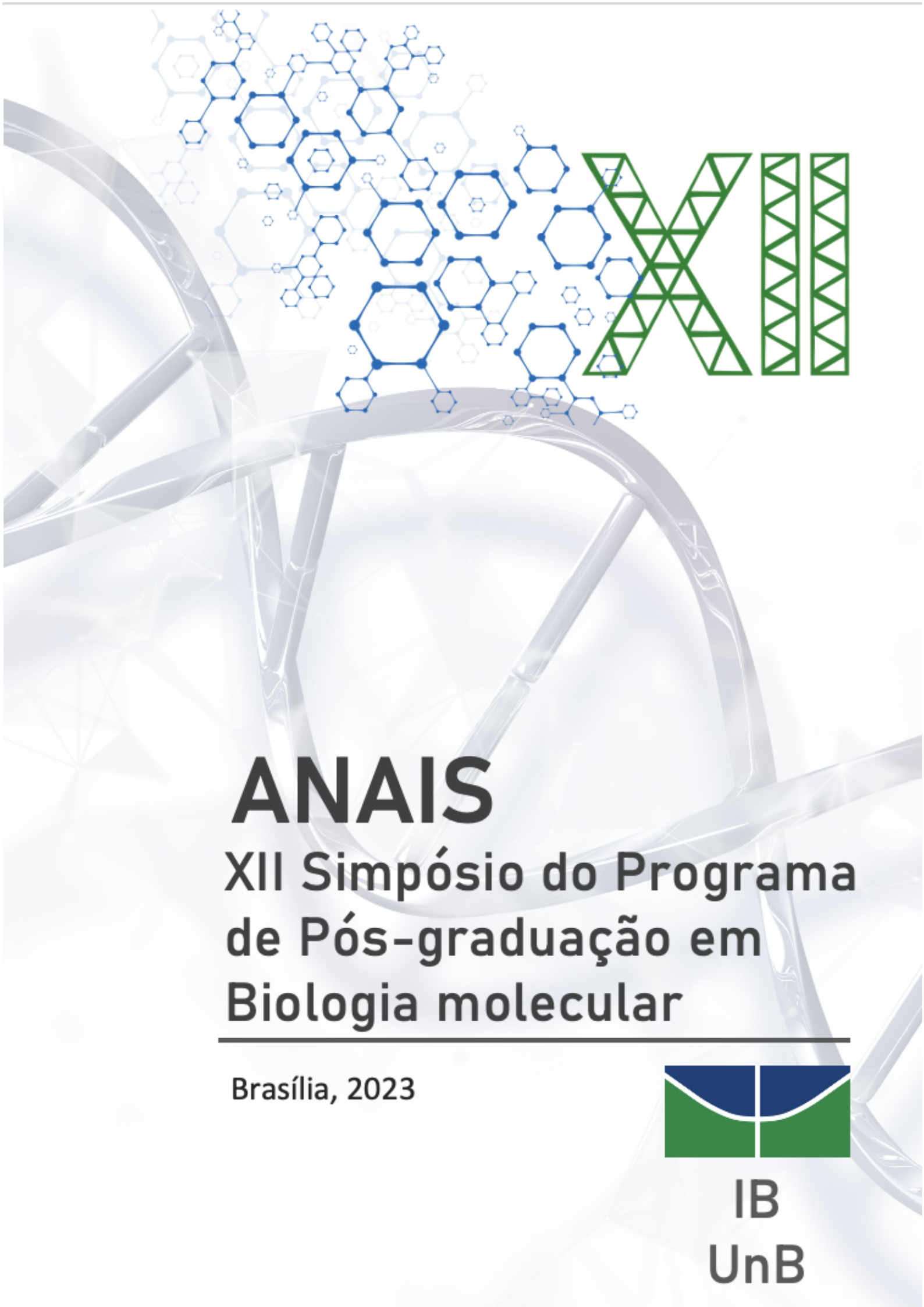

ANAIS

XII Simpósio do Programa de Pós-graduação em Biologia molecular

Brasília, 2023

IB
UnB

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

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

Brasília
Dezembro de 2023

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Sumário / Summary

Apresentação

O XII Simpósio do Programa de Pós-Graduação em Ciências Biológicas (Biologia Molecular) (PPGBioMol) da Universidade de Brasília (UnB) foi celebrado nos dias 7 e 8 de dezembro de 2023 em Brasília-DF. O simpósio contou com a participação de alunos, professores e pesquisadores da UnB e convidados. Este ano, as palestras e discussões foram divididas em dois eixos temáticos: *Imunologia – Terapia, Vacinas e Interação patógeno hospedeiro* e *Genômica, Biologia Estrutural* e *IA aplicada a química medicinal*.

O PPGBioMol possui diferentes linhas de pesquisa. A fim de organizar a diversidade de conteúdos, 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 microrganismo. Adicionalmente, uma quinta área foi criada para avaliar os trabalhos dos estudantes de graduação. Foram 74 resumos submetidos. Um trabalho de cada área foi escolhido para apresentação oral em 8 de dezembro. Estes Anais contêm os resumos dos alunos e reflete o engajamento dos estudantes e professores com o desenvolvimento e fortalecimento da pesquisa científica em nossa universidade.

Presentation

The X Symposium of the Graduate Program in Biological Sciences (Molecular Biology) (PPGBioMol) at the University of Brasilia (UnB) was held on December 7th and 8th, 2023 in Brasília-DF. The symposium was attended by students, professors, and researchers from UnB as well as invited guests. This year, the lectures and discussions were divided into two thematic axes: *Immunology – Therapy, Vaccines, and Host-pathogen Interaction and Genomics, Structural Biology*, and *AI applied to Medicinal Chemistry*.

PPGBioMol has different lines of research. To organize the diversity of contents, the submitted abstracts were classified into four major areas: (01) Cell Biology, Development, and Cancer; (02) Biochemistry, Biophysics, and Structural Biology; (03) Genetics, Genomics, and Evolution; (04) Cellular and Molecular Biology of Microorganisms. Additionally, a fifth area was created to evaluate the work of graduate students. Seventy-four abstracts were submitted. One work from each area was chosen for oral presentation on December 8th. These Proceedings contain the abstracts of the students and reflect the engagement of both students and professors in the development and strengthening of scientific research at our university

Coordenador do programa de Pós-Graduação em Biologia Molecular

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Graduandos

Beatriz Abreu Vasoncelos

Guilherme Santana Vieira

Maria Eduarda

Secretária do PPG-Biomol

Ana Hilda Tiberti

Programação / Program

07 de Dezembro

Tema: Imunologia – Terapia, Vacinas e Interação patógeno hospedeiro

8:30 – 09:15 – Registro dos participantes e entrega de material
09:15 – 09:30 – Abertura
09:30 – 10:25 – Palestra de abertura – Prof Martin Bonamino – (INCA e Fiocruz do Ceará)
Título: Desenvolvimento de Terapias com Linfócitos Geneticamente Modificados
10:25 – 11:10 – Palestra – Prof Fátima Ribeiro Dias (Universidade Federal de Goiás)
Título: Polimorfismos nos genes da via da vitamina D: resistência e suscetibilidade à leishmaniose tegumentar americana.
11:10 – 11:25 – Coffee Break
11:25 – 12:10 – Palestra – Helton da Costa Santiago (Universidade Federal de Minas Gerais)
Almoço
14:00 – 17:00 – Sessão de Pôsteres

08 de Dezembro

Tema: Genômica, Biologia Estrutural e IA aplicada a química medicinal

Mesa redonda: Biologia estrutural e Química medicinal

09:00 – 10:30
Aisel Valle Garay (Pesquisador Colaborador PPGBiomol – IB-UnB)
Título: Uma jornada de 30 minutos pelas raízes históricas centenária da Ultracentrifugação Analítica e suas perspectivas atuais para sua compreensão científica e tecnológica
Prof Bruno Neves (Universidade Federal de Goiás)
Título: Improving Drug Discovery and Toxicity Assessment with Multitask Learning: An Artificial Intelligence Approach
Brenno Amaro da Silveira Neto (Instituto de Química – UnB)
Título: Bioimageamento com sistemas fluorescentes: sintetizado na Química, aplicado na Biologia
10:30 – 10:45 – Coffee Break
10:45 – 11:30 Prof Dario Grattapaglia (Embrapa-DF)
Título: Genômica e genética quantitativa convergem para a predição de características complexas: Fisher já sabia de tudo 100 anos atrás
11:30 – 11:50 – Workshop BS DIAGNÓSTICA
Título: Técnicas de Biologia Molecular – Aplicações e Inovações. Esta apresentação abordará as técnicas de Biologia Molecular mais recentes e inovadoras que revolucionam a forma como compreendemos e tratamos doenças, além de discutir suas aplicações práticas em um contexto clínico e de pesquisa.
Almoço
14:00 – 14:45 – Professora Homenageada: Prof Maria Sueli Felipe
14:45 – 17:00 – Apresentação oral dos melhores trabalhos e premiação

Palestras / Invited talks

7 de Dezembro
Tema: Imunologia – Terapia, Vacinas e Interação patógeno hospedeiro

P1 - Desenvolvimento de Terapias com Linfócitos Geneticamente Modificados
Prof. Dr. Martin Bonamino
INCA e Fiocruz do Ceará

P2 - Polimorfismos nos genes da via da vitamina D: resistência e suscetibilidade à leishmaniose tegumentar americana
Profa. Dra. Fátima Ribeiro Dias
Universidade Federal de Goiás

P3 – Desenvolvimento clínico da SpiN-Tec: uma vacina brasileira contra Covid - 19
Prof. Dr. Helton da Costa Santiago
Universidade Federal de Minas Gerais

8 de Dezembro
Tema: Genômica, Biologia Estrutural e IA aplicada a química medicinal

Mesa redonda: Biologia estrutural e Química medicinal
Título: Uma jornada de 30 minutos pelas raízes históricas centenária da Ultracentrifugação Analítica e suas perspectivas atuais para sua compreensão científica e tecnológica
Dr. Aisel Valle Garay (Pesquisador Colaborador PPGBiomol – IB-UnB)

Título: Improving Drug Discovery and Toxicity Assessment with Multitask Learning: An Artificial Intelligence Approach
Prof. Dr. Bruno Neves (Universidade Federal de Goiás)

Título: Bioimageamento com sistemas fluorescentes: sintetizado na Química, aplicado na Biologia
Prof. Dr. Brenno Amaro da Silveira Neto (Instituto de Química – UnB)

P4 - Genômica e genética quantitativa convergem para a predição de características complexas: Fisher já sabia de tudo 100 anos atrás
Prof. Dr. Dario Grattapaglia
Embrapa-DF

Apresentações orais / Oral presentations

1. Biologia Celular, desenvolvimento e câncer (A1)

Título: Therapy-induced bone loss prevention by solid lipid nanoparticle treatment
Aluno: Marina Arantes Radicchi

2. Bioquímica, biofísica e biologia estrutural (A2)

Título: Method for detecting SARS-CoV-2 viral RNA using Ribozymes and associated DNA hairpins in hybridization chain reaction with fluorescent resonance energy transfer
Aluno: Leonardo Ferreira da Silva

3. Genética, genômica e evolução (A3)

Título: Biotech strategies for root-knot nematode control in soybean
Aluno: Náttany Souza Costa

4. Biologia celular e molecular de microorganismos (A4)

Título: Delivery of anti-cd3 scfv by *Saccharomyces boulardii* decreases inflammation in dss-induced colitis in mice
Aluno: Sylvia Barbosa Pinhate

5. Graduação

Título: NLRP3 inflammasome activation-dependent pyroptosis modulates carcinogenic parameters in human gastric cancer cells
Aluno: Julia Perin Manchine

O conteúdo destas apresentações encontra-se na seção Resumos.

Melhor Poster

1. Biologia Celular, desenvolvimento e câncer (A1)

Título: Modulation of melatonin on carcinogenic parameters and pyroptotic cell death in human pancreatic cancer cells
Aluno: Sarah Pinho Bezerra

2. Bioquímica, biofísica e biologia estrutural (A2)

Título: Integration of computational and experimental strategies in the discovery and characterization of Thimet oligopeptidase inhibitors from *Trypanosoma cruzi*
Aluno: Verônica Lucas Sequeira da Silva

3. Genética, genômica e evolução (A3)

Título: Ship: an annotation-based program for the identification of putative genomic safe harbors in eukaryotic model organisms
Aluno: Matheus de Castro Leitão

4. Biologia celular e molecular de microrganismos (A4)

Título: Unraveling new roles of the apoplastic soybean Germin-like protein 10 in land plants: from development to resistance against root-knot nematodes
Aluno: Valdeir Junio Vaz Moreira

5. Graduação

Título: The function of omega-3 dha in modulating white and brown adipose tissue and its impact on carcinogenic parameters in melanoma cells.
Aluno: Nathalia Cristina Silva Lago

O conteúdo destes pôsteres encontra-se na seção Resumos.

Resumos / Abstracts

1. Biologia Celular, desenvolvimento e câncer (A1)

A1.R1 - Intermittent Cold Exposure Reversed High-Fat Diet-Induced Metabolic and Cognitive Dysfunctions in Mice

Wellinghton de Medeiros Barros
Universidade de Brasília (UnB)

Recent studies have shown that the consumption of a high-fat diet (HFD) is a good model for generating obesity and cognitive impairments and it is important to seek non-pharmacological alternatives that can function as strategies for the treatment of both metabolic dysfunctions and behavioral effects induced by HFD. Thus, the objective of this study is to evaluate the effect of chronic and intermittent exposure to cold on HFD-induced metabolic and cognitive changes. Methods: Male and female C57Bl/6 adult mice were divided into 8 groups exposed to different diets and temperature conditions (HFD or control diet (CD) and cold (4oC) or room temperature (RT) (22oC) exposure for 10 weeks. The Δ of body mass, brown adipose tissue (BAT) temperature and oxygen consumption as well as behavior were evaluated after the experimental period. Results: HFD increased the body mass in females (CD+RT x HFD+Cold $p = 0.02$) and males (CD+RT x HFD+RT $p < 0.0001$), however, cold exposure was able to reverse this metabolic outcome (male HFD+RT x HFD+Cold $p = 0.01$). Cold exposure increased BAT temperature (female: CD+Cold x HFD+RT $p = 0.0005$; male CD+RT x CD+Cold $p = 0.0005$, CD+RT x HFD+Cold $p = 0.024$, HFD+RT x HFD+Cold $p = 0.035$). Regarding oxygen consumption, UCP-1-linked respiration decreased in the female mice fed a HFD (CD+RT x HFD+RT $p = 0.04$), however, cold exposure reverted this effect (HFD+RT x HFD+Cold $p = 0.03$). Interestingly, we observed that the consumption of HFD caused recognition memory deficits in both female ($p = 0.0728$) and male ($p = 0.51$) mice that were reversed by cold exposure (female: $p = 0.04$, and male: $p = 0.002$). Discussion/Conclusion: With the present data we can infer that cold stimulation can increase the activity of BAT, thereby mitigating the harmful effects of HFD on cognition. In summary, chronic and intermittent cold exposure has proven capable of attenuating the deleterious metabolic and cognitive effects induced by HFD consumption in mice.

A1.R2 - Evaluation of the antitumor activity of copper oxide nanorods in murine mammary carcinoma

Giovanna de Carvalho Nardeli Basílio Lôbo
Universidade de Brasília (UnB)

Cancer, the second leading cause of death worldwide, represents a significant public health challenge. Among cancers, breast cancer stands out as a major threat to females, with increasing mortality rates and relevance to public health in Brazil. This disease presents clinical, morphological and genetic heterogeneity, influencing therapeutic responses. Nanotechnology emerges as a potential treatment for cancer, enhancing the pharmacological properties of compounds used in diagnosis and treatment. Nanoparticles offer advantages such as reduced side effects, targeted cellular delivery and controlled drug release. Copper oxide nanoparticle (CuO NPs), specifically in the form of nanorods (CuO-nr), stand out for their thermophysical, catalytic and antibacterial properties. CuO-nr, coated with citrate, is stable and presents favorable characteristics for therapeutic use. Therefore, the present project aims to focusing on carcinogenesis and associated cellular and inflammatory mechanisms. CuO-nr-cit nanostructures exhibited a hydrodynamic diameter of 107.1 ± 0.67 nm and a zeta potential

of -23.8 ± -1.87 mV over 90 days. For in vitro assays, two cell lines were chosen: NIH-3T3 (non-tumor model) and 4T1 (tumor line), culture according to ATCC guidelines. The Alamar Blue reagent was used to asses cell viability after treatment with different concentrations of CuO-nr-cit (1.56, 3.125, 6.26, 12.5, and 25 µg mL-1) for 24, 48 and 72 hours. Statistical data were analyzed using Graphed Prism 5.0, subjected to specific tests with 95% statistical confidence, including the Two-Way Anova Test. Preliminary results demonstrated remarkable selectivity of the nanostructure in reducing viability in the 4T1 tumor line at three of the five concentrations, with minimal impact on the non-tumor NIH-3T3 line. The study aims to deepen the understanding of the potential of CuO-nr-cit nanoparticles in breast cancer treatment.

A1.R3 - Efeitos do peptídeo crotamp14 sobre a expressão gênica em linhagem de câncer de mama triplo negativo

Michel Lopes Leite
Universidade de Brasília (UnB)

Triple negative breast cancer (TNBC) accounts for 10% to 20% of all breast cancer diagnoses. TNBC is characterized by the absence or low expression of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). Its prognosis can be unfavorable due to a high metastasis rate and lack of targeted therapies. Therefore, the search for new, more selective and less harmful therapeutic strategies for patients is needed. One promising alternative is the use of antimicrobial peptides (AMPs) with potential antitumor effects, as these molecules can selectively target the plasma membrane of neoplastic cells or intracellular targets. Many studies have been conducted on the membranolytic action mechanisms of these molecules, but few have focused on the modulation of gene expression in neoplastic cells exposed to AMPs. Therefore, the aim of this study is to evaluate the changes in gene expression in the MDA-MB-231 breast adenocarcinoma cell line exposed to synthetic crotAMP14, which has shown antitumor activity in in vitro tests. Nuclear magnetic resonance spectroscopy studies have shown that crotAMP14 adopts an α-helix structural conformation in a membrane-mimetic environment. Relative gene expression analysis by qRT-PCR suggests that crotAMP14 is capable of inducing the expression of p53, a central protein in the process of cellular apoptosis.

A1.R4 - Therapy-induced bone loss prevention by solid lipid nanoparticle treatment

Marina Arantes Radicchi
Universidade de Brasília (UnB)

Breast cancer leads to a crisis in the natural balance of bone resorption and its formation, within conventional treatments is doxorubicin, a chemotherapy drug that has several collateral effects such as therapy-induced bone loss [1-3]. 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. The association of doxorubicin to a nanostructure guarantees chemotherapy advantages, delivering towards the tumor, promoting specific action, reducing side effects, increasing the drug's effect by reducing its contact surface, and enhancing its biodistribution [4]. With drug delivery, bone is protected, preventing bone loss and breast cancer progression. To avoid bone loss in breast cancer treatment, a solid lipid nanoparticle containing doxorubicin (SLNDox) was used in a tumor-bearing mice model analyzed by an in vivo imaging system and scanning electron microscopy. 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. Bone loss prevention was observed when SLNDox was administered to no-tumor and tumor-bearing female

Balb/c mice. Imaging techniques were used to obtain information about bone loss in the femur of tumor-bearing mice treated with nanostructured doxorubicin, indicating prevention of bone loss in the cancellous portion and thinner growth plate in the Dox-HCl group of animals. In addition, the nanostructured treatment maintained the radiodensity of cortical bone similar to healthy animals in computed tomography analysis.

A1.R5 - Melatonin Modulates Carcinogenic Parameters, Mitochondrial Dynamics, and Attenuates Inflammatory Cell Death in Human Gastric Cancer Cells

Sabrina Azevedo Machado
Universidade de Brasília (UnB)

Introduction: Melatonin is molecule with numerous biological activities. It is mainly produced in response to darkness. There is an increasing focus on melatonin in oncology since this molecule has onconstatics effects. However, the role of melatonin in gastric cancer is poorly understood. Therefore, this work aimed to analyze the role of melatonin in the modulation of the carcinogenic parameters, mitochondrial function, oxidative stress, and cell death in the gastric cancer cell line (AGS). Methods: AGS cells were stimulated with melatonin at concentration of 0.625, 2.5, and 5mM at different times. Cell proliferation was assessed by the CFSE staining. Mitochondrial viability and function were assessed by MTT assay and high-resolution respirometry, respectively. Oxidative stress was assessed by DCF-DA staining. Cell death profile was assessed by annexin-V/propidium iodide. The enzyme lactate dehydrogenase (LDH) release was evaluated by the CyQUANTTM kit. Cell cycle and membrane pore formation were assessed by the PI probe staining. Pro-caspase-1 and GSDMD abundance were assessed by Western blot. Results: Both melatonin at 2.5 and 5mM promoted a reduction in mitochondrial viability, cell proliferation, oxidative respiration, and ROS production. In addition, these concentrations significantly increased apoptotic death compared to unstimulated cells. Nevertheless, attenuation of the inflammatory death, pyroptosis, was observed by reducing key parameters such as LDH release, pyroptosis-related membrane pore formation, pro-caspase-1 and GSDMD abundance. Conclusion: Taken together our data showed that melatonin, from 2.5mM, 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 data highlight that the melatonin can promote pyroptosis attenuation in gastric cancer cells which could be crucial in the use of melatonin in therapeutic approaches.

A1.R6 - Analysis of the effects of fatty acid stimulation on carcinogenic parameters of pancreatic adenocarcinoma

Ramon Buson Lima Paiva
Universidade de Brasília (UnB)

Adenocarcinoma pancreatic cancer annually is responsible for nearly 460 thousand deaths worldwide. This mortality rate occurs due to its highly metastatic potential, delayed diagnosis, and inefficient treatment. In this context, lipids arise as potential molecules that impact cancer progression. After a literature search and an assay screening, we selected three fatty acids to analyze their effect in Mia-Paca-2 cell line, derived from human adenocarcinoma pancreatic cancer. Docosahexaenoic acid (DHA) is an omega-3 unsaturated fatty acid that has the potential as an adjuvant treatment against different types of neoplasms. Arachidonic acid (ARA) is an omega-6 fatty acid that plays dubious roles in neoplasms. Palmitic acid is a saturated fatty acid associated with cancer establishment and progression. In pursuit of assessing the impact of these lipids in human adenocarcinoma pancreatic cancer, Mia-paca-2 cells were cultured and stimulated with one of the following: DHA at concentrations of 25 µm and 50 µm; ARA at concentrations 50 µm or 100 µm; PA at 50µm or 200µm concentrations. Cell viability

was analyzed by using MTT assay. Our results, until them, showed that at 24 hours, ARA reduced mitochondrial cell viability in a dose-dependent manner. At 48 hours, DHA decreased cell viability at 50µm concentration, demonstrating a dose-dependent effect; ARA reduced cell viability by a dose-dependent way; while PA increased cell viability at a higher dose. At 72 hours, DHA did not affect cell viability, while ARA caused a dose-dependent reduction, and PA reduced cell viability at the 50µm.To sum up, our study contributes to the literature by showing the differents effects that those lipids exercise in adenocarcinoma pancreatic cancer cells, reflecting on the importance of a healthy dietary intake in the quality of life.

A1.R7 - Modulation of melatonin on carcinogenic parameters and pyroptotic cell death in human pancreatic cancer cells

Sarah Pinho Bezerra.
Universidade de Brasília (UnB)

Introduction: Pancreatic cancer is one of the most lethal types of cancer, characterized by an immunosuppressive tumor microenvironment and resistance to conventional therapies, with no effective treatment options for advanced disease stages. Previous studies have pictured melatonin, a natural indoleamine, as potential tool for adjuvant treatment in cancer therapy. While it has been well described that melatonin can present anti-proliferative effects and modulate cell death by apoptosis in different cell lines, other cell death pathways that could be triggered by this molecule is still under investigation. In this context, this project aimed to characterize the effects of melatonin on the modulation of carcinogenic parameters and characterizers cell death by pyroptosis in the human pancreatic adenocarcinoma cell line (PANC-1). Methods: PANC-1 cells were cultured and stimulated with distinct melatonin concentrations for different time points. Flow cytometry was employed to assess cell death, cell proliferation, cell cycle progression, nuclear fragmentation, caspase-1 activation, and lipid droplet biogenesis. Mitochondrial cell viability, membrane pore formation, lactate dehydrogenase (LDH) enzyme release, and reactive species (RS) generation were investigated using spectrophotometry. Results: Our data revealed that melatonin induced cytotoxicity in PANC-1 cells in a dose-and time-dependent manner. It led to augmented nuclear fragmentation, G0/G1 cell cycle phase arrest, and a reduction in cell proliferation and lipid droplet biogenesis. Additionally, melatonin treatment resulted in increased RS production, LDH release, caspase-1 activation, and a decrease in plasma membrane integrity, correlating with the occurrence of pyroptotic cell death. Conclusion: This study demonstrates the potential antitumor effect of melatonin against pancreatic cancer cells in vitro, unveiling new therapeutic possibilities that could be applied for pancreatic cancer treatments.

A1.R8 - Analysis of the potential antitumor action of metformin alone or in combination with omega-3 DHA in human ovarian cancer cells

Milena Nascimento Verdam de Araújo
Universidade de Brasília (UnB)

INTRODUCTION: Cancer, a chronic genetic disease, is characterized by the uncontrolled proliferation of abnormal cells. While it has a genetic basis, 80-90% of cases are associated with environmental and lifestyle factors, thus making them preventable. Among the major risk factors for ovarian cancer, the second most common gynecological neoplasm in Brazil, chronic metabolic disorders, and hormonal disturbances such as obesity, type II diabetes, and polycystic ovary syndrome are prominent. It has been described that metformin, a drug used in the treatment of such disorders, also exhibits antitumor effects. We hypothesize whether DHA, an omega-3 fatty acid

that has distinguished itself by presenting antitumor properties in various cancer types, potentiates the effects of metformin on human ovarian cancer cells in vitro. METHODS: Human ovarian cancer cells of the A2780 lineage were properly cultured and treated with different concentrations of Metformin and DHA diluted in the culture medium. After treatment periods of 24, 48, and 72 hours, the cells were processed for initial analyses of mitochondrial viability using the MTT assay, cell death profile through flow cytometry using Annexin and P.I., as well as cellular morphology examination by Transmission Electron Microscopy. RESULTS: Treatment with DHA reduced cell viability at 24 and 48 hours; there was no significant difference between cells treated with Metformin in any of the three analysis periods. Morphological analysis suggests that Metformin treatment appears to induce greater formation of intracellular vesicles and alter mitochondrial morphology. CONCLUSION: In line with previous literature data, DHA shows initial evidence of modulating the cell viability of ovarian cancer cells, promoting a reduction in viability dependent on mitochondrial activity. It has not yet been possible to determine the specific effect of Metformin on this tumor type, but microscopy data suggest modulation of mitochondrial dynamics.

A1.R9 - Evaluation of Immune System Activation Through Methylene Blue Associated with Nanostructures for Breast and Ovarian Cancer

Ana Luísa de Gouvêa da Silva
Universidade de Brasília (UnB)

The role of immune system in cancer treatment has promote the development of numberless works in the theme of immunotherapies. Breast cancer, the second most common type, and ovarian cancer, which has the highest lethality when compared to other types of cancer on the female genital system, are those that stand out in women. Nanobiotechnology has been developed and is earning prominence for targeted drug delivery, in addition to being able to promote an immune response based on the activation of dendritic cells. Previous studies demonstrate efficacy, in vitro, of the use of maghemite nanoparticles associated with methylene blue [MAGCIT-MB] for the treatment of breast and ovarian cancer. Thus, the present work included the application of MAGCIT-MB to promote anti-tumor immune responses for the treatment of breast and ovarian cancer in vitro. MAGCIT-MB exhibited a hydrodynamic diameter of 60.93 nm. After 6 hours of treatment, it is possible to observe a large amount of internalized nanoparticles moving towards the cell interior in well-defined structures, similar to lysosome and/or endosome, and moving into the cell, as show in transmission electron microscopy [TEM] analysis. Panotipic staining indicate the internalization of nanoparticle and morphology alterations after treatment. It can be observed, by flow cytometry, a modulation in biogenesis of lipid droplets and a reduction of mitochondrial transmembrane potential after treatment with MAGCIT-MB. The maturation of dendritic cells was analyzed after treatment with the supernatant of treated tumor cells by flow cytometry. 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, which can also be see in light microscopy.

A1.R10 - PmiR-Select - a computational approach to plant pre-miRNA identification in genomes

Deborah Ribeiro Bambil
Universidade de Brasília (UnB)

pre-miRNAs are precursors of microRNAs (miRNAs). They are longer sequences that are less used in silico to mine miRNAs. Meanwhile, miRNAs are non-coding RNAs that regulate gene expression. This study aimed to mine plant pre-miRNAs and miRNAs in genomes, analyzing the redundancy in pre-miRNAs (from 95 to 70%; 5% intervals). Thus,

a computational tool (PmiR-Select®) based on covariance and hidden models was developed to identify new pre-miRNAs. At miRBase, 8677 pre-miRNAs and 10491 plant miRNAs were filtered from 2942 families. The second filter preserved pre-miRNAs from 70 to 300 nucleotides, which resulted in 8045 pre-miRNAs from 2623 pre-miRNA families. Based on redundancy analysis, the third filter used the Weka tool to classify color similarity with the deep learning (DL) algorithm. It eliminated pre-miRNAs with 80% redundancy, leading to a 58% reduction in pre-miRNA sequences. After these filters, angiosperms (ANG) retained the highest number of exclusive pre-miRNA sequences and families (2514 and 2165), followed by gymnosperms (GYM - 278 and 238), bryophytes (BRY - 149 and 105), and algae (78 and 82). Furthermore, 37 conserved pre-miRNA families were identified after those filters among the three land clades. Algae did not share any pre-miRNA with other clades. The highest number was consistently found in ANG for these conserved pre-miRNAs, except for a unique pre-miRNA that prevailed in GYM. Around 50% of these pre-miRNAs have already been identified as conserved miRNAs. pre-miRNAs' use reinforces their potential to identify conserved and new miRNAs more confidently. Applying PmiR-Select® on the rice genome, 8470 new pre-miRNAs homologous to 36 families were identified. Seventeen families already existed in the miRBase and 19 new families for rice, representing a 5% increase in deposited pre-miRNA families (nFam: 341). These new pre-miRNAs and their families contribute to designing and analyzing results in benchtop or computational experiments.

2. Bioquímica, biofísica e biologia estrutural (A2)

A2.R1 - Resolution of artificial protein-protein interactions from statistically optimized sequence landscapes

João Antonio Alves Nunes
Universidade de Brasília (UnB)

Coevolutionary theories describe the probability distribution of interacting proteins in terms of a Boltzmann statistical model. As a result of selective pressures, that distribution is expected to sharply deviate from uniformity by featuring a relatively small number of highly probable sequences across the entire sequence space. While that statement must be true for interacting protein systems in general, their sequence distributions may have not been fully shaped by selective pressures opening the possibility that novel protein-protein interactions could be selected from artificially generated lower entropy distributions. The aim of this work was to investigate the physical meaning of protein-protein interactions selected from artificial fitness landscapes, optimized with statistical criteria. For that, we explore a Genetic Algorithm, which solves the optimized distributions by maximizing the statistical coupling, starting from the native multi-sequence alignments and exploring the space of scrambled multi-sequence alignments. We also solve a distribution by minimizing statistical couplings through random shuffling of multiple sequence alignment. Once likely artificial sequences are selected from maximized and minimized distributions, their binding free-energies at a fixed native bound state are evaluated according to free energy calculations based on the MM/PBSA method. To evaluate the physical meaning of native and artificial sequences, we calculated the selection temperature in relation to random sequences of the same composition.

A2.R2 - Quantification of Error Sources Accounting for Misidentification of Protein Partners in Coevolutionary Approaches

José Antonio Fiorote Santos
Universidade de Brasília (UnB)

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 work, we have shown that the 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). Degenerate solutions with two error sources arise from mismatches among (i) similar and (ii) non-similar sequences. Once this problem remains unsolved, we contribute here with 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 (n') at a maximum MI content. In this scope, this framework allows us to estimate a trajectory probability in GA simulations, as well as shows us the dependence of trajectories that achieved higher n with a higher MI gap between fully scrambled and native arrangement. Lastly, take into consideration that GA simulations in real systems showed low final n values, we discuss the reassessment of sequences based on their similarity as alternative for enhancing n values.

A2.R3 - Characterization of the 5-enolpyruvylshikimate-3-phosphate synthase from *Escherichia coli*

Daniel Vieira Neves
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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 transfers the enolpyruvyl moiety from phosphoenolpyruvate (PEP) to the 5-hydroxy position in shikimate-3-phosphate (S3P), thus producing 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate (Pi). Also, this enzyme is the only molecular target known for the broad-spectrum herbicide glyphosate. Although there is not 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 characterization of the E. coli EPSPS aiming at the development of a glyphosate SPR-based biosensor. The protein is expressed in E. coli BL21 (DE3), through a pET-M11 construct, in ZYM-5052 medium. Then, a two-step purification follows through immobilized metal (Ni^{2+}) ion affinity liquid chromatography and size exclusion chromatography. The percentage of secondary structures and the stability in different conditions of pH and temperature were determined via circular dichroism. The colorimetric enzymatic assay, which detects the Pi liberated during the reaction, presented the following enzymatic parameters: Km (S3P) = 0.055 mM; Km (PEP) = 0,148 mM. These enzymatic parameters coincide with those described in the literature. In conclusion, the next steps are to determine the inhibition parameters for glyphosate; and, to immobilize the protein to the surface of the biosensor to allow for glyphosate sensibility and specificity tests.

A2.R4 - Short-term high-fat diet feeding induces cognitive decline, aggressiveness and anxiety-like behavior in adult zebrafish (*Danio rerio*)

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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.

A2.R5 - Targeting SARS-CoV-2 main protease (Mpro) with structurally diverse inhibitors

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Although COVID-19 is no longer a global public health emergency, concerns in the scientific community regarding the potential SARS-CoV-2 resistance to available therapeutics still persist. The main protease of SARS-CoV-2 (Mpro) is the main target on the development of antivirals, due to its crucial role in viral replication. To obtain Mpro, E. coli BL21(DE3) cells were transformed with the pGEX-6P1 plasmid, encoding a GST-Mpro-6xHis fusion construct with PreScission protease cleavage sites between Mpro and the fusion tags. After expression, the lysate was used to purify Mpro by sequential GST-glutathione and Ni-Affinity chromatographies. Mpro with the native C- and N-termini was produced after cleavage by PreScission followed by size exclusion chromatography. Mpro activity was measured by fluorescence kinetic assays using the substrate MCA-AVLQSGFR-Lys(Dnp)-Lys-NH2, with excitation and emission wavelengths set at 320 nm and 405 nm. Assays were performed in 100 µL using 96-wells plates on the SpectraMax M3 spectrofluorometer. Kinetic parameters were determined by fitting initial velocities to the Michaelis–Menten equation with GraphPad Prism 9 software, resulting in a Km value of 3.54 µM, consistent with literature values. Inhibition assays for potential Mpro inhibitors were carried out under the same conditions, with incubation for 30 minutes at 37°C. Compounds with different structural nature were assessed, including: a small protein of 9kDa called Bowman-Birk trypsin and chymotrypsin inhibitor (BTCI); two short cyclic peptides derived from BTCI inhibitory loops (PepChy and PepTry) and a Schiff base organomettalic complex (OM-P1). Nirmatrelvir, a potent Mpro inhibitor, was used as a positive control, exhibiting a remarkable half-maximal inhibitory concentration (IC50) of 18.56 nM similar to other reports. The results showed that PepTry and BTCI did not inhibit Mpro activity, while PepChy and OM-P1 inhibited with IC50 values of 5 µM and 34 µM, respectively.

A2.R6 - The effect of a high-fat diet on zebrafish on metabolism and behavior

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The ingestion of high amount of fat and sugar is associated with metabolic dysfunction and cognitive impairment in rodents and humans. The zebrafish has emerged as a translational research model to elucidate the biological mechanisms involved in these impairments. Thus, this study aims to decipher the metabolic and behavioral changes induced by a high-fat diet (HD) in zebrafish. Twenty-four animals were divided into two groups, one fed a

control diet (CD) and the other with HD for 15 days. Morphological parameters, including weight, body length, and abdominal length, were assessed, with fasting blood glucose serving as a metabolic parameter. The following behavioral tests were conducted: (i) the novel tank test, to evaluate motor activity and anxious-type behavior; (ii) the T-maze test, to assess learning and memory; and (iii) the mirror-induced aggression test, to evaluate aggressive behavior. It was observed that the HD led to an increase in body weight and length ($p < 0.001$ and $p < 0.01$) and fasting blood glucose ($p < 0.05$), but there was no alteration in abdominal length. The HD did not impact locomotor parameters, such as total distance traveled and absolute turning angle in the novel tank test. In the same test, a trend of reduced exploration of the top zone in the HD group was observed, indicating anxious-type behavior. In the T-maze test, animals treated with HD spent significantly more time exploring the already familiar arm ($p < 0.05$), demonstrating cognitive decline in spatial recognition memory. Finally, no difference was observed between groups in the total time of aggressive behavior. In conclusion, the HD induced morphological and metabolic changes accompanied by cognitive impairment in zebrafish.

A2.R7 - Study of a snake venom serine protease crafted through ancestral sequence reconstruction technique

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Snake venom serine proteases (SVSPs) are toxins that act on the prey's hemostatic system. Despite their high sequence homology, SVSPs act on a wide variety of substrates, showing, among others, kallikrein-like and thrombin-like activity. The evolution that resulted in such diversification can be studied through the ancestral sequence reconstruction technique. In this approach, ancestral sequences are built from modern sequences, enabling us to understand the properties of extinct proteins. Therefore, this work aims to study the evolution of SVSPs through reconstruction of the Viperidae ancestral sequence and characterize it biochemically and biophysically. The phylogenetic tree and the ancestral sequence were built using the MEGA-X software. The enzyme was expressed in *Komagataella phaffii* cells and purified through affinity chromatography (Ni2+). Protein deglycosylation was performed by enzymatic breakdown and confirmed by SDS-PAGE. Enzymatic tests were performed using the glycosylated and deglycosylated enzyme. The reconstructed sequence showed good statistics values. The protein expressed has a different size than expected, which was confirmed to be caused by N-glycosylations, a prevalent post-translational modification in SVSPs. Activity tests showed that the enzyme has activity against fibrinogen only when deglycosylated, probably due to a non-native glycosylation made by *K. phaffii.* The cleavage of fibrinogen did not generate clot formation, indicating that the enzyme acts in the fibrinogen depletion. The enzyme showed activity against a synthetic kallikrein substrate when glycosylated and deglycosylated, in agreement with the belief that kallikrein is the ancestral of SVSPs. Esterase activity, a common SVSP activity, was not observed. The structural characterization by circular dichroism and fluorescence is being finalized to comprehend the structural characteristics of the enzyme. With this study, we expect to expand the knowledge of the evolution of SVSPs.

A2.R8 - Novel antimicrobial peptides isolated from the central dwarf frog *Physalaemus centralis* (bokermann, 1962) skin secretion

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Bacterial infections pose a global threat to human health, highlighting the imperative for worldwide research and development of compounds with antibacterial properties, particularly to address infections caused by multidrug-resistant pathogens. The escalating number of resistant organisms stands as one of the major threats to humanity in the 21st century. Studies project that in the coming years, 300 million people may succumb to diseases caused by resistant organisms, with profound economic repercussions. Antimicrobial Peptides (AMPs) constitute the primary line of immune defense for many organisms. AMPs are compounds effective against microorganisms resistant to various commercially available drugs. They act on the bacterial plasma membrane, complicating the development of resistance to these compounds. Additionally, AMPs exhibit mechanisms of action at the intracellular level, leading to the inactivation of specific cellular processes. The aim of this study is to identify and characterize the chemical and biological components of cutaneous secretions from the anuran *Physalaemus centralis*, with a focus on prospecting antimicrobial peptides for therapeutic applications. Four new peptides (PEP1, PEP2, PEP4, and PEP5) were isolated and characterized, demonstrating antimicrobial activity against common and resistant Gram-negative and Gram-positive pathogenic bacteria, as well as a fungal species. The peptides in this study share similarities with peptides from the nattererin family, previously isolated from *Physalaemus nattereri*. In comparing their effects on bacterial growth, the four new peptides proved to be more efficient, reducing the necessary concentration to completely inhibit bacterial growth to 2 µM in some cases. Furthermore, recent findings indicate that these compounds also exhibit activity against cancer cells, showing promising potential in the quest for new active compounds with applications beyond their efficacy against resistant bacteria.

A2.R9 - Evaluation of the role of redox metabolism in the tolerance of tardigrades to anhydrobiosis

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Tardigrades are highly resilient microinvertebrates that endure extreme environments through cryptobiosis. Biochemical and physiological adaptations are crucial for their survival amidst the environmental stresses they encounter. This study aims to comprehend the adaptive mechanisms of these organisms, particularly in response to desiccation, emphasizing the potential essential role of endogenous antioxidants in surviving and recovering from anhydrobiosis. Our investigation involves the use of two inhibitors - aminotriazole (ATZ) and buthionine sulfoximine (BSO) - targeting the antioxidant activity/capacity of tardigrades from the genera Paramacrobiotus and Milnesiumm collected at the university campus. Previous results indicated that ATZ (1 mM) pre-incubation causes tardigrade death following 24 hours of anhydrobiosis, with no observed effect in the control group. Currently, we are currently determining the activity of antioxidant enzymes catalase and glutathione S-transferase, as well as measuring levels of lipid peroxidation and carbonyl proteins. This study is expected to contribute to a deeper understanding of the tardigrade redox adaptive mechanisms involved in their survival strategies under adverse environments.

A2.R10 - An improved expression and purification protocol enables the structural characterization of Mnt1, an antifungal target from *Candida albicans*

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Abstract

Objective: To isolate the Mnt1 protein from *Candida albicans* (CaMnt1) with a high degree of purity and characterize its structural properties. Results: We describe new ways to obtain high yield of recombinant CaMnt1 in *Komagataella phaffii* using flask induction. The purified protein's identity was confirmed by MALDI-TOF/TOF mass spectroscopy.

The Far-UV circular dichroism (CD) spectra demonstrates that the secondary structure of CaMnt1 at pH 7.0 has a prevalence of pronounced negative dichroic bands at 208 nm and 218 nm, compatible with a protein formed by α-helices (208 nm and 222 nm) and β-sheets (218 nm). The fluorescence spectroscopy results show that the tertiary structure of CaMnt1 is pH-dependent, with a greater intensity of fluorescence emission at pH 7.2. The results from molecular modeling corroborate the hypothesis that Tyr209 stabilizes the formation of an oxocarbenium ion-like intermediate during nucleophilic attack of the donor sugar, ruling out the hypothesis that it performs a nucleophilic attack in a double displacement mechanism. Conclusions: This methodology can substantially improve the yield of recombinant proteins expressed in flask-grown yeasts. In addition, we describe the structural characterization of a fungal mannosyltransferase that can be exploited for the development of new antifungal drugs.
Keywords: α-1,2-mannosyltransferase, Candida albicans, glycosylation, Mnt1/Kre2, structural analysis, drug target.

A2.R11 - Proteomics of macrophages in response to infection with *Trypanosoma cruzi* and the induction of innate trained immunity by *Phytomonas serpens*.

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The protozoan *T. cruzi* is the causative agent of chagas disease. Macrophages are phagocytes that are part of the first line of defense that the parasite faces when invading the vector host. The present work aims to characterize the proteomic variations of macrophages in response to in vitro infection with *T. cruzi* and the induction of innate trained immunity by the trypanosomatid phytoparasite *Phytomonas serpens*, which shares antigens with *T. cruzi*. Macrophages were produced by differentiation of THP-1 monocytes. Several experiments were carried out to optimize conditions to test the following conditions of macrophage interaction with trypomastigote forms of *T. cruzi*: 1- macrophages without contact with *T. cruzi* or *P. serpens* (negative control), 2- Macrophages after infection with T. . cruzi, 3- Macrophages incubated with *P. serpens*, 4- Macrophages incubated with *P. serpens* followed by infection with *T. cruzi*, 5- Macrophages incubated with LPS (positive control). In these experiments, the strains of *T. cruzi:* Y and the transformed CL Brener strain expressing the td Tomato fluorescent protein were used, which were produced from metacyclic epimastigote forms, in an aged culture with LIT medium. The results of these assays are being or will be monitored by optical microscopy, fluorescence microscopy and proteomic analysis.

A2.R12 - Scanning protein structure databases for 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 on 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. The structures deposited in the Protein Data Bank and AlphaFold Protein Database were analyzed by an algorithm that measured the distances 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. Some of those triads had the two arrangements that are not found in serine proteases, so their accessible surface area was compared to the pattern found in serine protease catalytic residues. The only triad that met this requirement was accessible to solvent but far from the protein surface and, therefore, inaccessible to peptides. To model a serine protease with an undocumented triad using this protein would involve removing part of the protein so peptides can reach the triad, and then including an oxyanion hole and substrate binding residues, so this protein was discarded as a model.

A2.R13 - Integration of computational and experimental strategies in the discovery and characterization of Thimet oligopeptidase inhibitors from *Trypanosoma cruzi*

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Thimet oligopeptidase (TOP) is a metalloprotease that belongs to the M3 family and has a zinc-binding motif, composed of the amino acid sequence HEXXH, which is highly conserved among this family. Here we aimed to perform a biochemical and structural characterization of TOP from *Trypanosoma cruzi* and evaluate how its overexpression or absence can impact on the life cycle of the parasite that causes Chagas' disease. The three-dimensional structure of TOP was predicted using different servers, and the structure chosen for docking was that obtained using AlphaFold, which uses artificial intelligence in its prediction. Molecular docking was carried out using Glide Maestro software and visual inspection analysis was conducted using PyMOL or UCSF Chimera software. TOP was expressed using a heterologous expression system in bacteria using the E. coli BL21 Ai strain. The protein was purified using affinity chromatography, its purity and confirmation were verified by SDS Page and Western Blot. We produced specific antibodies against the enzyme which were localized close to the parasite's flagellar pocket, a site of intense exchange of molecules with the extracellular environment. In addition, we observed that the enzyme is active against the substrate Mca-Pro-Leu-Gly-Pro-D-Lys(Dnp) and that its activity is zinc-dependent, as expected. Although its inhibition is not affected by EDTA, the enzyme is inhibited by Cpp-Ala-Ala-Phe-pAb with an IC50 of 54.29 nM. Our work shows the potential for the development of TOP inhibitor molecules and how this enzyme could be an important therapeutic target for Chagas disease which is a neglected tropical disease with a major socio-economic impact in the countries where it occurs.

A2.R14 - Caracterização da modulação de canais iônicos dependentes de voltagem por anestésicos gerais injetáveis e voláteis com a técnica de eletrofisiologia celular patch clamp.

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Over the decades, new compounds have been described, but the exact molecular mechanism for inducing therapeutic unconsciousness, muscle relaxation, and analgesia is not fully elucidated. This is due to the diversity of molecular targets already described and the molecular characteristics of anesthetics, which are often small molecules with hydrophobic properties. The ability to disturb cell membranes, attributed for many decades to the lipophilic properties of anesthetics, was considered the mechanism of action. However, in recent decades, direct interaction with membrane proteins has been characterized. This study focuses on characterizing the activity of volatile and injectable general anesthetics at clinical concentrations on ion channels using the patch clamp in

vitro cellular electrophysiology technique. TCE showed preferential inhibition of voltage-dependent potassium channels (Kv) in SH-SY5Y cells. With activity on the Kv1.3 channel, an EC50 of 26.68 mM was described, and inhibition of 10.53% on Kv1.2WT. In the mutated Kv1.2-FRAKT channel, a current potentiation was characterized, indicating a possible mutation in the interaction site. Among the subtypes tested with TCE at 5 mM, the Kv3.1 channel showed the highest rate of current amplitude inhibition (±20%) and had an effect on the channel's opening kinetics, increasing the time to reach maximum conductance. Propofol was evaluated at concentrations of 10, 30, and 100 uM, showing dose-dependent effects on steady-state inactivation in all tested channels, with variations of up to 30mV for NaV1.4. A reduction in current amplitude was also observed in the first 50s, ranging from 10 to 30%, with no modulation in recovery from inactivation. Action potentials recorded in SH-SY5Y cells differentiated with acid retinoic expressing Kv1.2 showed hyperpolarization after pronounced depolarization with sevoflurane perfusion, an effect in line with the increase of maximum conductance in the presence of the anesthetic.

A2.R15 - Method for detecting SARS-CoV-2 viral RNA using Ribozymes and associated DNA hairpins in hybridization chain reaction with fluorescent resonance 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 ribozymes recognize the SARS-CoV-2 RNA that is 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 in vitro transcribed to produce the target viral RNA and three ribozyme molecules that were used in the Ribozyme cleavage assays. In addition, DNA hairpins labeled with Cy3/Cy5 fluorophores were designed and used in FRET-HCR assays with DNA and RNA target sequences. Currently, detection experiments involving all stages of the process were conducted in order to validate its complete effectiveness. The results indicated that two engineered ribozymes were able to identify and cleave the in vitro transcribed target of viral RNA and the DNA hairpin molecules were efficient in HCR reactions and the fluorophores in FRET reactions with DNA and RNA target sequences, with the best results showing fluorescence intensity 1.34 ± 0,11 times higher than the negative control, thus highlighting the potential of the proposed method. Additionally, work is being done on the design and prototyping of the FRET detection device by processing images taken by smartphones.

3. Genética, genômica e evolução (A3)

A3.R1 - Development and application of scalable bioinformatics pipelines for comprehensive bacterial genomics analyses

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The 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 efficient and standardized computer pipelines that comprehend the quality control of raw sequencing data, genome assembly, and extensive gene annotation with graphical reports. In particular, we provide an annotation module specialized for bacteria involved in nosocomial infections, which identifies antibiotic resistance genes (ARG), virulence factors, prophages, and integrative elements. To demonstrate their use, we sequenced and analyzed three *Klebsiella pneumoniae* samples from the University Hospital of Brasilia. The annotation revealed that they belong to the worldwide threat sequence type, ST 11, one of the major carbapenem-resistant *Klebsiella pneumoniae* (CRKP) lineages. We detected the presence of NDM-1, CTX-M-15, and OXA beta-lactamases genes in all three isolates, as well as several other ARGs distributed among chromosomes and plasmids. Additionally, virulence genes frequently related to hypervirulent strains, such as Salmochelin, have also been detected. Synteny analyses showed that besides the highly similar genome content, their genomes are structurally different, with several rearrangements observed. Finally, we carried out a retrospective comparative genomics analysis using other CRKP lineages isolated from Brasilia and other Brazilian locations. The results indicate that the resistance phenotype is shifting, with recent isolates displaying co-existence of multiple carbapenemases and other ARGs in their genomes. In conclusion, the results reiterate alerts about the emergence of high-risk clones due the convergence of resistance and virulence genes, reinforcing the need for pathogen surveillance programs for combating the spread of such high-risk characteristics.

A3.R2 - Genetic Transformation of Common Bean for RNAi-Mediated Silencing of Whitefly (*Bemisia tabaci*) Genes

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The common bean (Phaseolus vulgaris L.) is a crop of significant food importance in Brazil, but it faces a substantial challenge represented by the whitefly *Bemisia tabaci*, which transmits the Bean golden mosaic virus (BGMV), resulting in significant losses in bean production. The intensive use of chemical insecticides presents limitations in terms of cost, environmental impact, and the development of resistance in insect populations. Therefore, the central objective of this project is to develop bean lineages resistant to whiteflies by silencing the acetylcholinesterase (AChE) and ecdysone receptor (EcR) genes of the insect, using RNA interference (RNAi) technology. This strategy aims to reduce the survival of whitefly nymphs and adults, thus contributing to a more sustainable crop management. To achieve this goal, the project encompasses various stages, from the preparation and multiplication of DNA and vectors for transformation to the obtaining of transgenic plants by biolistics. The identification of transformed plants is carried out through DNA extraction and PCR for the detection of the selection marker. Furthermore, progeny tests are conducted to assess the inheritance of the transgene, as well as the analysis of siRNA production in transgenic plants. The performance of transgenic plants is evaluated in bioassays with insects, covering aspects such as whitefly mortality, feeding preference, oviposition, and the quantification of genes in whiteflies. These combined steps aim to develop new whitefly-resistant bean lineages, with the potential to promote a more sustainable management of this vital crop.

A3.R3 - A novel strategy for GmPR10 overexpression via CRISPR/Cas9 aiming at increasing tolerance to nematodes in soybean

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The root-knot nematodes (RKN), *Meloidogyne spp.*, are considered one of the most economically important plant pathogens, impacting both yield and quality of soybeans. Previous transcriptome and proteomic studies of contrasting soybean genotypes (BRS133- susceptible, and PI595099- highly tolerant) searched for candidate genes that may be directly related to greater tolerance of soybean to RKN. Among these candidates, we identified genes encoding proteins that inhibit or degrade the enzymes of the digestive tract and cuticles of these pathogens, as the protein of class 10 related to the pathogenesis (GmPR10). The overexpression of GmPR10 in the tobacco model plant promoted reductions in the number of galls (51.6- 57.8%) and in nematode reproduction factor (40.4-48.7%), compared to wild-type plants. These results point to the GmPR10 gene as a promising candidate to be engineered. The editing of upstream open reading frames (uORFs) has recently emerged as a strategy for gene overexpression via CRISPR/Cas9 technology. uORFs affect the translation of associated downstream primary ORFs, not always in a positive way; identifying and even editing them can increase the translation of the GmPR10. Initially, the two uORFs present in the 5'-UTR sequence of the gene were identified, cloned, and site-directed mutated to delete its start codon. The validation of these predicted uORFs was carried out in transformed protoplasts of *Nicotiana benthamiana* with a dual-luciferase reporter vector in which it is possible to analyze the expression of luciferase by that of renilla luciferase. As expected, expression analysis at mRNA level indicated that there is no statistically significant difference among mutated and non-mutated uORF variants. As for the results of the expression of the reporter proteins (LUC/REN), double mutations (uORFs 1 and 2) increase 3.5-fold approximately in LUC/REN activity that indicates both uORFs for GmPR10 editing via CRISPR/Cas9 the overexpression of GmPR10.

A3.R4 - Ship: an annotation-based program for the identification of putative genomic safe harbors in eukaryotic model organisms

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Targeting foreign genes for insertion into specific genomic regions that do not affect endogenous gene expression is an ideal strategy for integrating them into host cells. These regions, known as Genomic Safe Harbors (GSHs), can be identified through diverse approaches, including viral insertion site analysis, examination of gene function loss, or similarity to GSHs found in other organisms. Most available GSHs are situated in regions of high gene density, raising concerns about unstable expression and unpredictable phenotypes. As synthetic biology continues to move towards investigating polygenic modules rather than single genes, there is an increasing demand for tools that can systematically and efficiently identify GSHs. To expand the repertoire of GSHs available, we present SHIP, a software designed to detect potential GSHs in eukaryotic organisms. SHIP uses a set of rules to target intergenic regions minimizing potential gene expression disruption. We employed the program to evaluate GSHs in Saccharomyces cerevisiae. The program detected six possible GSHs, which were manually curated, with five of them chosen for further in vivo analysis. Promising regions were identified based on sequencing, cytometry, stability test, RT-qPCR, and RNA-Seq data. These 5 regions displayed a high cell count accumulating the reporter green fluorescent protein (UkG) and transgene stability after ten days of cultivation. Although further experiments will be required to confirm these regions as genuine GSHs, our results indicated that SHIP provides a list of

promising candidates to assist in the experimental assessment of GSHs for any organism with an annotated genome.

A3.R5 - Genome-wide SNP allelotyping and genetic diversity analysis of the Brazilian maize core collection and indigenous traditional varieties

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The core collection of the Brazilian Maize germplasm collection (BMCC) with 300 accessions was established to represent the genetic diversity of more than the 4000 accessions of the overall Embrapa collection organized into four races: indigenous, old commercial, recent commercial and exotic commercial. This classification, based on morphology and geography could be validated, enhanced or better understood by genomic data to clarify its genetic structure, diversity and relationships. To enable reduced-cost and faster characterization of maize accessions, each one corresponding to an outbred diverse population, we adopted an allelotyping strategy of DNA pool to estimate allele frequencies of each accession for 3526 SNPs genotyped on the EMBRAPA 65KMultispecies chip. Initially three pool assembly methods were evaluated for their precision by comparing the estimates of allele frequencies obtained by the normalized allelic intensity ratio of the two SNP alleles in the pool, with the estimate from genotyped individuals. The methods were: (1) Equimolar pool of individually extracted nanodrop measured DNA; (2) same as (1) but fluorescence measured DNA; (3) DNA extraction from bulked leaf tissue samples weighed to the nearest 0.1 gram. The treatment that provided the lowest RMSD (Root Mean Square Deviation) was the bulk leaf extraction method, with an average RMSD across accessions of 5.7%. DNA pools of 287 BMCC accessions were alellotyped and 64 of them in replicates. Reproducibility assessed by the Wright fixation index (Fst= 0.0047) indicated that accurate estimates can be obtained with minimal sampling error. A k-means clustering based on Fst among all 287 accessions (Fst from 0.0017 to 0.57) detected six genetic groups. We are now expanding the study, to 204 indigenous traditional Brazilian maize varieties to jointly analyze them with the BMCC data to understand their divergence, structure, and potential genomic signatures of domestication.

A3.R6 - Characterization of human antibodies using third generation sequencing methodology for processing and analysis

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The immune system is divided into two main pathways: the innate immune system (IIS) and the adaptive immune system (AIS). In the AIS, T and B lymphocytes are stimulated to multiply and produce identical cells with specific receptors for recognizing pathogens. B lymphocytes undergo B-cell receptor (BCR) rearrangement during maturation, and the receptors are released after these cells differentiate into plasma cells. The immune repertoire is the set of BCR and TCR receptors rearranged by lymphocytes during the EIS response. Understanding immune repertoires in different contexts is important for immunotherapy, clinical diagnosis and the discovery of therapeutic targets. Antibody phage display (APD) technology uses functional antibody fragments to identify specific ligands to the antigen being tested. Next-generation sequencing (NGS), such as Illumina sequencing, is used to identify the enriched domains after selection with phage display, but this methodology has some disadvantages as it does not allow simultaneous reading of the VH and VL region of the molecule, hindering enrichment analysis, a process that verifies an increase in antibodies binding to the antigen. The third generation

of sequencing allows larger DNA fragments to be read, but it does present some barriers. In the case of sequencing with the MinION device (nanopore, oxford), a high error rate (~10%) means that additional bioinformatics processes are needed to correct errors and identify enriched sequences in the samples. Two sequencing methods were used, the first was native sequencing of the samples after DNA linearization, the other was the use of phi29 DNA polymerase for rolling circle amplification, allowing the generation of concatamers, which will be used to form consensus with a lower error rate.

A3.R7 - Research of genetic variants in biological female patients who have syndromic intellectual disability

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Intellectual disability (ID) corresponds to incomplete mental development, which leads to certain limitations in mental abilities, intellectual functions, adaptive behavior and motor skills, when compared to the abilities existing in individuals of the same age, gender and culture. These limitations create difficulties in the social and domestic life of the affected person, which causes the need for physical and emotional support from the family and those close to them, as well as qualified professional help to better guide relatives on how to deal with the situation. The objective of this project is to increase the existing knowledge of syndromic ID in women by searching for variants that explain their condition. Karyotype, Microarray, Fragile X syndrome and Exome Analysis tests were carried out in an attempt to determine the cause of the clinical condition. The exomes were analyzed on the genetics platform Franklin. In total, 23 female patients participated in the research and 9 of those have had their exome analyzed so far. Of the 23 patients, 13 had family with history of ID or some other learning disorder, with only 3 of these families having a case of consanguinity. The phenotype presented by the patients was extremely important for the association of ID with a specific syndrome. The variants that were found in the exomes demonstrated the heterogeneity of ID.

A3.R8 - Proteomic changes caused by *Hemileia vastatrix* in coffea arabica plants during a susceptible interaction

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Brazil ranks first in coffee production. The species *Coffea arabica* and *C. canephora* are the best known, especially C. arabica, for having a higher market value. On the other hand, this species is more susceptible to diseases, including coffee rust, caused by the fungus *Hemileia vastatrix*. This disease affects coffee plantations all over the world, causing great economic losses. The primary method of control involves using copper-based fungicides, however this not only raises production expenses but also disrupts non-target species. Hence, the search for alternative methods is necessary for a more environmentally friendly and efficient control. An alternative to agrochemicals has been the gene silencing of susceptibility genes (S-genes). During a compatible interaction, S-genes are activated, aiding the pathogen's colonization. In this context, the present study aimed to identify potential S-genes through proteomic studies. For this study, a total of 18 coffee plants 6 months old were used, of which 9 were subjected to infection with *H. vastatrix* and 9 were non infected (control condition). On the tenth day of infection, the plants were collected, subjected to total proteins extraction and analyzed by LC-MS/MS. In total, 288 differentially abundant proteins were identified when the inoculated condition was compared with the control condition, in which 150 were increased and 138 decreased. Interestingly, there was a large increase in the abundance of proteins involved in carbohydrate metabolism, such as Beta-glucosidase, potentially involved in the

release of Beta-glucans present in fungal cell walls, favoring their pathogenicity and successful colonization. In addition to proteomic analysis, RT-qPCR was performed to validate potential candidates for S-genes. The results obtained in this study revealed important candidates to be used in the future as targets for gene silencing or knockout with the aim to obtain rust-resistant coffee plants.

A3.R9 - Bioinformatics workflows for extrachromosomal circular DNA analysis

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Extrachromosomal circular DNAs (eccDNAs) are double stranded circularised DNA molecules found in all eukaryotic organisms investigated so far, including humans. From their discovery in the 1960s, their independent replication outside of chromosomes, wild variation in sizes, and diverse genetic content have since been uncovered. eccDNAs have gained the spotlight as potential cancer and ageing research targets due to their apparent correlation with genomic instability - their investigation is limited, however, by a lack of accurate, easily accessible bioinformatics tools. Raw data processing in eccDNA research is a multi-step process, thus necessitating the deployment of computational workflows, or pipelines, which connect the necessary steps as a means to minimise errors and aid reproducibility. However, those unfamiliar with the command line may have trouble installing or running such programs. Furthermore, the output of most available pipelines is direct to command line, simple text, or distributed within directory trees, making data interpretation harder for the final user. The incorporation of several programs within a single, easy-to-use pipeline and generation of a consensus report using their individual predictions aims to facilitate the interpretation of eccDNA-related research. Comparison between four different published eccDNA detection tools (FLEC, ecc_finder, cyrcular-calling and CReSIL) showed the number of predicted eccDNAs varied wildly for the same human fibroblast samples. An intersection between all of their predicted regions, however, highlighted those predicted by all of them, creating a consensus which can be used for annotation and further biological interpretation. Visual aids such as ideograms, upset plots and Venn diagrams were generated to aid with output comparison between programs. In the future, we hope to implement this workflow, with the addition of automated functional annotation tables and dynamic reports, as a containerized pipeline.

A3.R10 - Development and Validation of a Screening Test for Quantitative Detection of TRECs and KRECs in Capillary Blood Conditioned on Filter Paper

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During lymphocyte maturation, DNA circles are formed and excised as a result of the differentiation of lymphocyte receptor chains, referred to as TREC (T lymphocytes) and KREC (B lymphocytes). Quantification of these products enables the screening of diseases such as SCID, which can lead to death if left untreated. Early diagnosis and monitoring of newborns are essential for the effectiveness of treatment, making the quantification of TREC and KREC a potential addition to the newborn screening tests in Brazil. The aim of this study is to develop and validate a quantitative real-time PCR screening method and establish a reference value model. Standardization includes the following studies: accuracy, precision/reproducibility, reportable range, analytical sensitivity, and analytical specificity. An In-house qPCR was standardized on the LightCycler II thermocycler (Roche) using primers specific for TREC and KREC synthesized by IDT. RNAseP amplification was used as an internal control. Optimal primer and probe concentrations (0.5 µM and 0.25 µM, respectively) and reaction conditions (95 °C/30s, followed by 45 cycles

of 95°C/5s and 60°C/30s) were established. Two reaction kits were evaluated, the 2X TaqPath ProAmp Multiplex Master Mix (Thermo) and DNAMaster (Roche), with the latter showing better performance. For comparison against the in-house method, the RUO TaqMan® SCID/SMA NBS Assay kit (Thermo) was validated following the manufacturer's instructions. Using a calibration curve composed of six 1:10 dilution points from positive controls, a standard curve for this assay was constructed ($R2 \geq 0.99$, Slope -3.31, y-intercept ~38, and error ≤ 0.05). The stability of the standard curve was also assessed and showed minimal variation between assays. The prospects of this work include completing analytical precision tests for the in-house test and defining the reference value, thus enabling the availability of a low-cost screening test for neonatal screening in Brazil.

A3.R11 - Biotech strategies for root-knot nematode control in soybean

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Soybeans are the most important crop grown in Brazil and among the challenges in its production, the parasitism by root-knot nematodes (RKN) of the genus Meloidogyne stands out. This endoparasite feeds on the roots of economic crops and produces giant cell clusters called galls that interfere with the plant's uptake of water and soil nutrients. RKN is controlled by nematicides, which are inefficient and highly toxic, crop rotation, and moderately resistant cultivars derived from a single genetic source in soybean. Therefore, the use of biotech approaches to incorporate new sources of resistance into elite cultivars is promising. Here, two biotechnological strategies were used simultaneously to RKN control in soybean: (1) Overexpression of the AdEXLB8 gene; (2) RNAi-mediated silencing of nematode genes involved in its primary metabolism or plant infection process, such as those encoding cysteine protease, isocitrate lyase, splicing factor, and the effector 16D10. Genetically modified (GM) plants were obtained using the Agrobacterium-based transformation method. These plants were screened by transgene amplification by PCR and enzyme-linked immunosorbent assays to the molecular characterization. Plants from three independent transformation events at T2 generation were selected for challenge assays against *Meloidogyne incognita*. Fifteen-day-old plants were inoculated with 1000 J2 juveniles of *M. incognita*. After 60 days, the GM plants showed a significant reduction in the number of galls per gram of root (22.0-34.0%), in the number of egg mass per gram of roots (46.0-50.0%), in the number of eggs per gram of roots (59.0-59.6%), and in nematode reproduction factor (30.0-50.0%) compared to wild-type plants. So far, the pyramiding strategy appears effective in controlling *M. incognita* and can be applied to soybean breeding programs as a complementary source of resistance to RKN.

A3.R12 - CRISPR Editing of a Gene Associated With the "erect leaves" Phenotype in Micro-Tom tomato

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The broad efficiency associated with the advent of the CRISPR system, as an adaptive immunological memory mechanism, raised this system to a level of global importance for biotechnology as a tool for genome editing, largely, due to the high specificity of Cas9 endonucleases in editing target regions in the genome of commercially valuable crops, such as tomatoes, *Solanum lycopersicum*. Tomato is considered one of the five main commercial crops in the world and a basic component of the diet in several countries. However, in contrast to the increase in tomato productivity over the last few decades, breeding programs have faced difficulties in advancing the development of improved varieties to the main standards of agricultural production, with emphasis on disease

resistance, as well as increased yield agricultural. Among the various factors capable of compromising the commercial production of tomatoes, the limitation caused by the density of this crop in the field, as well as events that compromise their photosynthetic efficiency, stands out as a guiding factor requiring the search for strains carrying profiles genes characterized by presenting a phenotypic profile marked by plant architecture that allows greater density, as well as better photosynthetic yields, a characteristic that in recent decades has been widely associated with genes coding for called auxin transport proteins. Based on the data cited here, we propose the "knockout" of a gene of agricultural importance in tomatoes, which will not be mentioned here for reasons of intellectual protection. This gene, in turn, has been widely described in the literature in mutants characterized by the "erect leaves" plant architecture phenotype. The editing of this gene will be entirely mediated by the advent of the CRISPR technique.

4. Biologia celular e molecular de microrganismos (A4)

A4.R1 - Characterization of extracellular vesicles of the mycoparasitic fungus *Trichoderma harzianum*

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Trichoderma harzianum is a filamentous fungus that can act as a mycoparasite, saprophyte, or a plant symbiotic. It is widely used as a biological control agent against phytopathogenic fungi and can also be used for plant growth promotion and biofortification. The interaction between *T. harzianum* and phytopathogenic fungi involves mycoparasitism, competition, and antibiosis. The mechanism by which *T. harzianum* communicates and interacts with its hosts is not well understood; however, recent studies have shown that extracellular vesicles (EVs) play an important role in this process. EVs are secreted by the cell membrane and vary in size from 50 to 1000 nm. They transport proteins, lipids, and microRNAs, and play important roles in the communication between cells and organisms. In this study, we characterized the extracellular vesicles of *T. harzianum* produced in response to S. sclerotiorum mycelia and identified proteins that mediate communication and interaction between the fungus and its hosts.

A4.R2 - Delivery of Anti-CD3 scFv by *Saccharomyces boulardii* Decreases Inflammation in DSS-Induced Colitis in Mice

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Introduction. Inflammatory Bowel Diseases, such as Ulcerative Colitis (UC), are chronic inflammatory disorders that affect the gastrointestinal tract (GIT). UC is characterized by recurrent inflammation and damage to the colon region, and treatment is based on anti-inflammatory drugs and monoclonal antibodies. *Saccharomyces boulardii* is a probiotic yeast widely used to treat intestinal disorders, and studies suggest beneficial effects in UC. Additionally, it can be genetically modified for heterologous expression and adapted for the oral delivery of molecules in the GIT. Here, we investigate the therapeutic effects of *S. boulardii* as a delivery vehicle of an immunomodulatory anti-CD3 scFv antibody fragment in a Dextran Sodium Sulfate (DSS) induced colitis mouse model. Methods and Results. ScFv expression in yeast was monitored by western-blot and Cytometry. All the diseased groups were given 3% DSS in water for the first five days. During the 11-day experiment, mice were orally

gavaged daily with recombinant yeast, for the treated group or 0.9% saline, for the control group. Weight, fecal consistency, and rectal bleeding were monitored to evaluate the disease activity index, and on the day of euthanasia, the colon was measured and weighed. The anti-CD3 treated group showed similar inflammatory parameter values to the healthy group and significant differences from the non-treated group. Conclusion. Despite low antibody detection in transformed yeast in flow cytometry tests, their oral administration improved disease symptoms. Next, we are working to make the antibody more available on the yeast surface and maximize its beneficial effects to test again in an in vivo model.

A4.R3 - Unraveling new roles of the apoplastic soybean Germin-like protein 10 in land plants: from development to resistance against root-knot nematodes

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Root-knot nematode (*Meloidogyne spp.*), have evolved infection mechanisms that involve the secretion of effector proteins into host plants to suppress immune responses and facilitate parasitism in a large range of land plants. During co-evolutionary process some host acquired different resistance mechanism to overcome RKNs infections, between them, the PI 595099 soybean genotype. Previous omics approaches performed in our research group identified Germin-like protein subfamily 1 member 10 as a pivotal candidate resistance gene in view of high expression 30 day after inoculation. Some point mutations in GmGER10 promoter was capable of increasing the cis-element composition and reinforcing its expression in a specific way to M. incognita-induced feeding sites. Their subcellular localization confirmed that this protein is located in the extracellular matrix and DAB staining of transgenic tobacco's leafs overexpressing GmGER10OE drastically reduced the H2O2 content. In turn, two independent knockout ger10 into *A. thaliana* genome, were also investigated at this study, and showed the orthologous germin loss-of-function increase the susceptibility against this RKNs. Although of possible "trade-offs" between GmGER10OE and in both model plants, transient expression in soybean hairy roots showed more than 55% in reduction in galls/plant. Galls-induced in transgenic tobacco lines showed 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. Our data provided the first evidence of GmGER10 in resistance against *M. incognita* and will be investigated as one target in crop protection.

A4.R4 - Protein characterisation of trypanosomatid subcellular compartments using APEX-2 and proteomic approaches

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Trypanosomatids are a group of parasitic protozoa belonging to the Kinetoplastida class, including species from the genera Trypanosoma and Leishmania. These microorganisms possess unique organelles that play a crucial role in their nutrition and survival. An example is the glycosome, an organelle related to the peroxisome, known to contain the majority of enzymes related to the glycolytic pathway and other vital metabolic pathways. Due to the presence of enzymes in pathways considered essential for the survival and nutrition of various trypanosomatids, many glycosomal enzymes have been promising targets for the development of new drugs. So far, the analysis of the overall glycosomal proteome in trypanosomatids has been primarily approached through conventional organelle enrichment methods. However, this approach may result in limited coverage of identified proteins. To

overcome this limitation, proximity labeling methods have been extensively investigated for proteomic mapping of different subcellular compartments. Yet, their application to trypanosomatid glycosomes had not been evaluated. In our studies, we aimed to determine whether the APEX2-based proximity labeling strategy could be effectively employed for analyzing the proteomic composition of glycosomes and nucleus in L. infantum and T. cruzi. To achieve this, we fused APEX2 with the glycosomal targeting signal peptide (PTS1), as well as with the nuclear localization signal (NLS). The results indicated that APEX2- PTS1 and APEX2-NLS directed specific biotinylation of the glycosome and nucleus, respectively, in L. infantum. Our findings establish the potential feasibility of applying APEX2 in the biochemical context of L. infantum. We believe that this approach will allow us to reveal metabolic adaptation mechanisms employed by this group of parasites, while also identifying potential pharmacological targets for the treatment of diseases associated with trypanosomatids.

A4.R5 - Caracterização funcional e estrutural dos peptídeos antimicrobianos sintéticos Grammistidinas hibridizados com motivos ligação metálica ATCUN

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Antimicrobial peptides (AMPs) have attracted the attention of researchers, as they have shown promise as substitutes or assistants to conventional antibiotics, because they act on bacterial membranes, reducing their possibility of resistance. Though they exhibit intriguing properties, certain physical-chemical aspects may challenge their commercialization. Therefore, rational design strategies must be employed to optimize and produce more effective antimicrobial agents without causing toxicity. Thus, the goal of this work was to characterize the chemical and biological properties of two peptide analogs of Grammistidin. The peptides were synthesized in a solid phase and purified using High-Performance Liquid Chromatography (HPLC). With a minimum inhibitory concentration (MIC) for both peptides ranging from 4 to 8 µM, the peptides demonstrated remarkable efficacy in inhibiting the development of the Gram-positive bacterium S. aureus and its resistant version. However, a range of 64 to 128 µM was observed for Gram-negative bacteria including E. coli and K. pneumoniae, along with resistant bacteria of P. aeruginosa and K. pneumoniae. The carbapenem-resistant Acinetobacter baumannii, on the other hand, also displayed MIC values of 4 and 8 µM. After the tests, the bacteria were sown in a solid medium, where we could observe the bactericidal action of the peptides studied. Except for resistant bacteria and E. coli, for which testing could not be performed, all of them demonstrated the death of 99.9% of the bacteria at 32 to 64 µM. At a dosage of 16 µM, the ATCUN-Pp2a peptide exhibited hemolysis above 50%, while the remaining peptides showed elevated hemolytic activity. In summary, the data demonstrate that the peptides have modest hemolytic activity at therapeutic dosages and strong bactericidal efficacy against certain bacteria, especially resistant strains.

A4.R6 - In vitro antiviral activity of Brazillian Cerrado plant extracts from *Eugenia dysenterica* and *Erythroxylum suberosum* against Chikungunya virus

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Chikungunya virus (CHIKV), transmitted by Aedes mosquitoes, belongs to the Togaviridae family and the Alphavirus genus. Clinically, the disease caused by CHIKV resembles dengue with symptoms like fever, headache, and arthralgia. It significantly impacts public health in many neotropical regions, causing frequent outbreaks. A promising antiviral strategy involves exploring plant-based compounds for disrupting viral infection cycles.

Secondary metabolites from various plants, including alkaloids, saponins, flavonoids, and coumarins, exhibit antiviral potential. Flavonoids stand out among these. Eugenia dysenterica (ED) and Erythroxylum suberosum (ES), medium-sized Cerrado trees, rich in flavonoids, are traditionally used for various purposes, including as an antirheumatic, antiasthmatic, diuretic, anti-inflammatory, and antimicrobial activity. To assess the potential of ethanolic leaf extracts against CHIKV, preliminary in vitro analyses were conducted. The cytotoxicity profile of two leaf ethanolic extracts on Vero cells was determined through lysosomal viability analysis, using the neutral red assay. Simultaneously, the antiviral potential was determined though plaque assays at different treatment steps. Cytotoxicity assays indicated that the concentration toxic to 50% (CC50) of the Vero cells at 48 h was 10,461 for ED and 1,623 mg/mL for ES extracts. Antiviral assays revealed that ED ethanolic extract inhibited 69.4% of CHIKV activity at 80 µg/mL, while ES inhibited over 79% at 40 µg/mL during post-treatment step. Additionally, ED extract inhibited 85.4% at 100 µg/mL during pre-treatment. Ultimately, ED inhibited 59% at 80 µg/mL, and ES exhibited 96.8% inhibition at ten µg/mL concentration during co-treatment step. Eugenia dysenterica and Erythroxylum suberosum emerge as potential sources for antiviral compounds against CHIKV infections. Ongoing studies further investigate their antiviral activity.

A4.R7 - Development of an expression platform for peptides in *Komagataella phaffii*

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Antimicrobial peptides (AMP) are cationic and amphiphilic molecules found in a wide range of organisms from bacteria to vertebrates, plants and fungi. These molecules present microbicidal activity and tend to show low toxicity to eukaryotic cells, making then possible candidates to substitute conventional antibiotics. Considering this rational, DS 01 is a 29 amino acid residues long AMP (M+H+=2793.39 Da), which disrupts the cell membrane of targeted microorganisms in vivo. DS 01 is a member of the dermaseptin family firstly identified, isolated, and characterized from the skin secretion of frogs from the Phyllomedusinae subfamily. The present work aims the development of a platform for the heterologous production of peptides in the yeast Komagataella phaffii (previously Pichia pastoris) based on the yeast´s secretion and protein processing apparatus. The production of DS 01 was primarily chosen as a proof-of-concept, since it represents a valuable academic challenge. Initially, Escherichia coli was transformed with plasmid containing the expression signals for the production of correctly processed yeast alpha mating factor (αMF), a 13-amino-acid peptide. DS 01 was cloned as four identical 29-amino-acid modules interspaced by Kex2 and Ste13 cleavage sites and preceded by the αMF secretion signal. Plasmid DNA was isolated using a midiprep kit (Qiagen) followed by digestion with XhoI and NotI restriction enzymes overnight at 37°C. The expression cassettes were purified from agarose gel and ligated to two Pichia expression vectors: one of them with the constitutive glycolytic PGK1 promoter PGK, and the other with the methanol-induced promoter from AOX1. The resulting plasmids were named pKDS01 and pPICZalphaA_DS01. Both vectors were linearized with SacI prior to yeast electroporation. K. phaffii transformants were obtained on selective plates. The next step will consist on the expression and purification of the peptide.

A4.R8 - Genome-resolved metagenomic analysis of Great Amazon Reef System sponge-associated Latescibacterota and their potential contributions to the host sponge and reef

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The Great Amazon Reef System (GARS) is a vast biogenic reef impacted by a sediment plume layer, which creates a light-reduced environment and affects physicochemical properties as well as living organisms, including sponges. The sponge's microbiome plays many ecological roles, helping the sponge to thrive and contributing to the ecosystem. It is also a rich source of commercial and industrial bioactive compounds. In this context, ten metagenome-assembled genomes (MAG) of the candidate phylum Latescibacterota, derived from GARS sponge microbiota, were analyzed to predict their ecological role and were prospected for biotechnological traits. GARS sponge tissues were obtained, and their metagenomes were sequenced and assembled, yielding 1,054 MAGs. Ten MAGs were chosen for further investigation based on their taxonomic classification and the abundance of the Latescibacterota in GARS sponges. The procedure included MAG's quality definition, metabolic reconstruction, and search for bioactive compounds. Metabolic reconstruction from medium to high-quality MAGs revealed potential roles for GARS sponge-associated Latescibacterota. These bacteria may help the host survive through aid in nutrient consumption by producing essential nutrients and breaking complex substrates; host protection via defense systems; and harmful substances detoxification. Also, genes related to persistent organic pollutant degradation, like glyphosate, and biogeochemical cycle reactions such as ammonification, sulfate reduction, and phosphorus remineralization, were identified, implying participation in bioremediation and nutrient cycling. Finally, the examined MAGs contain genes for numerous bioactive substances, including industrial enzymes, secondary metabolites, and biologically active peptides, which may have biotechnological significance. In conclusion, GARS sponge-associated Latescibacterota may contribute in multiple ways to the sponge and the reef and can be used to acquire new bioproducts.

A4.R9 - Serine-integrase controlled genetic switches for permanent memory: from minimized bacterium *Mycoplasmycoides* JCVI-Syn3B to plant model *Nicotiana benthamiana*

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The ability to regulate gene expression in response to external cues is one of the central mechanisms of the differentiation and maintenance of life in nature, as well as one of the main goals of scientists in efforts to control and reprogram organisms. The availability of molecular tools that allow genetic manipulation is crucial for such advances in synthetic biology, especially when creating intricate genetic circuits and activation cascades to work as synthetic regulatory networks. Serine-integrases (Int) are strong candidates for these applications due to their ability to rearrange DNA through insertion, excision, or inversion of a target sequence without the need of host factors. Taking advantage of their plasticity and robustness, here we propose the application of serine-integrases in the assembly of genetic circuits integrated into the genome of two models of opposing complexity: *Mycoplasma mycoides* JCVI-Syn3B, a synthetic bacterium hosting a minimized prokaryotic genome, and *Nicotiana benthamiana*, a model plant with its complex eukaryotic genome. In *M. mycoides* JCVI-Syn3B, Int9 and Int13 were tested as effectors in genetic switches capable of inverting a reporter gene sequence, but only Int9 activation resulted in a reporter gene expression increase. As for *N. benthamiana*, a more complex switch was assembled. Named Int-Plex@ (Integrase-Plant Expression), this genetic switch system consists of a reporter gene sequence flanked by a combination of att sites of Ints Bxb1, PhiC31, Int 13, and Int9 and can be divided into two modules: the inversion module, activated by Int9 or Int13, and the excision module controlled by ints phiC31 and BxB1. All four Ints were able to edit *N. benthamiana* genome successfully. This memory switch system can be used in future genetic circuits

to engineer and modulate plant metabolic pathways of economic and environmental importance, while also addressing transgene biocontainment issues given the possibility of cassette excision.

A4.R10 - Expression and occlusion of dengue virus NS1 peptides inside polyhedra of a cypovirus for serological diagnosis

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The dengue virus (DENV) is an arbovirus of global health concern. Existing serological diagnostic kits in the market primarily rely on detecting the highly immunogenic NS1 antigen, which is identifiable during the initial 5 days of infection. However, these kits face challenges including delayed detection of IgG/IgM antibodies and susceptibility to cross-reactivity with other serotypes and different arboviruses. Cypoviruses, members of the Spinareoviridae family, stand out for their ability to form highly stable and protease-resistant crystallized protein structures known as polyhedra. These structures hold promise for the development of serological diagnostic tests by incorporating recombinant proteins within them. This study aims to express both multi-epitope and individual epitopes from the NS1 region of the four dengue virus serotypes enclosed within polyhedra. To achieve this, a chimeric gene encompassing multiple epitopes was designed, incorporating segments of the NS1 protein from all four DENV serotypes and subsequently synthesized. Individual epitopes of each serotype were amplified using PCR. These constructs were them separately individually cloned into a plasmid containing the cypovirus polyhedrin, controlled by a baculovirus promoter and the sequence of the alpha-helix 1 (H1) of polyhedrin. The H1 has been demonstrated to associate with the polyhedrin protein to form polyhedra. Following cloning, the genes were fused to the to the H1. Recombinant baculoviruses containing these various constructs were generated and used them to infect insect cells. Infected insect cells exhibited the presence of polyhedral crystals. The expected molecular mass of the recombinant proteins were analyzed and confirmed by SDS-PAGE and Western-blot techniques. These recombinant crystals will undergo further purification and be assed as diagnostics inputs employing serum from patients previously diagnosed with dengue by RT-PCR and/or viral isolation.

A4.R11 - Uso do riboswitch de tetraciclina para controle on/off de expressão proteica em *Komagatella phaffii*

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The methylotrophic yeast *Komagataella phaffii* is one of the most important microbial platforms to produce recombinant proteins. Despite its importance in the context of industrial biotechnology, the use of synthetic biology approaches in *K. phaffii* is hampered by the fact that few genetic tools are available for precise control of gene expression in this system. In this work, we used an RNA aptamer activated by tetracycline to modulate protein production at the translational level. Using lacZ as gene reporter, we have demonstrated significant reduction of the heterologous protein upon addition of tetracycline. Furthermore, this genetic control device was applied for the control of Ku70p. This protein is involved in non-homologous recombination and the control of its production paves the way for the development of strains exhibiting higher rates of homologous recombination.

A4.R12 - Effects of Ap6, 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 have purified the peptide Ap6 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 chelicera and is fractionated by reversed-phase liquid cromatography (RP-HPLC). The fractions of interest are submitted to a second RP-HPLC so the Ap6 is purified. The effects of Ap6 on Kv-channels are being evaluated by patch-clamp. To date, 48,22ug of Ap6 have been purified by HPLC and electrophysiological tests were performed on HEK 293T cells that expressed Kv 1.1 channels and L292 cells expressing Kv 1.3 channels.

A4.R13 - Transcriptional dynamics in transgenic plants overexpressing genes related to plant immunity

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The effects of biotic and abiotic stresses on agricultural productivity cause considerable risks to food security. The objectives of this study were to identify and characterize the behavior of plant defense genes under different stress conditions using *Arabidopsis thaliana* as a model system. Through genetic transformation, phenotyping, and transcriptome sequencing, the behavior of different categories of candidate genes were investigated when plants were exposed to damage caused by different plant pathogens (Meloidogyne incognita*, Fusarium oxysporum f. sp. conglutinans*). In the various systems studied, overexpressed genes AdEXLB8 and AsTIR19, which originated from wild Arachis species, caused statistically significant transcriptional modifications in transgenic plants and altered the manner in which the model species responded to the applied stresses. Transgenic Arabidopsis plants with the *Arachis stenosperma* NLR gene (AsTIR19) exhibited increased tolerance *to F. oxysporum f. sp. conglutinans*. By comparing transgenic OE and WT plants were identified 455 upregulated and 323 downregulated differentially expressed genes (DEGs) when compared to wild-type plants. Gene Ontology (GO) categories were most enriched in the biological categories of response to abiotic or biotic stimulus, response to stress and signal transduction, while receptor binding or activity and extracellular were also enriched. For the expansin gene (AdEXLB8) originating from *Arachis duranensis*, *A. thaliana* overexpressed a total of 485 significantly DEGs when comparing against the wild type. Analysis revealed significant GO biological processes enriched in the differentially expressed gene set, including jasmonic acid biosynthetic process, response to hypoxia, defense response to fungus, and regulation of response to stress. This global transcriptomic analysis revealed insights into the molecular mechanisms underlying the role of these genes in plant stress responses.

A4.R14 - Development of a molecular toolbox for genome editing of the minimal cell by crispr/cas9

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Conceptually, a minimal cell is an organism with a reduced genome in which all genes are essential for homeostasis, replication and survival. The objective of creating a minimal organism is to understand the requirements for life and to build new programmable cells. With this objective, the bacterial strain JCVI-syn3.0 was created. Based on the natural genome of Mycoplasma mycoides, the JCVI-syn3.0 cell has the smallest genome ever reported, a synthetic genome with only 438 genes (531 kb). However, around 17% of its genes are still unknown. To better understand its gene content, new molecular tools are necessary since, as its natural precursor cell, the JCVI-syn3.0 has limited known mutagenic mechanisms. Our proposal to circumvent this problem is to introduce a CRISPR/Cas9 machinery coupled to the homologous recombination system, RecET, which will enable guided modifications. For the introduction of Cas9 and RecET into the JCVI-syn3B genome, a JCVI-syn3.0 derivative strain that has cre/lox recombination sites, an integrative plasmid was designed containing the recombinases genes under the control of the tetracylline inducible promoter. Once the strain capable of expressing Cas9-RecET is established, we will silence a gene with an already known phenotype (JCVISYN3B_0004, kgsA, which confers Kasugamycin antibiotic sensibility) to confirm the activity of the system. For that, a suicide plasmid carrying the guide RNA and a homology sequence will be inserted into the synthetic bacteria. We will delete the gene or replace it with a reporter gene. Both mutations will be confirmed by sequencing, phenotypic characterization assays, and finally, expression analysis through qPCR. The introduction of this mechanism into the minimal cell might be used for guided mutagenesis, allowing the functional study of unknown genes, contributing to the development of programmable cells.

A4.R15 - Analysis of miraculin genes in the incompatible interaction of *Meloidogyne incognita* with resistant *Coffea arabica*

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Meloidogyne incognita is a very aggressive nematode especially on coffee (*C. arabica*) crops even leading to plant death. Brazil, as the world's largest coffee producer, experiences severe impacts on its coffee production due to the presence of this pathogenic nematode. Controlling *M. incognita* has become an escalating challenge, with conventional chemical nematicides experiencing diminishing effectiveness. The consumer markets for coffee increasingly demand greener technologies, emphasizing biotechnological solutions. The molecular-level understanding of the *M. incognita*-coffee interaction is limited, highlighting the crucial need for such knowledge in the development of these technologies. Differential gene expression analysis are a good path to start these technologies' development. Here, we studied the gene expression profile of the early interaction between resistant coffee roots and *M. incognita*. The Kunitz superfamily and miraculin-like genes showed an evident modulation in their expression in this scenario. 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 interacting with resistant coffee genotypes, demonstrating that miraculin genes are related to RKN early infection. GO enrichment analysis has brought to light some candidate genes for molecular strategies to promote plant health. Furthermore, other pathogen-systems involving nematodes and other pathogens have shown deregulation at the miraculin gene´s expression, making those a possible target for molecular control of these pathogens.

A4.R16 - Analysis of the impact of reduced cholesterol synthesis on viability and oxidative stress parameters during microglial infection with the Zika Virus

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The Zika virus (ZIKV) belongs to the Flavivirus genus, which cause a significant health impact, especially in tropical countries. Viruses induce molecular and metabolic changes to facilitate viral replication, and among these alterations, those in lipid metabolism, particularly in cholesterol metabolism, play a role in various stages of the viral cycle. Cholesterol modulation can interfere with viral entry, replication and viral particle assembly. The Zika virus is known for its tropism for the central nervous system, capable of causing damage and neurological dysfunction not only in neonates but also in adult hosts, including harmful microglial activation in post-infection recovery. Therefore, this study aims to analyze whether reducing cholesterol synthesis via statins modulates parameters of damage and microglial activation in ZIKV infection. The cell lineage and virus isolate utilized were C20, a human microglial cell line, and ZIKV PE243. The cholesterol synthesis-reducing agents used were simvastatin and rosuvastatin. Mitochondrial viability was measured using the MTT assay, and reactive oxygen species (ROS) in the mitochondria and cytosol were assessed using the probes CELLROX Green and CELLROX deep Red, respectively. It was observed that the reduction in mitochondrial viability by ZIKV was restored in the presence of rosuvastatin but not simvastatin. In the CELLROX assays, it was observed an attenuation in cytosolic ROS only in the presence of rosuvastatin. The response difference between both statins may be, at least partially, explained by differences in absorption, IC50, and cytotoxicity. The reduction in parameters associated with damage in the infection indicates a promising effect of cholesterol reduction in the context of ZIKV infection in microglia. Moreover, the generation of ROS, a marker of microglial activation, was reduced in the presence of rosuvastatin, potentially ameliorating in case of a dysfunctional microglial activation phenotype.

A4.R17 - Evaluation of the mCipA protein as an enhancer of fungal cellulase activity in the deconstruction of brachiaria

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Brazil has remarkable potential to boost its bioeconomy through the exploration of its microbial biodiversity for the production of biological inputs. Focusing on animal nutrition, we highlight the use of enzymes to optimize digestive processes, promoting the health and productivity of livestock. In the degradation of lignocellulose, the bacterium *Clostridium thermocellum*, notable for its efficiency, stands out for organizing enzymes into complexes called cellulosomes. The study focuses on characterizing the secretomes of *T. reesei* RUT-C30 and *T. Harzianum* TR274, investigating possible synergy with mCipA from *C. thermocellum* expressed in E. coli BL21 (DE3). The hypothesis is that the presence of CBM3 and two cohesin I domains in mCipA enhances the cellulolytic activity of fungal secretomes in the deconstruction of brachiaria, an important carbon source in animal nutrition. Liquid cultivation in a minimal medium using brachiaria as a carbon source is the adopted strategy for obtaining the secretome. FPase and CMCase assays on the secretome of T. reesei RUT-C30 indicated that brachiaria induces the production of cellulolytic enzymes. Biochemical characterization, including optimal pH and temperature, highlights the adaptability of these enzymes. The expression of mCipA by autoinduction is followed by protein analysis through SDS-PAGE and WesternBlot, with quantification performed in a BCA assay. Protein purification occurred through affinity chromatography. Hydrolysis assays of brachiaria and Avicel are being conducted to evaluate synergy between purified mCipA and fungal secretomes. Future steps include new constructions of mCipA for specific evaluation of its domains in synergy with fungal cellulases and new phases of biochemical characterization of the secretomes..

A4.R18 - Unravelling the Baculovirus-Orthomyxovirus connection: insights from the GP64 gene transfer and host specificity analysis

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The Baculoviridae family includes insect-infecting viruses with large circular double-stranded DNA genomes. The Alphabaculovirus genus specifically infects lepidoptera and is divided into Group I and Group II. Each group has a major envelope glycoprotein, GP64 and F protein, respectively, crucial for viral budding and cell entry. GP64 shares structural and sequence similarity with the envelope glycoprotein (GP) of Thogotovirus, a distant virus belonging to Family Orthomyxoviridae (ssRNA-), suggesting the horizontal gene transfer (HGT) from an orthomyxovirus ancestor. However, more evidence is needed to support this hypothesis. To explore this connection, and if thogotovirus GPs can be functional homologs of GP64, we replaced the gp64 gene of AcMNPV baculovirus with the GP of Apis thogotovirus 1 (ATHOV-1), sequenced in our lab from honeybees samples. In vitro experiments involving viral passage, virus titration, western blot, and qPCR showed that this modified GP rescued baculovirus infectivity in lepidopteran cell lines. Additionally, it functioned as a fusogen, as confirmed by pH-sensitive syncitia formation assay. Furthermore, fluorimetry and cell cytometry assays revealed that this GP significantly enhanced baculovirus entry and gene transduction in mosquito cell lines. We also conducted cryo-electron microscopy analysis of these recombinant viruses that indicate diferential incorporation GPs to the viral envelope, a possible determinant of functional homology between these proteins. In this work we also characterized the genome and phylogeny of a novel thogotovirus from a lepidopteran insect, obtained by datamining in the SRA database, which clusters with baculovirus gp64 in the GP-based phylogeny. Although phylogenetically more closely related to baculoviruses, this virus GP was not able to rescue the infectivity in the absence of gp64.

A4.R19 - 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 any others commodities, being recognized for years for its importance in animal and human nutrition. Recently, the consumption of soybean proteins has been increasing due to its beneficial effects on human health and the growing interest in alternative sources of more environmentally responsible and cruelty-free meat, such as plant-based meat. Leghemoglobin is an oxygen-binding hemoglobin present in legume nodules, that shows a great potential to be the base of the development of a plant-based meat with a more “pleasant” taste. Despite that, soybean also produces allergenic proteins, harmful secondary metabolites, and carcinogenic elements. Although many of these nutritive proteins are lost during soybean industrial processing to produce animal food and other products. Here we show the establishment of a cell-free expression system for the production of synthetic soybean proteins as an interesting tool to produce specific 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. We have expressed five different soybean proteins of interest, and five leghemoglobin variants, that were purified by protein-linked polyhistidine tag affinity chromatograph. Experiments have been carried out for the utilization of soybean residues as liposomal structures to encapsulate synthetic proteins. 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 in a greater rate than in the original organism.

A4.R20 - Fungal enzyme biopropection for application in animal nutrition

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Brazil has privileged conditions to develop its bioeconomy, as it possesses an enormous microbial biodiversity that can be a source of different bioproducts. The microbiological biodiversity of the Cerrado, the predominant biome in the central region of Brazil, has been studied to prospect for commercially relevant bioinputs. In this sense, among various biological raw materials and application areas, enzymes from filamentous fungi in Cerrado soil can be used as zootechnical additives (enzyme cocktails) in ruminants. This work focuses on the bioprospection of cellulases from seven fungal isolates from Cerrado soil, as well as the molecular and morphological identification of four of these isolates (1, 15, 16 and 19), in order to select and characterize the secretomes with the best efficacy in degrading the target substrate (Brachiaria brizantha cv. Marandu). The fungi were grown in liquid medium with Brachiaria as a carbon source to induce the synthesis of cellulolytic enzymes. From the secretome, collected through centrifugation and filtration, microenzyme assays were conducted to detect the activities of endoglucanase (CMCase), total cellulases (FPase), and xylanase. The results of these assays were used as parameters to select the fungi that will continue in the research (Trichoderma harzianum 274 and isolate 19). Regarding molecular identification, the DNA of the four isolates was extracted, amplified (calmodulin, β-tubulin, and ITS genes), and purified. In this context, in order to explore the specific objectives of the project, the next steps are: to sequence the purified DNA samples, identify morphologically the isolates, characterize the selected secretomes, analyze the in vitro digestibility of Brachiaria, optimize the conditions for inducing enzyme synthesis (in a low-cost cultivation medium).

A4.R21 - Use of Chicken Eggshell Membrane (EM) for Treatment of Osteoporosis in Wistar Rats (*Rattus norvegicus*)

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1. INTRODUCTION

Osteoporosis is considered a global 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. Chicken eggshell membrane - EM (eggshell membrane) has demonstrated effectiveness in relieving pain and joint stiffness. Eggshell is a rich source of calcium, and has potential as an industrial raw material for use in applications such as bone metabolism. This study aims to evaluate the effectiveness of EM for the treatment of osteoporosis in ovariectomized Wistar rats (*Rattus norvegicus*), which may contribute to future therapeutic application. 2. MATERIALS AND METHODS In this study, an EM nanoparticle will be developed. Wistar rats (*Rattus norvegicus*) will be used. The treatment will consist of performing intragastric gavage for 40 days (1x/day), with the compounds: serum (200µL), and eggshell membrane nanoparticles (NEM) (20 mg/mL/day). The animals will be divided into 6 groups: G1 - Control Group (saline); G2 - Control group with induction of osteoporosis by inflammation (Dexamethasone) (NEM); G3 – Control group with induction of osteoporosis due to menopause (Ovariectomy) (saline solution); G4 - Group without osteoporosis induction (NEM); G5 - Group with induction of osteoporosis due to inflammation (Dexamethasone) (NEM); G6 - Group with induction of osteoporosis due to menopause (Ovariectomy) (NEM). In all experimental groups, Bone Mineral Density Analysis and microcomputed tomography will be performed. 3. 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 effectiveness in the treatment of osteoporosis.

A4.R22 - Characterization of two alphabaculoviruses isolated from the looper caterpillar, *Chrysodeixis includens* and identification of a cypovirus in mixed infection

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The looper caterpillar, *Chrysodeixis includens*, is a defoliating pest that causes economic damage to several crops in Brazil. Currently, the caterpillar can be controlled using transgenic plants, chemical insecticides and biological insecticides such as baculoviruses. Baculoviruses are highly specific viruses that are lethal to insects and allow for a reduction in the use of chemical insecticides, thus reducing environmental pollution. However, baculoviruses have limitations in their use, such as taking a long time to kill the target insect and sensitivity to abiotic factors such as solar radiation. In this research, two viral isolates of a baculovirus that kills *C. includens* will be analyzed, the ChinNPV-CNPSo168 isolated and the ChinNPV-TB isolated. The aim will be to characterize the pathogenicity of the isolates and to study their genomes at a molecular level in order to associate virulence and genomics. The viruses were amplified in laboratory populations, quantified and used for bioassays. In addition, the viral DNA has been purified and will be subjected to high-performance sequencing to characterize the genome. Early bioassay results of the ChinNPV-TB isolate showed that even at relatively low concentrations of 1 x 106 Obs/ mL, the virus was able to kill 68% of the larval population tested. Also, after nucleic acid extraction, some samples of ChinNPV-168 and ChinNPV-TB showed contamination with small RNA segments, along with baculovirus viral DNA. It was postulated that these segments must belong to a virus of the genus Cypovirus (CPV), which has already been found in mixed infection with baculoviruses. Although they form polyhedra as a protective structure for the virus, as occurs with baculoviruses, CPV infection occurs exclusively in the cytoplasm of the infected cell. Hence, we also intend to sequence this new contaminating CPV that has been identified, at the genomic and ultrastructural level, since no CPV has ever been characterized in *C. includens*.

A4.R23 - Does genetic silencing and Bt genes, used to control the "cotton boll weevil", affect bees?

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The cotton boll weevil (CBW) is one of the pests limiting the expansion of cotton in Brazil. To overcome this challenge, Genetically Modified (GM) cotton resistant to CBW is being developed using RNA interference (RNAi) and Bt (*Bacillus thuringiensis*) technology. However, GM crops may have a negative impact on biodiversity and non-target organisms, such as bees, which are important pollinators. Therefore, biosafety tests are necessary to risk assessment before the commercialization of a new GM organism. This study aims to investigate the lethal and sublethal effects of double-stranded RNAs (dsRNA: dsCHS2 and dsVG) and entomotoxin (CRY23/37Aa) present in GM cotton pollen and ingested by larvae and adults of two bee species: *Scaptotrigona postica* (native) and A*pis mellifera* (exotic). Worker and princess bees (immature and adult) will be evaluated under controlled conditions at the Plant-Pest Molecular Interaction Laboratory (LIMPP) of EMBRAPA CENARGEN. For larvae, the tests consist of three treatments (triplicates): Larval diet (LD) + dsRNA or CRY; LD + distilled and autoclaved water; and pure LD. These tests will be repeated for each dsRNA and CRY. There will be 20 larvae per plate (96 wells and natural food) for *S. postica* and 20 larvae (48 wells plate and artificial food) for *A. mellifera*, totaling 1080 larvae for each bee species. For adults, the tests consist of the same treatments, but instead of LD, syrup (water + sugar) will be used, in quadruplicates, with 10 bees in each one-liter circular container. A total of 720 adult bees of each species will be used. The effects evaluated in larvae will include mortality, developmental time of immatures, and in newly

emerged bees, body mass, cephalic capsule width, and intertegular distance. The effects evaluated in adults will include mortality and locomotion activity. With this work, we hope to contribute to achieving sustainable agriculture with Genetically Modified Organisms in Brazil.

A4.R24 - Occurrence of viruses in vanilla plants in Brazil

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Vanilla (V. spp) is an orchid that is sold worldwide due to its aromatic fruits with high added value. Of the more than 100 described species of the genus Vanilla, 30 occur in Brazil, of which 15 have aromatic fruits. However, Brazil has no tradition in cultivating this orchid, despite the market being very attractive and with high commercial potential. At Embrapa, there is currently an active germplasm bank with around 70 accessions of different species of the genus Vanilla. One of the main phytosanitary problems of using a species for crop production is the emergence of diseases caused by viruses that can cause great damage to the crop and also affect other susceptible species with potential viral spillage. Currently, there are no records of the occurrence of viruses in vanilla in Brazil. The present project aims to investigate the occurrence of RNA viruses in Brazilian vanilla without symptoms of infection using the high-performance sequencing (HTS) method. The project began with the analysis of Vanilla planifolia samples collected from commercial plantations and private collections without apparent symptoms of infection. Total RNA was extracted from *V. planifolia* leaves and subsequent HTS sequencing of a pool of samples was performed. For bioinformatics analyses, the CLC Genomics® program were used to de novo assembly sequences, and from the assembled reads (contigs) a local search was carried out in a RefSeq bank of RNA viruses in the Geneious® program using the BLAST tool. Some contigs showed high identity with members of the genus Allexivirus (family Alphaflexiviridae). Experiments are underway to detect these new ones in individual plants.

A4.R25 - Could *Cryptococcus neoformans* melanin or laccase 1 be involved in inflammation and macrophage death?

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Cryptococcus neoformans is the most common cryptococcosis agent. Annually, there are about 152 thousand new cases of cryptococcosis, 73% of which lead to death. *C. neoformans* produces melanin in a reaction that is catalysed by laccase 1 (LAC1). Although *C. neoformans* melanin has a role in fungus survival in its environment niche, this pigment is also an important virulence factor during the interaction with mammalian host, favouring the infection. The *C. neoformans* capsule, its most critical virulence factor, has already been shown to interfere with inflammation and cell death. However, the role of the melanin and laccase 1 in inflammation and death of the host cells remains unclear. Thus, in this work we study whether melanin and LAC1 modulate inflammatory mediators produced by human macrophages and, consequently, alter their death. Melanin decreased the proportion of macrophages that ingested *C. neoformans* cells. In contrast, LAC1 increased phagocytosis by macrophages in the beginning of infection (2 h.p.i), although the opposite happened in late infection (24 h.p.i). *C. neoformans* promoted NF-kB and IRF pathway activation, however, melanin and LAC1 did not interfere with this process. We could notice that more macrophages died when exposed to *C. neoformans* in comparison with phagocytes that had not been incubated with the fungus; melanin and LAC1 exacerbated this cell death, mainly in late infection, when lithic death is predominant and apoptosis almost inexistent. Caspase 8 (CASP8) is cleaved and activated at the beginning of infection, whereas its cleavage disappeared in late infection. Conversely, Caspase 1 and Gasdermin D were cleaved

in late infection. The activation of these proteins probably explains the higher apoptosis rates seen in the beginning and lithic death. Therefore, melanin and LAC1 from *C. neoformans* could affect inflammation and macrophage cell death.

5. Graduação (A5)

A5.R1 - The Function of Omega-3 DHA in Modulating White and Brown Adipose Tissue and Its Impact on Carcinogenic Parameters in Melanoma Cells

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Introduction: Long-chain polyunsaturated fatty acids of the n-3 series (n-3 PUFAs), such as docosahexaenoic acid (DHA), have protective mechanisms against the development of inflammatory diseases, among them obesity and cancer. DHA was described as an inductor of pyroptosis cell death in certain types of tumor cells. However, the role of n-3 PUFAs in the communication between melanoma and adipose tissues (AT) is unclear. This study aimed to investigate the role of n-3 PUFAs in modulating AT and its function on the carcinogenic parameters of melanoma, particularly in the induction of pyroptosis. Methods: C57/BL6 mice were supplemented or not with omega-3 at a concentration of 1g/kg. Serum, peritoneal lavage, AT, liver, and spleen were analyzed. Additionally, the AT-conditioned medium (CM) obtained from those animals was used to stimulate the B16F10 melanoma cell line in vitro. After stimulation, cell viability and death, and cytokine quantification were assessed. Additionally, the human melanoma cell line MeWo was stimulated with DHA for 48 hours in vitro. Secretion of lactate dehydrogenase (LDH), membrane pore formation, and caspase-1 activation were analyzed. Results: Our data demonstrated that supplementation with omega-3 reduced AT weight and led to an increase in lipid droplet biogenesis of peritoneal cells, in addition to a reduction of reactive oxygen species (ROS). Furthermore, stimulation of B16F10 cells with brown adipose tissue (BAT)-CM from omega-3 DHA-supplemented mice resulted in decreased cell viability and increased cell death. Moreover, the treatment with DHA induced LDH release, increased membrane pore formation, and triggered caspase-1 activation. Conclusion: This study demonstrated the potential of omega-3 supplementation in modulating AT, as well as suggesting the ability of DHA to induce pyroptosis in melanoma cells in vitro. Thus, it provides new perspectives for the use of omega-3 DHA as an adjuvant in the treatment of melanoma.

A5.R2 - NLRP3 inflammasome activation-dependent pyroptosis modulates carcinogenic parameters in human gastric cancer cells

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Introduction: Pyroptosis is a type of programmed lytic cell death that was first discovered in immune cells, but it has garnered significant attention within the field of oncology. In tumor cells, cell death pathways are inhibited as an evasion mechanism. However, the induction of pyroptosis in cancer cells leads to a dual effect on tumor progression. Thus, the aim of this work is to unravel the role of NLRP3 inflammasome activation dependent-pyroptosis in human gastric cancer cells. Methods: AGS cells were stimulated with lipopolysaccharide (LPS) (1µg/mL) for 24h and nigericin (20µM) for 2h and replaced with culture medium (short stimulus - SS), or continuously stimulated with LPS and nigericin (long stimulus - LS), according to the period of the analysis. The mitochondrial viability was assessed by MTT assay. The enzyme lactate dehydrogenase (LDH) release was evaluated by the CyQUANTTM kit. The cell death profile was assessed by annexin-V/propidium iodide (PI). The membrane pore

formation was assessed by PI staining. Cell proliferation was assessed by CFSE staining. Results: It was observed that both SS and LS reduced cell viability in AGS cells, in addition to inducing an increase in lytic cell death, whose effect was most prominent in LS. Moreover, both stimuli reduced the AGS cell proliferation, which was intensified in SS. Both stimuli induced pore formation, but it was more notable in LS, which also showed augmented LDH release in AGS cells. Conclusion: Our data collectively demonstrate that pyroptosis induction exerts an anti-tumor effect on carcinogenic parameters, including the reduction of proliferation and cell viability, increase in lytic cell death and LDH release, and induction of pore formation in human gastric cancer cells. These findings provide novel insights into potential therapeutic strategies against gastric cancer.

A5.R3 - Evaluation of the chemical composition of raw grains from coffea arabica accesses cultivated in an irrigated system

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Coffee, Coffea arabica, is a product that presents differences in the chemical characteristics of the beans, which can generate different quality drinks. Breeding research aims to develop cultivars that are superior in quality. Thus, the objective was to evaluate the influence of genetic variability on the chemical composition of genotypes present in the Embrapa Cerrados germplasm bank. Plants from 20 accessions were grown in an irrigated production system at 1050 m altitude. The fruits were harvested at the cherry stage. Subsequently, the samples were crushed to obtain the concentration of Chlorogenic Acid (5-CQA) and Caffeine (CAF), using the NIRS FOSS 5000 System II type 461006. To determine the Protein (PR) content, the samples were subjected to digestion in perchloric solution, in digester blocks, at 250°C for 30 minutes. The samples were then evaluated by Flow Injection Analysis (FIA). ANOVA was applied, with Scott Knott's test at 5%. The C. arabica genotypes formed eight groups when analyzing the PRs. Genotypes 9 and 15 showed higher significant values, with 10.04% and 9.74%, respectively. On the contrary, genotype 1 presented a value of 5.57%. The PRs contribute strongly to the aroma and flavor of coffee. For 5-CQA, individuals formed 13 groups, with genotypes 5, 11, 10, 8 and 15 having the lowest levels, between 1.09% and 2.07%. The highest value was 7.76%, from individual 19. 5-CQA contributes to the astringency of coffee. As for CAF, the individuals formed 11 groups, with genotypes 13, 1, 15, 12, presenting the lowest significant values, between 0.62% and 0.69%. The highest value was also from individual 19, with 1.42%. The CAF is one of the compounds responsible for the bitter taste of coffee drinks. Therefore, considering the chemical characteristics above, access 15 (IAC 1) has greater potential for producing superior quality beverages.

A5.R4 - Heterologous Expression and Purification of the Cryptococcus neoformans SMT and Structural Characterization of the Aspergillus fumigatus and Candida auris SMTs

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Invasive fungal diseases (IFDs) are a rising global health concern, affecting an estimated 300 million people per year, notably those immunocompromised, and resulting in 1.6 million deaths. Treatment is further complicated by the increasing therapeutic failure of the broadly used antifungals. A putative new pharmaceutical target has been identified in the sterol C24-methyltransferases (SMTs), which are part of the fungal-specific synthesis pathway of ergosterol, acting on the methylation of the carbon 24. However, knowledge about SMTs from species of medical interest remains incipient and no resolved structures are currently available, curbing the exploration of this target.

Therefore, this work aims to characterize the SMTs of three prominent human pathogenic species: *Aspergillus fumigatus* (AfSMT), *Candida auris* (CauSMT) and *Cryptococcus neoformans* (CnSMT). The erg6 genes coding their SMTs were inserted into pET-28a(+) vectors which were then cloned into E. coli B21 (DE3) cells. AfSMT and CauSMT were expressed in self-inducing media and purified through immobilized metal affinity chromatography with nickel columns. The enzymes were used in both their free and ligand-bound states in assembling screening and refinement sitting drop crystallization plates. Crystals of AfSMT and CauSMT were retrieved from these plates and then submitted to X-ray diffraction, which confirmed their proteic nature but produced only low resolution diffraction patterns. CnSMT was assayed for expression and solubility in DE3 cells grown in IPTG supplemented media and a predictive model was produced using Alphafold. SDS-PAGE gels showed CnSMT to be highly expressed in the tested conditions but to not be present in the soluble fractions of the cell lysate, suggesting the formation of inclusion bodies during expression. Further optimization assays will be carried out for the better crystallization of AfSMT and CauSMT and for the expression of soluble CnSMT.

A5.R5 - Structural and kinetic studies of venom serine proteases from animals of the Toxicofera clade

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Most snakes have venoms that can be lethal to several species, and among the bioactive compounds found in them, Snake Venom Serine Proteases (SVSPs) stand out. However, little is known about lizard venoms, but their toxinological profile is known to include serine proteases similar to those found in snake venoms. These animals are grouped into a monophyletic group, the Toxicofera clade, whose name reflects the presence of venom. These enzymes have a broad biotechnological potential and could be used in developing drugs for hemostatic and inflammatory disorders, depending on which stage of the coagulation cascade they interfere with. Consequently, there is a great interest in studying the catalytic properties, structural characteristics, and the evolution of this class of enzymes. Therefore, three serine proteases were chosen for this project: an ancestral SVSP from the Viperidae family (AnV_tox), constructed using ancestral sequence reconstruction technique, Hs_tox1 from Gila Monster (*Heloderma suspectum*) venom, and Vk_tox1 from Komodo Dragon (*Varanus komodoensis*) venom, aiming to characterize them enzymatically and structurally. The methodology involves transforming *Komagataella phaffii* cells via electroporation, heterologous expression assays, and purification using a HisTrap column. Once the protein is pure, crystallography assays will be conducted through hanging drop vapor diffusion, detection of possible plasminogen activation activity using a synthetic fluorogenic substrate, and assays with blood plasma, varying the enzyme concentrations, observing whether clot formation occurs or not. The project continues the doctoral project of a student within the program. Thus, there are preliminary results such as the construction of the ancestral SVSP sequence, cell transformation containing genes for AnV_tox and Vk_tox1 production, purification protocols, and some activity assays.

A5.R6 - Paradigmas do uso da oxibenzona e seu impacto ambiental no ecossistema de Brasília

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Due to the great incentive of use, about 14,000 tons of protectors reach the oceans annually and although each filter has a maximum permitted concentration, in recent years, studies have reported the toxic effect that the accumulation that UV filters can have in different ecosystems, having its widespread occurrence in the

environment. Among these solar filters, oxybenzone (oxybenzone, also known as benzophenone-3 or BP-3) is now among the most widespread environmental pollutants, often detected in plants, fish, fish, poultry and microorganisms. Being identified as a phototoxic agent, genotoxic and hormone deregulation and coral whitening. This research has as its problems to resolve the existence of UV filters, such as oxybenzone, in the water systems of Brasilia and whether such amounts found of this phototoxic substance represent a threat to the environment and health of the population. Evidence of research indicates that this substance has impacted the ecosystem of other countries in which bioaccumulated was found; Therefore, there is a strong need to investigate this problem that in Brazil that, being a country with a high index of solar incidence, has a high consumption of products that has this molecule active ingredient. Thus, the objective of this research is to qualitatively and quantitatively verify the presence of benzophenone in the waters of Brasilia and whether such quantities found a threat to ecosystem and human health directly or indirectly.

A5.R7 - Metabolic Syndrome: The use of computational tools in the search for new molecular targets

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Introduction: Molecular research often makes use of computational tools coupled with in silico analysis, using proteins stored in genomics and proteomics databases to identify new bioactive molecules, thus building bridges between clinical biomedical data and pharmaceutical research. Objective: To describe the metabolic alterations and the resulting pathophysiological mechanisms in Metabolic Syndrome, as well as to identify and select, using computational tools, new possible inhibitory molecular targets for PTP1B. Methods: Bibliographic research using the Scielo, Lilacs and Pubmed databases. Research in a virtual environment, based on the strategies of virtual screening and Target fishing in the fishing and mapping of bioactive targets and ligands. Results: 8,378 ligand molecules were fished through the PubChen and CHEMBL platforms and molecule banks, resulting in 120 ligands after applying the established criteria. 1056 targets were recognized by applying reverse docking and selecting the complexes. Conclusion: PTP1B is a highly validated target by the scientific community, concluding its interaction with other molecules and the possibility of new pharmacological studies on its complexes.

A5.R8 - Uso do teste do cometa para avaliar o dano ao DNA em *Saccharomyces cerevisiae*

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DNA damage arises from a complex interaction between endogenous and exogenous factors, which include physical and chemical agents and errors inherent in cellular replication. In the context of chemical agents, hydrogen peroxide (H2O2) is considered a relevant oxidizing agent in eukaryotic cells, capable of triggering oxidative stress and DNA damage. The precise assessment of this damage is carried out using advanced techniques, notably the comet test. The fundamental principle of the comet test involves the destruction of cell membranes, followed by electrophoresis of the nucleic acid in a thin layer of agarose. This study, in particular, focused on optimizing the comet test for yeast cells, enabling accurate detection of damage induced by oxidizing agents such as H2O2. Optimization encompassed determining the enzyme concentration for cell wall digestion (300 U/mL), cell optical density at 600 nm (0.8), the variable effect of H2O2 concentrations (5 and 10 mMol) and adjustments. in the electrical quantities of electrophoresis, established at 0.3 V/cm. The results showed the effectiveness of these modifications by revealing a proportional increase in the "comet tail" according to the increase in H2O2 concentration, indicating

greater damage to DNA. The possibility of evaluating damage and repairs in the DNA of yeast cells not only provides a more refined view of genotoxicity, but also opens new horizons for research related to DNA repair. This methodology, based on meticulous adjustments of the comet test protocol, stands out as a significant advance in understanding the mechanisms underlying genetic damage at the molecular level. By employing these adaptations that have refined the sensitivity and precision of the assay, it is possible to consolidate the comet test as a robust tool in the investigation of the complex phenomena involved in DNA damage and repair.

A5.R9 - Isolation and identification of pollen grains fermenting microorganisms

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Meliponiculture consists of the rational breeding of stingless honey bees, represented by the taxonomic tribe Meliponini. This group of insects inhabits tropical and subtropical regions and has eusocial habits. In Brazil, approximately 250 species are described as stingless, among them, the species *Frieseomelitta varia*, popularly known as yellow marmalade, stands out. Products derived from bees, such as honeys, propolis and pollens, are known to have a rich microbiota, the diversity of which includes lactic and alcoholic fermenting species. The main bacteria associated with stingless bees are Lactobacillus, Bacillus, Streptomyces, Clostridium, Staphylococcus, Streptococcus, Enterobacter, Ralstonia, Pantoea, Pseudomonas, Fructobacillus, Lysinibacillus and Neisseria. On the other hand, the most frequently described yeast species isolated from stingless bees belong to the Starmerella genus. In addition, the fermentation of bacteria and yeasts is the main way of preserving honey and transforming pollen into bee bread (stored food). The project aims to develop fermented food products from bee derived products, in order to apply academic knowledge to the needs of the Brazilian beekeeping sector. Therefore, the aim is to develop cerratense products with standardization and characteristics that make it possible to include them in the market. In view of the microorganisms present in bee products, it is important to characterize and identify them, as well as cultivate them, in order to know them morphologically and physiologically, since their metabolites will strongly influence the characteristics of fermented beverages. To this end, experimental and theoretical data (feasibility studies) will be generated for the different honeys, pollens and propolis, based on the collections made, and proposed routes for better economic use for the sector will be studied.

A5.R10 - Produção de proteínas Enhancinas de baculovírus na forma de cristais para o uso como controle biológico de pragas agrícolas

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The Baculoviridae family consist of viruses that infect exclusively insects, and are widely used for the biological control of agricultural and forest pests. They are also known for their biotechnological applications as heterologous protein expression vectors and potential gene therapy vectors. Alphabaculovirus and Betabaculovirus are a genera from this family that encode a metalloprotease named Enhancin, a protein that facilitate baculovirus infection by degrading insects intestinal peritrofic matrix. The aim of the present study is to produce Enhancin proteins incorporated into protein crystals, under a novel crystallization strategy, for the stable formulation and use as an adjuvant for baculovirus based biopesticides. Chimeric proteins containing a peptide coding for the alpha helix (H1) of Thyrinteina arnobia cypovirus (TharCPV) polyhedrin gene, co-expressed with the full polyhedrin protein, allows for their crystalization. Primarily, we have extracted the genomic DNA of the Spodoptera frugiperda granulovirus

(SfGV) from the occlusion bodies of a viral isolate. Specific oligonucleotides were designed and used to amplify and isolated the Enhancin 1 and 2 genes of SfGV by polymerase chain reaction (PCR). The DNA fragments containing the genes will be cloned in a modified plasmid vector, genetically fused with the H1 crystalization domain of TharCPV. The work aims to produce such proteins in insect cells though the construction of recombinant baculovirus using the Bac-to-Bac expression system. Finally, we aim to produce and purify polyhedrin crystals containing SfGV Enhancins and conduct tests that measure their ability to enhance the pathogenicity of baculovirus against insects, attempting to increase even more their biopesticide capability.

A5.R11 - Caracterização do efeito imunológico da produção de anticorpos terapêuticos por leveduras sobre a mucosa em modelos animais

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Anti-CD3 is a monoclonal antibody that binds to the CD3 molecule on the surface of T lymphocytes and stimulates their proliferation. Anti-CD3 partially mimics stimulation by antigen-presenting cells by binding to the receptor, producing an initial signal that activates intracellular signaling events, leading to cell expansion and immunoregulation. Ulcerative colitis is an inflammatory bowel disease that causes tissue damage in the intestinal mucosa, resulting from an imbalance in the populations of CD4+ T cells, which mediate intestinal inflammation. Regulatory T cells are responsible for regulating this imbalance and inflammation by acting on pathogenic T cells. Intravenous treatment with anti-CD3 was effective in stimulating the production of IL-10, acting on pathogenic cells and regulatory T cells Tr1 and Foxp3+ Treg. However, it presented side effects due to the exacerbated release of cytokines at the application site, which could lead to an insufficient immune response. Administration of probiotic organisms can generate beneficial effects in the intestine and restore the enteric microbiota. Various genera of bacteria and yeasts are associated with this probiotic effect. *Zymomonas mobilis* is a Gram-negative, facultative anaerobic bacterium. *Z. mobilis* has stood out in the context of therapeutic applications, as doctors in the city of Recife used *Z. mobilis* AG11 and CP1 to treat patients with intestinal disorders, mainly chronic colitis. Here, we explore *Z. mobilis* bacterium as a potential recombinant probiotic, due to its probiotic characteristics, ease of genetic manipulation, and its efficiency in the heterologous production of proteins. This work aims to adapt a lymphocyte activation protocol by probiotic bacteria for genetically modified microorganisms that produce anti-CD3 antibodies.

A5.R12 - Análise do Resistoma em Bactérias em Amostras de Água do Pantanal: Identificação e Caracterização dos Genes de Resistência

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Antimicrobial resistance in bacteria in the environment is a threat to human and animal health that raises concern. Antibiotic overuse and selective pressure contribute to an increase in the resistome - the collection of all antibiotic resistance genes from pathogenic and non-pathogenic bacteria in a microbial community. Interpreting the resistome is important to monitor the problem with antimicrobial resistance to adopt strategic measures appropriately. Water and aquatic ecosystems are an important reservoir for the horizontal spread of resistance genes, as they allow the continuity and transfer of resistant bacteria. The Pantanal is one of the largest humid areas on the planet and is located in the center of South America, in the Upper Paraguay river basin, an area of

138,183 km², with 65% of its territory in Mato Grosso do Sul. This biome has a wide biodiversity of species in general and bacterial species. It is an important example of an advantageous environment for the dissemination of resistance genes, which can affect the balance of the Pantanal's aquatic ecosystem, compromising water quality and local biodiversity, capable of causing a harmful impact on the environment, human health and animal. This project aims to investigate the resistome of bacteria in water samples from the Pantanal, with the aim of identifying, characterizing, and monitoring the resistance genes present in this ecosystem, with the possibility of mitigating risks to human health and the environment. Bacterial DNA sequencing will be carried out in water samples collected in different locations in the Pantanal. The investigation seeks to contribute to the understanding of antimicrobial resistance in aquatic ecosystems, specifically in the Pantanal, in Mato Grosso do Sul.


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