ANAIS

XI Simpósio do Programa de Pós-graduação Biologia Molecular

15 e 16 de Dezembro, 2022

Anais do XI Simpósio do Programa de
Pós-graduação em Ciências Biológicas (Biologia Molecular)
da Universidade de Brasília

Annals of the XI Symposium of the
Graduate Program in Biological Sciences (Molecular Biology)
of the University of Brasilia

Brasília
Dezembro de 2022

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Sumário

Apresentação

O X Simpósio do Programa de Pós-Graduação em Ciências Biológicas (Biologia Molecular) (PPGBioMol) da Universidade de Brasília (UnB) aconteceu nos dias 15 e 16 de dezembro de 2022. O evento desse ano teve ainda um intuito comemorativo, uma vez que o PPGBioMol completou 50 anos em 2022. Além disso, foi um importante passo na retomada de eventos presenciais, que ficaram suspensos devido à pandemia da COVID-19. O simpósio foi aberto para estudantes e profissionais com produção acadêmica ou que tinham interesse em temas moleculares das ciências da vida, e contou com a participação de convidados e alunos, pesquisadores e professores.

Tendo como base que o PPGBioMol compreende inúmeras linhas de pesquisas, os resumos dos inscritos foram classificados em quatro grandes áreas: (01) Biologia celular, desenvolvimento e câncer; (02) Bioquímica, biofísica e biologia estrutural; (03) Genética, genômica e evolução; (04) Biologia celular e molecular de microrganismos. Os trabalhos foram apresentados oralmente ou no formato de pôster.

Presentation

The X Symposium of the Graduate Program in Biological Sciences (Molecular Biology) (PPGBioMol) of the University of Brasilia occurred on December 15th and 16th, 2022. This year's event also had a commemorative purpose, since PPGBioMol completed 50 years in 2022. In addition, it was an important step in the resumption of face-to-face events, which were suspended due to the COVID-19 pandemic. The symposium was open to students and professionals with academic production or who were interested in molecular themes of the life sciences, and had the participation of guests and students, researchers and professors.

Since the PPGBioMol comprises numerous research lines, the abstracts of subscribers were categorized in four major areas: (01) Cellular Biology, development, and cancer; (02) Biochemistry, biophysics, and, and structural biology; (03) Genetics, genomics, and Evolution; (04) Cellular and molecular biology of microorganisms. The research works were presented orally or in poster format.

Comissão organizadora

Professores do Departamento de Biologia Celular

Dra. Andréa Queiroz Maranhão (Coordenadora PPGBioMol)

Dr. Marcelo de Macedo Brígido

Pós-doutorandos e pesquisadores colaboradores

Dr. Aisel Valle Garay

Dra. Jacyelle Medeiros Silva

Dra. Paula Maria Quaglio Bellozi

Dr. Pedro Henrique Miranda Bürgel

Dra. Raquel das Neves Almeida

Doutorandas

Ma. Andreza Henrique Vidal

Ma. Izadora Cristina Moreira de Oliveira

Ma. Patrícia de Souza da Silva

Mestrandas

Luane Tomé de Paula Campos

Verônica Lucas Sequeira da Silva

Comissão avaliadora

Iniciação Científica

Ma. Andreza Henrique Vidal

Ma. Izadora Cristina Moreira de Oliveira

Biologia Celular, desenvolvimento e câncer

Dra. Jacyelle Medeiros Silva

Genética, genômica e evolução

Dr. Pedro Henrique Miranda Bürgel

Bioquímica, biofísica e biologia estrutural

Dra. Paula Maria Quaglio Bellozi

Dr. Raquel das Neves Almeida

Biologia celular e molecular de microrganismos

Dr. Aisel Valle Garay

Programação

15 de Dezembro – Quinta-feira	
09:00 – 09:20	**Mesa de abertura**
09:20 – 10:00	**O uso da Ressonância Magnética Nuclear no estudo de biomateriais** Dra. Maria Carolina Bezerra di Medeiros Leal Pesquisadora associada ao FoRC (Food Research Center – Centro de Inovação, Pesquisa e Difusão apoiado pela FAPESP)
10:30 – 11:10	**Uma visão geral da patogênese do Calazar: da infecção à morte** Prof Dr. Carlos Henrique Nery Costa Professor Titular da Universidade Federal do Piauí
11:10 – 11:50	**Innate Immunity, SARS-CoV-2, and COVID-19. What did we learn from this dangerous combination?** Prof Dr. Dario Zamboni Professor Titular da Universidade de São Paulo
14:00 – 17:30	**Sessão de Pôsteres**

16 de Dezembro – Sexta-feira	
09:00 – 09:30	**A Ciência e seus caminhos para a Inovação no Brasil** Prof Dra. Maria Sueli Felipe Professora e Pesquisadora da Universidade Católica de Brasília
09:30 – 10:30	**Mesa Redonda** **Spodoptera frugiperda fall armyworm virus and its biological control applications** Dr. Leonardo Assis AgBiTech **Diversificando o sistema de fomento à ciência: a experiência do Serrapilheira no apoio privado a jovens cientistas** Dra. Cristina Caldas Diretora de Ciência, Instituto Serrapilheira, Rio de Janeiro **Biologia Molecular e o Desenvolvimento de Bioprocessos** Prof Dr. João Ricardo M. de Almeida Pesquisador da Embrapa Agroenergia, Brasília.
11:00 – 12:00	**Homenagem aos 50 anos do PPGBioMol** Prof Dr Carlos Medicis Morel Professor Emérito da Universidade de Brasília

14:30 – 16:00	**Apresentações Orais dos Trabalhos Selecionados** **Iniciação Científica** Secretion from white adipose tissue lacking Dicer triggers mitochondrial dysfunction, cell death, and inflammation in insulinoma cancer cells Milena Nascimento Verdam de Araújo **Biologia Celular, desenvolvimento e câncer** THE ROLE OF MELATONIN ON THE MODULATION OF MITOCHONDRIAL FUNCTION, INFLAMMATION, AND CARCINOGENIC PARAMETERS OF HUMAN GASTRIC CANCER CELLS Sabrina Azevedo Machado **Bioquímica, biofísica e biologia estrutural** Molecular docking approaches of the interaction between ferulic acid and endoxylanase HXYN2 from Humicola grisea var. thermoidea Izadora Cristina Moreira de Oliveira **Genética, genômica e evolução** Obtaining resistent soybean cultivars from whitefly with interferindo RNA strategy Juliane Costa Cabral **Biologia celular e molecular de microrganismos** Analysis of metagenome-assembled genomes from a lignin-enriched microbial community Vitória Pinheiro Balestrini **Premiação dos melhores trabalhos no formato pôster** **Iniciação Científica** Effect of transient expression of proteins tyrosine phosphatase from Cotesia flavipes Bracovirus in insect cellsAndrews Alexander Fredéric Monvoisin Santos Fisch **Biologia Celular, desenvolvimento e câncer** THE ROLE OF OMEGA-3 IN WHITE AND BROWN ADIPOSE TISSUE MODULATION AND THE FUNCTION OF THESE TISSUES ON THE CARCINOGENIC PARAMETERS OF MELANOMA CANCER Débora Santos da Silva **Bioquímica, biofísica e biologia estrutural** Impact of sexual dimorphism on the mitochondrial function of LDL receptor knockout mice Nathasha Maria Corrêa Prado Lopes

	Genética, genômica e evolução Ship: Annotation-based program for identifying Genomic Safe Harbours in eukaryotic model organisms Matheus de Castro Leitão **Biologia celular e molecular de microrganismos** Discovery of novel Thogotovirus (family Orthomyxoviridae) and the functional analysis of their role in the emergence of baculovirus GP64 envelope protein Bruno Milhomem Pilati Rodrigues
16:00 – 16:30	**Encerramento**

Palestras

P1 - O uso da Ressonância Magnética Nuclear no estudo de biomateriais
Dra. Maria Carolina Bezerra di Medeiros Leal
Pesquisadora associada ao FoRC (Food Research Center – Centro de Inovação, Pesquisa e Difusão apoiado pela FAPESP)

P2 - Uma visão geral da patogênese do Calazar: da infecção à morte
Prof Dr. Carlos Henrique Nery Costa
Professor Titular da Universidade Federal do Piauí

P3 - Innate Immunity, SARS-CoV-2, and COVID-19. What did we learn from this dangerous combination?
Prof Dr. Dario Zamboni
Professor Titular da Universidade de São Paulo

Mesa Redonda

M1 - Spodoptera frugiperda fall armyworm virus and its biological control applications
Dr. Leonardo Assis
AgBiTech

M2 - Diversificando o sistema de fomento à ciência: a experiência do Serrapilheira no apoio privado a jovens cientistas
Dra. Cristina Caldas
Diretora de Ciência, Instituto Serrapilheira, Rio de Janeiro

M3 - Biologia Molecular e o Desenvolvimento de Bioprocessos
Prof Dr. João Ricardo M. de Almeida
Pesquisador da Embrapa Agroenergia, Brasília.

Apresentações orais

1. Iniciação Científica

Secretion from white adipose tissue lacking Dicer triggers mitochondrial dysfunction, cell death, and inflammation in insulinoma cancer cells
Milena Nascimento Verdam de Araújo

2. Biologia Celular, desenvolvimento e câncer (A1)

THE ROLE OF MELATONIN ON THE MODULATION OF MITOCHONDRIAL FUNCTION, INFLAMMATION, AND CARCINOGENIC PARAMETERS OF HUMAN GASTRIC CANCER CELLS
Sabrina Azevedo Machado

3. Bioquímica, biofísica e biologia estrutural (A2)

Molecular docking approaches of the interaction between ferulic acid and endoxylanase HXYN2 from Humicola grisea var. thermoidea
Izadora Cristina Moreira de Oliveira

4. Genética, genômica e evolução (A3)

Obtaining resistent soybean cultivars from whitefly with interferindo RNA strategy
Juliane Costa Cabral

5. Biologia celular e molecular de microorganismos (A4)

Analysis of metagenome-assembled genomes from a lignin-enriched microbial community
Vitória Pinheiro Balestrini

O conteúdo destas apresentações encontra-se na seção Resumos.

Resumos

1. Biologia Celular, desenvolvimento e câncer (A1)

A1.R1 - OMEGA-3 DHA TRIGGERS PYROPTOSIS CELL DEATH DURING MICROGLIAL ZIKV INFECTION: A POTENTIAL MECHANISM LINKED TO AN ANTIVIRAL RESPONSE

Fernanda Gomes Lago; Heloísa Antoniella Braz De Melo; Kelly Grace Magalhães
Universidade de Brasília, Departamento de Biologia celular, Brasília-DF, Brasil

Introduction: ZIKA virus (ZIKV) is a neurotropic arbovirus associated with the establishment of microglial neuroinflammation in the Central Nervous System. Although ZIKV-induced cell death in neuronal cells is well known,the occurrence of pyroptosis in microglial cells during ZIKV infection is still not well understood.Previously,our group have demonstrated the neuroprotective and antiviral effects of omega-3 docosahexaenoic acid (DHA) in neuronal cells. However, little is known about the role of DHA during ZIKV-induced pyroptosis in microglial cells.Thus, this project aimed to investigate the occurrence of pyroptosis during neuroinflammation induced by ZIKV in microglial cells and the role of DHA within this process. Methods:Human microglial cells were pre-treated with DHA and infected with ZIKV. Cell viability was assessed by LDH release assay. Cell death was assessed by Annexin-PI staining. Caspase-1 activation was evaluated by FAM-FLICA staining. Cytokines release (IL-1β,IL-6, and TNF-a) was evaluated by ELISA. Viral load was accessed by RT-qPCR. Results: Our data showed that ZIKV induced lytic cell death with LDH release of microglial cells,and, unexpectedly, DHA enhanced this event. Moreover, DHA treatment decreased ZIKV viral load.Beyond that,DHA treatment induced higher Caspase-1 activation and IL-1β secretion during ZIKV infection.In contrast, ZIKV infection alone did not impact these inflammatory parameters. When microglial cells were treated with recombinant IL-1β, we observed an augmented microglial proliferation and activation markers. Conclusion: Our data suggests that DHA-induced controlled microglial pyroptotic cell death during ZIKV infection, which could be an important factor linked to its antiviral function against this arbovirus, beyond the inflammatory role of this pathway.

A1.R2 - Secretion from white adipose tissue lacking Dicer triggers mitochondrial dysfunction, cell death, and inflammation in insulinoma cancer cells.

MILENA NASCIMENTO VERDAM DE ARAÚJO[1], GABRIELLA SIMÕES HEYN[1], GABRIEL PASQUARELLI-DO-NASCIMENTO[1], HELOISA ANTONIELLA BRAZ-DE-MELO[1], SABRINA AZEVEDO MACHADO[1], PAULA BELLOZI[1], ANDREZA FABRO DE BEM[1], SONIA NAIR BÁO[1], MARCELO ALVES DA SILVA MORI[2], KELLY GRACE MAGALHAES[1]
[1]Laboratory of Immunology and Inflammation, Department of Cell Biology, University of Brasilia, Brasilia, DF, Brazil. [2]Laboratory of Aging Biology, University of Campinas, Campinas, SP, Brazil.

The adipose tissue (AT) is a complex and highly plastic endocrine organ with a heterogeneous composition, including immune cells. A growing number of studies have been placing ATs as key players in many physiological processes. It regulates metabolic homeostasis, inflammation, and immune response by secreting many several factors, as well as exosomal miRNAs, known as post-transcriptional silencers that may act as tumor suppressors or oncogenes. AT can benefit tumoral cell growth, sustaining their high demand to proliferate and spread. Herein, we aimed to analyze the carcinogenic and inflammatory effects of secretion products of AT in the mouse insulinoma cells, and whether the absence of Dicer in the AT, a crucial endonuclease for miRNAs biogenesis, could trigger a different effect on insulinoma survival and inflammation. MIN6 cells were stimulated with the secretion derived

from Brown and White AT from C57BL/6 adipose-specific Dicer-KO (Adicer) or C57BL/6 wild-type (WT) mice. After treatment, cell death, proliferation, mitochondrial respiration, and inflammatory parameters were evaluated. We found no differences between BAT and WAT stimuli in insulinoma viability, proliferation, and survival. However, the BAT stimulus induced a greater secretion of pro-inflammatory cytokines. Secretion from BAT-Adicer also promoted lytic cell death, DNA fragmentation, and decreased proliferation in insulinoma cells, but no effects were observed in inflammation, oxidative stress, and mitochondrial respiration. Secretion from WAT-Adicer promoted cell death, oxidative stress, inflammation, and triggered disturbances in mitochondrial function of MIN6 cells. Our data suggest that secretion products from WAT lacking miRNAs have the ability to promote mitochondrial dysfunction, exacerbated oxygen consumption, cell death, increased oxidative stress, and inflammatory mediators generation in insulinoma cancer cells which could be an important pharmacological target for anti-tumor therapies.

A1.R3 - Effects of omega-3 (DHA) on carcinogenic parameters of melanoma cells in vitro

Ramon Buson Lima Paiva; Débora Santos da Silva; Luana Borges Baptista; Heloísa Antoniella Braz De Melo; Kelly Grace Magalhães

Universidade de Brasília, Departamento de Biologia celular, Laboratório de imunologia e inflamação (LIMI), Brasília-DF, Brasil

INTRODUCTION: Ômega-3 is an essential fatty acid acquired from fish and plant oils. Among other ômega-3 fatty acids, docosahexaenoic acid (DHA) has been studied to prevent and treat several diseases, including cancer. Melanoma is a skin cancer derived from melanocytes, the cells that produce the pigment melanin. This cancer is a highly aggressive primary cutaneous malignancy and is responsible for a majority of skin cancer–related deaths. Considering this, the present project aimed to analyze the possible effects of ômega-3 stimulus on B16F10 cells, derived from murine melanoma. METHODS: B16F10 cells were stimulated or not with DHA at different times and concentrations. Mitochondrial viability was assessed by MTT assay and analyzed by spectrophotometry. Cell proliferation was evaluated by CFSE staining and analyzed by flow cytometry. Membrane pore formation was assessed by propidium iodide uptake and analyzed by fluorescence spectrophotometry. Lipid droplet biogenesis was analyzed by BODIPY staining and analyzed by flow cytometry. RESULTS: Melanoma cells treatment with DHA triggered cell viability loss in a time and dose-dependent manner. DHA at 50µM induced membrane pore formation, indicating an occurrence of DHA-induced lithic cell death in those melanoma cells in vitro. In addition, DHA was able to reduce melanoma cell proliferation at 50 µM concentration. None of the concentrations had a significant effect on lipid droplet biogenesis. CONCLUSION: Our data support the antineoplastic potential of DHA. Our results suggest that DHA can reduce the mitochondrial viability of melanoma cells, by the induction of a type of lithic cell death that still need to be clarified. Thus, this project shows novel effects of docosahexaenoic acid, DHA, on murine melanoma cells, supporting evidence on the potential adjuvant antitumor therapy of the ômega-3 DHA

A1.R4 - THE ROLE OF OMEGA-3 IN WHITE AND BROWN ADIPOSE TISSUE MODULATION AND THE FUNCTION OF THESE TISSUES ON THE CARCINOGENIC PARAMETERS OF MELANOMA CANCER

DÉBORA SANTOS DA SILVA[1], HELOÍSA ANTONIELLA BRAZ-DE-MELO[1], LUANA BORGES BAPTISTA[1], KELLY GRACE MAGALHÃES[1]

[1]Laboratory of Immunology and Inflammation, Department of Cell Biology, University of Brasília, Brasília, DF, Brazil

The n-3 long-chain polyunsaturated fatty acids (n-3 PUFAs) such as docosahexaenoic (DHA) have protective mechanisms against the establishment of metabolic syndromes, such as obesity and cancer. DHA not only has

important effects on adipose tissue, but also has the ability to induce pyroptosis cell death in tumor cells. However, the role of DHA in the crosstalk between melanoma cancer and adipose tissue is poorly known. In this way, this work aimed to investigate the role of ômega-3 in adipose tissue modulation and the function of these tissues on the carcinogenic parameters of melanoma, as well as the induction of pyroptosis. Mice (C57/BL6) were supplemented or not with DHA at a concentration of 1g/kg for 30 days. After this period, serum, peritoneal lavage, adipose tissues, liver, and spleen were analyzed. In addition, secretion products of adipose tissue from these DHA supplemented mice were isolated and used to stimulate melanoma cell line B16F10. Carcinogenic parameters such as cell viability, cell death, and cytokine quantification were evaluated. Moreover, B16F10 and MeWo were stimulated with DHA (12.5µM, 25µM, 50µM and 100µM) for 48h in vitro. LDH secretion and caspase-1 activation were analyzed. Our data demonstrated that DHA reduced the weight of adipose tissues. Peritoneal lavage cells from supplemented animals had increased LD biogenesis but reduced ROS formation. In addition, the stimulation of B16F10 with the secretion products of BAT from supplement animals led to a decrease in cell viability as well as increased cell death and reduced IL-6 secretion. Furthermore, the 25µM concentration induced LDH release and caspase-1 activation. Thus, this work demonstrated the potential of omega-3 supplementation to modulate adipose tissues immunological profile and function, and also suggest the ability of DHA to induce pyroptosis in the MeWo cell line. This study generates new perspectives for the use of omega-3 as adjuvants in the treatment of melanoma.

A1.R5 Production of a cancer marker protein using the Cell-free transcription-translation technique

Hermerson Sousa Maia; Lilian Hasegawa Florentino; Rayane Nunes Lima; Valquíria Alice Michalczechen; Elíbio Leopoldo Rech Filho
Universidade de Brasília (UnB); Embrapa Recursos Genéticos e Biotecnologia (CENARGEN)

Currently, cancer represents one of the causes of higher mortality worldwide and conventional therapy is invasive and has serious side effects. Immunotherapy is an alternative that aims at a more efficient and less toxic treatment. Synthetic biology made possible the development of techniques that allow the production of proteins in vitro, among them, the TXTL, a tool that allows the transcription and translation of a fragment of DNA. The GAGE family represents a set of 16 genes located on the X chromosome, these genes encode cancer and testicular antigens and their expression occurs only in germ cells, their high expression occurs in several types of cancer and because of this, these antigens are studied to diagnosis and candidates for immunotherapy. Our methodology starts with the synthesis of the Phis2008p vector with the GAGE-1 gene sequence for its expression in prokaryotes. The vector was later cloned into Escherichia coli to obtain DNA. The TXTL reactions occurred in specific parameters with 30nM of DNA, at 29° C for 24 hours, with a final volume of 50 uL. Reactions were analyzed on SDS-page and WesternBlot. Protein purification occurred through affinity chromatography using imidazole. ELIZA assays to confirm antigen specificity against ANTI-GAGE1 were performed. An expression kinetics of the protein fused to GFP was performed, showing a detection in 2 hours, remaining constant in up to 48 hours of incubation. In vitro cytotoxicity assays through MTT were performed with Hela and Hek293T cells using different concentrations of the purified protein. We report minimal cytotoxicity with 5uM and maximum with 30uM, and 30uM for Hela and Hek293T, respectively. Qualitative cytotoxicity assays using DAPI as a cell viability marker were also performed. Our results reinforce the immunotherapeutic potential of cancer/testis antigens (CT).

A1.R6 THE ROLE OF MELATONIN ON THE MODULATION OF MITOCHONDRIAL FUNCTION, INFLAMMATION, AND CARCINOGENIC PARAMETERS OF HUMAN GASTRIC CANCER CELLS

SABRINA AZEVEDO MACHADO; JÚLIA PERIN MANCHINE; PAULA MARIA QUAGLIO BELLOZI; ANDREZA FABRO DE BEM; KELLY GRACE MAGALHÃES.
Universidade de Brasília, Departamento de Biologia Celular, Brasília-DF, Brasil

Introduction: Melatonin is a pleiotropic molecule 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 this molecule can modulate cell growth. 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 the gastric cancer cell line (AGS). 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. The cell death profile was assessed by annexin-V/propidium iodide (PI). The enzyme lactate dehydrogenase (LDH) release was evaluated by the CyQUANTTM kit. Cell proliferation was assessed by the CFSE probe staining. The cell cycle was assessed by PI probe staining. Oxidative stress was assessed by DCF-DA staining. 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 increased ROS production. In addition, these concentrations significantly increased apoptotic death compared to unstimulated cells. On the other hand, a possible inhibition of the inflammatory death pyroptosis was described, once melatonin reduced LDH release and did not trigger pore formation. Conclusion: Our data showed that melatonin at the higher concentration was able to promote an antitumor effect by reducing mitochondrial viability, increasing cell death, and reducing oxidative phosphorylation in AGS gastric cancer cells. Importantly, our results highlight that the melatonin can promote the inhibition of pyroptosis in these cells, which could be crucial in the application of melatonin in therapeutic approaches.

A1.R7 The role of Melatonin in pancreatic cancer cells: modulation of carcinogenic parameters and cell death by pyroptosis

Sarah Pinho Bezerra[1], Kelly Grace Magalhães[1]
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Pancreatic cancer is one of the most lethal types of cancer, with a less survival rate. To date, no effective treatment options in advanced pancreatic cancer. Compared with other types of cancer, pancreatic cancer is resistant to conventional therapy and has an immunosuppressive tumor microenvironment. The melatonin, a natural indoleamine synthesized from tryptophan, is emerging as a potential tool for cancer treatment. The melatonin alleviates the side effects of chemotherapy and radiotherapy and has demonstrated anti-proliferative effects in different types of cancer, including pancreatic cancer. It has been well described that melatonin induces cell death by apoptosis in pancreatic cancer cell lines, but little is known about other cell death pathways in this context. The objective of this study is to characterize the effects of melatonin on the modulation of carcinogenic parameters as well as to investigate whether melatonin induces death by pyroptosis in pancreatic adenocarcinoma cells. Human pancreatic ductal adenocarcinoma cells (PANC-1) were cultured and stimulated with different concentrations of melatonin: 0.625mM, 1.25mM, 2.5mM, 3.75mM and 5mM for 24h, 48h and 72h. A mitochondrial cell viability assay was performed using MTT. To analyze the modulation of the cell cycle, the assay with propidium iodide was performed. Complementary analysis will also be carried out, such as: analysis of the profile of cell death, cell proliferation, LDH release, reactive species and cytokine dosage. Our data have shown that melatonin induces cytotoxicity in 24h from a concentration of 2.5mM. There was modulation of the cell cycle and an increase in DNA fragmentation from a concentration of 2.5mM in 48h.This work demonstrates the potential antitumor effect of melatonin in vitro and opens space for new applications in the treatment of pancreatic cancer.

A1.R8 Evaluation of immune system activation through methylene blue associated with nanostructures for breast cancer in vitro

Ana Luísa G. Silva[1]*, Leonardo G. Paterno[2], Cleber L. Filomeno[2] and Sônia N. Báo[1]

[1]Department of Cell Biology, Institute of Biological Sciences, University of Brasília, Brasília/DF – Brazil. [2]Laboratory of Research on Polymers and Nanomaterials, Chemistry Institute, University of Brasília, Brasília/DF – Brazil.

The role of immune system in cancer treatment has promote the development of numberless works demonstrating the important cellular characteristics that can be used for its therapy, such as the activation of dendritic cells and CD8+ T lymphocytes. One of the treatments that has gained importance in cancer area is the nanobiotechnology, the creation of materials at the nanometric scale directed for biology. Nanomaterials are earning prominence for early diagnosis and targeted drug delivery, in addition to being able to promote an immune response based on the activation of dendritic cells. Previous studies demonstrate efficacy of the use of metallic nanoparticles associated with methylene blue [MAGCIT-MB] for the treatment of breast cancer cell lines. Thus, the present work included the application of MAGCIT-MB to promote and evaluation of anti-tumor immune responses for the treatment of breast cancer in vitro. As MAGCIT-MB exhibited a hydrodynamic diameter of 60.93 nm with a polydispersion index of 0.199 and zeta potential of -20.9 mV. Wound healing assay exhibited that the nanoparticles interfere on the tumor cell migration. By flow cytometry, the maturation of dendritic cells was analyzed after treatment with the supernatant of treated tumor cells. The results demonstrate that, after stimulation, dendritic cells exhibited changes in cell morphology, acquiring an adherent fusiform shape with cytoplasmic extensions and an increase on the expression of CD80, CD86 and CD11c on their surface. To elucidate the cytolocalization and the process correlated to the cell death, confocal microscopy was realized. The results suggests that the nanoparticle was collocated with mitochondria.

A1.R9 Characterization of voltage-gated ion channel modulation by injectable and volatile general anesthetics using the patch clamp cell electrophysiology technique.

Diogo Vieira Tibery, Elisabeth Nogueira Ferroni.
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In 1846, the efficiency of general anesthesia with diethyl ether in surgical procedures was demonstrated for the first time, a process that has revolutionized medicine. Over the decades, new compounds have been described, but the exact molecular mechanism for inducing therapeutic unconsciousness, muscle relaxation and antinoception are not elucidated. The difficulty in elucidating the mechanism is due to the diversity of molecular targets described and physicochemical characteristics of anesthetics, which are often small molecules with hydrophobic characteristics. The ability to disrupt cell membranes, due to lipophilic properties of anesthetics, was for many decades believed to be the mechanism of action, however direct interaction with membrane proteins was characterized in ligand-gated channels (GABAA, NMDA) and voltage-gated channels (KV, NaV, CaV, K2P, Kca, HCN). This work focuses on characterizing the activity of volatile and injectable general anesthetics at clinical concentrations in ion channels using the in vitro patch clamp cell electrophysiology technique. The hypnotic/sedative compound trichloroethanol (TCE) showed preferential inhibition of voltage-gated potassium channels (Kv) in SH-SY5Y cells. In HEK293T-Kv1.2 and L929-Kv1.3 at 5 mM induces an inhibition of approximately 10% of the current amplitude and a EC50 of 26.68 mM in Kv1.3 was characterized. Among the subtypes tested with TCE at 5 mM, the Kv3.1 channel showed the highest current amplitude inhibition rate (±20%) and delay the opening kinetics of the channel, increasing the time to reach the maximum conductance. The injectable compound propofol at a concentration of 30 uM induces 5-10% inhibition of voltage-gated sodium channels (NaV1.3, Nav1.4 and NaV1.6), with changes in the availability of channels in more negative potentials (Δ 10-15 mV) and delay in channel recovery after inactivation.

A1.R10 ANALYSING THE INFLUENCE OF INTERMITTENT FASTING AND CAFETERIA DIET ON BREAST CANCER PROGRESSION: THE ROLE OF ADIPOSE TISSUE

GABRIEL PASQUARELLI DO NASCIMENTO[1]*; HELOÍSA ANTONIELLA BRAZ DE MELLO[1], GABRIEL RIBEIRO FARIAS[1], IGOR DE OLIVEIRA SANTOS[1], SABRINA AZEVEDO MACHADO[1], LAURA COX[2], RAFAEL MACHADO REZENDE[2], KELLY GRACE MAGALHÃES[1]

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Breast malignancies are the most common and lethal tumors among the female population. Triple-negative breast cancer (TNBC) associates with neoplasm augmented aggressiveness and metastasis. The evolution of this disease is influenced by the individual lifestyle, including the diet. The increasing consumption of cafeteria diet (CAF) associates with the alarming overweight and obesity statistics, phenotypes characterized by metabolic syndrome, inflammation, and adipose tissue (AT) dysfunction. In contrast, intermittent fasting (IF), an efficient obesity therapy, correlates with metabolic efficiency, diminished inflammatory processes, and AT adequate activity. Although the impact of dietary patterns on breast cancer is well documented, there is scarce information regarding the effects of TNBC on adipose depots and about the impact of AT modulated by diets on this tumor type. In the present study, we evaluated the influence of intermittent fasting and a cafeteria diet on triple-negative breast cancer progression and investigated the role of AT in this context. We used eight weeks female BALB/c mice to compare the effects of 24 h-period IF and CAF ad libitum on the progression of 4T1-mediated TNBC and showed that the latter feeding pattern coped with tumors with increased mass and size and correlated with systemic inflammatory processes, including in the brown AT (BAT). Considering that 4T1- impacted animals consuming cafeteria items show BAT inflammatory processes, we sought to investigate the effects of molecules secreted by ATs on 4T1 cells in vitro. We informed that molecules secreted by BAT presented higher cytotoxic effects on 4T1 cells compared to white AT (WAT), augmenting mainly apoptosis in these neoplastic cells. We described that this effect was enhanced in mice submitted to IF and diminished in rodents in CAF. Our data suggest that molecules secreted by BAT of animals submitted to IF may present antitumoral properties by affecting 4T1 cell death profile.

A1.R11 CYTOTOXICITY ASSAY OF LIPID NANOPARTICLES ASSOCIATED WITH DOCETAXEL IN GASTRIC ADENOCARCINOMA

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Stomach cancer is the most common neoplasm that affects the digestive system and can result from multiple factors, usually caused by interactions between the environment and genetic factors. It is the fourth most common neoplasm in men and the sixth most common among women; being the third the number of mortalities, accounting for about 8.8% of the total cancer deaths in Brazil. Docetaxel (DTX), one of the most used drugs for the treatment of adenocarcinomas, has a mechanism of action in inhibiting mitosis and cell division, which can cause hypersensitivity, nephrotoxicity, fluid retention and neutropenia. However, their encapsulation in solid lipid nanoparticles (SLN) may reduce these problems, improving their effectiveness and carrying out the drug directly to interact with tumor-specific cell. This work objective to evaluate the cytotoxic effect of the formulation of solid lipid nanoparticles (SLN) associated with DTX in gastric adenocarcinoma (AGS) in vitro. The cytotoxic action on the AGS was estimating with four treatment groups were performed: (1) SLN-DTX formulation containing 1 mg/mL of Docetaxel; (2) SLN; (3) Docetaxel and (4) Absolute ethanol. Cell viability test, morphology and cytoskeleton analysis, colony formation assay, cell death, cell cycle, protein expression, cytokine and cell proliferation assays were performed. With the results obtained through the MTT assay, it was possible to observe that SLN-DTX have cytotoxic activity from 10 ng/mL and DTX from 10 µg/mL in 24 hours; after 48 hours there is a reduction of more than 50% in both treatments, suggesting a dose and time-dependent effect. Through the morphology tests, it was possible to notice that the cells increased their volume and decreased the cytoplasmic projections after the treatment. The SLN-DTX and DTX induced damage to the microtubules resulting in a stop of the cell cycle in G2/M resulting in 73.5% and 66.5% of death by apoptosis, respectively. The SLN and Ethanol did not present significant toxicity in the AGS tumor line in any of the tests, behaving similarly to the untreated control. Therefore, with the results of this work, it was possible to conclude that the association of DTX with SLN proved to be efficient, presenting cytotoxic action in gastric adenocarcinoma cells, favoring the use of this formulation in the administration of medications. The

expectation is that the formulation developed should be efficient in promoting the reduction of the viability of stomach tumors in vivo studies of gastric cancer.

A1.R12 NANOSTRUCTURED DOXORUBICIN PREVENTS BREAST CANCER RELATED BONE LOSS

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Cancer stands out as a target of innovation and research, with a goal of reducing side effects, preventing metastases, better prognosis and survival rates, and improving the patient's quality of life. Recent approaches include treatments such as chemotherapies, surgeries, radiotherapies, immunotherapies, and hormone therapies. Chemotherapy remains a key part of the treatment of breast cancer, where its adverse effects reduce the patient's quality of life by promoting bone loss, heart damage, generalized inflammation, and reduced survival. Doxorubicin is a drug widely used as chemotherapy, and in breast cancer, this drug increases inflammation, promoting the progression of bone and lung metastases, which are responsible for high mortality, leading to a paradoxical treatment, where the antibiotic drug can promote the growth and generation of new tumors, due to the imbalance in bone metabolism, promoting bone resorption, releasing growth factors and cytokines that modulate the metabolism resulting in a context of bone loss capable of inducing tumor growth. The association of doxorubicin to a nanostructure guarantees chemotherapy advantages, delivering towards the tumor, promoting specific action, reducing side effects, increasing the effect of the drug by reducing its contact surface, and enhancing its biodistribution. With drug delivery, bone is protected, preventing bone loss and breast cancer progression. A solid lipid nanoparticle containing Doxorubicin was formulated, it is stable, with dimensions for viable biodistribution and drug delivery. Through in vivo experimentation, it is possible to observe the reduction in tumor progression of lung metastasis, the lower mortality rate, and drug concentration in organs of interest for the treatment of breast cancer. As partial results computed tomography and scanning electron microscopy indicate the prevention of bone loss in tumor-bearing mice treated with nanostructured doxorubicin.

A1.R13 Transcriptome analysis of breast cancer cell lines exposed to CrotAMP14 with potential antitumor activity

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The discovery of several tumor suppressor genes and oncogenes in the last two decades has allowed advances in cancer treatment. However, cancer remains a leading cause of death worldwide. Cancer is a term used to bring together in a single group more than one hundred types of diseases that share characteristics such as disordered cell growth, capacity to invade and spread to other organs and tissues. Breast cancer is the most common cancer type in women, with an estimated 1.3 million cases annually. Therapies conventionally used for breast cancer include surgical removal, radiotherapy, and chemotherapy. However, these treatment modalities are harmful to healthy cells, causing undesirable side effects. Therefore, the seeking for alternative treatments that can reduce side effects is of extreme clinical importance. Antimicrobial peptides (AMPs) may represent an alternative that can be used alone or combined with other drugs already used in breast cancer treatment. AMPs are small molecules

(5 up to 100 aa) with a broad spectrum of targets. In addition to eliminating neoplastic cells, binding to their plasma membrane, and forming pores alongside the membrane, AMPs also interact with intracellular targets, changing the gene expression profile of these cells. The RNA-seq transcriptome analysis of cells exposed to peptides with potential antitumor activity is a method that allows us to identify genes and/or gene networks that may be related to the emergence, maintenance, proliferation, and invasion of cancer. Therefore, the present work aims to evaluate the transcriptome of breast cancer strains exposed to peptides (with an antitumor potential) to find possible molecular markers related to the development of this neoplasm.

A1.R14 INDUCTION OF IMMUNOGENIC CELL DEATH BY VACCINATION IN AN EXPERIMENTAL MURINE MODEL OF MELANOMA AFTER THERAPEUTIC VACCINATION PROTOCOLS

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Melanoma treatments are usually systemic and present innumerable side effects. The immune system has tools for eliminating tumor cells before tumor tissue is formed. Still, many tumors manage to repress the immune system, allowing cancer to grow and preventing the immune system from exerting its role. Mushrooms are sources of beta-glucans that present biologically active properties, mainly immunoregulatory. In addition to activating the immune, the early presentation of tumor antigens can cause the maturation of the response against the tumor. It can improve the body response against a future neoplasm. The use of therapeutic vaccines has been gaining ground with its use in the treatment of malignant tumors resistant to drugs or therapies currently available. Prophylactic vaccines have been known for a long time and act by preparing the immune system for the encounter with the antigen or organism used in the vaccination, aiming for a more effective and faster treatment of pathogens. In vitro assays were performed with B16 (melanoma-derived cells) culture for 24 hours with chemotherapeutic agents or mushroom fractions at different concentrations. After 24 hours the supernatant was collected and used to determine the concentration of ATP and HMGB1 to prove immunogenic death. Mammal cells were added with treatment for more 24 hours to access cytokine production. To prove the same theory of cell death, fluorescence microscopy was done to show translocation of calreticulin to the membrane which along with the increase in ATP and HMGB1 concentration in the supernatant are known markers of immunogenic cell death. For the In Vivo experiments, the B16 cells were cultured for 24 hours with different treatments and administered as subcutaneous vaccines for 3 consecutive weeks prior or after tumor induction to access anti tumoral response. In vitro results show that when treated with doxorubicin, B16 cells experienced an immunogenic death. The treated B16 cells co-cultured with M1/M2 macrophages and dendritic cells induced a cytokine production modulation along with gene modulations. In vivo vaccination of C57bl6 mice before or after tumor induction shows different results depending on the treatment b16 cells received. The treatment that causes immunogenic death plays a more interesting role in tumor prevention through vaccination. Based on the results, immunogenic death is an important step in activating the immune response and can be used in cases of melanoma.

A1.R15 27-hydroxycholesterol modulates brain's acetylcholinesterase homeostasis: a preclinical evidence

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Alterations in cholesterol metabolism within the brain have a significant role in Alzheimer's Disease (AD) pathophysiology. Previous studies demonstrated that Swiss mice fed a high cholesterol diet displayed short-term spatial memory impairment, and it was correlated with an increase in the acetylcholinesterase (AChE) catalytic

activity in the prefrontal cortex and hippocampus (HP) brain areas. Even though cholesterol cannot transpass the blood-brain barrier (BBB), its oxidized metabolite, 27-hydroxycholesterol (27OH), can freely cross the BBB and it has been observed that its high concentrations mediate the disruption of several systems within the brain, promoting cognitive decline and neurodegeneration. However, the relation between high levels of 27OH and AChE remains unknown. Therefore, this study aimed at elucidating the involvement of 27OH in the homeostasis of brain's AChE. We evaluated the gene expression, protein density, and catalytic activity of AChE in HP and cortico-hippocampal neurons in culture [treated with 27OH (0.5 or 1µM) or DMSO (1 µM) for 6 hours], as well as in cortical and HP cells of 3-month-old male (n = 4) and female (n = 6) CYP27A1 overexpressing mice (with 5 times higher concentrations of 27OH in their brains) in comparison to age-matched male (n = 5) and female (n = 4) wild-type C57Bl/6 mice. Protocol nº 4884-2019. We observed that the treatment with 27OH (0.5µM) increased AChE's protein density in HP neurons [$F(2,14) = 9.11$, $p = 0.002$, $p \leq 0.001$]. Moreover, both concentrations of 27OH increased AChE's catalytic activity in cortico-hippocampal cultured-neurons [$F(2,79) = 6.60$, $p = 0.002$, $p \leq 0.05$]. Cyp27Tg mice showed constitutive alterations in AChE's gene expression (Female: presented an increase in cortical ($t = 2.22$, $df = 8$, $p \leq 0.05$) and a decrease in HP cells ($t = 2.87$, $df = 8$, $p \leq 0.05$); Male: showed increase in HP cells ($t = 3.18$, $df = 7$, $p \leq 0.01$) in comparison to sex-matched wild-type mice. Regarding to AChE's protein density, we observed a tendency of an increase in the HP cells of the male mice ($t = 2.25$, $df = 6$, $p = 0.06$) and a decrease in the cortical cells of the female mice ($t = 3.771$, $df = 7$, $p = 0.007$) in comparison to their respective control groups. Notably, high levels of 27OH have been found in the brains of early-onset and sporadic AD patients (Heverin et al., 2004). Hence, these results might help us understand why high levels of this oxysterol are associated with mild cognitive impairment seen in AD patients. Overall, the present findings suggest 27OH as a modulator of the AChE homeostasis within the brain.

2. Bioquímica, biofísica e biologia estrutural (A2)

A2.R16 Viability evaluation of the recombinant NS3 protease activity, as a future research target for dengue treatment

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Here we aimed to develop antiviral compounds to control dengue virus. NS3 enzyme has been used as a target once it plays a role in the replication and assembly of new host cell viruses . Previously, this enzyme was synthesized in a bacterial system to use its structure as a model for inhibitory peptides. After that, some tests were necessary to identify serine-protease activity viability . For NS3 heterologous expression, 2YT medium with added kanamycin antibiotic [25µg.mL-1] was necessary. The recombinant NS3 induction was performed by IPTG [0.2µM]. The NS3 expression kinetics was evaluated in a polyacrylamide gel. Production increase of the target was confirmed to be 5 mg.L-1 in 4 h, approximately. After extraction, the purification was performed by affinity chromatography on a columna packed with nickel resin (ProBondTM Resin, Invitrogen, EUA) followed by an ion exchange column (Hitrap QFF – 5 mL). SDS-PAGE showed that the NS3 enzyme was purified with a single protein of 74,304 kDa. After thrombin excision, the next step was to perform a new evaluation of the proteolytic activity of NS3 (37ºC by 5 hours), this time using various concentrations of enzyme and substrate. The reaction was monitored continuously in an ELISA reader (405nm) as the pNA substrate released a colorimetric signal. A constant increase in absorbance resulting from substrate cleavage by the recombinant NS3 enzyme was observed, confirming its proteolytic activity. Thus, further tests will allow the extraction of kinetic data of the protease activity of NS3. These data will facilitate the calculation and further analysis of the enzyme after testing it on inhibitory compounds.

A2.R17 Redox metabolism in Hymeniacidon heliophila (Porifera, Demospongiae) under influence of tide and solar radiation

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Many animals endure seasonal conditions associated with oxidative stress when oxygen is limited or unable to be extracted by organisms due to environmental conditions. Some well-known mechanisms that can explain hypoxia tolerance include high constitutive levels of antioxidants, tolerance to oxidative damage, increase of antioxidants due to the increase of ROS, and preparation for oxidative stress (POS). The Porifera phylum, which origin is estimated to be more than 800 million years ago, is a good biological model for the evolutionary analysis of metabolic adaptions, such as the POS mechanism. The sponge Hymeniacidon heliophila is commonly found inhabiting coastal environments, rarely seen totally submerged throughout the whole day. The species developed several adaptations that allow it to survive the functional anoxia caused by the low tide and the high solar radiation. Considering these two stressors (aerial exposure and solar/UV radiation), expeditions were made along the coast of São Sebastião (SP) to collect H. heliophila sponges. To verify the occurrence of POS in the field under totally natural environmental conditions, day and night samplings were made, with submerged and exposed sponges (high and low tides), on 3 different days. The whole-body homogenates of H. heliophila were used to measure the activity of antioxidant enzymes, levels of glutathione, and oxidative stress markers of sponges. We also compared the redox effect of air exposure, the solar/UV radiation impact during the day and night, and the temperature influence during summer and spring. Results showed an influence of seasonal variations in environmental conditions on redox metabolism. Moreover, we observed the occurrence of a "redox depression" during the aerial exposure, without POS mechanism activation. This research discusses the role of redox metabolism as part of the biochemical adaptations of H. heliophila sponges to survive and cope with tidal and solar radiation stresses.

Keywords: aerial exposure; ultraviolet radiation; antioxidants; Porifera; oxidative stress; glutathione

A2.R18 Crystallization of recombinant phytocystatin from peach (Prunus Persica) in complex with papain

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Introduction: Phytocystatins are proteins that act in the inhibition of cysteine proteases, regulating several physiological processes in plants. Thus, these proteins have a great biotechnological potential. The present work aims to carry out a structural characterization, by means of bioinformatics, of a phytocystatin from peach (Prunus persica) at the level of primary, secondary, tertiary and quaternary structures. Methodology: A multiple sequence alignment was performed with phytocystatins with more than 55% identity with peach phytocystatin. The secondary structure was predicted by PSIPRED and models of tertiary and quaternary structures were generated using Modeller and AlphaFold. The models were evaluated by Mol Probity, Verify 3D and PROSA-web. Results: From the alignment, conserved sequences in the phytocystatin of interest were identified. In addition, a secondary structure was identified predominantly formed by beta sheets connected by loops, with only one alpha helix, in the N-terminal portion. The three-dimensional models generated by both methodologies presented, in general, positive scores by the validation software. Conclusion: Phytocystatins have a conserved structure, at primary, secondary, tertiary and quaternary levels. It was observed that when modeling dimers, the association occurs by domain swapping, impacting the biological activity of the protein. Finally, biochemical and biophysical assays are needed to determine how the interconversion between forms takes place, as well the structural elements that influence this process.

A2.R19 Optimization assays for the heterologous expression of a sterol C24-methyltransferase from the human pathogenic fungus Cryptococcus neoformans

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Cryptococcosis is a systemic ringworm infection caused by Cryptococcus spp. fungi, notably C. neoformans. This opportunistic pathogen affects mostly those immunocompromised, frequently being deadly. Furthermore, most widely used antifungal drugs results in side effects due to interactions with human metabolism. Ergosterol, the main sterol found in fungi, is characterized by a β methyl group on the carbon 24. Analogously to cholesterol in animal cells, it is a structural cell membrane component, regulating fluidity, besides acting as a signaling molecule. Its methylation is promoted by enzymes known as sterol methyl transferases (SMT), which represents a major difference in the metabolic pathways of animals and fungi, making them a standout target for antifungal drugs with expected diminished side effects. However, no 3D structures of SMTs are currently available, hindering their structure-oriented development. This study aims to optimize a protocol for the heterologous expression of soluble SMT from C. neoformans (CnSMT) to allow protein purification. A structural model of CnSMT was predicted using alphafold and it's to be used in studies of ligand-interaction specificity. A pET-28a(+) vector containing the erg6 gene from C. neoformans was cloned into E. coli B21 (DE3) cells for protein expression using a medium supplemented with IPTG. Cell growth was routinely monitored through optical density and protein expression and solubility were then gauged through SDS-PAGE. Preliminary results showed that although present, the protein is largely non-soluble in standard expression conditions. Due to this, it has been proposed that CnSMT is contained within inclusion bodies, restricting the attainment of the pure protein. New trials, including variations in the protocol, are underway to diminish or eliminate the formation of these inclusion bodies. Moreover, crystallographic, enzymatic, and inhibition studies are planned to aid in the development of new and selective drugs.

A2.R20 Construction and characterization of serine proteases with novel catalytic triads

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The catalytic mechanism of serine proteases, the most abundant group of proteolytic enzymes, is based in three structural components: the catalytic triad, the oxyanion hole, and the specificity pockets. The catalytic triad residues (serine, histidine, and aspartate) are arranged in four different ways in the polypeptide sequence of classical serine protease clans, indicating at least four different evolutionary origins of this machinery and allowing separation of these enzymes according to their triad layout. However, in theory, the triad residues can be disposed in six different orders. The reason for the other two possible arrangements not being present in known serine proteases has not been discovered. Therefore, to investigate this phenomenon, the purpose of this work is to find and/or to construct serine proteases that possess novel catalytic triads, produce these enzymes, and perform their biophysical and biochemical characterization. The structures deposited in the Protein Data Bank (PDB) were analyzed by an algorithm that measured the distance between specific atoms of all serine, histidine and aspartate residues throughout the protein structure and compared the results with the cut-off distance value calculated from the mean distances found in the active sites of known serine proteases. Triads that met this criterion were separated according to the order in which the three residues appear in the protein sequence, and 3579 triads, found in 807 protein structures, had the two arrangements that are not found in serine proteases. In addition to proximity, the triad residues must have a particular accessible surface area (ASA) to allow catalysis, so the novel triads are being screened for one that has ASA similar to that of a serine protease catalytic residues. When such protein is found, the possibility of proteolytic activity generated by its triad will be analyzed to use the structure as a model to design an enzyme that has the undocumented triad arrangement.

A2.R21 Cloning, expression, purification and initial characterization of the 5-enolpyruvylshikimate-3-phosphate synthase from Escherichia coli

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The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, EC 2.5.1.19) is the sixth enzyme on the shikimate pathway, which produces chorismate, the precursor of tryptophan, phenylalanine and tyrosine in plants and some

microorganisms. The EPSPS is the only molecular target for the broad-spectrum herbicide glyphosate. Although there isn't a scientific consensus on the carcinogenic effects of glyphosate on humans, the vigilance of this pesticide is essential for further public health discussions. Therefore, this work focused on the production and characterization of the E. coli EPSPS aiming at the development of a glyphosate biosensor. The EPSPS gene was amplified and cloned into pET-M11 and pET-SUMO plasmids, adjusted for the "Ligation Independent Cloning" system that eliminates the use for restriction enzymes and DNA ligase. Then E. coli BL21 (DE3) e E. coli Rosetta (DE3) cells were transformed with the plasmids containing the gene of the protein of interest. In order to evaluate the ideal expression condition, two protocols were tested for each vector: i) expression in LB medium induced by IPTG; ii) expression in ZYM-5052 medium induced by lactose. The pET-M11 construct, expressed in E. coli BL21 (DE3), in ZYM-5052 medium, was chosen due to the greater amount of soluble protein recovered after cell lysis. A two-step purification was optimized for obtaining the purified protein: immobilized metal (Ni2+) ion affinity liquid chromatography followed by size exclusion chromatography. The polidispersity and hydrodynamic size of the purified protein sample obtained were determined through dynamic light scattering (DLS). The DLS results indicated that the protein is most likely in a monomeric condition. In conclusion, it's possible now to analyze biochemical and biophysical aspects of the EPSPS. And in time, the immobilization of the protein to the surface of the biosensor will be performed to allow for glyphosate sensibility and specificity tests.

A2.R22 Elucidation of the primary structure of the TfTx peptide, isolation of the scorpion Tityus fasciolatus and evaluation of activity in ion channels Kv 1.1, Kv 1.2 e Kv 1.4.

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Among the different channels are the K+, channels, responsible for maintaining cell excitability. This group houses a great diversity of channels, divided into four families: the voltage-dependent channels (Kv), Inwardly Rectifying Potassium (Kir), Two-Pore Potassium Channel (K2P) and Calcium-activated Potassium Channel (KCa). The Kv and Kca are the main targets of scorpion toxins between potassium channels, acting mainly on the Kv1 channel subfamily, which are abundantly present in nervous tissues. The toxins that act on potassium channels, known as KTxs, are characterized by having 20 to 95 amino acid residues, stabilized by 2 to 4 disulfide bridges and are currently distributed in 7 different families, classified as: α, β, γ, δ, ε, λ and k-Ktx. where they are classified according to their chain of amino acids and function performed. The TfTx, a peptide isolated from the venom of Tityus fasciolatus, has a molecular mass of 3.583,64 Da [M+H]+ and its partial sequence revealed 19 amino acid residues with the presence of 3 disulfide bridges. In comparisons made in databases, it was observed that this peptide has a high degree of similarity (78%) with 2 other peptides: CllNtx isolated from the venom of Centruroides limpidus and 'Peptide A' isolated from Centruroides hirsutipalpus. This group of peptides has no physiological activity described and studies carried out with these molecules suggest that they compose an not yet described family of neurotoxins with possible activity in potassium channels.

A2.R23 Diagnostic method for SARS-CoV-2 based on nanostructured Ribozymes and DNA-hairpins associated in a hybridization chain reaction with fluorescence resonant energy transfer

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The SARS-CoV-2 pandemic demonstrated that rapid diagnosis by real-time detection of the viral genome (RT-qPCR Reverse Transcriptase quantitative Polymerase Chain Reaction) and by serological methods were essential to control its rapid spread. However, these methods have limitations of high cost, need for refrigeration, specialized equipment and professionals. The development of rapid, portable, simple and easy-to-use detection techniques in variable conditions without the need for refrigeration and specialized equipment is a priority for recent and future epidemics, which is in line with the present project. In this, designed and nanostructured ribozymes will recognize the SARS-CoV-2 RNA that will be cleaved into fragments that will carry out a hybridization chain reaction (HCR) with DNA hairpins containing fluorophore pairs with fluorescence resonant energy transfer potential (FRET) that can be detected by simple devices designed for this purpose. First, DNA sequences were designed and transcribed to produce the target viral RNA and three ribozyme molecules to be used in the Ribozyme cleavage assays. In addition, DNA hairpins with TAMRA/FAM and Cy3/Cy5 fluorophores were designed and used in FRET-HCR assays with DNA and RNA target sequences. The results indicated that two of the three designed ribozymes were successful in cleaving the target RNA. Moreover, DNA hairpins with Cy3/Cy5 pairs were the most efficient in detection of the target DNA, and were also effective in FRET-HCR assays to detect the target RNA. Currently, detection experiments involving all stages of the process are being conducted in order to validate its complete effectiveness. Additionally, work is being done on the design and prototyping of the FRET detection device by processing images taken by smartphones.

A2.R24 Impact of sexual dimorphism on the mitochondrial function of LDL receptor knockout mice

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Familial hypercholesterolemia (FH) is a dyslipidemia with a genetic origin, which is caused by a functional impairment in the low-density lipoprotein receptor (LDLR), with subsequent disturbance in lipoprotein metabolism and cholesterol accumulation in the bloodstream. The high level of circulating cholesterol existent in the low-density lipoprotein (LDL) induces harmful effects on cellular metabolism and tissue development. Mitochondrial dysfunctions have already been described in biological tissues in male FH models, however, there are few studies with females. Considering that the sex differently impact metabolic dysfunctions, the aim of in this study was to understand the influence of sexual dimorphism on mitochondrial function in a FH model. Male and female C57Bl/6 wild-type (WT) and LDLR-/- mice approximately 6 months old were anesthetized and euthanized for blood collection for lipid profile analysis. Hippocampi and BAT homogenates, as well as hepatic and cardiac isolated mitochondria, were used to assess oxygen consumption through high-resolution respirometry. Male and female LDLR-/- mice presented a significant increase in cholesterol and triglycerides levels, when compared to WT. In LDLR-/- males hippocampus, there was a significant decrease in O2 consumption related to complex I+II activity, oxidative phosphorylation and maximal respiratory capacity, when compared to WT animals. In LDLR-/- males BAT there was also a significant decrease in mitochondrial respiration related to UCP-1 activity, and in the respiratory reserve capacity, compared to WT animals. On the other hand, LDLR-/- females presented a significant decrease in the hepatic O2 consumption related I+II complex activity, when compared to WT ones. The results suggest that LDLR deletion affects the mitochondrial bioenergetics of several tissues, with a greater impact on males than females, reinforcing that sexual dimorphism should be considered in the outcomes of this disease.

A2.R25 Integration of computational and experimental strategies in the discovery of thimet oligopeptidase metalloprotease inhibitors from *Trypanosoma cruzi*

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Chagas disease (CD) affects more than 6 million people around the world according to the World Health Organization (WHO) and although there is treatment available, the results are variable among treated individuals, being ineffective in the acute phase of the disease. Furthermore, due to high toxicity and severe side effects, a portion of patients abandon treatment before completion. This scenario reveals the need and urgency for investment in Research and Development aimed at identifying new targets and therapeutic molecules for validating new drugs. In this context, this project is part of a line of research focused on pathogen proteases as potential therapeutic targets. The development of effective therapies that minimize adverse effects and encourage patients to adhere to treatment is crucial. Knowing that proteases are fundamental in different stages of metabolism and life cycle of parasites, thimet oligopeptidase from *Trypanosoma cruzi* is a promising molecular target to be explored for the development of inhibitors. This project aims to perform a virtual screening using computational approaches to identify hits with activity against the enzyme thimet oligopeptidase from *T. cruzi* (TOPTc), validate the inhibition on the enzyme, evaluate the trypanocidal effect of the selected compounds and elucidate the catalytic mechanism. The enzymatic characterization of the protease was carried out in a previous work, as well as the effect of two inhibitors known to inhibit metalloproteases. Structure prediction was done using distinct servers that work with different methodologies such as deep learning based algorithm, threading and deep neural network. Virtual screening based on molecular docking enabled the selection of different compounds that will be used in future steps against different forms of the parasite, intending to discover a possible function for this protein in *T. cruzi* in order to contribute to studies of the host-pathogen relationship.

Keywords: Thimet oligopeptidase, metalloprotease, virtual screening, docking, Chagas disease.

A2.R26 GENERATION OF GRAMMISTINS AND ODORRANAIN ANALOGUES WITH THE ADDITION OF ROS-PRODUCING AMINO-TERMINAL COPPER AND NICKEL BINDING (ATCUN) MOTIF

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Recent years have seen a rise in the emergence of pathogenic microorganisms that are multidrug-resistant, mostly as a result of the improper usage of available antibiotics. The search for novel compounds with antimicrobial properties against resistant pathogens is urgent because of antimicrobial resistance severely public health. The complex generated by the amino-terminal copper and nickel binding (ATCUN) motif is capable of producing reactive oxygen species (ROS) that may oxidatively damage lipids in the bacterial membrane, which is another intriguing possibility for the creation of novel antimicrobial agents. The current study aims to assess and characterize the biological activity of five synthetic peptides, including three novel analogs of the antimicrobial peptide Odorranain (identified from Odorrana grahami) and two Grammistins: Grammistin Pp1 and Grammistin Pp2a (isolated from Pogonoperca punctata) with the addition of the ATCUN motif. The work will involve manual peptide synthesis, antimicrobial assays with numerous Gram-positive and Gram-negative pathogenic bacteria, cytotoxicity tests with cancerous and normal cells, membrane calorimetry, scanning electron microscopy (SEM), and circular dichroism. This binding motif intends to boost the antibacterial activity when copper is present while decreasing the hemolytic and cytotoxic activity of the studied peptides. The addition of ATCUN motifs to antimicrobial peptides is a simple strategy that potentiates antibacterial activity, due to its double action giving rise to a new class of antibacterial agents. The peptide synthesis of all peptides was performed using Fmoc solid phase peptide synthesis. The peptides were purified by semi-preparative RP-HPLC using C18 columns and the fractions were evaluated by MALDI-TOF

mass spectrometry. Given the completion of the production procedure for the analogs of interest, the biological and physical-chemical characterization experiments will be conducted in the coming months.

A2.R27 Enzymatic, inhibition and crystallographic studies of C24-methyltransferase from human pathogenic fungi Candida auris and Aspergillus fumigatus

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Invasive fungal infections are a major cause of mortality in immunocompromised patients caused mostly by Candida and Aspergillus species. Candida auris stand out with high mortality rates. Ergosterol is an essential lipid for the cell viability due to its function in membrane fluidity and permeability. Cholesterol and ergosterol share the same metabolic pathway until the stage of zymosterol synthesis, from this point onwards they diverge and use different enzymes. The gene erg6 encodes a 24-C-methyltransferase (SMT) that adds a methyl group to C-24, converting zymosterol into fecosterol or, in an alternative, but similar reaction, converting lanosterol to eburicol. The SMT is a promising target because it is present in invasive fungi and absent in the human host. The soluble CauSMT (C. auris) and AfSMT (A. fumigatus) was purified by immobilized metal affinity chromatography. The effect of additives on the oligomeric state of the proteins was analyzed which showed a pentameric CauSMT and a tetrameric AfSMT. Circular dichroism analysis demonstrated that CauSMT and AfSMT were stable at acid and neutral pH. The melting temperature of both enzymes at the most stable pH was 59 °C. Enzymatic assays showed that CauSMT has specificity for zymosterol and AfSMT uses the alternative pathway converting lanosterol to eburicol. One molecule was identified as a potential inhibitor of CauSMT. Since no 3D structures of SMTs are currently available, crystallization assays were performed for both free and ligand-bound (co-crystallization) proteins. Crystals were obtained and evaluated at Brazilian synchrotron light source, Sirius, showing diffraction to ~7 Å. In pursuit of the development of potent and selective drugs, optimization of crystallization conditions to get high resolution diffracting protein crystals data and molecular docking studies are being carried out.

A2.R28 Enzymatic characterization of Aspergillus brasiliensis in co-culture with Trichoderma reesei RUT-C30

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Lignocellulosic biorefineries are sustainable industrial facilities that use agricultural waste as raw material to produce higher value-added products such as biofuels and chemical “building-blocks”. One of the major costs of biorefineries is the enzymes needed to break down the plant cell wall into fermentable sugars. These enzymes are obtained from microbial sources such as filamentous fungi. The current industry standard for enzyme production are fungal monocultures. This technique produces limited cocktails, as no organism is capable of secreting all the necessary enzymes in sufficient quantities. An alternative to be explored is to simulate the degradation of organic matter that occurs in nature and to use more than one filamentous fungus in the enzymatic production, a practice called co-cultivation. In this work, the enzymatic characterization of Aspergillus brasiliensis was performed and its co-cultivation with the fungus Trichoderma reesei RUT-C30 was evaluated using sugarcane bagasse as carbon source. The resulting enzymatic cocktails were characterized regarding the effect of strain inoculation time and the effects of pH and temperature on enzymatic activities. The results show that the profile of each enzymatic extract is highly dependent on the type of culture and the order in which the participating fungi were inoculated. Some of the co-cultures, under certain conditions, reached higher activities than their respective monocultures for enzymes such as CMCase, pectinase, β-glucosidase and β-xylosidase.

A2.R29 Molecular docking approaches of the interaction between ferulic acid and endoxylanase HXYN2 from Humicola grisea var. thermoidea

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HXYN2 is an endoxylanase of the GH11 family, produced by the thermophilic fungus Humicola grisea var. thermoidea, and its main characteristic is its high structural and functional stability, making it a promising enzyme for use in various industrial processes. The three-dimensional structure of HXYN2 endoxylanase was obtained by homology molecular modeling with GH11 endoxylanases using the trRosetta algorithm. Multiple sequence alignments between HXYN2 and templates were performed using the MEGAv7 program. Molecular docking prediction between model of HXYN2 and Ferulic Acid (FA) (PDB: 3NX2) was performed using the CB-Dock automatic method with the AutoDock Vina program. The multiple alignment of amino acid sequences presented highest sequence similarity in the β-strands that form the "palm" of endoxylanases. The three-dimensional structure of HXYN2 consists of a single domain of β-jelly-roll structure, composed of two twisted anti-parallel β-sheets forming a long and deep cleft. The unique α-helix is packed under the β-sheet B. The "thumb" between the β11- β12 β-strands shows a highly conserved consensus sequence "PSIXG" among GH11 xylanases. The two catalytic Glu residues and substrate-binding environment of the HXYN2 model are highly conserved among GH11 xylanases. Forty-five molecular docking solutions with favorable Gibbs free energies (-3.4 to -6.8 kcal/mol) were predicted and grouped into five regions of potential interaction o FA with HXYN2, each with nine docking solutions. The data suggest that FA interacts with greater probability in the aglycone region of HXYN2 in a non-inhibitory and non-competitive manner. This result corroborates the enzymatic kinectics data of HXYN2 in the presence of FA, which showed an increase in catalytic efficiency without modifying substrate affinity.

A2.R30 Polyethylene consumption as a source for polyhydroxyalkanoate production

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Polyhydroxyalkanoate (PHA) is a biopolymer produced by bacteria as an energy store. Its production occurs under stress in a limited essential nutrient environment and excess carbon source. Its monomeric structure gives physicochemical characteristics similar to synthetic polymers, making it a viable and clean alternative to petrochemical plastic. The advantages outweigh the plastic similarities, as it has non-toxicity and biocompatibility properties. Nevertheless, despite the biotechnological potential, high cost and toxic solvents for its extraction and purification constraints its large-scale manufacturing. In a previous study, Dr. Julianna Peixoto and coworkers isolated and identified in the soil plastic debris with three bacterial strains of the genera Comamonas PE63, Delftia PE138, and Stenotrophomonas PE591 that biodegrades polyethylene (PE), and identified PHA synthase genes in genomic analysis of these strains. Hence this research aims to analyze the bioplastic production from PE consumption, physicochemical characterization, and metabolic pathway analysis for subsequent optimization. Strains PE63 and PE138 were preferred for cultivation in a minimal medium with pure PE for PHA yield, extraction, purification, and characterization. PHA extraction was carried out over alkaline digestion with NaOCl, purification and dissolution with $CHCl_3$, and the PHA precipitation by CH3OH. Infrared Spectroscopy (ATR-FTIR) was used to evaluate PE biodegradation, identifying changes in the chemical structure and formation of new functional groups; ATR-FTIR was used in PHA identification and analysis. Subsequently, composition analysis will be carry out with Gas Chromatography, molecular mass, and diameter analysis with Dynamic Light Scattering (DLS) and Differential Scanning Calorimetry (DSC) to identify melting temperature, crystallinity degree, and thermal and oxidative stability.

A2.R31 NOVEL ANTIMICROBIAL PEPTIDES ISOLATED FROM THE CENTRAL DWARF FROG Physalaemus centralis (Bokermann, 1962) SKIN SECRETION

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Bacterial infections threaten human health revealing a worldwide need associated with research and development of compounds with antibacterial properties to treat infections, especially those caused by multidrug-resistant pathogens, as the growing number of resistant organisms is one of the greatest threats to humanity in the century XXI. Studies estimate that in the next years, 300 million people will die due to diseases caused by resistant organisms, under a huge economic impact. The first line of immune defense for several living beings is composed of Antimicrobial Peptides (AMPs). AMPs are compounds that demonstrate efficiency against microorganisms resistant to various commercially available drugs. AMPs have mechanisms of action on the plasma membrane of bacteria (which makes it difficult to select mechanisms of resistance to these compounds) and also mechanisms of action at the intracellular level, due to the inactivation of certain cellular processes. The present work aims to identify and characterize chemically and biologically compounds from the cutaneous secretion of the anuran Physalaemus centralis with an emphasis on antimicrobial peptides for therapeutic applications. Four new peptides were isolated (PEP1N4, PEP2N5, PEP4N6 and PEP5N7) and showed antimicrobial activity on Gram-negative and Gram-positive pathogenic bacteria and also a fungal species. The peptides characterized in the present study are similar to peptides of the nattererin family, isolated from Physalaemus nattereri. Comparing the effects in combating bacterial growth, these four new peptides have been shown to be efficient, reducing in some cases the concentration necessary to completely inhibit the bacterial growth to 2 µM. These AMPs have shown great activity against HeLa, B16F10 and MCF-7 cancer cells. In the next steps will be carried out assays to evaluate their antiviral, antiparasite, imunomodulatory and would healing properties.

A2.R32 Quantification of Error Sources Accounting for Misidentification of Protein Partners in Coevolutionary Approaches

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Finding the correct set of partners for a given pair of interacting protein families based on multi-sequence alignments (MSAs) is a problem which has received great attention over the years. In recent works, we have shown that he native contacts of two interacting proteins store the strongest mutual information (MI) signal and we have used this to discriminate MSA concatenations with the largest fraction of correct pairings (n) using a Genetic Algorithm (GA). We have found degenerate solutions with two error sources, arising from mismatches among (i) similar and (ii) non-similar sequences. Once this problem remains unsolved, we contribute here a statistical framework to describe the probability distribution of two interacting protein families with a large number of sequences that feature a unique “native” arrangement (‘) at a maximum MI content. Furthermore, we discuss statistical dependence of generating type-(i) and -(ii) solutions in space of parameters n and MI in GA simulations.

A2.R33 Study of a snake venom serine protease constructed through ancestral sequence reconstruction technique

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Snake venom serine proteases (SVSPs) are a group of toxins that acts on the hemostatic system of the prey. Despite having high sequence similarity among them, SVSPs act on a wide variety of substrates, showing, among others, kallikrein-like and thrombin-like activity. The divergent evolution that resulted in such diversification can be

studied through the ancestral sequence reconstruction technique. In this approach, putative ancestral sequences are built from modern sequences, enabling us to better understand enzymatic and structural properties of extinct proteins. Therefore, the goal of this work is to study the evolution of SVSPs through reconstruction of the Viperidae ancestral sequence and characterize it biochemically and biophysically. Vipers are the group that have the greatest diversity of SVSPs. The sequences were obtained using the HMMER tool with the MEROPS database. The phylogenetic tree and the ancestral sequence were built using the MEGA-X software. A 3D model of the protein structure was made using the Alphafold software. The gene was obtained commercially and used to transform Pichia pastoris cells. The enzyme, expressed in P. pastoris, was purified through affinity chromatography (Ni2+). Deglycosylation tests were performed and confirmed by MALDI-TOF analysis. The reconstructed sequence showed good statistical values and its structure is similar to others SVSPs. The expressed protein showed a higher molecular mass than expected in SDS-PAGE, which was confirmed to be caused by N-glycosylations, a very common pos-translational modification in SVSPs. Activity tests are being carried out in order to define whether the enzyme is active and which substrate it recognizes. The next steps to be taken include the enzymatic and structural characterization of the reconstructed enzyme. With this study we expect to expand the knowledge in functional and structural evolution of SVSPs.

A2.R34 Functional and structural characterization and mechanism of action insights of two mastoparan peptides

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Mastoparans are antimicrobial peptides that demonstrate pharmacological potential due to their multifunctional properties. Based on mastoparan-L, mastoparan-R1 and R4 were computationally modified, reducing their cytotoxicity, optimizing their antimicrobial activities, and improving their therapeutic potential. This work evaluates whether the peptides would maintain their activity against E. coli strains under different salt concentrations, and how the structure would interfere with the peptides' bioactivity. R1 and R4 preserved their activity under the conditions evaluated, with the analogs having points of improvement in antibacterial activity at physiological salt concentrations. The secondary structures of the peptides were investigated through circular dichroism (CD) in different solvents and temperatures. No significant changes were observed in the peptide's helicity, even under different temperatures, and they did not lose their structures in anionic mimetic conditions. The three-dimensional structures of the R1 and R4 peptides were elucidated through NMR spectroscopy. The structural calculations showed that the peptides adopted amphipathic α-helical segments between the residues Leu3-Ile13 (R1) and Leu3-Ile14 (R4). Surface plasmon resonance (SPR) results evaluated the binding affinity of the peptides with mimetic membranes, including pure POPC and POPC/POPG (4:1). We observed that mastoparan-L has a higher affinity for both constitutions, whereas its analogues have a lower potential for association, but greater specificity. This corroborates with our molecular dynamics simulations, which show greater interactions in POPC:POPG membranes for R1 and R4, demonstrating binding specificity. Despite presenting few a differences in

their functional and structural profiles, the R1 analogue stood out for presenting the highest activity at lower concentrations, greater bacteriostatic potential and for having promising affinity for anionic membranes.

A2.R35 Thermodynamic analysis of the partition process of small ligands into proteins

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General anesthetics are usually small molecules, which their main common feature is lipophicility. Such property is highlighted by the Meyer-Overton rule, which states that the potency of the anesthetic is directly correlated to its capacity of dissolving in lipids. But how this interactions happen in molecular scale? It has been shown that they interact with low-affinity to proteins, making very challengeful to describe the interaction using experimental methods. Indeed, the general anesthesia mechanism is still unknown. In this work we focus on redesigning the interactions between general anesthetics and protein receptors using a partition approach between two phases (protein surface and reservoir), using a sevoflurane-Kv1.2 atomic model and computational tools. To address the new model accuracy, its thermodynamic properties have been compared to the ones obtained by the classic methods. Also, we tested the behaviour of the partition model when the reservoir has many ligand concentrations. As shown in the results, the partition-based model exhibits good accuracy with the other methods and does not present any dependence in the concentration, which allows us to calculate many properties from a single concentration and extrapolate many properties to more diluted regimes.

A2.R36 MODULATION OF GUT MICROBIOTA IMPROVE METABOLISM OF WHITE ADIPOSE TISSUE AND GLUCOSE TOLERANCE IN C57BL/6 MICE

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Introduction: The gut microbiota is intimately involved in numerous aspects of normal host physiology, from nutritional status to behavior and stress response. Dysbiosis caused by obesity can modify the functioning of the intestinal barrier, host energy uptake, insulin resistance, inflammation and fat deposition. Antibiotic-induced changes in gut microbiota may affect the response to HFD-induced obesity. Our objective was to investigate the effect of gut microbiota modulation on metabolism function. Methods: Experiments were conducted in accordance with the ethical principles of animal experimentation and approved by the Committee on Ethics in the Use of Animals (CEUA) from the Institute of Biology of University of Brasilia, under protocol number 23106.110055/2021-87. Seven-week old C57BL/6 male mice were submitted to a Control diet (CD-15.8 kcal% of fat) or High-Fat Diet (HFD-57.2 kcal% of fat) for 8 weeks. They were randomly assigned into 4 groups to receive (i) CD + vehicle, (ii) HFD + vehicle, (iii) HFD + antibiotics, or (iv) HFD + antibiotics, followed by fecal transplantation from CD-fed donors. Gut microbiota was modulated with ciprofloxacin 0.2 g/L and vancomycin 0.5 g/L in drinking water from week 10 to 15. Feces from control group were collected for fecal transplantation through gavage in one sub-group fed with HFD on week 13. Then, mice were submitted to glucose tolerance test and adipose tissue histology. Feces for microbiota analysis were collected on weeks 10 and 15 and analyzed by16S rRNA gene sequencing. Results: Mice fed with HFD showed increased weight gain when compared to mice fed with CD. Antibiotic treatment did not alter weight gain. HFD-fed group presented decreased glucose tolerance when compared to the CD group. Treatment with antibiotics and fecal transplantation showed significant improvement in glucose tolerance in relation to the HFD group. Visceral and subcutaneous white adipose tissue from HFD group presented larger and more unilocular adipocytes

than animals fed with CD. Mice treated with antibiotics and fecal transplantation had significantly lower adipocytes when compared to mice in the HFD group. The composition of the intestinal microbiota showed significant segregation between the diversity of species among the different groups. Conclusion: Our findings suggest that changes in gut microbiota composition may be involved in the control of metabolic homeostasis dysregulated by high fat diet. We observed a reversal in glucose intolerance and decrease in adipocyte size in animals that received antibiotics and fecal transplantation. These results are consistent with evidences that the microbiota is intimately involved in several aspects of host physiology. However, there is a need to elucidate the possible mechanisms involved in this interaction.

A2.R37 Effects of epilepsy-associated mutations on hNav1.2 channel functioning and the electrophysiological characterization of Tst2 peptide from the scorpion Tityus stigmurus

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Epilepsy is a disease caused by exaggerated activity of neurons or abnormal brain synchrony. Infections, autoimmune diseases, acquired causes, and genetic mutations are the main causes. In literature, it can cite studies relating epilepsy and mutations in voltage-gated sodium channels, as a family with benign familial neonatal-infantile seizures, which after genetics analyses identified the presence of I1596S mutation. The study reporting the V1627M mutations that is associated with epilepsy of infancy with migrating focal seizures. Finally, a study reporting a patient with a mutation in the SCN2A gene (L1650P) described related to early childhood epileptic encephalopathy. Part of the population with epilepsy is resistant to the action of drugs. It is necessary to search for new ways of treating the disease such as compounds of scorpion's venom. The objective of this project is evaluate the effects of epilepsy-associated mutations (I1596S, V1627M and L1650P) on hNav1.2 channel functioning and the electrophysiological characterization of the Tst2 peptide from the Tityus stigmurus scorpion. The purification and identification of the peptide was done by HPLC and MALDI-TOF/TOF. The mutation will be performed by the directed mutagenesis system QuikChange II and the electrophysiological recordings are being made in whole-cell mode, using the EPC 10 Heka amplifier through the PatchMaster software. The purification occurred in three steps, the toxin eluted at 37,8% on crude venom chromatography and the monoisotopic mass was [M+H]+ 6985,74 Da. Electrophysiological characterization (Nav1.1-Nav1.7) of the peptide (100 nM) was made. For activation, the most affected channel was Nav1.1 (with prepulse) and Nav1.7 (without prepulse). Nav1.3 was the most affected in inactivation and Nav1.4 for recovery from inactivation. No change in the rapid inactivation was observed. Next steps: carry out the mutations in Nav1.2 and analyzing them electrophysiologically.

A2.R38 Effect of Tucum-do-Cerrado consumption on thermogenesis of brown adipose tissue and lipid metabolism in diet-induced obesity rats

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Tucum-do-Cerrado (Bactris setosa Mart.) is a typical fruit of the Brazilian biome, rich in phenolic compounds. These dietary compounds may have beneficial metabolic effects by modulating thermogenesis and lipid metabolism in brown adipose tissue (BAT). This study evaluated the effect of Tucum-do-Cerrado consumption on BAT thermogenesis and lipid metabolism, in diet-induced obesity rats. Rats were treated with: control (CT); control + Tucum-do-Cerrado (150 g freeze-dried Tucum-do-Cerrado/ kg of diet; CT/TUC); high-fat (HF) or high-fat + Tucum-do-Cerrado (150 g freeze-dried Tucum-do-Cerrado/ kg of diet) (HF/TUC) diet. The HF diet increased energy intake; weight gain; hepatic triglycerides (TGL) and total cholesterol (CLT); Ucp1, Pgc1a, Vegfr2, and Vegfa mRNA levels in BAT, and reduced total food intake; serum LDL; hepatic and serum HDL, and Fasn mRNA levels in BAT, compared to CT diet. The Tucum-do-Cerrado consumption increased hepatic CLT; Ucp1 and Vegfa mRNA levels in BAT and decreased serum TGL levels, regardless of the diet type. The high-fat diet with Tucum-do-Cerrado increased

Srebp1-c, Prkaa1, Prdm16 BAT mRNA levels, and reduced Acaca and Fasn BAT mRNA levels, in relation to a high-fat diet. The control diet with Tucum-do-Cerrado increased Vegfr2 and reduced Fasn mRNA levels in BAT compared with the control diet. No difference was observed in Ppary, Slc2a4, and Prkaa2 mRNA levels in BAT in all treatments. In conclusion, the consumption of Tucum-do-Cerrado may enhance thermogenesis in BAT, but does not affect body weight gain in a diet-induced obesity model. In addition, Tucum-do-Cerrado consumption combined with the high-fat diet improves lipid metabolism through the upregulation of fatty acid oxidation genes and the downregulation of fatty acid synthesis genes.

A2.R39 Optimization strategies in the heterologous production of the main protease of SARS-CoV-2 in Escherichia coli.

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Covid-19 pandemic is caused by the SARS-CoV-2 virus and has led to more than 6 million deaths worldwide. Despite successful global vaccination efforts, the risk of outbreaks by resistant variants and sub-variants demands more selective pharmacological treatments, such as those based on viral-protease inhibitors. The chymotrypsin-like Main Protease of SARS-CoV-2 (Mpro, also named 3CLpro) is a highly conserved and attractive antiviral target due to its essential function in proteolytic processing of the two pp1a and pp1ab polyproteins during viral replication. The black-eyed pea trypsin and chymotrypsin inhibitor (BTCI) is a small protein from the Bowman-Birk family isolated from Vigna unguiculata seeds that bifunctionally inhibits trypsin- and chymotrypsin-like proteases. Peptides that mimic BTCI reactive loops retain their inhibitory capabilities. Molecular docking with these peptides and BTCI within the Mpro substrate-binding pocket indicated a strong binding -affinity, indicating the potential of BTCI and derived peptides as Mpro inhibitors. In order to obtain Mpro, E. coli BL21(DE3) cells were transformed with pGEX-6P1 plasmid that encodes the fusion GST-Mpro-6xHis protein cleavable by PreScission protease for removal of GST- and 6xHis-tags. Expression was confirmed by western blotting, and the supernatant of the lysed cells was applied to Ni-Affinity for fusion protein purification. GST-PreScission protease was expressed and purified by GST-glutathione affinity chromatography. Cleavage by PreScission, combined with GST-glutathione and Ni-Affinity chromatography yielded Mpro without tags and higher purity. At the moment, Mpro purification and solubility are being optimized prior to the subsequent biochemical and biophysical characterization of the inhibition assays.

A2.R40 Bioprospection of Synthermes wheeleri gut microbiome using the bacteria metagenome for mining enzymes able to convert lignocellulose into chemicals with a high added value

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Termites, generally, consume 3 to 7 billion tons of lignocellulosic materials per year and therefore represent one of the most prolific and efficient lignocellulose decomposers on Earth. The bioconversion of cell wall polysaccharides by termites is a highly coordinated process achieved by the gut-resident microbial symbionts. In this work we aim to use the biotechnological potential of these microorganisms to obtain enzymes able to convert the polysaccharides into chemicals with a high added value. The process was carried out with the bacterial gut metagenome of Syntermes wheeleri, an endemic termite specie of Brazilian savanna (Cerrado). Here we developed an integrated bioinformatic analyze (ggKbase and Geneious) that show us clusters of proteins in phylogenetic trees with the potential of innovation. The domains were distributed in Glycosyl Hydrolases (GHs) - 3, 5, 9 and 10 with representatives of the phyla Firmicutes, Proteobacteria, Bacteroidetes and Spirochaeta, among others. In work to characterize and see the potential, we used E. coli BL21(DE3) to synthesize some of them. The biochemicals results

demonstrated 40-45ºC as the best temperature of activity for Exo 85-74 (exoglucanase) and β-G 72-26 (β-glucosidase), besides to present acid and basic pH as the optimum, respectively. Circular Dichroism complement demonstrating the pH dependence of secondary structure, according the α-helices and β-sheets quantities change. These characteristics provided the best conditions to saccharification. This process will be taken in distinct biomass, as sugar cane, using heat treatment as an environmentally friendly pretreatment process, compared with chemical pretreatment, to enhance the access of proteins to polysaccharides. FTIR (Fourier-transform infrared spectroscopy) is associated detecting the before and after of biomass bioconversion by bond patterns of products. All of this will elucidate the capability of enzymes from termite gut bacteria metagenome have in biotechnology processes of lignocellulose bioconversion.

A2.R41 Integration of top-down and bottom-up proteomics from microorganisms of medical and biotechnological relevance

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The most used proteomic approach, bottom-up, analyzes peptides resulting from proteolytic cleavage. On the other hand, top-down proteomics analyze intact proteins, being more adequate for the identification of proteoforms. Integration of both approaches has demonstrated its potential in the understanding of some pathogenic and biotechnological relevant bacteria, such as Neisseria meningitis and Escherichia coli. The present study aims at integrating bottom-up and top-down proteomic approaches to characterize proteoforms of the etiologic agent of Chagas disease, Trypanosoma cruzi, and Corynebacterium glutamicum, used in industries for the production of glutamate. C. glutamicum growth was performed in CGXII medium at 30 ºC. After 6.75 h, Penicillin-G (Pen-G) or Tween 40 were added to the media to induce glutamate production. Glutamate production were monitored using HPLC with amperometric detection. Cells grown for 9 h in Pen-G and control group were submitted to protein extraction for proteome analysis. T. cruzi epimastigote cells were grown in DMEM medium, under 37 ºC. Proteins of C. glutamicum and T. cruzi were submitted to GELFrEE fractionation and the first 12 fractions of each submitted to LC-MS/MS. Resulting raw files were analyzed by TopPic suite for proteoforms identification. Amino acid analysis revealed C. glutamicum production of glutamate after the induction using Pen-G and Tween-40, 4.44 g/L and 0.60 g/L, respectively. Because of the higher amount of glutamate, Pen-G induction was chosen to be compared with control through proteomic analysis. Top-down proteomics of C. glutamicum Pen-G condition identified 656 proteoforms, belonging to 281 proteins. A large number of proteoforms were identified with N-terminal acetylation. Bioinformatics analysis of C. glutamicum in control condition are in progress. Top-down proteomics of T. cruzi identified 166 proteoforms, from 106 proteins. N-terminal acetylation was also very present in T. cruzi proteoforms.

A2.R42 Proteomic analysis of Trypanosoma cruzi interaction with macrophages

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This doctoral thesis is part of the line of study of the host-parasite interaction during the innate immune response. More specifically, the project aims to study the interaction of T. cruzi trypomastigotes with macrophages. Therefore, in order to determine the molecular alterations in proteomic terms, a comparative proteomics approach is being used between macrophages, macrophages infected with T. cruzi and macrophages infected with T. cruzi previously infected with the phytopathogen Phytomonas serpens, Methodology: THP-1 monocytes (ATCC-TIB-202) were cultured according to the recommendations of the Rio de Janeiro Cell Bank (BCRJ), and later differentiated into macrophages using phorbol 12-myristate 13-acetate (PMA) at the concentration 100 nM. Interactions were carried out with the phytopathogen Phytomonas serpens and trypomastigotes forms of T. cruzi, obtained through culture with Helas cells. Cells were extracted in Tris pH8.0 8M urea solution and sonicated. Samples will be digested with

trypsin, reduced with DTT and alkylated with iodacetamide. The generated peptides will be submitted to nano UHPLC Dionex Ultimate 3000 coupled to an Orbitrap Elite mass spectrometer (Thermo Scientific). The generated spectra will be used for computational searches for protein identification (Peaks) and quantification (Progenesis) using a concatenated database of T. cruzi and H. sapiens sequences. Results: Standardization of cultures: THP-1 monocytes (ATCC-TIB-202), differentiation in macrophages with PMA, assays of interaction with parasites; Helas cells infection/production of T. cruzi trypomastigotes; culture of Phytomonas serpens. Standardization of the methodology for extracting and quantifying samples for proteomic analysis. LC-MS/MS runs are scheduled to take place in the next weeks.

A2.R43 Characterization of two oxidoreductases and their role in polyethylene biodegradation.

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Polyethylene is the synthetic polymer most widely produced. It is a material with great versatility, resistance, durability, and low cost, hence its popularity and recalcitrance, which can remain in the environment for over 100 years. Biodegradation is a sustainable way to manage this waste, as current post-consumer plastic management strategies are inefficient and generate an environmental impact. In previous work from the laboratory, Dr. Julianna Peixoto isolated 9 bacteria belonging to the genera Comamonas PE63, Delftia PE138, and Stenotrophomonas PE591 from plastic debris found in Cerrado soils, capable of degrading PE of high molecular weight (191,000 g.mol-1) without pre-treatment, and it was observed physical-chemical and structural modification on PE structure induced by biodegradation. In addition, genomic and transcriptomic analyses of the bacteria were performed. The objective of the project presented here is to study the polyethylene degradation metabolism due to the characterization of the effect of oxidoreductase enzymes. For this, two enzymes were chosen, Peroxiredoxin and Coniferyl aldehyde dehydrogenase, selected from genomic and transcriptomic data of Delftia PE138. First, a PCR was performed to obtain the gene sequence of each protein. Afterward, the sequences were inserted into the pET-24a plasmid, which was used to transform the E. coli BL21(DE3). For the expression of the enzymes, the bacteria were cultivated in an auto-induction medium containing lactose. To evaluate protein expression, samples were taken from the culture and analyzed by 15% SDS-PAGE conducted under denaturing conditions. The next steps of the work will be the purification and characterization of the enzymes, which is in process, and the experiments to analyze the effects of the enzymes in plastic degradation. Subsequently, the plastics will be analyzed in the ATR-FTIR device for analysis of the chemical profile of the PE films, to evaluate oxidation, product formation, chain scission, and other chemical changes in the PE structure. And the supernatant from the reaction plastic-protein will be analyzed using the technique of gas chromatography-mass spectrometry (GC-MS) for analysis of plastic degradation products.

A2.R44 Effect of high fat diet on neurochemical and behavioral parameters in zebrafish

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Obesity is a high- prevalence (13% of adult population in 2016), global health concern defined by a high body mass index (BMI). Several comorbidities are associated, including some affecting central nervous system (CNS), i.e. neurodegenerative diseases, cognitive deficit and psychobehavioral disturbs. Zebrafish has raised as a versatile and cheap model widely used to study human diseases, including obesity and neurological diseases. Therefore, our objective is verify the impact of a high-fat diet on the central nervous system (CNS) using well- stablished behavioral tests. Animals was feed according with three dietary groups. The standard diet group (SD) received only 7.5 mg/fish of dry food, while the high-fat diet groups received 5 mg/fish dry food plus 7.5 (HFD-7.5) or 15 mg/fish (HFD-15) of chicken egg yolk. Dietary fat content (w/w) was approximately 6.5%, 16.9% and 21.1%, respectively. After two weeks of diets ingestion, behaviors were assessed. Both HFD groups had obesogenic effects, indicated by increase on BMI, abdominal length and body weight compared with SD group. We show a HFD ingestion induced

aggressive and anxiety-like behavior on zebrafish, as measured by mirror-induced aggression and novel tank diving test, respectively. Also, the higher concentration of HFD (HFD-15) elicited cognitive deficit on inhibitory avoidance test while sociability was unaffected, as determined by the social preference test. Our results are in accordance with evidences in obese human and rodent models, suggesting similar effects of fat intake. Therefore, we highlight the unexplored potential of zebrafish to elucidate this study field.

3. Genética, genômica e evolução (A3)

A3.R45 scRNA-Seq Analysis of Individuals Vaccinated with different SARS-CoV-2 Vaccine plataforms

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The COVID-19 pandemic has been a global public health threat, having killed, according to official reports, 6.3 million individuals and infected approximately another 500 million worldwide. Massive immunization was the most efficient approach to fight off the pandemic and still is crucial in saving lives. Since the beginning of the pandemic, multiple vaccines have been produced, employing different design and development strategies. Although the approved COVID-19 vaccine has shown significant efficacy and safety, it is widely known that various vaccine platforms may display differences in elicitating immune effector responses and protection longevity. Consequently, understanding such differences might elucidate vaccine molecular mechanisms of action and provide valuable immunological insights for developing safer and more efficient vaccines. The main objective of the present study was to evaluate the global differential gene expression and antibody response dynamics of individuals vaccinated with different vaccine platforms through scRNA-Seq. The vaccine platforms considered were: CoronaVac, BNT162b2, and Ad5-nCov. Public databanks, such as the Sequencing Reading Archive (SRA) and Database Resources of the National Genomics Data Center, China, were investigated to gather the intended data. The data are being collected and processed using the 10x genomics platform cell ranger (version 7.0.1) software. The scRNA-Seq data quality control, normalization, dimensionality reduction, and differential gene expression will be executed through Seurat (v4) R programming language package. It is expected to establish specific transcriptional and immunological levels of responses in each vaccine technology analyzed in the present work.

A3.R46 Analysis of miraculin family members in Coffea arabica genomes after interaction with Meloidogyne incognita

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Root-knot nematodes (RKNs) are one of the most important pathogens in coffee crops that severely affected Brazilian production. Meloidogyne incognita is the most aggressive RKN to the coffee crop, even leading to plant death. Chemical control has been showing less efficiency each harvest in controlling RKN. Biotechnological solutions that guide to greener technologies have been required by the most demanding consumer markets. The coffee-RKN interaction at the molecular level leads to the knowledge generation to start the development of these strategies. Gene's relative expression studies in plant-pathogen interaction allow the analyses of genes related to plant host immune response. The aim of this work is to study the gene expression profile of the early interaction between coffee roots and M. incognita. We observed in the corresponding transcriptome generated by this interaction an evident modulation in the expression of genes annotated as members from the Kunitz superfamily

and miraculin-like genes. We performed a phylogenetic analysis of these genes and then compared their differential expression by RT-qPCR. Miraculin genes have shown deregulation when RKN was present at the resistant genotype, demonstrating that miraculin genes are related to RKN early infection. GO enrichment analysis has brought to light some genes that may play an important role in the response to nematode infection. We performed a phylogenetic analysis of these genes and then compared their differential expression by RT-qPCR. Miraculin genes have shown deregulation when M. incognita was infecting the resistant genotype, demonstrating that miraculin genes are related to early infection. Our results may increase the knowledge on the molecular basis of the incompatible interaction to nematodes and allow further studies to develop molecular approaches promoting plant health and resilience to RKN.

A3.R47 Ship: Annotation-based program for identifying Genomic Safe Harbours in eukaryotic model organisms

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Since the beginning of genetic engineering, the insertion of exogenous genes into host cells has presented a substantial challenge. With time and Synthetic Biology advent, the challenge became greater. Especially with the expansion from monogenic to polygenic traits of interest, leading to the necessity of metabolic pathways and entire genetic circuits being inserted into the genome of the target organisms. Targeting inserts to genomic regions allowing their expression without altering endogenous gene expression is a desirable design approach for exogenous genes integration into host cells. Such regions, classified as Genomic Safe Harbours (GSHs), have been identified by viral insertion site, analysis of loss of gene function, or similarity to GSHs from other organisms. Available GSHs are predominantly located in intragenic loci, with high genetic density and, in the human genome close to oncogenes, raising concerns about unstable expression and unpredictable or undesirable phenotypes. Given the ongoing analytical expansion, the necessity of tools for systematic and optimized identification of GSHs became clear. To expand the potential GSHs (pGSHs) available in eukaryotic models, the present work developed SHIP, a computer program to identify pGSHs in intergenic regions. Here, the genomes of 3 model organisms were analyzed, the program identified 6 pGSHs in Saccharomyces cerevisiae, 11 in Mus musculus, and 16 in Homo sapiens. As a chassis organism, we chose yeast for in vivo validation assays. Of the 6 regions, we selected, after extensive manual analysis, three for further evaluation. The correct insertion of the biobricks reporter cassettes occurred only for two pGSHs. Sequencing, cytometry, and stability data revealed promising regions with a high number of cells accumulating the reporter green fluorescent protein, GFP, and stability of the biobricks after ten days of cultivation. Nevertheless, more experiments will be necessary to validate these regions as truly GSHs. SHIP predicts GSHs and is a general-purpose tool to provide an investigative start by reducing the pGSHs that need to be evaluated.

A3.R48 Prospecting for transcriptional markers in Covid-19

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ALU transposable elements are among the most frequent repetitive elements in the human genome. When inserted in gene regions, they act regulating the production of transcript isoforms and in the half-life of these transcripts through different independent mechanisms, such as the interaction of these ALU elements with RNA-binding proteins. These elements form dsRNA that are able to trigger inflammatory processes through double-stranded RNA sensors. The editing of adenine-to-inosine (A-to-I) base RNAs performed by the ADAR protein is one of the main controls of this mechanism by destabilizing the double-strand formed by ALU-containing RNAs, and thus, decreased editing has been shown to increase the inflammatory response in viral diseases, such as in patients with COVID-19

and autoimmune diseases such as inflammatory bowel disease. The study aimed at bioinformatic analysis of monocyte transcripts from patients with mild or severe COVID-19, obtained by the Sequence Read Archive (SRA) database. From the edit analysis, an increase in RNA editing in monocytes of infected patients was identified, unlike the decrease in editing in other leukocytes seen in the literature. The decrease of editing in ALU elements leads to an increase in the antiviral response, on the other hand, other studies have shown multiple mechanisms of action by which the increase in ADAR expression and editing bases also leads to an increase in the inflammatory response from other mechanisms, but with a decrease in antiviral stimuli and the possibility of viral persistence, including activation and stabilization of the transcription factor STAT3, which has inflammatory action and inhibits gamma interferon (INF-γ). Several edits occur in genes related to the inflammatory response, and further studies are needed to verify their mechanisms of action. The editing targets detected in this study may serve to support the development of new interventions for the treatment of severe forms associated with COVID19.

A3.R49 Pre-miRNAs predicted by homology in pink ipê (Handroanthus impetiginosus Mart. ex DC Mattos)

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In the last few years, genetic studies have sought to identify and relate non-coding RNAs (ncRNAs), not to encode proteins but to express regulatory functions. Technological advances, are possible for the increase of ncRNA annotations. In particular, the ncRNAs small class, such as microRNAs (miRNAs), by 2020 reached 149 thousand publications in PubMed. The class of small ncRNAs has the miRNAs according to the miRNAs update criteria, pre-miRNAs <300 nt and mature miRNAs with lengths from 20 to 24 nt are considered true. Recently, the pink ipê (Handroanthus impetiginosus) had your noted genomes, were the first specie of the Bignoniaceae family. The pink ipê is a native species from the Brazilian Cerrado biome (Brazilian Savannah). The prediction of pre-miRNAs, was with input from the genome of the pink ipê available NCBI, total length 503289 Mb (N50=81316 bp), 13206 scaffolds, 13204 contigs, 31688 structures and 35479 messenger RNA transcripts, 13204 sequences wherein <1000 and >558523 nt, were prediction pre-miRNAs using homology-based approach, with the PmiRSelect (https://github.com/DeborahBambil/PmiRSelect). Were predicted 28 pre-miRNAs sequences, at six families of miRNAs in pink ipê, and never copy identifies. Sequences listed with chromosome and position from genome. The highest number 13 pre-miRNAs of miR482 family query match, related to resistance to diseases caused in plants. Following the miR7494 with six pre-miRNAs identified, accession of 8 miRNAs, this characterized by the salinity response The miR408, miR530, and miR1023 also identified two pre-miRNAs, miR408 your role inducing responses to plant nutrient deficiencies, miR530 responds to stress like high temperature and salinity, miR1023 have acted as a defensive role in the invasion of the pathogen. The miR5568 identified only one pre-miRNA, controlling the production of biomass and plant nutrients.

A3.R50 Development and application of scalable bioinformatics pipelines for comprehensive bacterial genomics analyses

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Recent advances in DNA sequencing technologies have reshaped bacterial genomics enabling chromosome-level assemblies at a fraction of cost and time. However, the full realization of this potential relies on computational resources to analyze the data. For this purpose, we have developed three computer pipelines to provide an efficient and standardized way of analyzing genomics data. These pipelines comprehend the quality control of raw sequencing data, genome assembly and evaluation, and extensive gene annotation with graphical reports. In the case of bacteria involved in nosocomial infections, the annotation identifies resistance genes, virulence factors, prophages, and integrative elements. To demonstrate it, we analyzed three MDR Klebsiella pneumoniae samples from the University Hospital of Brasilia. All the isolates were identified as the worldwide threat ST 11, one of the

major carbapenem-resistant Klebsiella pneumoniae (CRKP) lineages. We detected the presence of blaNDM-1, blaCTX-M-15, and blaOXA beta-lactamases genes in all three isolates, as well as several others distributed among chromosomes and plasmids. Moreover, virulence genes frequently related to hypervirulent strains (HvKp), such as Aerobactin, Salmochelin, and Yersiniabactin, have also been detected. Finally, we highlight the assembly of two complete chromosome sequences and the detection of more than ten plasmid incompatibility groups. In conclusion, the results of this work reiterate alerts about the emergence of high-risk pathogens due to the convergence of resistance and virulence genes and reinforce the need for pathogen surveillance programs.

A3.R51 Genotypic characterization of bacterial isolates obtained from the use of pelgipeptins as a selection mechanism: preliminary results

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Antimicrobial-resistant bacteria probably produce similar chemical compounds. Here we characterized bacterial isolates from Cerrado soil resistant to the antibiotic activity of P. elgii promoted by pelgipeptins. The DNA of isolates B7 and C6 were sequenced by Nanopore and Illumina tecnologies. B7 16S rRNA blast indicated greater similarity with K aureofaciens (99.24%). However, their 16S rRNA-based phylogenetic tree formed a unique subclade. The C6 blast indicated greater similarity with M. radiotolerans (99.86%) and their phylogenetic tree formed a subclade with it. The B7 genome draft showed 25 contigs, contamination of 1.32, coverage of 300x, n50 of 761556 bp, L50 equal to 4 and completeness of 99.47. The GC content was 72.8%. The C6 draft presented 24 contigs, contamination of 2.46%, coverage of 400x, n50 of 2073557 bp, L50 equal to 2 and completeness of 100%. The GC content was 70.36%. On the TYGS platform, the closest lineage to B7 is K. xanthocidica (dDDH:37.8%) indicating that it may be a new species. As for C6, it was confirmed to be M. radiotolerans (dDDH:94%). On the JspeciesWS platform, the ANIs were 86.86% (B7) and 97.51% (C6). An ANI <95% for B7 may indicate a species other than the genus Kitasatospora. Regarding secondary metabolism, 49 gene clusters were detected on the Antismash for B7 and 9 for C6. Among them, the NRPS (non-ribosomal peptide synthase) responsible for the production of pelgipeptins in P.elgii, found in B7 and C6. CAMPR4 was used to predict antimicrobial peptides. Isolate B7 presented 59 predictions and C6, 40, all with probability greater than 0.68 (scale from 0 to 1). Drug development demands a process that begins with the discovery and characterization of microorganisms with biotechnological potential. This study completed the whole genome characterization of microorganisms that produce antibacterial compounds, as well as attesting to the efficiency of antimicrobials for species selection with similar important traits.

A3.R52 Silver nanoparticles obtained by green synthesis exhibit antibacterial activity in vitro and enhance the resistance of cabbage to black rot

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Most metallic nanoparticles (MNPs) synthetic routes use traditional methods with the use of potentially toxic reagents that can cause negative impacts, both to the environment and to human health. A safer alternative for the eco-friendly production of MNPs is the use of plant extracts based on Green Chemistry concepts, where less harmful reagents and renewable sources are used. This approach has advantages such as biocompatibility, higher stability, sustainability, shorter reaction time, and cost effectiveness. The present study aimed at the green

synthesis of silver nanoparticles (AgNPs) using aqueous extracts of cabbage leaves, Arabidopsis, neem, and noni, as well as aqueous extracts of parts of the noni fruit (peel or pulp/seed), as reducers and stabilizers agents. AgNPs synthesis reactions were carried out at 6 different concentrations of extracts (10, 15, 20, 25, 30, and 60 mg/mL) in aqueous solutions of silver nitrate (AgNO3) at 1 mM. In total, 42 samples of AgNPs were produced, of which 14 were selected according to their hydrodynamic diameters (HD), polydispersity indexes (PdI), and Zeta potentials (ZP); and then tested in vitro to evaluate their antibacterial activity against Xanthomonas campestris pv. campestris (Xcc). AgNPs synthesized with aqueous extract of noni fruit peel (AENFP) at a concentration of 60 mg/mL, showed the lowest HD and highest antibacterial effect at a final concentration of 64 µM. Furthermore, plants of susceptible genotypes of B. oleracea were treated with AENFP-AgNPs, and positive modulation of defense-related genes was obtained by qRT-PCR. Finally, plants treated with AENFP-AgNPs at 64 µM when challenged with Xcc showed a more tolerant phenotype, highlighting that the application of AgNPs seems to trigger an effective plant defense response. The present study reveals the potential of AgNPs to target antibacterial activity and improve plant defense and finally proposes a promising alternative approach to combat black rot.

A3.R53 Obtaining resistent soybean cultivars from whitefly with interferindo RNA strategy

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The whitefly (Bemisia tabaci) is a cosmopolitan pest that causes damage to several crops, including Soybean (Glycine max (L.) Merrill) one of the most important legumes in the world. Genetic engineering techniques can be used to develop soybean resistant to B. tabaci. Gene silencing via RNA interference (RNAi) is a post-transcriptional effect in which a double-stranded RNA (dsRNA) specifically corresponding to a target gene is introduced into a target organism. Research on RNAi has demonstrated success as a strategy for obtaining pest-resistant plants with greater specificity. The aim of this work was to genetically transform soybeans to express small siRNAs that can be taken up by whiteflies and lead to the silencing of vital genes. A suppression vector was developed involving a DNA fragment with sequences repeated in tandem with homology to a region of the B. tabaci v-ATPase gene, under control of the CaMV35S promoter, the ATAHAS selection marker gene that confers resistance to imazapyr herbicides, and the EPSPS gene that confers tolerance to the ammonium glyphosate herbicide. The vector was used to transform soybean embryos by the biolistic method. To select the transgenic plants, the embryos were maintained in a culture medium containing the herbicide imazapyr. The integration of the transgene into the genome of soybean plants was confirmed by PCR amplifications and lateral flow immunoassay analysis for detection of the EPSPS protein. The vector developed was efficient to transform genetically soybean embryos, as the immunoassays detected the presence of the protein corresponding to the EPSPS marker gene. PCR analyzes confirmed the presence of the transgene with homology to a region of the B. tabaci v-ATPase gene.

A3.R54 FUNCTIONAL ANALYSIS OF GENES POTENTIALLY RELATED TO RESISTANCE OR SUSCEPTIBILITY TO WITCHES' BROOM IN CUPUASSU BY HETEROLOGOUS EXPRESSION IN TOMATO MICRO-TOM

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The comparative analysis of the gene expression of different genotypes of cupuassu, susceptible or resistant to witches' broom disease (WBD), in the early stages of infection by Moniliophthora perniciosa, allowed identifying molecular differences that may help explain the plant defense mechanisms against WBD. In addition to insights into the resistance process, the study generated a list of potentially important genes, including three candidate

genes: the transcription factor TgERF9, the thaumatin-like protein (TgTLP1) and the gene TgPR10.1. Here, we present the functional analysis of these genes by heterologous expression in tomato micro-Tom (MT). The transformed plants, expressing the gene of interest, were challenged with hemibiotrophic phytopathogenic fungi (Fusarium oxysporum fsp lycopersici race 3, Verticillium dahliae race 2) and a necrotrophic one (Sclerotinia sclerotiorum). MT-TgPR10.1 plants were challenged with M. perniciosa. All fungal species were able to colonize plant tissues, either transformed or non-transformed plants. However, despite the darkening into the stem of plants inoculated with verticillium and fusarium, wilt symptoms were not observed, even in a water deficit condition. Furthermore, plant growth and fruit production were not affected by infection. However, plants transformed with TgERF9 and TgTLP1 tended to be smaller and less productive. The detached leaf bioassays indicate that the expression of TgERF9 and TgTLP1 increased the susceptibility of MT to the fungus S. sclerotiorum. MT-TgPR10.1 plants showed moderate resistance to S. Scletoriorum. Expression of this gene did not affect the development of WBD in MT. MT-TgPR10.1 presented a height increase, indicating a change in hormonal balance. More detailed studies, such as measurements of phytohormones concentration, RNAseq and effects on endophytic organisms are necessary to better understand the function of these genes in the process of resistance and susceptibility to diseases.

A3.R55 Development of a serine-integrase based genetic switch system for plant genome editing in Nicotiana benthamiana

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Serine-integrases are a promising biotechnological tool to modulate plant metabolic pathways and regulate gene expression under controlled conditions. Originally present in nature as a mechanism for phage DNA integration into bacterial host genomes, these recombinases can rearrange long sequences of DNA in different ways, as excision or sequential inverting events turning on and off a target gene expression in a reversible and specific manner, according to the manipulation of their recombination sites pairs' sequences, positions and distribution on a synthetic construct. In this work we show the assembly and operation of a memory switch system consisting in the application of four distinct prophage serine-integrases as an input trigger mechanism for the inversion or excision of a target gene stably integrated into the genome of Nicotiana benthamiana. The memory genetic switch is formed by a mGFP gene sequence -silenced due to it being present in an antisense orientation relative to its promoter sequence – flanked by a combination of attachment sites for serine-integrases Bxb1, PhiC31, Int 13 and Int9 and can be divided in two modules: the excision module and the inversion module. The excision module is activated by Bxb1 or phiC31. In this case, the DNA sequence flanked by its attachment sites is excised from the genome. The inversion module is activated by Int9 or Int13. For this module, the activation results in mgfp sequence being flipped to its coding sequence and the switch output is the fluorescent reporter mGFP. Different plasmid delivery strategies for serine-integrase heterologous expression were successfully tested, although yielding varying levels of activation efficiency: leaf tissue agroinfiltration, biolistic system, and PEG-mediated protoplasts transfection. This memory switch system can be used in future genetic circuits to engineer and modulate plant metabolic pathways of economic and environmental importance.

4. Biologia celular e molecular de microrganismos (A4)

A4.R56 Evaluation of the factors that affect the differentiation in muriform cells of the fungus Fonsecaea

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Chromoblastomycosis (CBM) is a chronical fungal infection, classified as a Neglected Tropical Disease. It affects especially the lower limbs of agricultural workers, occurring in all of 5 regions in Brazil. CBM is mainly caused by Fonsecaea spp., including 3 main species: F. pedrosoi, F. monophora e F. nubica, in fact, according to epidemiological studies, F. pedrosoi holds the largest number of cases. Fonsecaea spp are polymorphic fungi: in environment, they are found as mycelium and conidia whereas at the stage of infection in humans they are transformed in muriform cells (MC). Several studies showed different methods to induce the differentiation of Fonsecaea spp. Although, the established protocols requires long periods of incubation (20 – 40 days) to generate MC. Therefore, this work has the objective to evaluate the factors that can affect the differentiation of Fonsecaea spp in MC. Our main goal is to define a protocol for obtaining MC in a shorter time than the previously described in the literature. The clinical isolate F. Nubica, was used in this work. Conidia were incubated on different formulations of minimal media, pH (2.5, 5.0, 7.0), temperature (28, 37oC) and carbon sources (dextrose – 5.5 mM to 55mM and sucrose – 87.6 mM) were tested. After 7 days of growth at 37oC, only the medium with pH 2.5 induced MC. None of formulations with pH 5 and 7 was able to induce MC. The best results were obtained after 7 days of growth in two media: one containing sucrose as carbon source and another containing glucose at 5.5 mM both in pH 2.5. Although both induced MC efficiently, they have different carbon sources, and CM6 better represents the physiological conditions. In conclusion, the experiments showed that acidic condition pH (2.5) is essential for differentiation and to maintain the MC. In addition, our protocol using a modified culture medium formulation was efficient to generate MC in a shorter period than the previous reported protocols.

A4.R57 Effect of transient expression of proteins tyrosine phosphatase from Cotesia flavipes Bracovirus in insect cells

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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 Protein Tyrosine Phosphatase (ptp) viral genes derived from CfBV genome for transient expression in insect cells. The plasmids were transfected into Trichoplusia ni cells (Tn5B) and we checked at 48 hours post transfection for morphological changes and effector caspase activity. Cells undergoing apoptosis were observed in transfections with 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 PTP proteins are sufficient to induce apoptosis in vitro. The functional redundancy within this set of genes correlates well with the presence of conserved phosphatase 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.

A4.R58 expression of the M structural protein of SARS-Cov 2 fused to the C-terminus of the Cry1C protein using the Baculovirus/Insect Cells expression system

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The structural membrane (M) protein of the SARS-CoV-2 coronavirus is a protein involved in viral particle assembly inside infected cells. Thus, this work aimed to express the SARS-CoV-2 M protein fused to the C-terminus of the Cry1C protein for possible use as a diagnostic input.Specific oligonucleotides were used to amplify the M protein gene by PCR. This was used for cloning in the transfer vector that has the 3' region of the Bt cry1C gene (1CT).Recombinant Baculovirus was constructed to infect insect cells. Analysis of recombinant protein expression and expression kinetics was performed by SDS-PAGE and Western-Blot, using Anti-his antibodies and sera from

patients tested positive for SARS-CoV-2. The presence of protein crystals was analyzed by scanning electron microscopy (SEM) and a semi-purification of this protein was performed.The SARS-CoV-2 M protein gene was amplified by PCR and inserted into the genome of a baculovirus successfully fused to the 3' portion of the cry1C gene of Bt. Recombinant virus containing the m_1ct gene was assembled. Insect cells were infected and expression of the recombinant M_1CT protein was confirmed by SDS-PAGE and Western-Blot. Immunoreactivity of the M-1CT protein was confirmed by Western-blot. The expression kinetics of the recombinant protein showed that the protein begins to be expressed from 24/48 h pi, forming stable protein crystals up to 144h.pi The SEM showed crystals of different shapes and sizes The expression of the SARS-CoV M protein -2 fused to the C-terminal region of the Bt Cry1C protein was successfully performed.The recombinant protein was expressed in the form of crystals of different sizes that stabilized it. Thus, it will be possible to use this protein in ELISA-type immunological tests for immunodetection of the SARS-CoV-2 virus, for a possible generation of serological tests and diagnostic kits.

A4.R59 Analysis of the exportome of the asexual stages of Plasmodium falciparum by lysis with Streptolysin O

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Malaria is a neglected disease of paramount epidemiological importance. One of the causative agents, Plasmodium falciparum, causes the severe form of the disease and has been shown to be resistant to antimalarial drugs. P. falciparum is an apicomplexan protozoan whose life cycle is asexual in human erythrocytes. The parasite develops in the red blood cell through four stages, exporting various proteins to the host cell (exportome). This study aimed to analyze this exportome by performing the selective lysis technique with streptolysin O (SLO), a Streptococcus pyogenes toxin capable of causing pores in the erythrocyte membrane and allowing the selective passage of molecules, in order to understand this exportome and discover potential targets for the development of new vaccines and drugs. The parasites were cultivated in a culture medium containing human erythrocytes, synchronized with 5% Sorbitol to obtain newer forms and enrichment of more mature forms with Percoll 63%. Upon reaching high parasitemia of more mature forms, the infected red blood cells were lysed using SLO and three important fractions were obtained: parasitic, erythrocyte membrane and cytoplasmic fractions. The fractions were individually extracted and digested and later analyzed in the mass spectrometer (MS). The samples were submitted to nanoelectrospray ionization MS and the data analyzed in software for protein identification and quantification. The study analyzed three samples of the membrane fraction (triplicate). The samples showed 45 human proteins and 355 P. falciparum proteins. Analyses were performed using Gene Ontology (GO) terms regarding biological processes, molecular functions and cellular composition performed by proteins. Data revealed a series of modifications induced to the host cell and the complex metabolism of the parasite in the asexual stages, having already been observed in previous studies.

A4.R60 Molecular cloning and expression of arboviral proteases using the baculovirus system and insect cells

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The Yellow Fever virus (family Flaviviridae), Chikungunya and Mayaro virus (family Togaviridae), are human viruses transmitted by arthropods (arboviruses), which cause morbidity and mortality, especially in tropical developing countries. Their RNA genomes encode both structural and non-structural proteins, important for replication and assembly of new viral particles, producing a single polyprotein that must be cleaved to produce individual mature proteins. This function is performed by proteases NS2A-NS2B (Flaviviridae) and nsP2 (Togaviridae) that have been explored as targets for the development of antiviral treatments. This work aimed to produce arbovirus proteases in insect cells using baculovirus as an expression vector. The NS2A-NS2B and nsP2 protease genes were amplified by PCR and cloned into the plasmid vector pFastBac1. This plasmid was then used for the construction of

recombinant baculoviruses through the Bac-to-Bac® system. The recombinant viruses were used to infect insect cells IPLB-Sf21-AE (Sf21) in culture and the expression of the recombinant proteins was analyzed at different hours post infection (h, p.i.) by polyacrylamide gel electrophoresis (SDS-PAGE) and confirmed by immunodetection with specific antibody by Western blot. The molecular cloning of the Yellow Fever virus NS2B-NS3 genes and the Chikungunya and Mayaro virus nsP2 genes in vector plasmids was confirmed through restriction analysis and sequencing. The construction of the recombinant baculoviruses was verified by PCR and the expression of the Chikungunya virus protease, with the expected molecular mass, was confirmed by SDS-PAGE and Western blot. The project will continue with the objective of completing the expressions of viral proteases and use the cell extract for purification. These proteins may then be used in the future as a platform for carrying out in vitro assays to assess the inhibitory potential of molecules with antiviral activity against these viral proteases.

A4.R61 Molecular identification and evaluation of the ability to produce muriform cells in vitro of clinical isolates of Fonsecaea sp.

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Chromoblastomycosis is a Neglected Tropical Disease (NTD) according to the World Health Organization (WHO), which is caused by filamentous dematiaceous fungi. Among the causative agents, Fonsecaea spp. is the main genus and Fonsecaea pedrosoi is the most common species responsible for the infections. The members of Fonsecaea spp. are polymorphic and share high similarity in morphological characteristics, which can lead to difficulties in identification during diagnosis, since the microscopic evaluation of the morphology is the mostly used method, making the molecular methods required for greater accuracy. The distinction of the species is important for epidemiological analyses and can direct the patient therapy, considering that variations of the species can generate different disease features. This work aimed to identify, through molecular methods, 16 clinical isolates from Brazil which were morphologically defined as F. pedrosoi: 5 from LACEN-DF, 3 from UFPR, 1 from UFMG, 2 from Fiocruz-RJ and 5 from Instituto Adolfo Lutz-SP. We analyzed one of the most important virulence factors of Fonsecaea spp., which is the ability of the conidia to differentiate in muriform cells — cells that are considered the pathognomonic feature of the disease when found in the patient tissue, being large, spherical, highly pigmented, with a thick cell wall and the presence of septa. After sequencing two loci, ITS and CDC42, it was concluded that 37.5% (6 isolates) were not F. pedrosoi, from those, four were Fonsecaea monophora, two Fonsecaea nubica, and one Rhinocladiella aquaspersa. In addition, all isolates showed, in vitro, structural changes consistent with the characteristics of muriform cells usually visualized in infected tissue, however, they differed in shape and size among the isolates analyzed. Furthermore, other aspects of the isolates were also examined by observation of the conidiophores structures and the growth rate of each isolate.

A4.R62 Development of a prophylactic Vaccine against Chagas disease.

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Chagas disease (CD), caused by the protozoan Trypanosoma cruzi, is among the neglected tropical diseases recognized by the World Health Organization. The most recent data suggest that between 6 and 7 million people are infected, mainly in Latin American countries, where the disease has a great impact on national economies and public health systems. With regard to treatment, nifurimox and benznidazole are the only chemotherapeutics currently available to treat T. cruzi infection. However, this medication does not have significant effects in the later stage of the disease, where most cases are diagnosed, in addition to causing several side effects and genotoxicity, resulting in discontinuation of treatment by the patient. Among the molecules of interest for the development of a

vaccine for CD, proteases are a promising group because they are involved in several important biological processes such as the physiological maintenance of the cell, regulation and signaling of important cellular aspects. One of the ways to combat CD is the development of prophylactic treatments. Recombinant protein vaccines are a very promising form of the vaccine industry designed to protect against infectious and chronic diseases. Current vaccine approaches consist of inserting sets of isolated natural or recombinant antigens to induce an immune response. In this follow-up, this project proposes the development of a vaccine composed of a cocktail of the proteases Prolyl oligopeptidase (POP), Thimet oligopeptidase (THOP) and Oligopeptidase B (OPB), previously validated as antigenic potential, and to evaluate its protective effect in an experimental murine model of CD.

A4.R63 Application of alpha-amylases in cotton textile processing

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The project aims production, characterization and application of enzymes in cotton textile processing. De-sizing applied to remove gum from fabrics is one of the processing steps, and is used to allow an efficient dying in textile finishing processes. Amylases are widely applied in de-sizing steps. An alpha-amylase from Paenibacillus barengoltzii, AMYPb, heterologous expressed in Escherichia coli DE-3 was biochemically characterized and will be further applied in processing of cotton textile. In addition, a comparison of enzymatic activity and catalytic efficiency of AMYPb with commercial alpha-amylases will be performed.

A4.R64 CHARACTERIZATION OF EXOPOLYSSACHARIDES OBTAINED FROM AURICULARIA AURICULA AND ITS ABILITY TO MODULATE MACROPHAGE'S CYTOKINE PRODUCTION

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Introduction: The concept of innate immune memory rose upon the observation that certain stimuli, either homologous or heterologous, could provide an enhance immune response of innate cells after a second infectious signal. Further studies provided more information of how this enhancement occurred not only increasing mechanisms of response of these cells towards the infection but it also provided some insights on how the previous stimuli could alter the cell response through metabolic change and epigenetic reprogramming. In the trained immunity scenario, β-glucans obtained from yeasts, such as Candida albicans, presented protective effects against a secondary infection. These glucose polymers are major components of yeast and mushrooms cell walls and they have played important role in immunomodulation, mostly up-regulating immune responses. Their highly complex structures are responsible for their various mechanisms of action, like the binding ability of (1⟶3)-β-glucans with dectin-1 receptor. Therefore, we aimed to evaluate whether these glucans were present in Auricularia auricula exopolysaccharides and if they could provide innate immune training. Methods and Results: Auricularia auricula exopolysaccharides were obtained from mycelial submerged culture in potato dextrose broth followed either by 24 hours stress in milliQ-water or by alkaline mycelial extraction. The obtained exopolysaccharides were fractioned based in its water-solubility and analysed in 13C-NMR in DMSO-d6 at 343 K (nuclear magnetic resonance), verifying the presence of (1⟶3)-β-glucan in the water-insoluble fraction. Bone-marrow macrophages obtained from c57BL6/J mice wild type, Dectin-1 and TLR4 Knock-out mice were treated 100-12,5µg/ml of the water-insoluble fraction of A. auricula for 24 hours. After 2, 12 and 24 hours of the interaction course, the supernatant was collected for cytokine analysis. Conclusion: In this study we observed that Auricularia auricula

exopolysaccharides contained (1⟶3)-β-glucan, and accordingly with previous results they showed their capability of modulating the cytokine production capability of bone-marrow macrophages.

A4.R65 Immunomodulation of exopolysaccharides from Auricularia auricula in an inflammatory model of colitis

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Introduction: In Asian countries, Auricularia auricula has been a well know medicinal and edible fungi for the last 2000 years. In highlight are its antioxidant, anticancer, and immunomodulatory proprieties, which are associated with the B-glucans found in the cell wall of this basidiomycete. There are several types of B-glucans depending on the size and type of their branches, the type most present in A. auricula is the (1-3), which is a shorter chain and is mainly recognized by Dectin 1, a pattern recognition receptor (PRR) that has a crucial dual role in the gut, in recognition of pathogen-associated molecular pattern (PAMPs). Colitis is an inflammatory disease, often without a specific cause, that affects the colon, causing diarrhea and bleeding in more severe cases. That disturbance in the colon can also cause dysbiosis. Methods and Results: The B-glucan was extracted and purified from Auricularia auricula grown in Potato Dextrose broth for seven days and stressed in ultrapure sterile water for 24 hours. The supernatant was freeze-dried, resuspended in ultrapure water, and centrifugated to separate the soluble and insoluble fractions. C57/BL6 mice and Dectin 1 knock-outs with the same background were separated into four groups with four animals; Control; DSS, which received 2% dextran sulfate sodium water ad libidum; B-glucan, received 100ug the purified exopolysaccharide by forced oral administration; Treatment group, received the dextran sulfate water and the B-glucan. Weight measurements and animal feces were collected daily to measure the disease progression and microbiota changes. On the eleventh day of the experiment, the animals were euthanized, and parts of the intestine localized after the cecum were sectioned and used for tissue histopathology, RNA extraction for qPCR, and cytokine quantification. All experiments, including animals, were made accordingly with CEUA Protocol nº 18/2019. Conclusion: Groups treated with B-glucan showed modulation of the immune response, and visually there is an inflammatory attenuation on the state of the intestine in the wild-type mice.

A4.R66 Evaluation of the ecological and biotechnological importance of 10 Latescibacterota MAGs recovered from Amazon Reef sponges' microbiome

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The Amazon Reef is an extensive biogenic reef characterized by the leakage of water and sediments from the Amazon River, creating a plume layer that impacts physical-chemical parameters and living beings. The place is the habitat of an impressive number of species, including sponges. Sponges and their associated organisms have ecological and biotechnological relevance due to their participation in biological processes and the production of bioactive compounds. Considering this, a bioinformatical analysis of genetic and metabolic features of 10 metagenomic-assembled genomes (MAG) recovered from Amazon Reef sponges' microbiome was executed to determine their potential contributions to the ecosystem and the host, as well as biotechnological ways to use them. These MAGs were selected based on preliminary taxonomic classification. All MAGs presented quality between average and high. Besides, they were assigned to the same candidate phylum (Latescibacterota) and class (UBA2968). Metabolic reconstruction demonstrated the presence of heterotrophy-related genes. The identification of genes involved in biogeochemical cycles and bioremediation pathways indicated possible utilities to the reef. Genes connected to the synthesis of valuable products, heavy metal detox, and chemical defense were also found, showing plausible advantages to the host. From a biotechnological perspective, MAGs have some sequences linked to secondary metabolite production, industrial enzymes, and biologically active peptides. Therefore, these

microorganisms showed possible benefits for the sponges and the reef while demonstrating different means to explore them biotechnologically.

A4.R67 Effects of Ap4, a peptide isolated from Acanthoscurria paulensis venom, on the conductance of voltage-gated potassium channels

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Ionic channels are transmembrane proteins that have properties such as recognition and selection of specific ions, opening and closing movement mediated by response to electrical, chemical or mechanical signals and the conduction of ions through the membrane. Potassium channels act in the regulation of cellular excitability, as determining the temporal course and the shape of action potentials, impacting neuron-mark activities and fulfilling the role of modulating action potentials regulating, consequently, the release of the neurotransmitters. The biochemical composition of the venom of the Theraphosidae spiders is characterized by the presence of low molecular mass compounds, being free amino acids, ions, organic acids, nucleotides and nucleosides, and of peptides and proteins. Among the peptides, there are compounds that have pharmacological interest due to interaction with voltage-dependent ionic channels found in the heart, skeletal muscles and central and peripheral nerve systems. In this project, we will purify the peptide Ap4 from the venom of the Theraphosidae spider Acanthoscurria paulensis aiming to evaluate its modulation of the potassium channels. The venom is extracted by electrostimulation of the telson and is fractionated by reversed-phase liquid cromatography (RP-HPLC). The fractions of interest are submitted to additional RP-HPLC until Ap4 is purified. The effects of Ap4 on Kv-channels will be evaluated by patch-clamp. To date, 9.85 ug of Ap4 have been purified by HPLC. For the next steps, we will perform a HEK cell culture that expresses Ionic Kv channels followed by electrophysiological tests.

A4.R68 Heterologous expression of antibody fragments anchored to the cell wall of Saccharomyces boulardii

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Inflammatory Bowel Diseases are chronic pathologies such as Crohn's Disease and Ulcerative Colitis. Ulcerative Colitis causes recurrent inflammation of the gastrointestinal mucosa, available treatments induce remission by promoting an anti-inflammatory status in patients. Drugs used in clinical patients hold extremely high cost of production, cause severe side effects and are not efficient for all patients. Saccharomyces boulardii is a probiotic yeast used in the clinic for the treatment of intestinal dysfunctions, probiotics have shown great benefits for those afflicted by chronic gut inflammation, and studies show excellent capability to synthesis and deliver oral therapies. Thus, S. boulardii could be a delivery tool of neutralizing antibodies to the gastrointestinal tract, helping repair gut homeostasis by developing an immune regulation in the site of inflammation, for the treatment of different diseases such as Ulcerative Colitis. The anti-inflammatory effects of an anti-CD3 recombinant molecule expressed anchored to the cell wall of the S. boulardii yeast were assessed in the murine model of gut inflammation induced by Dextran Sodium Sulfate. Key words: Saccharomyces boulardii; anti-CD3; neutralizing antibodies; Ulcerative Colitis.

A4.R69 Analysis of metagenome-assembled genomes from a lignin-enriched microbial community

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The current concern for more ecologically sustainable processes led to a demand for studies on the valuation of a material that commonly has its potential wasted, in this case the lignin present in plant biomass. The enzymatic recalcitrance caused by the complex and amorphous structure ends up being one of the limiting steps in the lignin recovery process and, therefore, this compound is usually burned to generate energy in the industries themselves. Despite this, microorganisms such as fungi and bacteria are known to be able to circumvent this recalcitrance through their enzymes, and bacteria are still a recent branch of research and represent several advantages over

fungi. In this study, the microbial diversity of three consortia enriched for microorganisms capable of degrading lignin with a focus on bacteria was analyzed. The consortiums were obtained from three types of soil, two commercial soils and one garden compost soil, cultivated at 30°C and enriched through successive passages in a culture medium in which the lignin extracted by the alkaline method was used as the only carbon source. The metagenomic DNA of the third pass of the enrichment was extracted, sequencing it and generating 232 assembled genomes, selecting 39 after quality criteria of completeness of at least 70% and contamination less than or equal to 10%. Of these 39 genomes, the most abundant phyla in the three consortia were proteobacteria, bacteroidetes and actinobacteria, with only two genomes having species taxonomy. The consortia presented functions consistent with the place of isolation, in this case the soil, in addition to possible metabolisms related to the degradation of lignin. 4 genomes were chosen based on taxonomy, for analyzes of lignin degradation focused on monolignols. The genomes of Actinobacteria BY 70_11 and 2_71_9; Alphaproteobacteria MG 62_16 and 3_66_13, in addition to not representing the same species, showed different metabolic pathways related to the degradation of monolignols, with Actinobacteria BY 70_11 having the highest number of associated genes, unfortunately, it is not possible to confirm the ability or not to degrade the lignin of these 4 genomes due to the lack of literature on these specific pathways. This work proved to be an initial step for others to be carried out on lignin degradation with the same genomes with the expansion of metabolic pathways and with more genomes for deeper analyzes of lignin metabolism.

A4.R70 Prospection of genes involved in biotic stress responses in the wild diploid species Arachis stenosperma and Musa acuminata Calcutta 4

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The plant defense system involves numerous receptor proteins, with the perception of an invasion signal by pathogens occurring in the host plasma membrane or cytoplasm. The two lines of plant defense, triggered by molecular patterns associated with pathogens (PTI) and by the secretion of pathogen effectors (ETI), both activate metabolic pathways that culminate in the production of several factors related to the defense response, such as production of secondary metabolites, an oxidative burst, transcription factor activation and hormonal regulation. The characterization of genes involved in defense responses is often complex, with the mechanisms of action by which these are activated and deactivated during interaction with the pathogen still unclear. In addition, many genes potentially involved in PTI and ETI plant immune responses possess similar domains and conserved structures, such that precise molecular biology and bioinformatics tools are required for accurate assembly of such target genes and their regulatory elements in these often-complex loci in germplasm of wild plants. The aim of this study is validated candidate defense genes from sequences in libraries enriched for these in banana (Musa spp.) and peanut (Arachis spp.) wild resistant genotypes. Both cultures have high nutritional value, play a fundamental role in food security and, in commercial cultivars, are affected by a wide range of pathogens that cause serious losses in productivity. Candidate genes were selected based on predicted function, based on differential expression data from transcriptome analyses during the host-pathogen interactions, as well as following RT-qPCR expression validation. Subsequently, genes are being tested for molecular and phenotypic effects in vivo using an Arabidopsis thaliana plant model system. Candidate genes identified in resistant M. acuminata Calcutta 4 (PTI genes: Endochitinase and SNF) are currently being use for transformation of A. thaliana, and validation in planta for function through over-expression and phenotype analysis during artificial pathogen infection. Candidate genes from resistant Arachis stenosperma are currently being validated through overexpression in A. thaliana and challenge with the vascular wilt pathogen Fusarium oxysporum f. sp. conglutinans. A. thaliana plants overexpressing the AsTIR19 gene showed a significant reduction in disease progression when compared to wild-type plants. Analysis of such candidate genes involved in plant immunity will

further our understanding of the adaptive response in Musa and Arachis and guide application in plant breeding for disease control.

A4.R71 The role of melanin and laccase 1 from Cryptococcus neoformans in inflammation and death of human macrophages

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Cryptococcosis is an infectious disease caused by fungi from the genus Cryptococcus, the infection with C. neoformans being the most common and globally disseminated. It is estimated that 152 thousand new cases of cryptococcosis appear annually, of which 73% copes with patient decease C. neoformans produces melanin and a capsule that are probably the most important virulence factors of the pathogen. Melanin production is possible due to the presence of the enzyme laccase 1(LAC1) in the fungal cell wall and facilitates infection and evasion from the host immune response. Capsule from C. neoformans was demonstrated as an important factor in inflammation and death of host cells, however, the role of the melanin and LAC1 in this context was not explored yet. Therefore, in this work we investigate whether melanin and LAC1 interfere with the production of inflammatory mediators by human macrophages and, consequently, their death. The presence of melanin and absence of LAC1 caused less yeast phagocytosis by macrophages. Moreover, C. neoformans is able to trigger cell death, although absence of LAC1 abolished this process. Investigating types of deaths caused by C. neoformans, the presence of melanin and the lack of LAC1 promotes less apoptosis after 5 hours post infection (h.p.i), still all types of yeast stimulate apoptosis compared to uninfected cells. After 24 h.p.i, lytic death is predominant in all conditions of infected macrophages. Evaluating proteins related to inflammasome formation, C. neoformans increases expression of sensors such as NLRP3 and AIM2, as well as inflammasome adapter ASC and caspases activators of death (CASP1 and CASP8). However, melanin and lack of LAC1 promotes CASP8 cleavage. Some products of these caspases, like Gasdermin D and IL-1β appears to be cleaved and activated only in the presence of melanin. Thus, melanin from C. neoformans is likely to promote more pyroptotic cell death and production of inflammatory mediators by human macrophages.

A4.R72 Evaluation of lactic acid production under aerobic cultivation by an Mpc1-deficient strain of Komagataella phaffii

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One of the main challenges during lactic acid production by microbial fermentation is to minimize competitive metabolic routes that lead to the formation of by-products to enhance the overall product yield. In lactate-producing strains of Komagataella phaffii developed by our group, expressing a lactate dehydrogenase (LDH) and the disruption of the pyruvate decarboxylase (PDC) has been shown to enable the conversion of glycerol to L-lactate. However, arabitol was a persistent by-product with the disruption of the arabitol dehydrogenase (ArdH) gene. The metabolism of glycerol in K. phaffii under oxygen-limited cultivation results in a surplus of one molecule of NADH which may lead to arabitol production. The aim of this study is to evaluate the paradigm of lactate production in a lactate-producing strain of K. phaffii under aerobic conditions. Since K. phaffii is an obligate aerobe and Crabtree-negative yeast, biomass is the main product under respiratory metabolism. For this reason, the disruption of the subunit 1 of the mitochondrial pyruvate carrier (Mpc1) located in the inner membrane of

mitochondria was used to evaluate the feasibility of this strategy in an obligate aerobic yeast. Mutant strains of S. cerevisiae with the knock-out MPC genes resulted in poor growth in a defined synthetic medium. For this reason, both the wildtype (X-33) and an mpc1-deficient (XLm: X-33 Δmpc1) strain were cultured in both synthetic media (YNB without amino acids) with and without amino acid supplementation and in rich media (YPD). The previously constructed strain GLp (GS115: BtLDH+ Δpdc1) was selected for MPC1 disruption, resulting the GLpm strain (GS115: BtLDH+ Δpdc1/Δmpc1). In flask cultivations, GLp and GLpm strains presented distinct growth curves in synthetic media. Also, the specific growth rate of GLpm was lower than the GLp, showing that the growth was affected by the distuption of MPC1. Conversely, in batch cultivation, the titer of lactic acid production was the same for both strains.

A4.R73 Use of integrases for the precise and efficient control of gene expression in the yeast Saccharomyces cerevisiae

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Synthetic circuits, a recent product of the evolution of synthetic biology, can be used to modulate gene expression in a very precise way, including the control of specific genes involved in a metabolic pathway. The utilization of molecular tools such as integrases for the construction of genetic circuits based on Boolean logic is already being carried out in bacteria, plants and mammalian cells. Recently, 11 orthogonal integrases have been characterized and there is no report on their use in the yeast S. cerevisiae so far. Previous work in our lab has shown that integrases 4 and 8 can be controlled under the control of a promoter responsive to galactose (pGal1/Gal10). Integrase 4 was able to activate he genetic switch, unlike integrase 8. In this work we tested integrase 13 (Bacillus cytotoxicus NVH 391-98). We have shown that integrases 4 and 13 were functional in S.cerevisiae, paving the way for their utilization as a new molecular tool for the development and construction of genetic circuits in yeasts.

A4.R74 Occurrence of two unreported viruses infecting cultivated and wild Passiflora spp. in Brazil

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Diverse virus-like symptoms were observed in Passiflora accesses (Passiflora spp.) in the Germplasm Bank "Flor da Paixão" situated at Embrapa Cerrados, Distrito Federal, and in cultivated passion fruit in several regions of Brazil. Ensuing the recent reports of viruses infecting passion fruit crops in the world, and the same viruses identified in Brazil infecting other crops, we speculated the occurrence of two viruses (virus PF1 and PF2) could be present in passion fruit crops in Brazil. We confirmed by RT-PCR essays and Sanger sequencing the presence of PF1 and PF2 in several Passiflora spp. A total of 114 surveyed plants were collected in multiple locations from 2016 to 2021, and PF1 was identified in 4 of 19 cultivated P. edulis plants from a commercial field in Distrito Federal. Moreover, while PF1 was detected in 4 of the 55 Passiflora accessions sampled at the BAG-FP, the PF2 was identified in 14 of these 55 Passiflora spp. accessions. We also identified co-infection of these two viruses in Passiflora species

from BAG-FP. This is the first record of PF1 in P. edulis in Brazil, and PF2 and PF1 in other wild Passiflora species in the world. Our results warrant further studies to evaluate these viruses' epidemiology, impacts, and interactions in the passion fruit crop.

A4.R75 Discovery of novel Thogotovirus (family Orthomyxoviridae) and the functional analysis of their role in the emergence of baculovirus GP64 envelope protein

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The genus Thogotovirus (Family Orthomyxoviridae) is composed of viruses that infect mainly arthropods, having been detected and isolated from ticks, mosquitoes, and other insects, while some can infect mammals. Registered cases of Thogotoviruses causing severe disease and death in humans also raise very relevant public health concerns. The enveloped viral particle contains a genome of 6 or 7 segments of single-stranded negative-sense RNA (ssRNA -), with segment 4 usually encoding the viral envelope glycoprotein (GP), responsible for cell attachment and entry. Interestingly, Thogotovirus GP bears very little amino acid identity to the GPs of other members of the Orthomyxoviridae family (e.g., hemagglutinin of influenza virus); instead, the most similar protein to Thogotovirus GPs is the GP64 envelope protein of the very evolutionary distant baculoviruses. The Baculoviridae is a family of arthropod-infecting viruses with large circular double-stranded DNA (dsDNA) genomes, with GP64 being essential for viral entry in cells. The most recently evolved group in the family (Group I in the Alphabaculovirus genus) is the only one to have the GP64 glycoprotein, with phylogenetic data indicating that this protein was acquired via horizontal gene transfer from a thogotovirus ancestor. In this work, we characterized the genome and constructed the phylogeny of two novel thogotoviruses, one sequenced from the western honey bee (Apis mielifera) and another assembled from an RNAseq database of the lepidopteran Melitaea didyma. The gene coding for the GP from the honey bee thogotovirus was synthesized and used to construct recombinant baculoviruses to verify the ability of the thogotovirus GP to rescue the infectivity of a baculovirus lacking the gp64 gene. Through infection and cell fusion assays, it was possible to observe the ability of the thogotovirus GP to function as a fusion protein during baculovirus infection, further corroborating the probable evolutionary origins of GP64.

A4.R76 CELLULAR AND MITOCHONDRIAL METABOLISM OF MACROPHAGES INFECTED WITH DORMANT CRYPTOCOCCUS NEOFORMANS

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Dormancy is an adaptation present in a wide range of cells and organisms, in which cells reduce their metabolism to stay alive under stressful conditions. It occurs with Cryptococcus neoformans (Cn), a fungus that causes cryptococcal meningitis in immunocompromised patients. Considering our previous results, in which different growth stages of this fungus induce a distinct gene expression profile in infected murine bone marrow-derived macrophages (BMDM), here we analyzed the influence of dormant cells of C. neoformans (DCn) in the metabolism of infected BMDM. We infected BMDM with either DCn, Cn in the stationary growth stage (Stat or ura5Δ - auxotrophic

uracil strain), and heat-killed + 1% Stat (HK) for 24h. Then, we analyzed mitochondrial metabolism by flow cytometry and oxymetry. Stat induced mitochondrial depolarization; All infections induced a higher proton leak and extracellular acidification rate, indicating an increase in glycolysis; HK induced higher basal respiration and ATP production; Stat induced higher non-mitochondrial respiration. Otherwise, infection with Stat fungus prevented the increased glucose uptake induced by pre-treatment with LPS and IFN-γ. Moreover, cells pre-treated and infected with DCn increased their fatty acid uptake. Avoidance of metabolic changes is in line with the stealth strategy used by Cn during infection and it should be mobilizing lipids as an alternative fuel source to permit their reactivation and proliferation inside the phagolysosome.

A4.R77 In vitro screening of selected plant extracts from Cerrado against Chikungunya virus

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Chikungunya virus (CHIKV) is an arbovirus transmitted through the bite of a female mosquito from the genus Aedes. CHIKV belongs to the Togaviridae family and the Alphavirus genus, and clinically, the disease caused by CHIKV is like dengue fever, characterized by fever, headache, and arthralgia. Due to frequent disease outbreaks and epidemics, CHIKV has a significant impact on public health in many Neotropical countries. The use of plant-based compounds that affect the infection cycles of these arboviruses has been proposed as a promising therapeutic strategy. Secondary metabolism products from various plant species have shown antiviral activity against several viruses. Examples of these metabolites are alkaloids, saponins, flavonoids, and coumarins. However, besides having high antiviral activity, metabolites also need to have low cytotoxicity. Among the metabolites with antiviral activity mentioned in the literature, flavonoids stand out. Eugenia dysenterica (ED), Myrtaceae family, and Erythroxylum suberosum (ES), Erythroxylaceae family, are medium-sized Cerrado trees rich in flavonoids. Leaf infusions are used in traditional medicine as antirheumatic, antiasthmatic, diuretic, anti-inflammatory, and antimicrobial agentsy. To evaluate the potential of the leaf's ethanolic extracts against CHIKV, preliminary analyses were carried out in vitro. The cytotoxicity profile of two leaf ethanolic extracts on Vero cells was determined by lysosomal viability analysis using the neutral red assay, while the antiviral potential was determined by plaque assay at different treatment steps. Our cytotoxicity assays showed that ED and ES extracts showed concentrations toxic to 50% (CC50) of the cells at 10,461 and 1,623 mg/mL, respectively, at 48 h post-treatment. Our antiviral assay revealed that ED ethanolic extract inhibited 69.4% of CHIKV activity at 80 µg/mL concentration, while ES inhibited more than 79% at 40 µg/mL concentration at the post-treatment step. ED ethanolic extract inhibited 85.4% of CHIKV activity at 100 µg/mL concentration at the pre-treatment step. Ultimately, the ED ethanolic extract inhibited 59% of the CHIKV activity at 80 µg/mL concentration, while the ES inhibited 96.8% at 10 µg/mL concentration at the co-treatment step. As with many natural products, Eugenia dysenterica and Erythroxylum suberosum are potential sources of antiviral compounds for drug development against CHIKV infections.

A4.R78 Characterization of microRNAs in Musa acuminata induced during interaction with Pseudocercospora musae

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Endogenous microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level by cleavage or repression of mRNA translation. MiRNAs in plants regulate diverse cellular

processes, including defense responses to biotic stresses. Banana (Musa spp.), a monocotyledonous crop cultivated throughout tropical regions, is susceptible to numerous diseases due to sterility and a narrow genetic background. Pseudocercospora musae, the causal agent of Sigatoka leaf spot disease, is an important fungal pathogen of banana, causing losses due to reduction in functional leaf area. Here, leaf RNA samples were extracted from Musa acuminata subsp. burmannicoides, var. Calcutta 4 (resistant), at 3 and 12 days after inoculation (DAI) with conidiospores. Following small RNA library construction, samples were sequenced using Illumina HiSeq 2500 technology. High quality sequences were mapped against the M. acuminata ssp. malaccensis var. Pahang reference genome and plant miRNAs were predicted using the programs Mireap and ShortStack. A total of 228 conserved miRNAs belonging to 30 miR-families were identified, together with 22 predicted novel miRNAs. At 3DAI, 48 miRNAs from 25 miR-families plus two novel miRNAs were significantly differentially expressed between inoculated and control samples. At 12DAI, 31 miRNAs from 18 different miR-families and two novel miRNAs regulated after inoculation. Potential host gene targets of miRNAs were predicted using TargetFinder. RT-qPCR validation of expression was conducted for 11 miRNAs and 8 target genes. Inverse expression profiles were observed for 4 combinations. The characterization of miRNAs in M. acuminata and their role in gene expression modulation during interaction with P. musae provides resources for the development of efficient methods for control of Sigatoka leaf spot disease.

A4.R79 Use of chicken eggshell (EM) membrane for the treatment of osteoporosis in ovariectomized Wistar rats (Rattus norvegicus).

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Introduction: Osteoporosis is considered a worldwide public health problem. It is estimated that 200 million people worldwide are affected by this condition. The incidence of osteoporosis is higher among postmenopausal women, whose estrogen levels are naturally reduced. The chicken eggshell membrane - EM (eggshell membrane) has been shown to be effective in relieving pain and joint stiffness. The eggshell is a rich source of calcium, and with potential industrial raw material for use in applications such as bone metabolism. This study aims to evaluate the efficacy of EM for the treatment of osteoporosis in ovariectomized Wistar rats (Rattus norvegicus), which may contribute to future therapeutic application. Methodology: In this study, an EM nanoparticle will be developed. Female wistar (Rattus norvegicus) will be used. The treatment will consist of carrying out the treatment by intragastric gavage for 40 days (1x/day), with the following compounds: serum (200 µL), and eggshell membrane nanoparticles (NEM) (20 mg/mL/day). The animals will be divided into 6 groups: G1 - Control Group (saline solution); G2 - Control group with osteoporosis induced by inflammation (Dexamethasone) (NEM); G3 - Control group with induced osteoporosis by menopause (Ovariectomy) (saline solution); G4 - Group without induction of osteoporosis (NEM); G5 - Group with osteoporosis induced by inflammation (Dexamethasone) (NEM); G6 - Group with induced osteoporosis by menopause (Ovariectomy) (NEM). In all experimental groups, Bone Mineral Density Analysis and computed microtomography will be performed. After 60 days, the animals will be euthanized and blood and bone tissue will be collected (for hematological, biochemical and histopathological analysis). Expected results: It is expected that, with this study, by using the formulation developed in a murine animal model, it will be possible to establish evidence related to the efficacy in the treatment of osteoporosis.

A4.R80 EXTRACELLULAR VESICLES SECRETED BY Trichoderma harzianum DURING INTERACTION WITH Sclerotinia sclerotiorum.

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Extracellular vesicles (EVs) are membrane particles released by cells into their environment and are considered key players in intercellular and intracellular communication. The fungus Trichoderma harzianum is used in biological control mediated with the process of mycoparasitism against species of fungi Sclerotinia sclerotiorum,

Fusarium solani, Fusarium oxysporum, Rhizoctonia solani. For a better understanding of the Trichoderma host interaction mechanisms, in the present work, analyzes of the EVs secreted by the fungus T. harzianum TR274 during cultivation in minimal medium and with glucose and cell wall of S. sclerotiorum were carried out. First, cultures of the fungus T. harzianum TR274 were carried out with minimal medium and glucose at 24, 48 and 72 hours, as well as cultivation with autoclaved mycelium of S. sclerotiorum at the respective times. The purification processes were carried out by ultracentrifugation and the characterization through the NTA technique. Analyzes of purified vesicle proteins were analyzed by SDS-PAGE gel and processed in the LTQ-Orbitrap Elite. 100 proteins were identified. Histone acetylation categories, fungal cell wall degradation enzymes, were found. This was the first study of extracellular vesicles purified from the fungus T. harzianum TR274 and we observed that proteins related to the process of interaction with fungal hosts are carried by the vesicles during their development.

A4.R81 Enzimatic and transcriptomic analysis of Trichoderma reesei and Aspergillus brasiliensis co-cultures in relation to enzymatic degradation of sugarcane bagasse

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Agro-industrial waste is a major pollutant in countries that are based on agriculture economic matrices. Numerous filamentous fungi offer an important source of holocellulases for application in biorefineries for degradation of lignocellulosic plant cell walls. Although in nature fungi are encountered as complexes of multiple species living together, in biorefineries their application is still predominantly based on monoculture systems. Co-cultures systems have now been described that amplify the variety of enzymes produced, leading to an increased efficiency of degradation of bagasse and improving cost-effectiveness. Analysis of gene expression modulation is appropriate to better understand fungal enzyme production and secondary metabolism. The aim of this study is to characterize enzyme production in T. reesei and A. brasiliensis co-cultures both by enzymatic essays and gene expression, together with a characterization of the fungal interactions based on secondary metabolites. Employing RNASeq and RT-qPCR, the study will focus on selecting promising candidate genes for lignocellulosic degradation, for downstream application in improvement of elite fungal strains. Data will further our understanding of fungal co-cultures and how their interaction can be advantageous to biorefineries.

A4.R82 Development of an on-off switch regulator for Komagataella phaffii gene promoters

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Komataella phaffii (former Pichia pastoris) has become one of the most used host for protein expression platforms. Its high productivity is due to the usage of the AOX1 promoter that allows for high biomass accumulation in low cost culture media and increased protein yield. Additionally, this yeast can make post translational modifications in proteins such as glycosylation and disulfide bonds, absent features on the most studied microorganism Escherichia coli (Schwarzhans et al. 2017). However, the current molecular biology tools available in the market for genetic modifications are not as efficient as in other conventional yeasts. The reason is the low homologous recombination (HR) rate found in K. phaffii (Näätsaari et al. 2012). Therefore, our project seeks to develop a transient expression system for the ku70 gene. This sequence is known in many microorganisms for its participation in the repair of double-strand DNA breaks. When deleted, Δku70 strains significantly increases HR events. On the other hand, on a long-term evaluation, Δku70 strains are known to increase DNA damage, lose control of cell cycle and even perform non-canonical DNA events such as recombination between chromosomes. With this in mind, we have developed a ku70 expression cassete under control of the ku70 native K. phaffii promoter with a tetracycline-binding aptamer (Suess et al. 2003). This aptamer was inserted in tandem with the start codon and will produce a regulation site in the mRNA synthesized. Thus, in the presence of the tetracycline, the modified ku70 mRNA will fold and form a secondary structure that will block the ku70 protein translation. The absence of the ku70 protein will increase HR rates temporarily. If tetracycline is missing, the ku70 mRNA will be correctly translated and the

ku70 protein will have no modification. This enables the development of more complex strategies based on genetic manipulation to be performed and therefore expand the usage of K. phaffii in biotechnological applications.

A4.R83 Prospecting of ruminal bacteria and evaluating their role in lignin deconstruction

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Lignin represents the main renewable source of phenolic compounds that can be used to produce products of industrial interest. However, the inherent structural complexity and recalcitrance of lignin make its conversion into valuable chemicals a challenge. A central step for the biological valorization of lignin is its deconstruction into low molecular weight aromatic compounds that can later be converted by microorganisms. The use of bacteria has attracted increasing attention due to their ability to adapt to different environments and biochemical versatility. The bovine rumen is a microaerobic environment that hosts a diverse and highly specialized microbiota in the degradation of lignocellulose. Bacteria are predominant in this microenvironment, representing approximately 95% of all microorganisms. To explore the potential of ruminal bacteria in the anaerobic degradation of lignin, bovine rumen samples were inoculated in culture media containing kraft lignin as a carbon source, with or without yeast extract, at 37 °C for four days, under anaerobic conditions. Identification of bacteria enriched under these conditions was performed by sequencing the 16S rRNA gene. Furthermore, the ability of the consortia to degrade kraft lignin into single metabolites was evaluated by SEM, FTIR, and GC-MS analyses.

A4.R84 Synthetic storage vacuole for soybean proteins

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Soybean (Glycine max) has the great ability to produce more edible proteins per hectare than other commodities, being recognized for years for its importance in animal and human nutrition. Populations with a diet high in soy protein and low in animal protein have a lower risk of developing prostate and breast cancer. In addition, soy has proteins with medicinal potential, such as antioxidants, anticarcinogens and antivirals. Although the soybean product this high and elevated nutritive proteins, also produces allergenic proteins, harmful secondary metabolites, and carcinogenic elements. In addition, many proteins are lost during soy processing to produce animal feed and other products. Thus, the development of a synthetic soy protein expression utilizing cell-free system might be an interesting tool to produce specific proteins. Recently, the consumption of soybeans has been increasing due to its beneficial effects on human health such as prevention and treatment of various chronic illnesses which include cardiovascular diseases and various forms of cancer. Moreover, many proteins are lost in the processing of soy to produce animal food and other products. Cell-free protein expression system is an alternative for protein output without bacterial contamination, this system allows a high control of the expression and high level of purified protein. Synthetic organelles are a step to fabric a synthetic cell and may be applied for the construction of synthetic vacuoles, that could accumulate interest proteins from a cell-free expression system. We have expressed the soybean Bowman Birk type proteinase inhibitor C II; 2S albumin; Tubulin β-chain; β-Amylase, α-subunit of β-conglycinin, heat shock 70kda protein and Lipoxygenase. These proteins were purified by protein-linked poly-histidine tag affinity chromatograph. Experiments have been carried out for the utilization of soybean residues as liposomal structures to encapsulate synthetic proteins.

A4.R85 Overexpression of the soybean glutathione S-transferase (GmGST) gene in Nicotiana tabacum L. indicates increases in tolerance against Meloidogyne incognita

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The root-knot nematode, Meloidogyne incognita, is one of the most important phytopathogens for the economy, impacting both the yield and the quality of the products of many agronomic crops of worldwide interest. Previous transcriptomics and proteomics studies of contrasting soybean genotypes (BRS133 – susceptible, and PI595099 – highly tolerant) infested with M. incognita identified candidate genes for tolerance of this pathogen. Specifically, we highlight the protein glutathione S-transferase (GmGST) as it belongs to a superfamily of metabolizing enzymes involved in detoxification against harmful agents in the environment and reactive oxygen species (ROS). Therefore, the GmGST molecule was ectopically overexpressed in tobacco (Nicotiana tabacum L. cultivar Petit Havana) and in hairy roots induced in soybean leaves by Agrobacterium rhizogenes to evaluate the effects on the reproduction of M. incognita. Non-transformed tobacco plants (NTs) and the transgenic events, Ev1.1, Ev2.2, and Ev7.2, were inoculated with 1,000 second-stage (J2) juveniles of M. incognita race 3. Similarly, hairy bioassays soybean roots were infested with 1,000 J2 of M. incognita race 1. Transgenic tobacco plants showed significant reductions in the number of galls per gram of root, reaching 56.0%, when compared to NTs. The number of eggs per gram of root indicated decreases of up to 49.0%, while the reproduction factor (FR) showed a decrease of up to 42.0% in the transgenic plants. Additionally, overexpression of the gene in soybean hairy roots showed a reduction of approximately 40.0%. All these data point to the GmGST gene as a promising target for transcriptional activation via dead-Cas9 (dCas9) in soybean, aiming at increasing tolerance against the phytopathogen M. incognita.

A4.R86 Dynamic interactome of Trypanosoma cruzi RBPs and RNAs during infection

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Chagas disease is a systemic, chronic, and potentially fatal pathology caused by the protozoan Trypanosoma cruzi. Endemic in 21 countries of Latin America, it's considered a neglected disease by World Health Organization (WHO). Despite this, intense population migratory movements contributed to its distribution to other continents, affecting mainly economically active people. WHO estimates that 6 to 7 million people are infected worldwide. The current treatment medication, benznidazole, presents low efficacy in chronic phase and strong side effects, urging the development of new medicines. The high complexity of biological and interaction processes of the parasite with the host cell are challenges to be faced. Its life cycle and the extensive phenotypic changes and adaptations observed in it are due to massive modulations of gene expression. In this context, RNA-binding proteins (RBPs) are crucial to regulating several cellular processes such as mRNA metabolism (its stabilization, degradation, and protection of transcripts). Thus, the present study aims to elucidate the interactions and the constituents of RBP-RNAs complexes in the initial moments of infection in a host cell, as well as their importance to establish and maintain the process. Therefore, the new OOPS methodology (Orthogonal Organic Phase Separation) is being applied to perform in vitro infection assays in human cells to stabilize and purify RBP-RNA complexes. Infection assays with the technique are being standardized to generate sufficient material of RBPs and RNAs for proteomic and transcriptomic analysis by mass spectrometry and RNA sequencing. The results will be validated with biotechnological tools produced previously as recombinant proteins and polyclonal antibodies, using the traditional

techniques for RBP-RNAs complexes analysis. The expectation is to identify important constituents, contributing to increasing knowledge about the infection process and opening new research pathways.

A4.R87 PRODUCTION OF RECOMBINANT CHIKUNGUNYA VIRUS WITH VACCINATION POTENTIAL FROM A NON-INFECTIVE PROTOTYPE VIRUS IN MAMMALS

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A persistently infected Aedes aegypti cell line (Aag-2) was established with a mutant Chikungunya virus (CHIKV). The infectivity of this virus was evaluated in Aag-2, C6/36 (Ae. albopictus), Vero (monkey kidney) cells, and a primary culture of human fibroblasts. While this CHIKV isolate remained infective in mosquito cells (Aag-2 and C6/36), it has lost the ability to infect mammalian cells (Vero and fibroblasts). After high-throughput sequencing, the complete genome of the virus was sequenced and shown to have accumulated several non-synonymous mutations and two significant deletions in the coding sequence of the hypervariable domain of the nsP3 protein. Despite the specialization of this virus for mosquito cells, it showed low replication compared to its parent virus. This project aims to produce recombinant CHIKV viruses that have high replication levels in mosquito cells but that show attenuation or non-infectivity in mammalian cells. The CHIKV obtained from persistently infecting Aag-2, now called CHIKVAA, will be used as a prototype, as it has interesting biological characteristics for producing vaccine candidates. Recombinant viruses will be obtained by introducing desired CHIKVAA mutations into its parental CHIKV virus, resulting in infectious clones. With this work, we hope to validate the gain or loss of function of mutations present in CHIKVAA and produce vaccine candidates that are “half-infectious” successfully produced in mosquito cells but not infective in mammalian cells. KEYWORDS: Chikungunya virus, vaccine candidates, Aag-2, mosquito, Vero, CHIKV, recombinant virus.

A4.R88 Biotechnological potential of a dioxygenase isolated from Cerrado soil

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A soil microbiota presents a high diversity of microorganisms. Consequently, their unexplored ecological and physiological relationships have great potential for the discovery of new antibiotic-resistance genes (ARGs). The dissemination and surveillance of these genes produce a catastrophic effect on public health since multidrug-resistant pathogens are able to resist the antibiotics available, in order to impair the treatment of infectious diseases. The metagenomic strategy, allied to bioinformatic analysis, has the possibility to elucidate and understand the relation between the genes and their function in the cell. In this study, a new dioxygenase was characterized by metagenomic isolation from Cerrado soil. The enzyme was shown to have optimal activity under conditions of pH 7; a temperature of 30°C; and using iron ions as a cofactor for substrate cleavage. The catalytic dynamics of CRB2 showed values Vmax = 0.02281 µM/min and KM = 97.6. Its three-dimensional structure revealed the substrate binding to the cupin domain, where the active site is located. The analyzed substrates interact directly with the iron ion, coordinated by three histidine residues. Alternatively, changing the iron ion charge modifies the binding between the active site and the substrates. Currently, there is a demand for enzymes that have biotechnological activities of interest. Metagenomics allows analyzing the biotechnological potential of several organisms at the same time, based on sequence and functional activity analyses.

A4.R89 Development of neutralizing human antibodies for flavivirus infections and for COVID-19

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Recurrent outbreaks of flavivirus infections pose a challenge for scientific research. Severe clinical conditions, such as hemorrhagic dengue and congenital Zika syndrome, explain the importance of developing efficient treatments. In addition to these infections, a new disease has recently emerged, the COVID-19, caused by the SARS-CoV-2 virus that still remains in circulation. The context of new variants, the natural drop in immunity of the vaccines, and post-COVID complications sustain concern about this disease. Immunotherapy with human monoclonal antibodies (Ab) provides an advantageous alternative, exploiting the characteristics of neutralizing capacity of infection by high affinity binding. In this context, the present work aims at the development of neutralizing human Ab against conserved epitopes of flaviviruses and of SARS-CoV-2. We strategically designed mimetic antigens to regions involved in the infection process and that are conserved among flaviviruses and isolated human monoclonal Ab specific to these antigens using Phage Display technology. We were able to isolate different engineered Ab that showed different binding activities to ZIKV, YFV and DENV. The anti-flavivirus Ab also showed different structural characteristics. We show that selected anti-flavivirus Ab are able to neutralize ZIKV infection by plaque reduction neutralization test (PRNT) with Vero cells. These anti-ZIKA neutralizing Ab confirm the efficiency of our method of developing neutralizing Ab and these molecules, in a patent process, will be characterized in terms of their ability to neutralize in vivo against ZIKV, DENV and YFV. We also isolated successfully monoclonal Ab against conserved regions (Spike protein) among SARS-CoV-2 variants by Phage Display. The future characterization of the neutralizing capacity of the Abs against different flaviviruses and of the Abs against SARS-CoV-2 variants may demonstrate the therapeutic potential of these molecules.

A4.R90 Plant extracts with bactericidal activity and without cytotoxic activity towards eukaryotic cells

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Plant extracts are widely used in traditional medicine for several purposes due to their properties including antibacterial activity. For this application, extracts must have relevant bactericidal activity without decreasing viability of eukaryotic cells. In this sense, the aim of the present study was to screen 11 plant extracts and the liposomes produced with these extracts related to their possible bactericidal and cytotoxic activity in eukaryotic cells. For this, aqueous and ethanolic extracts of 11 plants were obtained at a concentration of 0.05 g/mL. For the assays, both aqueous and ethanolic extracts were concentrated to 2.5 g/mL, the ethanolic extract was roto-evaporated, lyophilized, and resuspended in distilled water. Escherichia coli and Staphylococcus aureus bacteria strains were grown to an optical density OD 600 =0.05 and exposed to the extracts at a concentration of 0.25 g/mL in Luria Bertani (LB) medium and kept at 37°C for 48 h. In order to prove the growth or not of bacteria, 10 µL of the suspensions of control bacterial or exposed to the extracts were inoculated in a solid LB medium and left for another 24 h under the same conditions and the possible growth of bacteria was evaluated. Eukaryotic cells viability was evaluated on JJ773 cells from ethanolic extracts and liposomes containing the same extracts. JJ773 cells were placed at a concentration of 1 x 104 cells/well in complete Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 1% antibiotic. The samples were tested at the same concentrations as performed in the test with bacteria and incubated for 24 h and the cellular activity was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. It was observed that extract G showed bactericidal activity for aqueous or ethanolic; and extracts A, E, F, J, and K presented activity only for ethanolic extracts. From these extracts that exhibited bactericidal activity, only E, F, and J extracts did not influence the cell viability of eukaryotic cells, with results statistically similar to the negative control, E and J the free or nanostructured extract, and F only nanoestructured. It appears that traditional medicine is correct and that nanostructuring can be an effective way to obtain bactericidal activity without decreasing cell viability.

A4.R91 The overexpression of the apoplastic soybean germin protein GmGLP10 enhances resistance to Meloidogyne incognita in transgenic tobacco plants and soybean hairy roots

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The root-knot nematodes (RKNs) represent one of the most harmful plant pathogens in world agriculture. The acquisition of effector genes into RKN's genomes of the genus Meloidogyne spp., is currently constituted as an important evolutionary strategy explored by these species for their establishment in several land plants. In turn, there are some host in nature that are highly tolerant to these plant parasitic nematodes, among them the soybean genotype PI 595099. Our bioassays revealed that this soybean genotype promoted more than 70% in reduced of galls/plant when infected with Meloidogyne incognita, while the egg masses/plant reduced more than 85%. To understand which resistance factors are associated with this phenotype, transcriptomic and proteomic approaches were recently performed in our research group and several resistance genes against multiple biotic stresses were identified. Among the differentially expressed genes (DEGs), the water-soluble glycoproteins Germin (GmGER10) was shown to be highly expressed in the PI 595099 genotype. Interestingly, we found some point mutations in GmGER10 promoter capable of increasing the cis-element composition and reinforcing its expression in a specific way to M. incognita-induced feeding sites. We verified more than 4 times of fluorescent intensity in root elogation when transgenic lines transformed with pGmGER10::eGFP was incubated in NEMAWATER solution. The subcellular localization of GmGER10 confirmed that this protein is located in the extracellular matrix and DAB staining of transgenic tobacco's leafs overexpressing GmGER10OE drastically reduced the H2O2 production. Furthermore, the lines GmGER10OE significantly decreased both leaf stickness as well as mesophyll area. Although we verified possible "trade-offs" between GmGER10OE and leaf development, transient expression in soybean hairy roots showed more than 55% in reduction in galls/plant. We verified abortion in feeding sites, which refleted in high reduction in galls diameter and feeding sites area. The same situation was also confirmed in galls-induced in transgenic tobacco lines where we observed a big delay in adult female development, a low expansion in citoplasmic content in feeding sites and once again typical events associated with programmed cell death. More study will be conducted to identify which downstream factors are modulated by GmGER10OE in plants. Furthermore, their potential in plant of agronomic interest will now be investigated in all transgenic tobacco generated in this present study. Our data provided the first evidence of GmGER10 in resistance against M. incognita and will be investigated as one target in crop protection.

A4.R92 Isolation and characterization of soil bacteria

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Colégio Militar de Brasília

Soil microbiome presents a fundamental role in decomposition of organic matter, biogeochemical transformations, nutrient cycling and degradation of toxic compounds. In addition, soil microorganisms produce compounds with antimicrobial activity and enzymes with industrial application. In this context, the aim of this work, performed by students from Colégio Militar de Brasília, was isolate and characterize soil bacteria. Eight samples were selected and analyzed regarding the macroscopic appearance of the colony, cell morphology, cell wall composition and

motility. The production of catalase, urease and amilase enzymes by soil bacteria's, as well the sensibility to antibiotics, was also evaluated. In addition to the biotechnological potential of the results, this work contributed to the development of the investigative spirit and the argumentative ability of high school students.

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