CHAIN REACTION (HCR), WITH THE POTENTIAL TO TRIGGER FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET). RIBOZYMES HAVE BEEN STUDIED FOR AROUND 40 YEARS AND HAVE ALREADY BEEN USED IN THE TREATMENT OF DISEASES CAUSED BY RIBOVIRUSES. AFTER IDENTIFYING A CONSERVED REGION AMONG DIFFERENT DENGUE VIRUS SEROTYPES IN THE NS5 GENE, ALL MOLECULES NECESSARY FOR THE METHOD WERE DESIGNED AND SYNTHESIZED. THE RESULTS INDICATED THAT THE CATALYTIC ACTIVITY OF THE RIBOZYME ON THE TARGET, BASED ON THE FLUORESCENCE INTENSITY ANALYSIS THROUGH THE FRET-HCR ASSAY METHOD, WAS SUCCESSFUL. SEVERAL TRIPLICATE ASSAYS WERE ALSO PERFORMED TO DETERMINE THE LIMIT OF DETECTION (LOD) AND IDENTIFY THE LOWEST VIRAL LOAD CONCENTRATION CAPABLE OF INITIATING THE HCR REACTION. THESE RESULTS DEMONSTRATED THAT, COMPARED TO THE USE OF THE SAME METHOD FOR DETECTING THE SARS-COV-2 VIRUS, THERE WAS A FOUR-FOLD DECREASE IN THE LIMIT OF DETECTION. THEREFORE, THIS IS A PROMISING METHOD FOR DETECTING THE DENV VIRUS, AND FUTURE PERSPECTIVES INCLUDE INCORPORATING THE RECYCLING OF THE TRIGGERING FRAGMENT TO ENHANCE ITS SENSITIVITY.

VALORIZAÇÃO DE PRODUTOS DA CADEIA DO ALGODÃO: EXPRESSÃO HETERÓLOGA DE AMILASE PARA O BENEFICIAMENTO DE TECIDOS

AUGUSTO SOARES LEITE RIBEIRO; ELIANE FERREIRA NORONHA; MÔNICA CARAMEZ TRICHES DAMASO

A INDÚSTRIA TÊXTIL É ALTAMENTE POLUENTE, DESDE O CULTIVO DE FIBRAS ATÉ A BAIXA REUTILIZAÇÃO DE ROUPAS. SEGUNDO A FUNDAÇÃO ELLEN MACARTHUR (2017), A PRODUÇÃO TÊXTIL CONSOME 93 BILHÕES DE METROS CÚBICOS DE ÁGUA POR ANO, DESTACANDO A URGÊNCIA DE TECNOLOGIAS MAIS SUSTENTÁVEIS NO BENEFICIAMENTO DE TECIDOS. AS AMILASES, AMPLAMENTE USADAS NA HIDRÓLISE DE AMIDO EM TECIDOS COMO JEANS, TÊM DEMANDA CRESCENTE PARA PRODUÇÃO NACIONAL. O USO DE ENZIMAS PODE MELHORAR A EFICIÊNCIA INDUSTRIAL E TORNÁ-LOS MAIS SUSTENTÁVEIS, MUITOS OCORRENDO EM ALTAS TEMPERATURAS, COMO A DEGOMAGEM DE JEANS. ESTE TRABALHO ESTUDOU CONDIÇÕES PARA OTIMIZAR A EXPRESSÃO DA AMILASE RECOMBINANTE AMYPB EM E. COLI E ANALISOU FIBRAS TRATADAS COM A ENZIMA. OS GENES DA AMILASE AMYPB DE PAENIBACILLUS BARENGOLTZII FORAM CLONADOS NO PLASMÍDEO PET21A E EXPRESSOS EM ESCHERICHIA COLI BL21 (DE3). AS CÉLULAS FORAM CULTIVADAS EM MEIOS TERRIFIC-BROTH E LURIA-BERTANI, INDUZIDAS COM IPTG OU LACTOSE, LISADAS, E A ENZIMA COLETADA POR CENTRIFUGAÇÃO PARA ENSAIOS DE ATIVIDADE (FUWA). A ENZIMA FOI APLICADA EM TECIDO JEANS NAS CONDIÇÕES IDEAIS DE PH E TEMPERATURA, COM ANÁLISE DAS FIBRAS POR MICROSCOPIA ELETRÔNICA DE VARREDURA. O PROJETO OTIMIZOU A EXPRESSÃO DA AMILASE RECOMBINANTE AMYPB EM E. COLI, COM DESTAQUE PARA OS MEIOS LURIA-BERTANI E TERRIFIC-BROTH AUTOINDUTORES. A ENZIMA FOI EFICAZ NA DEGOMAGEM DE TECIDOS JEANS, E A ANÁLISE POR MEV REVELOU DESEMPENHO SIMILAR À AMILASE COMERCIAL BIOKEY BSLH, REFORÇANDO SEU POTENCIAL INDUSTRIAL.

VIROLOGIA E MICROBIOLOGIA MOLECULAR

EXPRESSÃO HETERÓLOGA EM SISTEMA LESXY DAS DINAMINAS 1 E 2 DE PLASMODIUM FALCIPARUM PARA AVALIAR NOVOS POTENCIAIS INIBIDORES

LAÍS BOAZ DE LIMA, SÉBASTIEN OLIVIER CHARNEAU

MALARIA IS CAUSED BY A PARASITE OF THE GENUS PLASMODIUM. IN HUMANS, FIVE SPECIES ARE KNOWN TO BE RESPONSIBLE, WITH P. FALCIPARUM BEING THE ONE MOST ASSOCIATED WITH MORTALITY FROM THE DISEASE, WITH THE MAJORITY OF CASES IN CHILDREN UNDER 5 AND PREGNANT WOMEN. IN ADDITION, THERE ARE RECORDS OF P. FALCIPARUM STRAINS RESISTANT TO ANTIMALARIALS USED TO TREAT THE INFECTION, SUCH AS CHLOROQUINE AND ARTEMISININ. ON THE OTHER HAND, THE AVAILABLE VACCINE HAS LOW EFFICACY AND A SHORT-LASTING EFFECT.THE DYNAMIN-LIKE GENES REPRESENT A SUPERFAMILY OF GTPASES, WHICH ACT MECHANOCHEMICALLY AND PARTICIPATE IN VARIOUS CELLULAR PROCESSES SUCH AS ENDOCYTOSIS, FISSION AND FUSION OF ORGANELLES, CYTOKINESIS AND INTRACELLULAR TRAFFIC. THREE DYNAMIN-LIKE ENZYMES HAVE BEEN IDENTIFIED IN THE P. FALCIPARUM GENOME: PFDYN1, PFDYN2 AND PFDYN3, THE LAST OF WHICH IS A HYPOTHETICAL GENE. NONE OF THESE ENZYMES HAS YET BEEN PROPERLY CHARACTERIZED. THE PFDYN1, PFDYN2 DYNAMIN-LIKE ENZYMES ARE THE FOCUS OF THIS WORK. CONSIDERING THE NEED TO IDENTIFY NEW THERAPEUTIC TARGETS AND NEW DRUGS BY MEANS OF HETEROLOGOUS EXPRESSION OF THE PROTEIN AND FUTURE PURIFICATION, THE AIM IS TO TEST NEW DYNAMIN INHIBITORS. FINALLY, USING THE LEISHIMANIA TARANTOLAE EXPRESSION SYSTEM (LEXSY), WHICH HAS SIMILAR PATTERNS OF POST-TRANSLATIONAL MODIFICATIONS BECAUSE IT IS AN EUKARYOTIC ORGANISM SIMILAR TO PLASMODIUM. HEREFORE, WE HOPE TO PRODUCE A GREATER QUANTITY AND SIMILARITY TO DYNAMINS IN ORDER TO TEST INHIBITORS.PROCESSES SUCH AS ENDOCYTOSIS, FISSION AND FUSION OF ORGANELLES, CYTOKINESIS AND INTRACELLULAR TRAFFIC. THREE DYNAMIN-LIKE ENZYMES HAVE BEEN IDENTIFIED IN THE P. FALCIPARUM GENOME: PFDYN1, PFDYN2 AND PFDYN3, THE LAST OF WHICH IS A HYPOTHETICAL GENE. NONE OF THESE ENZYMES HAS YET BEEN PROPERLY CHARACTERIZED. THE PFDYN1, PFDYN2 DYNAMIN-LIKE ENZYMES ARE THE FOCUS OF THIS WORK. CONSIDERING THE NEED TO IDENTIFY NEW THERAPEUTIC TARGETS AND NEW DRUGS BY MEANS OF HETEROLOGOUS EXPRESSION OF THE PROTEIN AND FUTURE PURIFICATION, THE AIM IS TO TEST NEW DYNAMIN INHIBITORS. FINALLY, USING THE LEISHIMANIA TARANTOLAE XPRESSION SYSTEM (LEXSY), WHICH HAS SIMILAR PATTERNS OF POST-TRANSLATIONAL MODIFICATIONS BECAUSE IT IS AN EUKARYOTIC ORGANISM SIMILAR TO PLASMODIUM. HEREFORE, WE HOPE TO PRODUCE A GREATER QUANTITY AND SIMILARITY TO DYNAMINS IN ORDER TO TEST INHIBITORS.

MODULATION OF CELLULAR PARAMETERS OF MICROGLIA BY THE NUCLEOCAPSID (N) PROTEIN OF SARS-COV-2.

CÉZAR JÚNIO JERÔNIMO MARQUES, RAQUEL DAS NEVES ALMEIDA, HILLARY DA SILVA VELAME, BRENDA RABELLO CAMARGO, LEONARDO ASSIS DA SILVA, KELLY GRACE MAGALHÃES, BERGMANN MORAIS RIBEIRO.

SARS-COV-2 INFECTION CAN LEAD TO SEVERE RESPIRATORY SYMPTOMS AND HAS BEEN SHOWN TO AFFECT THE CENTRAL NERVOUS SYSTEM BY CROSSING THE BLOOD-BRAIN BARRIER, CAUSING PRE-ACTIVATION OF MICROGLIAL CELLS UPON CONTACT. THIS STUDY AIMED TO EVALUATE WHETHER THE NUCLEOCAPSID (N) PROTEIN OF SARS-COV-2 INFLUENCES SPECIFIC CELLULAR PARAMETERS OF HUMAN MICROGLIA C20 LINEAGE. OUR RESULTS DEMONSTRATED THAT VARYING CONCENTRATION OF THE N PROTEIN DID NOT ALTER CELL VIABILITY, AS MEASURED BY THE MTT ASSAY. ADDITIONALLY, CELL DEATH PROFILE MODULATION, CMICROGLIA WITHOUT CAUSING OXIDATIVE STRESS OR CELL DEATH, SUGGESTING ITS POTENTIAL AS A TARGET FOR SPECIFIC THERAPIES. FURTHER INVESTIGATIONS ARE REQUIRED TO UNDERSTAND HOW THE N PROTEIN AFFECTS MICROGLIAL FUNCTION AND THE IMPLICATIONS FOR DISEASE PROGRESSION OR RECOVERY.

STRAWBERRY VIROME IN CENTRAL BRAZIL (GOIÁS STATE): CANDIDATE VIRUSES OF QUARANTINE AND ECONOMIC RELEVANCE AND IDENTIFICATION OF POTENTIAL VIRAL ETIOLOGY OF REDDENING

TIAGO BARROS FERNANDES, GABRIEL RAPÔSO CIARLINI, ENZO CARDOSO VAZ RIBEIRO, FELIPE FOCHAT SILVA MELO, RITA DE CÁSSIA PEREIRA CARVALHO, MÁRCIO MARTINELLO SANCHEZ, TATSUYA NAGATA, MARILIA SANTOS SILVA PATRIOTA

STRAWBERRY CULTIVATION HAS HIGH PRODUCTIVITY IN BRAZIL, HOWEVER, THERE ARE CHALLENGES RELATED TO POOR PHYTOSANITARY QUALITY OF SEEDLINGS, WHICH INTRODUCE VIRAL INFECTIONS. IN THIS STUDY, SYMPTOMATIC STRAWBERRY LEAF SAMPLES WERE COLLECTED FROM COMMERCIAL AREAS IN 2022, IN CENTRAL BRAZIL (GOIÁS STATE). VIRUSES

WERE PURIFIED BY ULTRACENTRIFUGATION, FOLLOWED BY VIRAL RNA EXTRACTION. A CORRESPONDING CDNA LIBRARY, DEPLETED OF RIBOSOMAL RNA, WAS SEQUENCED USING HIGH THROUGHPUT SEQUENCING (HTS) ON THE ILLUMINA PLATFORM. BLASTX ANALYSIS OF THE 70.398 RESULTING CONTIGS (GENIOUS PRIME SOFTWARE) IDENTIFIED FOUR CANDIDATE VIRAL SPECIES HIGHLY LIKELY TO BE OF QUARANTINE (TYMOVIRUS LATANDIGENUM-ANDEAN POTATO LATENT VIRUS-APLV) AND ECONOMIC IMPORTANCE (POTEXVIRUS FRAGARIAE-STRAWBERRY MILD YELLOW EDGE VIRUS-SMYEV, CUCUMOVIRUS CMV-CUCUMBER MOSAIC VIRUS-CMV, AND MARAFIVIRUS MAYDIS-MAIZE RAYADO FINO VIRUS-MRFV). THROUGH HTS, THE COMPLETE SEQUENCE OF TWO SMYEV STRAINS WAS OBTAINED. ONE STRAIN BELONGED TO THE SAME CLADE AS A GERMAN STRAIN (GENBANK AJ577359), WHILE THE OTHER REPRESENTED A CLADE WITH NO HOMOLOGS (DIVERGENT FROM STRAINS FROM CANADA, CHINA, GERMANY, ARGENTINA, AND JAPAN). THE FULL RNA 2 AND RNA 3 SEQUENCES OF CMV WERE ALSO OBTAINED VIA HTS, MARKING THE FIRST REPORT OF CMV OCCURRENCE IN STRAWBERRIES IN BRAZIL. ADDITIONALLY, BWYV WAS DETECTED IN A REDDENING SAMPLE VIA ELISA, SUGGESTING THAT REDDENING IS AN ADVANCED SYMPTOM OF MARGINAL CHLOROSIS CAUSED BY A SYNERGISTIC VIRAL COMPLEX INVOLVING PLRV AND/OR SMYEV. VALIDATION OF THESE CANDIDATE VIRUSES DETECTED BY HTS AND ELISA IN CENTRAL BRAZIL (GOIÁS STATE) IS UNDERWAY THROUGH PCR OF THE ORIGINAL SAMPLES. THIS IS THE FIRST SURVEY DOCUMENTING GENETIC SEQUENCES OF STRAWBERRY VIRUSES IN CENTRAL BRAZIL. THE RESULTS PAVE THE WAY FOR MORE EFFECTIVE VIRUS CONTROL MEASURES AND CONTINGENCY ACTIONS AGAINST QUARANTINE VIRUSES.

EXPRESSION AND CHARACTERIZATION OF A CRYSTALLIZED SARS-COV-2 MEMBRANE (M) PROTEIN FUSED WITH THE C-TERMINAL PORTION OF THE BACILLUS THURINGIENSIS CRY1C FOR ENHANCED STABILITY AND CELLULAR RESPONSE ANALYISIS

GABRIEL RAPÔSO CIARLINI, TIAGO BARROS FERNANDES, ENZO CARDOSO VAZ RIBEIRO, GIOVANA CURCIO GUIMARÃES, RENATO DE OLIVEIRA RESENDE, MÁRCIO MARTINELLO SANCHEZ, TATSUYA NAGATA, MARILIA SANTOS SILVA PATRIOTA

THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-COV-2) TRIGGERED A GLOBAL PANDEMIC STARTING IN 2019, RESULTING IN MILLIONS OF DEATHS WORLDWIDE. CONSEQUENTLY, UNDERSTANDING BOTH STRUCTURAL AND NON-STRUCTURAL PROTEINS OF THE VIRUS HAS BECOME CRUCIAL FOR DEVELOPING DIAGNOSTIC KITS AND VACCINES. THIS STUDY FOCUSED ON EXPRESSING THE STRUCTURAL M PROTEIN FUSED TO THE C-TERMINAL OF THE CRY1C PROTEIN, NATURALLY PRODUCED BY BACILLUS THURINGIENSIS, RESULTING IN A CRYSTALLIZED PROTEIN POTENTIALLY OFFERING GREATER STABILITY. USING SPECIFIC OLIGONUCLEOTIDES, THE M PROTEIN GENE WAS AMPLIFIED VIA POLYMERASE CHAIN REACTION (PCR) AND CLONED INTO THE TRANSFER VECTOR PFAST1CT TRANSFER VECTOR, WHICH CONTAINS THE 3'END SEQUENCE OF THE CRY1C GENE. EMPLOYING THE BAC-TO-BAC® BACULOVIRUS EXPRESSION SYSTEM (THERMOFISHER SCIENTIFIC), WE ACHIEVED HE EXPRESSION OF THE FUSED M PROTEIN IN INSECT CELLS, AS CONFIRMED BY WESTERN BLOT ANALYSIS. ADDITIONALLY, THE PROTEIN'S CRYSTALS' MICROSTRUCTURE WAS EXAMINED USING SCANNING ELECTRON MICROSCOPY (MEV). TO ASSESS THE CELLULAR EFFECTS OF PROTEIN M PROTEIN, THE MTT COLORIMETRIC ASSAY INDICATED THAT M PROTEIN DID NOT INDUCE CELL DEATH. FURTHER EVALUATION OF CELLULAR PARAMETERS, INCLUDING LIPID BODY BIOGENESIS AND REACTIVE OXYGEN SPECIES GENERATION, WAS CONDUCTED UPON PROTEIN UPON SOLUBILIZATION. THE FINDINGS REVEALED THAT THE M PROTEIN WAS NON-CYTOTOXIC AND DID NOT CAUSE OXIDATIVE STRESS. HOWEVER, IT DID ACTIVATE LIPID BODY PRODUCTION, INDICATING CELLULAR ACTIVATION. THUS THE CRYSTALLIZATION OF THE M PROTEIN NOT ONLY SUGGESTSENHANCED STABILITY BUT ALSO PROVIDE A FOUNDATION ANALYSIS THAT COULD BE INSTRUMENTAL IN FUTURE DIAGNOSTIC AND VACCINE DEVELOPMENT EFFORTS, AS HIGHLIGHTED BY PREVIOUS RESEARCH.

EVALUATION OF THE VIROME OF STRAWBERRY PRODUCING AREAS IN CENTRAL BRAZIL (FEDERAL DISTRICT) BY HIGH THROUGHPUT SEQUENCING (HTS)

GABRIEL RAPÔSO CIARLINI, TIAGO BARROS FERNANDES, ENZO CARDOSO VAZ RIBEIRO, GIOVANA CURCIO GUIMARÃES, RENATO DE OLIVEIRA RESENDE2, MÁRCIO MARTINELLO SANCHEZ, TATSUYA NAGATA, MARILIA SANTOS SILVA PATRIOTA

THE STRAWBERRY CROP IS TRADITIONAL AND OF HIGH PRODUCTIVITY IN CENTRAL BRAZIL, OCCURRING MOSTLY IN FAMILY AREAS IN A CONVENTIONAL NON-ORGANIC SYSTEM, BUT THERE ARE CHALLENGES OF POOR PHYTOSANITARY QUALITY OF SEEDLINGS THAT INTRODUCE VIRUSES. AMONG TWENTY-FOUR SPECIES OF VIRUSES DESCRIBED IN STRAWBERRY PLANTS IN THE WORLD, IN BRAZIL, THERE ARE REPORTS OF OCCURRENCE OF SEVEN SPECIES OF THE GENERA CYTORHABDOVIRUS, SADWAVIRUS, CAULIMORIVUS, CRINIVIRUS, POLEROVIRUS AND POTEXVIRUS. IN THE PRESENT WORK, FROM SYMPTOMATIC STRAWBERRY LEAF SAMPLES COLLECTED IN COMMERCIAL AREAS (2019 TO 2023), VIRUSES WERE PURIFIED BY ULTRACENTRIFUGATION, THEN THE VIRAL RNA WAS EXTRACTED AND THE CORRESPONDING CDNA LIBRARY, DEPLETED OF RIBOSOMAL RNA, WAS SEQUENCED BY HIGH THROUGHPUT SEQUENCING (HTS) ON AN ILLUMINA PLATFORM. THE RESULTING CONTIGS (GENIOUS PRIME) SHOWED AT LEAST THREE CANDIDATE VIRAL SPECIES: HIGHLY PROBABLE OF QUARANTINE (IPOMOVIRUS BRUNUSMANIHOTIS-CASSAVA BROWN STREAK, VIRUS-CBSV) AND COMMERCIAL (POTEXVIRUS FRAGARIAE-STRAWBERRY-STRAWBERRY, MILD YELLOW EDGE VIRUS-SMYEV; ANULAVIRUS PZSV-PELARGONIUM ZONATE SPOT VIRUS-PZSV). BY HTS, THE COMPLETE SEQUENCE OF ONE STRAIN OF SMYEV, WIDELY PRESENT IN STRAWBERRY PLANTS IN THE COUNTRY, WAS OBTAINED, THIS STRAIN BEING FROM THE SAME CLADE OF STRAIN FROM CHINA (GENBANK OK562581). THE VALIDATION OF THESE CANDIDATE VIRUSES DETECTED BY HTS IN CENTRAL BRAZIL (FEDERAL DISTRICT) IS BEING CARRIED OUT BY PCR ON THE ORIGINAL SAMPLES. THIS IS THE FIRST SURVEY WITH A RECORD OF GENE SEQUENCES OF STRAWBERRY VIRUS IN CENTRAL BRAZIL. THE RESULTS OPEN PERSPECTIVES FOR ADEQUATE VIRUS CONTROL, QUARANTINE VIRUS CONTINGENCY, DEVELOPMENT OF DIAGNOSTIC KITS NOT COMMERCIALLY AVAILABLE AND SUPPORT FOR STRAWBERRY GENETIC IMPROVEMENT PROGRAMS FOR THE DEVELOPMENT OF STRAWBERRY RESISTANT TO VIRUSES.

INVENTORY OF GRAPEVINE VIROME IN VINEYARDS IN CENTRAL BRAZIL (FEDERAL DISTRICT) BY HIGH THROUGHPUT SEQUENCING (HTS)

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THE VINE IS AN EMERGING CROP IN THE FEDERAL DISTRICT-DF, CENTRAL BRAZIL, WHERE ALMOST 75 HA ARE PLANTED AND PRODUCTION (2021) OF MORE THAN 2 THOUSAND TONS. IN ADDITION TO THE PRODUCTION OF TABLE GRAPES, THE REGION IS ALSO DEDICATED TO THE PRODUCTION OF GRAPES FOR WINE AND WINE TOURISM. THERE ARE AT LEAST 36 SPECIES OF VIRUSES DESCRIBED IN GRAPEVINES IN THE WORLD, BELONGING TO 18 VIRAL GENERA. AMONG THESE VIRUSES, IN BRAZIL, THERE ARE REPORTS OF OCCURRENCE OF 13 SPECIES OF THE GENERA ALFAMOVIRUS, ALPHACARMOVIRUS, AMPELOVIRUS, CLOSTEROVIRUS, CUCUMOVIRUS, ILARVIRUS, MACULAVIRUS, NEPOVIRUS, SOBEMOVIRUS, TOBAMOVIRUS, VITIVIRUS. AMONG THE VIRUSES DESCRIBED IN GRAPEVINES IN THE WORLD, 10 SPECIES ARE REGULATED QUARANTINES, BEING 08 SPECIES OF THE GENUS NEPOVIRUS, 01 STRALARIVIRUS AND 01 TOMBUSVIRUS. IN THE PRESENT WORK, 54 SAMPLES OF SYMPTOMATIC VINE LEAVES WERE COLLECTED IN DF VINEYARDS (2024), FROM 08 ADMINISTRATIVE REGIONS (SÃO SEBASTIÃO, PLANALTINA, PARANOÁ, RIACHO FUNDO, SAMAMBAIA, SOBRADINHO, BRAZLÂNDIA, GAMA) AND WERE CATEGORIZED INTO 06 TYPICAL VIRAL SYMPTOMS (VEIN CHLOROSIS, VARIEGATION, REDDENING, LEAF DEFORMATION, MOSAIC, MARGINAL CHLOROSIS). VIRUSES FROM THESE SAMPLES WILL BE PURIFIED BY ULTRACENTRIFUGATION, THEN THE VIRAL RNA WILL BE EXTRACTED AND THE CORRESPONDING CDNA LIBRARY WILL BE SEQUENCED BY HIGH THROUGHPUT SEQUENCING (HTS) ON THE ILLUMINA PLATFORM. THE READINGS OBTAINED WILL BE ANALYZED BY BIOINFORMATICS AND BLASTX OF THE RESULTING CONTIGS WILL POINT OUT PROBABLE CANDIDATE VIRAL SPECIES OF QUARANTINE AND COMMERCIAL EXPRESSION. THE VALIDATION OF THESE CANDIDATE VIRUSES DETECTED BY HTS IN CENTRAL BRAZIL (DF) WILL BE PERFORMED BY PCR/ELISA ON THE ORIGINAL SAMPLES. THIS IS THE FIRST SYSTEMATIC SURVEY OF GRAPEVINE VIRUS GENE SEQUENCES IN CENTRAL BRAZIL, SINCE THERE ARE ONLY 206 GRAPEVINE VIRUS ACCESSIONS IN BRAZIL IN GENBANK CURRENTLY. THE RESULTS OPEN PERSPECTIVES FOR ADEQUATE CONTROL OF VIRUSES AND CONTINGENCY OF QUARANTINE VIRUSES.

FIRST REPORT OF MOROCCAN WATERMELON MOSAIC VIRUS IN PUMPKIN PLANTS IN BRAZIL

STEPHANNY BARRETO DOS SANTOS CÁRDENAS, BRUNO ARCANJO SILVA, HELENA MOTA, PALOMA DE SOUZA QUEIROZ, AMANDA BATISTA, CATERYNNE MELO KAUFFMANN, DÉBORA MARIA SANSINI FREITAS,TATSUYA NAGATA

THE CUCURBITACEAE FAMILY COMPRISES 97 GENERA AND AROUND 950 PLANT SPECIES. IN BRAZIL, THE MAIN CULTIVATED SPECIES BELONG TO THE GENERA CITRULLUS (WATERMELON), CUCUMIS (CUCUMBER, GHERKIN, AND MELON), CUCURBITA (PUMPKIN, SQUASH, AND ZUCCHINI), LAGENARIA (BOTTLE GOURD), AND SECHIUM (CHAYOTE). VIRAL INFECTIONS STAND OUT AS THE MAJOR DISEASES AFFECTING CUCURBITS, OFTEN OCCURRING AT HIGH INCIDENCES AND VARYING LEVELS OF SEVERITY, LEADING TO SIGNIFICANT REDUCTIONS IN BOTH TOTAL YIELD AND FRUIT QUALITY. IN BRAZIL, APPROXIMATELY FOURTEEN VIRUSES HAVE BEEN REPORTED INFECTING CUCURBITS, AND THESE VIRUSES CAN OCCUR IN SINGLE OR MIXED INFECTIONS. THEREFORE, THE AIM OF THIS STUDY WAS TO IDENTIFY NEW VIRUSES IN A POOL OF CUCURBITS (CITRULLUS SPP., CUCUMIS SPP., CUCURBITA SPP., AND SECHIUM SPP.) USING HIGH-THROUGHPUT SEQUENCING (HTS). THE SAMPLES WERE SEMI-PURIFIED, AND TOTAL RNA WAS EXTRACTED FROM A PELLET OBTAINED BY CENTRIFUGATION ON A SUCROSE CUSHION, USING A QUICK-RNATM PLANT MINIPREP KIT (ZYMO RESEARCH, IRVINE, USA). A CDNA LIBRARY WAS CONSTRUCTED AND SEQUENCED USING A NOVASEQ SYSTEM AT A 10G SCALE. READS WERE TRIMMED, AND CONTIGS WERE ASSEMBLED USING MEGAHIT. A BLASTX SEARCH WAS PERFORMED USING THE VIRAL PROTEIN DATABASE (REFSEQ 2023) IN THE GENEIOUS PRIME PLATFORM. THE ANALYSES REVEALED THE PRESENCE OF NINE VIRAL GENERA: CARLAVIRUS, CUCUMOVIRUS, BADNAVIRUS, CAULIMOVIRUS, CLOSTEROVIRUS, COGUVIRUS, POTYVIRUS, POLEROVIRUS, AND ORTHOTOSPOVIRUS. ADDITIONALLY, TWO CONTIGS SHOWED HIGH AMINO ACID SEQUENCE IDENTITIES (95.4% OVER 3,124 AA IN POLYPROTEIN AND 87.2% OVER 203 AA IN P1 PROTEIN) WITH MOROCCAN WATERMELON MOSAIC VIRUS (MWMV) FROM THE GENUS POTYVIRUS IN THE FAMILY POTYVIRIDAE, A VIRUS NOT PREVIOUSLY REPORTED IN BRAZIL. THE COMPLETE GENOME WAS ASSEMBLED BY MAPPING THE READS TO THE CONTIGS AS REFERENCES. THE ASSEMBLED COMPLETE GENOME OF MWMV (LC775353) WAS 9,713 NT, EXCLUDING THE POLY(A) TAIL, WITH 217,278 READS ALIGNED TO THE GENOME, RESULTING IN AN AVERAGE COVERAGE OF 3,369.6 AND A PAIRWISE IDENTITY OF 99.0%. THE ASSEMBLED GENOME ENCODED A POLYPROTEIN WITH A HIGH AMINO ACID SEQUENCE IDENTITY OF 97.82% WITH THE MOROCCAN ISOLATE (OQ847413). TO CONFIRM THE PRESENCE OF THIS VIRUS, RT-PCR WAS PERFORMED USING SPECIFIC PRIMERS TARGETING THE CYLINDRICAL

INCLUSION GENE (EXPECTED AMPLICON SIZE: 598 BP). ELEVEN FIELD SAMPLES OF PUMPKIN PLANTS (SIX FROM A FIELD IN THE JUAZEIRO REGION AND FIVE FROM THE PETROLINA REGION) WERE ANALYZED BY RT-PCR, WITH ONE SAMPLE FROM JUAZEIRO AND FIVE SAMPLES FROM PETROLINA TESTING POSITIVE FOR MWMV. ONE REPLICON FROM EACH REGION WAS SEQUENCED (JUAZEIRO, OR338305; PETROLINA, OR338306) AND SHOWED HIGH NUCLEOTIDE IDENTITIES OF 97.0% WITH EACH OTHER AND 96.4% AND 97.7%, RESPECTIVELY, WITH THE MOROCCAN ISOLATE (OQ847413). THIS IS THE FIRST REPORT OF THE OCCURRENCE OF MWMV IN BRAZIL AND SOUTH AMERICA, ASSOCIATED WITH SYMPTOMS OF MOSAIC, BLISTERING, AND YELLOWING IN PUMPKIN PLANTS.

EXPRESSION AND OCCLUSION OF HDV PROTEIN IN CYPOVIRUS POLYHEDRA FOR USE IN SEROLOGICAL DIAGNOSIS

FERNANDA MUNDIM GOMES, PATRÍCIA DE SOUZA DA SILVA E BERGMANN MORAIS RIBEIRO

THE HEPATITIS DELTA VIRUS (HDV) IS A HIGHLY PATHOGENIC PUBLIC HEALTH CONCERN THAT CAN CAUSE SEVERE LIVER DISEASES, WITH A HIGH LIKELIHOOD OF PROGRESSION TO HEPATOCELLULAR CARCINOMA. AS A DEFECTIVE VIRUS, IT RELIES ON THE HEPATITIS B VIRUS (HBV) TO OPERATE IN HEPATOCYTES. SEROLOGICAL DIAGNOSIS OF HDV IS BASED ON DETECTING ANTI-HDV ANTIBODIES, COMBINED WITH CLINICAL EVALUATION AND HBV POSITIVITY. HOWEVER, THERE ARE LIMITATIONS IN THE AVAILABILITY AND ACCESSIBILITY OF DIAGNOSTIC RESOURCES. THIS STUDY AIMS TO ACHIEVE THE EXPRESSION AND OCCLUSION OF THE HDV PROTEIN THROUGH CYPOVIRUS POLYHEDRAL CRYSTALS, A BIOTECHNOLOGICAL MODEL THAT, DUE TO ITS STABILITY AND ABILITY TO ENCAPSULATE PROTEINS, IS USEFUL FOR DEVELOPING DIAGNOSTIC IMMUNOASSAYS TO DETECT HDV INFECTION. TO ENABLE THE PRODUCTION OF THE RECOMBINANT PROTEIN FOR DETECTION, RECOMBINANT PLASMIDS CONTAINING THE HDV AND THYRINTEINA ARNOBIA CYPOVIRUS ALPHA-HELIX (H1) GENES WERE SYNTHESIZED AND AMPLIFIED VIA POLYMERASE CHAIN REACTION (PCR). THE CHOSEN VECTOR WAS PFASTBAC™DUAL, CONTAINING PROMOTERS FOR THE EXPRESSION OF THE RECOMBINANT HDV + H1 PROTEIN AND CYPOVIRUS POLYHEDRIN. AFTER ENZYMATIC DIGESTION, THE FRAGMENTS WERE CLONED AND TRANSFORMED INTO ESCHERICHIA COLI DH10B, WITH CONFIRMATION BY PCR. THE COLONIES WERE SUBJECTED TO THE BAC-TO-BAC® PROCESS TO GENERATE

RECOMBINANT BACMIDS, WHICH WERE USED TO TRANSFECT SF9ET CELLS WITH CATIONIC LIPOSOMES, FOLLOWED BY INFECTION IN TN5B CELLS. FOR PROTEIN PURIFICATION, CELL LYSIS WAS PERFORMED BY SONICATION, AND THE PELLET WAS STORED FOR USE IN WESTERN BLOTTING TECHNIQUE TO CONFIRM AND PROCEED WITH DIAGNOSTIC KIT CONSTRUCTION. THE RESULTS OBTAINED SO FAR INDICATE THAT HDV PROTEIN EXPRESSION WAS SUCCESSFUL, WITH SATISFACTORY CONCENTRATION AND PURITY LEVELS, REQUIRING FURTHER CONFIRMATION OF EXPRESSION. THE SUCCESSFUL PURIFICATION OF THIS PROTEIN OPENS POSSIBILITIES FOR DEVELOPING VARIOUS SEROLOGICAL DIAGNOSTIC FORMATS FOR HDV DETECTION.

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF TST2 PEPTIDE ISOLATED FROM THE SCORPION TITYUS STIGMURUS IN VOLTAGE-GATED SODIUM CHANNELS NAV 1.3 AND NAV 1.7

Nathalia Cristina Silva Lago 1, Sarah Pinho Bezerra1, Clarissa Maria Alves Portacio Santos 1, Felipe Diego Medeiros de Sousa 1, Kelly Grace Magalhães1THE PEPTIDE TST2 WAS PURIFIED FROM THE VENOM OF TITYUS STIGMURUS, A SCORPION SPECIES PREDOMINANTLY FOUND IN THE NORTHEASTERN REGION OF BRAZIL, THROUGH THREE CHROMATOGRAPHIC METHODS USING REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC). NEXT, MALDI-TOF MASS SPECTROMETRY WAS PERFORMED TO CONFIRM THE MOLECULAR MASS, WHICH WAS FOUND TO BE [M+H]+ = 6984.74 DA, AND TO ASSESS THE PEPTIDE'S PURITY. THE ACTIVITY ON VOLTAGE-GATED SODIUM CHANNELS (NAV) OF SUBTYPES NAV1.3 AND NAV1.7 WAS EVALUATED USING THE PATCH-CLAMP TECHNIQUE, ASSESSING CURRENT TRACES, OPEN PROBABILITY DURING ACTIVATION AND INACTIVATION AND RECOVERY FROM INACTIVATION.

THE ROLE OF MELATONIN IN MODULATING MITOCHONDRIAL FUNCTION IN PANCREATIC CANCER CELLS (PANC-1)

NATHALIA CRISTINA SILVA LAGO, SARAH PINHO BEZERRA, CLARISSA MARIA ALVES PORTACIO SANTOS, FELIPE DIEGO MEDEIROS DE SOUSA, KELLY GRACE MAGALHÃES

ACCORDING TO THE WORLD HEALTH ORGANIZATION, CANCER IS THE SECOND LEADING CAUSE OF DEATH WORLDWIDE (PAHO & WHO, 2020). AMONG THE DEADLIEST CANCERS, PANCREATIC CANCER STANDS OUT, WITH PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) BEING THE MOST COMMON SUBTYPE. IN THIS CONTEXT, MELATONIN HAS EMERGED AS A PROMISING ADJUNCTIVE THERAPEUTIC APPROACH, DISPLAYING ANTITUMOR PROPERTIES AND INDUCING CELL DEATH IN VARIOUS CANCER TYPES (TALIB ET AL., 2021). IT HAS RECENTLY BEEN DEMONSTRATED THAT MELATONIN IS ALSO SYNTHESIZED IN CELLULAR MITOCHONDRIA, ACCOUNTING FOR UP TO APPROXIMATELY 95% OF TOTAL PRODUCTION (REITER ET AL., 2021). MELATONIN IS PRESENT IN HIGH CONCENTRATIONS IN THE MITOCHONDRIA OF CELLS IN HOMEOSTASIS; HOWEVER, IN CANCER CELLS, MITOCHONDRIAL MELATONIN PRODUCTION IS OFTEN DYSFUNCTIONAL (REITER, R. J., 2021). THUS, MITOCHONDRIA-TARGETED COMPOUNDS, SUCH AS MOLECULES THAT INCREASE ROS PRODUCTION, ARE PROMISING IN THE TREATMENT OF SEVERAL TYPES OF CANCER, INCLUDING PANCREATIC CANCER, BY SELECTIVELY AFFECTING MITOCHONDRIAL FUNCTION IN CANCER CELLS (GOSH ET AL., 2020). MELATONIN PLAYS A REGULATORY ROLE WITH THE POTENTIAL TO SIGNIFICANTLY ENHANCE THE CLINICAL TREATMENT OF CANCER PATIENTS, ACTING BOTH AS AN INDUCER OF REACTIVE OXYGEN SPECIES (ROS)—PROMOTING A TUMOR-TARGETED ATTACK PATHWAY—AND AS A POTENT ANTIOXIDANT, PROTECTING HEALTHY TISSUES FROM OXIDATIVE DAMAGE DURING AGGRESSIVE TREATMENTS (SÁNCHEZ-SÁNCHEZ, A. M. ET AL., 2011). THUS, IN THIS STUDY, WE DEMONSTRATE THE IMPACT OF MELATONIN ON MITOCHONDRIAL METABOLISM IN HUMAN PANCREATIC ADENOCARCINOMA CELLS (PANC-1), HIGHLIGHTING ITS ANTITUMOR EFFECT THROUGH THE REGULATION OF MITOCHONDRIAL FUNCTION BY MAINTAINING OXIDATIVE STRESS AND MITOCHONDRIAL DYNAMICS.

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE ISOLATED PEPTIDE TS2 FROM THE SCORPION TITYUS SERRULATUS ON VOLTAGE-GATED SODIUM CHANNEL NAV 1.7

MARCELLA ALARCON IZAIAS PITEL, ISRAEL FLOR SILVA DE ARAUJO, LUIS FELIPE MENEZES, ADOLFO CARLOS BARROS DE SOUZA, DIOGO V. TIBERY, FELIPE DE PINA CAVALCANTI, MANUELLA PAIVA BATISTA MARTINS, ISAÍAS PEREIRA DIAS FILHO, ELISABETH NOGUEIRA FERRONI

THE PROJECT CONSISTED ON THE PURIFICATION OF THE PEPTIDIC NEUROTOXIN TS2, EXTRACTED FROM THE VENOM OF THE SCORPION TITYUS SERRULATUS, USING REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC). THE TS2 TOXIN IS A CHAIN PEPTIDE OF 62 AMINO ACIDS AND IT HAS FOUR DISULFIDE BONDS AND ONE AMIDATION (UNIPROT P68410). THE PRESENCE OF DISULFIDE BONDS IN THE CHAIN INDICATES A HIGH SPECIFICITY TO VOLTAGE-GATED CHANNELS, THIS SPECIFICITY IS PARTICULARLY RELEVANT FOR NEUROPHARMACOLOGICAL STUDIES.TS2 IS A PEPTIDE NEUROTOXIN THAT HAS A HIGH IDENTITY WITH SODIUM VOLTAGE-GATED CHANNELS, AS 72% TS1 (BETA-TOXIN) AND 100% TST2 (BETA-TOXIN), THE CLASSIFICATION OF TOXIN THAT PRESENTS EFFECTS ON SODIUM VOLTAGE-GATED CHANNELS ARE TWO: BETA-TOXINS, OPENS THE CHANNEL IN MORE HYPERPOLARIZING DIRECTION, AND ALPHA-TOXINS INHIBITS/DELAYS THE RAPID INACTIVATION OF THE CHANNEL. IN THE FIRST PAPER DESCRIBING TS2, IT WAS CLASSIFIED AS A BETA-TOXIN, BECAUSE OF THE HIGH IDENTITY WITH TS1A (MANSUELLE ET AL. EM 1992). CURRENTLY TS2 IS CLASSIFIED AS AN ALPHA-TOXIN, DUE ITS ELECTROPHYSIOLOGICAL ACTIVITY, THE NEW CLASSIFICATION WAS MADE BY COLOGNA ET AL (2012). THE PURIFICATION PROCESS INVOLVED THREE CHROMATOGRAPHIC STEPS EMPLOYING DIFFERENT ACETONITRILE (ACN) GRADIENTS TO SEPARATE VENOM COMPONENTS. THE MOLECULAR MASS OF THE TOXIN WAS CONFIRMED USING MALDI-TOF MASS SPECTROMETRY, YIELDING A MONOISOTOPIC MASS OF 6985 DA FOR A SINGLE CHARGE. THE RESULTS CONFIRMED THE IDENTIFICATION OF THE TS2 NEUROPEPTIDE, ESSENTIAL FOR FUTURE ELECTROPHYSIOLOGICAL ASSAYS USING THE PATCH-CLAMP TECHNIQUE ON VOLTAGE-GATED SODIUM CHANNELS. THE NAV 1.7 IS A VOLTAGE-GATED SODIUM CHANNEL RELATED TO NOCICEPTION, THE PURIFIED TOXIN TS2 WAS TESTED IN THIS CHANNEL, WITH THE PATCH-CLAMP TECHNIQUE, AND IT ELECTROPHYSIOLOGICAL ASSAYS, HAS SHOWN IT ABILITY TO MODULATE THE CHANNEL, BY DELAYING THE RAPID INACTIVATION (ALPHA-TOXIN) AND ALSO BY CHANGING THE CHANNEL ACTIVATION (BETA-TOXIN).

OVEREXPRESSION OF THE TRANSCRIPTION FACTOR HAA1 TO ENHANCE KOMAGATAELLA PHAFFII RESISTANCE TO INHIBITORY COMPOUNDS

MARIANA JARDIM PEDROSA, LETÍCIA MARIA MALLMANN FERREIRA, BÁRBARA GOMES PAES, DIO-GO KEIJI NAKAI, LÍVIA TEIXEIRA DUARTE BRANDÃO, JOÃO RICARDO MOREIRA DE ALMEIDA

THE USE OF RENEWABLE FEEDSTOCKS, LIKE LIGNOCELLULOSIC BIOMASS, IS GROWING RAPIDLY IN INDUSTRIAL BIOTECHNOLOGY BECAUSE OF ITS USAGE IN BIOPROCESSES. THIS BIOMASS ORIGINATES, PRINCIPALLY, FROM AGROINDUSTRY RESIDUES AND CAN BE USED TO OBTAIN SUBSTANCES SUCH AS XYLONIC ACID. UNFORTUNATELY, ONE OF THE BIGGEST OBSTACLES FOR THE USAGE OF LIGNOCELLULOSIC BIOMASS IS THE MICROORGANISM RESPONSE TO INHIBITORY COMPOUNDS FOUND IN RENEWABLE BIOMASSES, AFTER THEY ARE PRE-TREATED USING HYDROLYSES, SUCH AS ACETIC ACID AND LIGNIN DERIVED AROMATIC COMPOUNDS. THOSE CAN INHIBIT OR EVEN COMPLETELY BLOCK THE CELL'S METABOLIC ACTIVITY. IN THE PAST, GENETICALLY ENGINEERED STRAINS OF SACCHAROMYCES CEREVISIAE HAVE SHOWN HIGH TOLERANCE TO LIGNOCELLULOSE-DERIVED INHIBITORS. THEREFORE, THE OBJECTIVE OF THIS WORK IS TO EVALUATE THE INHIBITOR TOLERANCE OF KOMAGATAELLA PHAFFII ENGINEERED FOR HAA1 OVEREXPRESSION COMPARING THESE GENETIC ENGINEERED STRAINS TO A WILD TYPE STRAIN. K. PHAFFII HAS BEEN EMPLOYED IN SEVERAL BIOPROCESSES AND GAINED ATTENTION FOR THE PRODUCTION OF BIO-BASED CHEMICALS AND THE TRANSCRIPTION FACTOR HAA1 WAS CHOSEN SINCE HIS ACTIVATION EXPRESS A SERIES OF CELLULAR RESPONSES AGAINST THOSE METABOLIC INHIBITIONS. THE EXPERIMENT RESULTS DEMONSTRATE THAT THE OVEREXPRESSION OF THE TRANSCRIPTION FACTOR HAA1 IMPROVES THE YEAST'S RESISTANCE TO ACETIC ACID. INTERESTINGLY, YEAST CULTIVATION ALLOWED INCREASED CONSUMPTION OF ACETIC ACID AND A RISE IN EXTRACELLULAR PH AND CELLULAR GROWTH. THEREFORE, THOSE GENETIC MODIFICATIONS IN K. PHAFFII PROVIDE A BETTER EFFICIENCY OF THIS MICROORGANISM IN CULTURES THAT CONTAINS INHIBITORY COMPOUNDS AND, CONSEQUENTLY, ALLOWS THE YEAST TO GROW ON LIGNOCELLULOSIC BIOMASSES.

EXPRESSÃO DE ANTICORPO NEUTRALIZANTE ANTI-ZIKV EM CÉLULAS DE MAMÍFEROS

MARIA CLARA MOURA PINHEIRO, IGOR CABRAL STUDART, RENATO KAYLAN ALVES DE OLIVEIRA FRANÇA, MARCELO DE MACEDO BRIGÍDO E ANDREA QUEIROZ MARANHÃO

INFECÇÕES POR FLAVIVIRUS, ESPECIALMENTE PELOS VÍRUS DA DENGUE, DA ZIKA E DA FEBRE AMARELA, POSSUEM GRANDE IMPACTO NO BRASIL. DEVIDO

AO ALTO NÚMERO DE CASOS E À COMPLEXIDADE DAS MANIFESTAÇÕES CLÍNICAS DE HEMORRAGIA, SÍNDROME NEUROLÓGICA E MICROCEFALIA CONGÊNITA, É CRUCIAL DESENVOLVER FORMAS DE PREVENÇÃO E DE ATENUAÇÃO DOS SINTOMAS. UMA ALTERNATIVA PROMISSORA É O DESENVOLVIMENTO DE ANTICORPOS NEUTRALIZANTES. ASSIM, ANTICORPOS MONOCLONAIS HUMANOS ESPECÍFICOS PARA O PEPTÍDEO DE FUSÃO DO ENVELOPE VIRAL, UM EPÍTOPO CONSERVADO ENTRE OS FLAVIVÍRUS, FORAM SELECIONADOS PELA TÉCNICA DE PHAGE DISPLAY. ESSE TRABALHO TEVE COMO OBJETIVO A EXPRESSÃO DESSES ANTICORPOS EM UM SISTEMA HETERÓLOGO DE CÉLULAS DE MAMÍFEROS NO FORMATO DE FVFC (SCFV FUSIONADO À FC DE IGG1). AS CÉLULAS CHO-K1 E EXPI293 FORAM TRANSFECTADAS COM PLASMÍDEOS CONTENDO A SEQUÊNCIA FVFC, IN TANDEM COM O GENE GFP (PROTEÍNA VERDE FLUORESCENTE) E UM GENE DE RESISTÊNCIA À PUROMICINA, INTERCALADO POR SEQUÊNCIAS IRES. A PUROMICINA FOI UTILIZADA COMO MARCADOR DE PRESSÃO SELETIVA E A EFICIÊNCIA DA TRANSFECÇÃO FOI ANALISADA PELA PORCENTAGEM DE CÉLULAS GFP POSITIVAS POR CITOMETRIA DE FLUXO. APÓS A EXPRESSÃO DA PROTEÍNA RECOMBINANTE, O SOBRENADANTE DE CULTURA FOI PURIFICADO POR CROMATOGRAFIA DE AFINIDADE EM UMA COLUNA DE PROTEÍNA A. AS PROTEÍNAS PURIFICADAS FORAM ANALISADAS POR SDS-PAGE E WESTERN BLOTTING. A ANÁLISE DA EXPRESSÃO DE GFP POR CITOMETRIA DE FLUXO NAS CÉLULAS UTILIZADAS APRESENTARAM PORCENTAGEM DE CÉLULAS GFP POSITIVAS DE APROXIMADAMENTE 40%. A PRODUÇÃO E CONFIRMAÇÃO DA PURIFICAÇÃO DO ANTICORPO AZ1P, REPRESENTADAS POR UMA BANDA DE APROXIMADAMENTE 55 KDA, FOI OBSERVADA EM SDS-PAGE E WESTERN BLOTTING. A CAPACIDADE DE EXPRESSÃO DO ANTICORPO AZ1P FOI DEMONSTRADA E OS RENDIMENTOS DAS CÉLULAS EXPI293 E CHO-K1 FORAM SIMILARES (0,48 MG/L E 0,41 MG/L, RESPECTIVAMENTE). A HABILIDADE DA NEUTRALIZAÇÃO DA INFECÇÃO PELO ZIKV SERÁ DETERMINADA POR ENSAIOS PRNT.

PURIFICAÇÃO E CARACTERIZAÇÃO ELETROFISIOLÓGICA DO PEPTÍDEO TF4 ISOLADO DO ESCORPIÃO TITYUS FASCIOLATUS NO CANAL DE SÓDIO DEPENDENTE DE VOLTAGEM NAV 1.2

MANUELLA PAIVA BATISTA MARTINS, FELIPE DE PINA CAVALCANTI, ISAÍAS PEREIRA DIAS FILHO, MARCELLA ALARCON IZAIAS PITEL, ADOLFO CARLOS BARROS DE SOUZA, DIOGO VIEIRA TIBERY, ISRAEL FLOR SILVA DE ARAUJO, LUIS FELIPE SANTOS MENEZES, ELISABETH NOGUEIRA FERRONI

IN THE ANIMAL KINGDOM, ARTHROPODS OF THE CLASS ARACHNIDA, SUCH AS SPIDERS AND SCORPIONS, ARE THE MAIN CONTRIBUTORS TO MOST HUMAN INCIDENTS CAUSED BY ANIMALS WORLDWIDE. SPECIES IN THE BUTHIDAE FAMILY, SUCH AS TITYUS FASCIOLATUS, ARE IMPORTANT FOR THE STUDY AND CHARACTERIZATION OF SCORPION TOXINS. CHARACTERIZATION OF SCORPION TOXINS. THE VENOM OF THESE ANIMALS COMPRISES A DIVERSE ARRAY OF BIOACTIVE COMPONENTS, WITH PEPTIDES BEING THE KEY ELEMENTS RESPONSIBLE FOR THE SYMPTOMS MOST COMMONLY OBSERVED IN SCORPION STING INCIDENTS. THESE PEPTIDES CAN BE CLASSIFIED AS NDBPS (NON-DISULFIDE BOND PEPTIDES) AND DBPS (DISULFIDE BOND PEPTIDES). DBP-TYPE PEPTIDES ARE REGARDED AS THE PRIMARY AGENTS OF THE NEUROTOXIC ACTIVITY IN SCORPION VENOM, ESPECIALLY THOSE THAT MODULATE NA+ AND K+ CHANNELS. SODIUM CHANNELS ARE TRANSMEMBRANE PROTEINS THAT FORM A PORE ALLOWING THE PASSAGE OF SODIUM IONS, AND THEY ARE FOUND IN EXCITABLE CELLS, SUCH AS MUSCLES AND NEURONS. THEY PLAY A CRUCIAL ROLE IN MEMBRANE POTENTIAL CHANGES NEAR THE THRESHOLD FOR FIRING, INITIATION AND PROPAGATION OF ACTION POTENTIALS, AND THE GENERATION OF PRE- AND POSTSYNAPTIC GRADED POTENTIALS. THIS STUDY PRESENTS THE PURIFICATION OF THE TF4 PEPTIDE, ISOLATED FROM THE VENOM OF THE SCORPION TITYUS FASCIOLATUS, ALONG WITH ELECTROPHYSIOLOGICAL ASSAYS PERFORMED ON HEK 293 (HUMAN EMBRYONIC KIDNEY 293) CELLS EXPRESSING VOLTAGE-GATED SODIUM CHANNELS NAV 1.2.

EVALUATION OF THE ROLE OF MELATONIN IN THE MODULATION OF LIPID DROPLETS IN PANCREATIC CANCER CELLS PANC-1

CLARISSA MARIA ALVES PORTACIO SANTOS, SARAH PINHO BEZERRA, NATHALIA CRISTINA LAGO, FERNANDA GOMES LAGO, KELLY GRACE MAGALHÃES

CANCER IS A DISEASE CAUSED BY CELLULAR ALTERATIONS IN WHICH CELLS PROLIFERATE UNCONTROLLABLY, ACQUIRE A COMPETITIVE ADVANTAGE OVER OTHER CELLS, AND OBTAIN THE ABILITY TO INHIBIT CELL DEATH PATHWAYS. CANCER IS THE SECOND LEADING CAUSE OF DEATH WORLDWIDE DUE TO THE COMPLEXITY OF DIAGNOSIS AND TREATMENT. IN THIS CONTEXT, PANCREATIC CANCER IS AMONG THE SIX MOST LETHAL CANCERS IN THE WORLD, WITH PANCREATIC DUCTAL ADENOCARCINOMA (PDA) BEING THE

MOST COMMON SUBTYPE IN THIS GLAND. POOR PROGNOSIS, EARLY INVASIVENESS, AND NONSPECIFIC SYMPTOMS CONTRIBUTE TO THE WORSENING OF THE DISEASE AND ITS HIGH MORTALITY RATES. IN ADDITION, PDA IS RESISTANT TO CONVENTIONAL THERAPIES DUE TO ITS IMMUNOSUPPRESSIVE MICROENVIRONMENT. IN THIS PERSPECTIVE, ONE OF THE WAYS THAT CANCER CELLS SUSTAIN HIGH PROLIFERATION RATES, ESCAPE TREATMENTS, AND WITHSTAND STRESS IS BY REPROGRAMMING THEIR LIPID METABOLISM. THIS REPROGRAMMING IS DIRECTLY RELATED TO THE UPTAKE AND SYNTHESIS OF LIPIDS FOR ENERGY PRODUCTION AND STORAGE IN ORGANELLES. LIPID DROPLET (LD) IS A DYNAMIC ORGANELLE AND IS INVOLVED IN LIPID STORAGE AND SEVERAL PROCESSES FOR CARCINOGENESIS, SUCH AS ACTIVATION, MIGRATION, PROLIFERATION, AND CELL DEATH, IN ADDITION TO BEING ABLE TO AID IN TREATMENT EVASION. THUS, THE INVESTIGATION OF LD IS ESSENTIAL FOR INHIBITING THE PROGRESSION OF SEVERAL CANCERS. FURTHERMORE, MELATONIN IS AN EMERGING MOLECULE AS A MODULATOR OF LIPID METABOLISM, WHICH IS ALSO KNOWN AS AN ADJUVANT IN CANCER TREATMENT. THEREFORE, IN THIS PROJECT, WE SOUGHT TO ANALYZE THE ROLE OF MELATONIN IN LIPID BODIES OF HUMAN PANCREATIC ADENOCARCINOMA CELLS OF THE PANC-1 CELL LINE. THROUGH QUANTITATIVE AND QUALITATIVE METHODOLOGIES, OUR RESULTS SHOWED A DOSE- AND TIME-DEPENDENT DECREASE IN THE QUANTITY AND SIZE OF LIPID BODIES STIMULATED WITH MELATONIN IN THE PANC-1 CELL LINE. WITH THESE RESULTS, WE HYPOTHESIZE AN ANTITUMOR POTENTIAL OF MELATONIN IN PANCREATIC CANCER RELATED TO THE REDUCTION OF LIPID DROPLETS BIOGENESIS.

DESENHO RACIONAL PARA REDUÇÃO GENÔMICA DE ORGANISMOS PROCARIOTOS

GUILHERME BARBOSA MIRANDA GRIJÓ, PEDRO FELIPE SOUSA QUEIROZ, MATHEUS DE CASTRO LEITÃO, SOPHIA GARCIA DE RESENDE, VICTORIA RAFAELA MUNIZ DOS SANTOS, GABRIELA TOGAWA, ROBERTO COITI TOGAWA, CÍNTIA MARQUES COELHO

SYNTHETIC BIOLOGY, A RELATIVELY NEW SCIENTIFIC FIELD, HAS MADE SIGNIFICANT CONTRIBUTIONS TO VARIOUS SECTORS INCLUDING INDUSTRY, HEALTH, AND AGRICULTURE. TO ENGINEER ORGANISMS WITH NOVEL FUNCTIONS, RESEARCHERS HAVE BEEN CHALLENGED TO INTRODUCE ENTIRE

METABOLIC PATHWAYS OR COMPLEX GENETIC CIRCUITS INTO HOST ORGANISMS. HOWEVER, HIGH-LEVEL PRODUCTION OF DESIRED BIOMOLECULES IS OFTEN HINDERED BY THE ENERGY DEMANDS OF THESE ORGANISMS. TO ADDRESS THIS, GENOME REDUCTION HAS EMERGED AS A PROMISING STRATEGY TO MINIMIZE ENERGY CONSUMPTION IN PRODUCTION STRAINS. TRADITIONAL METHODS FOR DEVELOPING MINIMAL GENOMES HAVE BEEN LABOR-INTENSIVE AND TIME-CONSUMING, RELYING ON THE DELETION OF INDIVIDUAL GENES AND DETAILED FUNCTIONAL ANALYSIS. MOREOVER RECENT STUDIES, SUCH AS THOSE INVOLVING THE REDUCTION OF CHROMOSOME III IN SACCHAROMYCES CEREVISIAE AND THE CONSTRUCTION OF A MINIMAL MYCOPLASMA MYCOIDES GENOME, HAVE REVEALED LIMITATIONS IN ACCESSING FUNCTIONAL SYNTHETIC GENOMIC REDUNDANCY. CONSEQUENTLY, INITIAL ATTEMPTS TO CREATE MINIMAL SYNTHETIC GENOMES HAVE BEEN UNSUCCESSFUL. THIS PROJECT AIMS TO DEVELOP A RATIONAL DESIGN FOR GENOME REDUCTION IN PROKARYOTES USING COMPARATIVE GENOMICS. BY COMPARING THE MINIMAL MYCOPLASMA MYCOIDES GENOME (JCVI SYN 3.0) TO A MODEL PROKARYOTE, ESCHERICHIA COLI, WE SEEK TO IDENTIFY NON-ESSENTIAL GENES THAT CAN BE DELETED. A PYTHON-BASED COMPUTATIONAL PROGRAM IS BEING DEVELOPED TO FACILITATE THIS PROCESS. THE PROGRAM LEVERAGES COMPARATIVE GENOMICS TO CLASSIFY GENES IN E. COLI AS ESSENTIAL, QUASI-ESSENTIAL, OR NON-ESSENTIAL BASED ON THE DELETED GENES IN JCVI SYN 3.0. THIS APPROACH WILL ENABLE THE CREATION OF MORE EFFICIENT PRODUCTION STRAINS FOR VARIOUS BIOTECHNOLOGICAL APPLICATIONS.

PURIFICATION, DYNAMIC LIGHT SCATTERING AND ANTINEOPLASTIC ACTIVITY OF ENTEROLOBIN

BRUNO MATHEUS FERREIRA DE PAULA, GUILHERME SANTA VIEIRA, JOÃO AUGUSTO PACHECO DA CRUZ, NUNO MANUEL DOMINGUES, WAGNER FONTES, MARCELO VALLE DE SOUSA, SÔNIA MARIA DE FREITAS, AISEL VALLE GARAY, FÁBIO MORATO DE OLIVEIRA, CARLOS ANDRÉ ORNELAS RICART

ENTEROLOBIN IS A 54,806 DA CYTOLYTIC PROTEIN EXTRACTED FROM THE SEEDS OF ENTEROLOBIUM CONTORTISILIQUUM, A TREE FOUND IN BRAZILIAN TROPICAL FORESTS AND IN THE CERRADO BIOME (HERINGER, 1978). HERE, ENTEROLOBIN WAS PURIFIED AS DESCRIBED BY SOUSA AND MORHY (1997). SILVER NITRATE STAINED SDS-PAGE DEMONSTRATED A HIGH PURITY OF ENTEROLOBIN AFTER THE PURIFICATION PROCESS. HEMOLYSIS ASSAYS WERE

USED TO EVALUATE ITS ACTIVITY AS PURIFICATION PROGRESSED AND SHOWED AN INCREASE IN ACTIVITY DURING PURIFICATION. ION EXCHANGE FPLC CHROMATOGRAPHY USING MONO-Q COLUMN, SHOWED DIFFERENT INTERACTIONS OF ENTEROLOBIN WITH THE COLUMN ACCORDING TO SAMPLE TEMPERATURE PROBABLY RELATED TO ITS OLIGOMERIZATION STATE. THE TENDENCY TO FORM OLIGOMERS RELATED TO THE FORMATION OF THE ENTEROLOBIN PROTEIN PORE WAS EVALUATED BY THE DYNAMIC LIGHT SCATTERING (DLS) METHOD USING THE ZETASIZER NANO ZS LASER ANALYZER AT 633NM WAVELENGTH. ENTEROLOBIN SOLUTION DISPLAYED DIFFERENT POPULATIONS OF AGGREGATES ACCORDING TO TEMPERATURE. CONCERNING ENTEROLOBIN ACTIVITY, IN THIS WORK WE EXPLORED THE ANTINEOPLASTIC PROPERTY THE PROTEIN ON TUMOR LINES OF CHRONIC MYELOID LEUKEMIA, ACUTE PROMYELOCYTIC LEUKEMIA, GLIOBLASTOMA, PROSTATE AND BREAST CANCER. CELL VIABILITY WAS EVALUATED BYMETHYLTHIAZOLETETRAZOLIUM (MTT) ASSAYS. OVERALL, THERE WAS A REDUCTION IN CELL VIABILITY IN ALL TUMOR LINES TREATED WITH ENTEROLOBIN. THE SET OF DATA GENERATED CORROBORATES THE BIOTECHNOLOGICAL POTENTIAL OF ENTEROLOBIN AND PROVIDED NEW DATA ON THE STRUCTURAL PROPERTIES OF THE PROTEIN.

CARACTERIZAÇÃO ELETROFISIOLÓGICA DO PEPTÍDEO TF3 ISOLADO DA PEÇONHA DE TITYUS FASCIOLATUS NOS CANAIS DE SÓDIO DEPENDENTES DE VOLTAGEM $NA_v1.2$, $NA_v1.6$ E $NA_v1.7$

ISAIAS PEREIRA DIAS FILHO, FELIPE DE PINA CAVALCANTI, MANUELLA PAIVA BATISTA MARTINS, MARCELLA ALARCON IZAIAS PITEL, ADOLFO CARLOS BARROS DE SOUZA, DIOGO VIEIRA TIBERY, ISRAEL FLOR SILVA DE ARAÚJO, LUIS FELIPE SANTOS MENEZES E ELISABETH FERRONI SCHWARTZ

THE STUDY OF SCORPION VENOM HAS REVEALED A RICH SOURCE OF BIOACTIVE PEPTIDES WITH THERAPEUTIC POTENTIAL, ESPECIALLY IN THE MODULATION OF ION CHANNELS. AMONG THE PEPTIDES, THERE ARE THOSE WHO HAVE DISULFIDE BRIDGES IN THEIR CONSTRUCTION, FEATURE THAT GRANTS THEM THE MODULATION CAPACITY ON VOLTAGE-GATED CHANNELS. THOSE NEUROTOXINS, AS THEY ARE KNOWN, CAN BE DIVIDED INTO TWO DISTINCT GROUPS ACCORDING TO THEIR STRUCTURE AND ACTION ON THOSE CHANNELS, THE A-TOXINS AND B-TOXINS. THIS WORK AIMED TO CHARACTERIZE THE ELECTROPHYSIOLOGICAL PROPERTIES OF THE

NEUROTOXIN TF3, AN A-TOXIN, EXTRACTED FROM THE VENOM OF THE SCORPION SPECIES TITYUS FASCIOLATUS, FOCUSING ON ITS INTERACTION WITH THREE DIFFERENT ISOFORMS OF VOLTAGE-GATED SODIUM CHANNELS (NA_v) FOUND ON MAMMALS' CELLS. USING PATCH-CLAMP TECHNIQUES IN CELLS EXPRESSING THESE CHANNELS, THE EFFECTS OF THE PEPTIDE ON THE ACTIVATION AND INACTIVATION KINETICS OF NA_v, AS WELL AS ITS IMPACT ON SODIUM CURRENTS, WERE INVESTIGATED. THE RESULTS OBTAINED PROVIDE NEW INFORMATION ON THE SPECIFICITY AND POSSIBLE MECHANISM OF ACTION OF THE TF3, CONTRIBUTING TO THE DEVELOPMENT OF POTENTIAL SODIUM CHANNEL MODULATING AGENTS, WITH APPLICATIONS IN THE TREATMENT OF NEUROLOGICAL DISORDERS AND ANALGESICS.

CHARACTERIZATION AND CYTOLYTIC ACTIVITY OF ENTEROLOBIN

GUILHERME SANTANA VIEIRA; BRUNO M. F. PAULA ; JOÃO AUGUSTO PACHECO DA CRUZ; BRUNA R. B. GOMES; RAQUEL TAKAYA; CARLOS J. C. SANTANA; CONSUELO M.R. LIMA, WAGNER FONTES; MARCELO V. DE SOUZA; FABIO M. OLIVEIRA E CARLOS A. O. RICART

ENTEROLOBIN, THE FIRST CYTOLYTIC PROTEIN DISCOVERED IN PLANTS, IS ISOLATED FROM THE SEEDS OF ENTEROLOBIUM CONTORTISILIQUUM. INITIALLY IDENTIFIED FOR ITS HEMOLYTIC ACTIVITY, IT CAN ALSO LYSE VARIOUS CELL TYPES AND EXHIBITS PRO-INFLAMMATORY AND INSECTICIDAL PROPERTIES. INTERESTINGLY, IT SELECTIVELY TARGETS LEUKOCYTES, LYSING MOST CELL TYPES WHILE PRESERVING T LYMPHOCYTES, SUGGESTING SIGNIFICANT BIOTECHNOLOGICAL POTENTIAL. THE PROTEIN'S PRIMARY SEQUENCE, DETERMINED BY EDMAN DEGRADATION, CONSISTS OF 485 AMINO ACIDS AND SHOWS HOMOLOGY WITH PORE-FORMING PROTEINS FROM THE AEROLYSIN FAMILY OF BACTERIA. PURIFICATION OF ENTEROLOBIN INVOLVED EXTRACTION WITH 0.9% SALINE, AMMONIUM SULFATE PRECIPITATION (FRACTION F1), ION EXCHANGE (DEAE-CELLULOSE, FRACTION F2), AND ANION EXCHANGE CHROMATOGRAPHY (MONO-Q, FRACTION F3). THE PURITY OF FRACTION F3 WAS CONFIRMED BY SDS-PAGE AND HEMOLYSIS ASSAYS. THE F1 AND F2 FRACTIONS EXHIBITED EXCEPTIONAL ANTIPROLIFERATIVE ACTIVITY AGAINST HUMAN LEUKEMIA, GLIOBLASTOMA, PROSTATE, AND BREAST CANCER CELL LINES. ADDITIONALLY, ENTEROLOBIN WAS TESTED IN HUMAN NEUTROPHILS, WHERE IT INDUCED REACTIVE OXYGEN SPECIES (ROS) PRODUCTION AND CELL ACTIVATION. ENTEROLOBIN WAS ALSO TESTED

AGAINST MURINE MACROPHAGES AND GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA, WITH NO EFFECT ON CELL VIABILITY OBSERVED. CURRENTLY, FURTHER ANTI-PROLIFERATIVE ASSAYS ARE ONGOING, USING MARKERS FOR CELL DEATH AND ASSAYS WITH NORMAL FIBROBLASTS. ITS OLIGOMERIZATION ACTIVITY IS BEING INVESTIGATED THROUGH SDS-PAGE AND BN-PAGE, WHILE PROTEOLYTIC DIGESTION FOLLOWED BY LC-MS/MS ANALYSIS IS BEING USED TO CONFIRM ITS PRIMARY STRUCTURE AND IDENTIFY POST-TRANSLATIONAL MODIFICATIONS. THESE STUDIES ARE BEING CONDUCTED IN PARALLEL WITH ANALYSES OF GUMIFERIN, A CYTOLYSIN ISOLATED FROM E. GUMMIFERUM SEEDS.

APPLICATION OF XYLANASES FROM CLOSTRIDIUM THERMOCELLUM AND THE INFLUENCE OF PHENOLIC COMPOUNDS ON THEIR KINETICS FOR BIOMASS CONVERSION

ARTUR CARVALHO STRANZ; ELIANE FERREIRA NORONHA

BRAZIL, A MAJOR PRODUCER OF AGRO-INDUSTRIAL WASTE, FACES CHALLENGES IN FULLY UTILIZING THESE RESIDUES IN SUSTAINABLE BIOTECHNOLOGICAL PROCESSES. LIGNOCELLULOLYTIC ENZYMES, SUCH AS ENDO-B-1,4-XYLANASES, ARE KEY FOR CONVERTING PLANT BIOMASS INTO VALUE-ADDED PRODUCTS. XYNA AND XYNZ XYLANASES FROM CLOSTRIDIUM THERMOCELLUM ARE NOTABLE FOR THEIR RESISTANCE TO PHENOLIC COMPOUNDS AND ABILITY TO FORM ENZYME COMPLEXES, ENHANCING HEMICELLULOSE DEGRADATION. THIS STUDY AIMED TO PURIFY AND KINETICALLY CHARACTERIZE THESE ENZYMES AND EVALUATE THEIR EFFICIENCY IN HYDROLYZING DIFFERENT BIOMASSES.

XYNZ WAS EXPRESSED IN KOMAGATAELLA PHAFFII GS115 PLASMID AND PARTIALLY PURIFIED USING ULTRAFILTRATION AND CHROMATOGRAPHY. XYNA WERE EXPRESSED IN E.COLI CLONES WITH THE PET21A-XYNA AND PURIFIED VIA DEAE CHROMATOGRAPHY. HYDROLYSIS OF BRACHIARIA BRIZANTHA WAS PERFORMED WITH SEMI-PURIFIED XYLANASES AND A COMMERCIAL ENZYME (PROVEG). KINETIC ANALYSIS WAS ALSO CONDUCTED WITH FERULIC ACID. BOTH ENZYMES COULD NOT BE FULLY PURIFIED, THE KINETIC ANALYSIS SHOWED XYNZ HAD A KM OF 2.331 AND A VMAX OF 0.002241, INDICATING GOOD AFFINITY BUT LOW CATALYTIC EFFICIENCY, POSSIBLY DUE

TO ITS CONFORMATION. SURPRISINGLY, FERULIC ACID ACTED AS AN ACTIVATOR, IMPROVING XYNZ'S CATALYTIC EFFICIENCY. BOTH ENZYMES WERE ABLE TO DEGRADE BRACHIARIA, WITH XYNA SHOWING SYNERGISTIC ACTION WITH PROVEG, ENHANCING DEGRADATION EFFICIENCY. DIFFERENCES IN ENZYME FORMS SUGGEST THE EXPRESSION SYSTEMS MAY NOT HAVE BEEN OPTIMAL, AFFECTING PURIFICATION AND CHARACTERIZATION. TO OPTIMIZE RESULTS, PRODUCING ENZYMES DIRECTLY IN CLOSTRIDIUM THERMOCELLUM OR DESIGNING STABLE VARIANTS MAY BE NECESSARY. DESPITE THESE CHALLENGES, BOTH ENZYMES SHOWED POTENTIAL FOR BIOMASS DEGRADATION, WITH XYNA PARTICULARLY EFFECTIVE IN ENHANCING COMMERCIAL ENZYME EFFICIENCY. FURTHER RESEARCH IS NEEDED TO UNDERSTAND XYNZ'S BEHAVIOR WITH FERULIC ACID AND EXPAND ITS APPLICATION IN BIOMASS CONVERSION.

EFFECTS OF OMEGA-3 (DHA) AND PALMITIC ACID (PA) ON LIPID DROPLETS IN HUMAN PANCREATIC CANCER CELLS (MIA PACA-2)

RAMON BUSON LIMA PAIVA; NICOLAS SÁ RODRIGUES MAIA; KELLY GRACE MAGALHÃES

PANCREATIC CARCINOMA IS A DISEASE RESULTING FROM THE ACCUMULATION OF GENETIC MUTATIONS THAT DISRUPT THE BODY'S HOMEOSTASIS. IN THE ELUCIDATION OF TUMOR MECHANISMS, THE LIPID DROPLET (LD) IS AN IMPORTANT ORGANELLE, MODULATING TUMOR RESISTANCE AND SURVIVAL, IN ADDITION TO BEING RESPONSIBLE FOR LIPID METABOLISM. IN THIS CONTEXT, DOCOSAHEXAENOIC (DHA) AND PALMITIC (PA) FATTY ACIDS ARE IDENTIFIED IN THE LITERATURE AS PROMISING MOLECULES, WITH POTENTIAL ADJUVANT OR DELETERIOUS EFFECTS IN THE TUMOR SETTING, CAPABLE OF MODULATING LIPID DROPLETS (LD). METHODS: THE MIA-PACA-2 CELL LINE WAS USED FOR CELL CULTURE, WITH CELLS STIMULATED OR NOT FOR DIFFERENT TIME INTERVALS USING A CULTURE MEDIUM CONTAINING VARIOUS CONCENTRATIONS OF DHA OR PA. FOR LD ANALYSIS, IMAGES WERE ACQUIRED UNDER AN OPTICAL MICROSCOPE USING SLIDES WITH CELLS STAINED WITH OIL RED-O, A DYE THAT IDENTIFIES NEUTRAL LIPIDS, AND HEMATOXYLIN, A MARKER OF ACIDIC CELLULAR SUBSTANCES. THE LIPID DROPLET (LD) BIOGENESIS ASSAY INVOLVES DATA COLLECTION THROUGH CONFOCAL FLUORESCENCE MICROSCOPY AND FLOW CYTOMETRY USING THE BODIPY PROBE (LIFE TECHNOLOGIES), ANALYZED

WITH FLOWJO 10 SOFTWARE, EMPLOYING T-TESTS AND ANOVA (P = 0.05). RESULTS: PA INDUCED LIPID DROPLET ACCUMULATION AT 48- AND 72-HOURS AT HIGHER CONCENTRATIONS. ADDITIONALLY, BOTH DHA AND PA INCREASED THE NUMBER OF LIPID DROPLE